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Genetic impacts of stocking on indigenous brown trout populations

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Steve Killeen

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Head of Science

Executive Summary

There has been concern about the impacts of extensive stocking of fertile farm-reared brown trout on the genetic integrity of wild trout populations. This report surveys the evidence of genetic impacts on indigenous brown trout populations in Europe, caused by introductions of fertile farm-reared trout. The likely effects of such genetic changes are examined in the context of trout fisheries management in England and Wales.

Native wild brown trout, species complex *Salmo trutta*, are genetically diverse. British and Irish trout populations form a geographical mosaic derived from one or more of at least six lineages that evolved separately during the last Ice Age. Colonisation occurred after the ice retreated, around 14,000 years ago, and populations have diverged further since then as a result of natural selection and genetic drift.

The effective management and conservation of brown trout and other salmonids requires the recognition and conservation of genetic diversity within and among populations. Genetic diversity is essential for populations and species to be able to respond to both short-term and long-term environmental challenges. Loss of genetic diversity leads to lowered abundance, lowered recruitment and greater uniformity in life history characteristics. Genetic diversity results in phenotypic diversity and thus in diversity of angling opportunity and experience. Genetic diversity is also an integral component of biodiversity.

There is no evidence of reduced genetic diversity within and among brown trout populations in England and Wales compared to other areas in Scotland, Ireland, and north west Europe where little if any supplemental stocking has been undertaken. There is therefore no evidence that previous supplemental stocking with farm-reared brown trout has resulted in widespread decline in native genetic diversity in England and Wales.

The first brown trout farms in Britain were set up (1868-1873) by Armistead at Solway (nr Dumfries, Scotland) and by Maitland at Howietoun (nr Stirling, Scotland) and were based substantially on Loch Leven (Scotland) broodstock. Many current farm strains of brown trout are derived, directly or indirectly, from these Solway and Howietoun strains, although some appear to have a genetic influence from Denmark or elsewhere in north west Europe. Due to common origin and subsequent interchange of trout, many current farm strains of brown trout are genetically similar.

Farm-reared brown trout differ from wild brown trout in three main ways. They differ genetically due to founding effects and to subsequent domestication in culture, involving artificial selection, relaxed natural selection and genetic drift. They differ phenotypically in behaviour, physiology and morphology. They also differ in their learning opportunities especially in relation to feeding and anti-predator behaviour.

Domestication resulting in genetic changes can occur in the first generation of farm-rearing and substantial changes can occur in a few generations with survival and breeding ability of farmed fish in the wild being reduced by more than 50%. In some cases farm brown trout strains have been in culture for more than 30 generations.

Given the many changes that occur during farm-rearing and domestication of brown trout, it is not surprising that supplemental stocking with such trout often has little or

no impact on a wild population, either in terms of increasing fish numbers or in producing genetic changes. However, this does not mean that stocking can continue with impunity. The more that stocking is carried out the greater is the likelihood of detrimental genetic changes occurring. In addition, stocking can result in a reduction of fitness in a wild population due to low fitness of hybrids but without causing significant genetic changes.

Supplemental stocking of farm-reared brown trout has had a lesser genetic impact on wild populations than might be anticipated for many reasons including: number stocked relative to wild fish; reduced survival in the wild; transport and stocking technique; natural environmental conditions; physiological differences; morphological differences; altered territorial, feeding and predator-avoidance behaviours; greater angling susceptibility; reduced breeding ability; assortative mating; reduced survival of hybrid offspring. Difference in time of spawning (a high heritability trait) of farm-reared and wild brown trout is also a major factor in reducing introgression.

The extent of genetic changes in wild brown trout populations due to interbreeding with farm-reared strains of brown trout has been studied in many European countries, especially Denmark, France and Spain. The extent of this introgression is variable and unpredictable. It shows no obvious relationship to the extent of stocking. Introgression, in most cases, is much less than might be anticipated given the scale of supplemental stocking and the fact that it has been carried out for over 100 years in some cases. In some situations, in spite of extensive stocking history, no detectable introgression is present or it is very low (<5%). A majority of populations shows less than 25% farm-gene introgression while a minority of populations shows extensive introgression (50-80%) and a few appear to be 100% farm origin.

Stocked farm-reared brown trout result in greater introgression in the freshwater component in a river compared to the anadromous (sea trout) component, as a result of farm-reared brown trout that became anadromous experiencing high mortality at sea. Given that anadromy is a threshold quantitative trait (i.e heritable), stocking with farm-reared brown trout is likely to increase the freshwater component in a river and reduce the sea trout run.

Although domesticated farm-reared brown trout have poor survival in the wild, some individuals do survive and breed successfully. This has been erroneously taken as an indication that stocking is beneficial. The fact that some stocked fish survive does not mean that the total number of fish in the water is increased. The stocked fish may survive and breed at the expense of an equal, or even greater, number of the wild fish. That is, even though a proportion of the angling-catch consists of stocked fish or their offspring, the total catch could be reduced as a result of negative interactions between the stocked and wild fish.

While reduced survival of farm-reared brown trout is not a problem in itself, the damage occurs when individuals that do survive interbreed with wild brown trout and these hybrids backcross to the wild population in subsequent generations. As hybrids have lower survival and reproduction (outbreeding depression) than pure wild individuals this results in a lowering of fitness in the wild population and a reduction in the number of individuals available for exploitation and breeding. This lowering of fitness can occur as a result of stocking without significant genetic changes being detectable in the wild population. Indeed the lower the fitness of the hybrids the greater the reduction in population fitness but the lower the genetic change will be as introgression requires that the hybrids backcross with wild individuals.

There is no empirical evidence to support the hypothesis that introgression from farm-reared brown trout improves the fitness of wild brown trout populations. For a few populations that have lost genetic variability due to small size and physical isolation or severe bottlenecks it may be advantageous to introduce farm-reared or other non-native brown trout. Such introductions should not exceed one or two individuals per generation.

Reduction in fitness as a result of interbreeding between farm-reared and wild brown trout does not require that there are adaptive differences among wild populations but only that, as a result of genetic changes in farm-reared brown trout during founding and domestication, hybrids between wild and farm-reared trout have lower fitness than wild fish. However, the extent of fitness reduction will be increased due to local adaptive differentiation.

As most stocking involves effectively a small number of farm strains, breeding of stocked fish results in genetic homogenisation of wild populations. This can result in the loss of local adaptations and loss of overall genetic variability, which is likely to be important in allowing brown trout to continue to adapt to changing environmental conditions such as global warming and new diseases.

Local adaptation can be based on changes at a relatively small number of gene loci and can occur within a few generations. In brown trout, precise timing of spawning and emergence, and timing and directional aspects of migration are important in ensuring fitness under the environmental conditions specific to individual waters. Consequently these aspects often differ among wild brown trout populations.

Effective straying rates, in terms of gene flow, among most brown trout populations are probably less than 1%. This low level of gene flow is advantageous as it prevents loss of genetic variability in small populations, and allows the spread of favourable alleles, without preventing differentiation among populations due to local selection and drift. As with many things, a little bit does good while a lot is harmful!

This report advocates supportive breeding, a form of stocking in which the broodstock is taken from the native wild population each generation, and reared in a hatchery. This avoids introducing non-native genes into the population. The high survival to the fry, parr or smolt stage in the hatchery relative to that in the wild is often used as argument in favour of hatchery intervention. However, hatchery intervention is only justifiable when the overall life cycle survival of introduced fish is significantly higher, that is there is a net survival advantage from egg to egg over naturally produced fish. Protocols are recommended to reduce genetic impacts on the wild population, including imposing a factorial mating system, and keeping time in the hatchery as short as possible.

Supportive breeding is only recommended where there is an identifiable problem preventing the natural population from reaching its full capacity. Habitat restoration should take priority over stocking, if at all possible.

In summary, the extent of introgression in any stocked population is impossible to estimate from its previous stocking history and can only be determined by genetic analyses on the specific population concerned. It cannot be assumed, therefore, that just because a population has been stocked extensively in the past that the native genetic composition has been lost and that stocking with fertile farm-reared brown trout can continue in the future without further detriment. The assumption, until such times as proven otherwise, for each population should be that it has not been substantially changed in its genetic make-up as a result of previous stocking and should be protected from further stocking. The genetic changes caused by stocking with farm-reared brown trout are almost always detrimental to the fitness and survival of individual populations and potentially to the long-term survival of the species. Therefore, it is recommended that all supplemental stocking (see definition) with fertile farm-reared and non-native brown trout in England and Wales is prohibited. The only forms of stocking permitted should be supportive breeding, or stocking with sterile triploid fish.

In some situations of self-sustaining wild brown trout populations it may be necessary for non-biological reasons (for example angling) for supplemental stocking with fertile farm-reared brown trout to continue, at least in the short term. If so, such stocking should be undertaken with takeable (legal) sized trout, as these fish are the least likely to survive to breed. All fertile farm-reared brown trout should be physically marked before stocking. Anglers should be encouraged to remove all farm-reared trout caught and practice catch-and-release with wild trout.

Nomenclature used in this report

Many of the following terms are used with different meanings by various authors. The meanings used in this report are defined here, to avoid confusion. See the glossary in Appendix I for other definitions.

The name **brown trout** is used in the taxonomic sense and indicates all life history types within the species complex *Salmo trutta*. It includes **sea trout** as well as **freshwater trout** (from rivers and lakes).

Wild brown trout refers to trout that have resulted from natural reproduction in the wild.

Native wild brown trout refers to wild brown trout that have resulted from natural colonization of the water system.

Feral wild brown trout refers to wild brown trout that have resulted from stocking of farm-reared trout in a previous generation. In some literature, these are referred to as **naturalized**.

Farm-reared brown trout refers to brown trout that have resulted from artificial reproduction and closed culture for more than one generation. A permanent broodstock line is maintained on the farm.

Hatchery-reared brown trout are fish whose broodstock is taken from the wild each generation. Offspring are reared under culture conditions until the fry or later stage before release. The term hatchery is used by some authors even when a permanent broodstock line is maintained.

Non-native refers to brown trout that are the result of direct translocation without hatchery rearing beyond the eyed egg stage.

Stocking refers to the artificial release of brown trout that have been reared in a farm or hatchery for a period of time. A number of different types of stocking are relevant to brown trout (other fish stocking can be classified in different ways, see for example Utter and Epifanio, 2002):

1. **Put-and-take stocking** where stocking takes place into a water with little or no natural spawning, such as artificial reservoirs and dams. The aim is usually to produce a viable angling fishery.
2. **Stocking to restore** a self-sustaining population in a water where the previous population had become effectively extinct, or to establish a self-sustaining population in a water where brown trout did not previously exist.
3. **Supplemental stocking** with brown trout of farm or non-native origin to supplement a wild population with the aim of increasing the number of trout available for angling or conservation:
    - To increase directly the number of fish available where the natural production is insufficient to meet angling demand (harvest supplementation).
    - To augment a depleted population where the reason for the decline has been identified and rectified. This requires that the stocked fish contribute to offspring recruitment in subsequent generations without diminishing the recruitment of the existing wild population (population supplementation).

4. **Supportive breeding.** Stocking with first generation hatchery-reared offspring of wild native trout, again with the aim of increasing the number of trout, generally where there is an identifiable life history bottleneck to wild production, such as a lack of spawning grounds but suitable habitat for later stages.

Only supplemental stocking and supportive breeding are considered in this report. All subsequent references to stocking refer to supplemental stocking.

Fitness refers to the contribution of an individual to future generations. Fitness involves both overall life cycle survival and reproductive success, including mating and offspring survival to reproductive age. **Population fitness** is the mean fitness of all members of a population.

Introgression is the introduction of genes (alleles) from one population (or strain) into another by means of hybridisation and backcrossing of hybrids to parental types. Some papers use it in a more general sense as the proportion of farm genotypes in a wild population, thus including farm x farm offspring as well as all hybrids. Since it can be difficult to differentiate between these in some cases, introgression is used in the looser sense here.

Unless otherwise stated, all references to farm-reared brown trout and other salmonids refer to **fertile, diploid fish**. While the main focus of this report is on brown trout, information on other salmonids is included where appropriate. The other species cited are: Atlantic salmon (*Salmo salar*), rainbow trout, including the anadromous form, steelhead (*Oncorhynchus mykiss*), coho salmon (*O. kisutch*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tshawytscha*) and lake char (trout) (*Salvelinus namaycush*).

Contents

1 Genetic diversity in the brown trout

There has been concern about the impacts of extensive stocking of fertile farm-reared brown trout on the genetic integrity of wild trout populations (for example, Laikre 1999). The Environment Agency will review its policy on trout stocking in 2007, as part of its National Trout and Grayling Fisheries Strategy. This report was commissioned to contribute to this policy review. It surveys the evidence concerning the genetic effects of stocked brown trout on indigenous populations in Europe and aims to assess whether those effects are detrimental.

1.1 Origins of brown trout genetic diversity

The native range of the brown trout is from Iceland and north west Russia south to the Atlas Mountains of Morocco and east to the Caspian and Aral Sea drainages in Asia (MacCrimmon and Marshall, 1968). It is typified by remarkable variation in its external appearance, behaviour, ecology, life-history and other aspects of its biology. This variation led nineteenth century naturalists to describe over 100 different species and sub-species of brown trout, including about 20 in Britain and Ireland. The recognition that brown trout could change its appearance to some extent under different environmental conditions led Regan (1911) to group all of these forms into a single polytypic species *Salmo trutta*. While multiple species are again scientifically recognized in particular localities (for example, Kottelat, 1997; Ferguson, 2004; Duguid *et al.*, 2006), insufficient genetic information is currently available to allow a comprehensive taxonomic revision throughout the range. However, whether we regard brown trout as a single species, or as a complex of species, the important consideration is that there is enormous genetic diversity within and among populations. This genetic diversity is fundamentally important for survival, reproduction, productivity and angling diversity, as well as being an important component of salmonid fish biodiversity. Thus the biological population, and not designated species or other taxa, or life-history forms, should in most situations be the focus for management and conservation.

Because of its extensive genetic variability (for example, Ferguson, 1989), brown trout has attracted considerable attention from geneticists. Recent studies looking at brown trout throughout the natural range (Bernatchez, 2001; Antunes *et al.*, 2002; Duguid, 2002) have demonstrated that there are at least five major evolutionary groups comprising:

- Atlantic drainages;
- Black, Caspian and Aral Seas (Danubian);
- Mediterranean;
- Adriatic;
- *marmoratus* trout of the northern Adriatic.

These groups have been separated for some 500,000 to 2 million years (Bernatchez, 2001), with Lake Ohrid and the Balkan peninsula being the probable epicentre of brown trout diversity. In considering England and Wales, only the Atlantic group is relevant.

During the last ice age, which started about 75,000 years ago, and had its maximum extent about 18,000 years ago, most of England and Wales, in common with much of the rest of Britain and north west Europe, was ice covered (Denton and Hughes 1982). It is unlikely that brown trout could have survived in freshwater in Britain during this time except possibly in southern regions during periods of partial glacial retreat. By 14,000 years ago the ice was in final retreat and recolonisation could commence. However, even at maximum glaciation, ice cover was not complete throughout north west Europe and there were several refuge areas where brown trout could have survived. Current genetic evidence (Ferguson and Fleming 1983; Hamilton *et al*. 1989; Hynes *et al*. 1996; Bernatchez, 2001; Duguid, 2002; McKeown, 2005) suggests that, as well as a refuge in the ice free area from France southwards, there were at least five separate refuges in the northern part of the range. Some of these refuges may only have existed for part of the last glaciation, but it was a sufficient period of time for genetic differentiation of the brown trout isolated in them.

As a result of genetic isolation in these refuges, two major distinct ancestral groups (south and north) evolved, with at least five separate lineages in the northern group. Based on mitochondrial DNA (mtDNA) evidence, at least six lineages appear to have independently colonized Britain and Ireland in the postglacial period (McKeown, 2005). Since the same diversity of lineages occurs in Ireland, which did not have any postglacial freshwater connection with the rest of Europe, as in England (McKeown, 2005), it is clear that all colonization of Britain and Ireland was by anadromous brown trout (sea trout). Thus the change from sea trout to freshwater (river and lake) trout has occurred independently in each water system. The six lineages reflect at least 75,000 years of evolution and it is likely that the two major lineage groups (south and north) differentiated at an earlier time, possibly during the previous glaciation. In some areas interbreeding of lineages has occurred, resulting in mosaic patterns of genetic diversity. In other cases lineages have remained discrete resulting in sympatric, reproductively isolated populations (for example in Lough Melvin and other Irish and Scottish lakes and rivers, Ferguson, 2004; Duguid *et al*., 2006). The environmental conditions that brown trout inhabits are highly variable. Natural selection has undoubtedly resulted in genetic differentiation among populations as they have adapted to these conditions in the 14,000 years since colonisation. Genetic drift has also resulted in random genetic changes especially in small populations.

1.2 Studying brown trout genetic diversity

Before considering the extent of genetic variation in brown trout populations in England and Wales and the genetic impact of stocking farm-reared brown trout, it is important to consider the methods used for studying genetic variation. Many misconceptions, even in the scientific literature, have arisen through failure to understand what the various techniques can show and, more importantly, what they cannot show. Broadly speaking there are two main ways to study genetic diversity: molecular genetic studies and quantitative genetic studies. The boundary between these two is currently breaking down.

1.2.1 Molecular genetic studies

Molecular genetic studies consider specific genes (gene loci). The actual genotype of an individual trout is determined at a sample of its gene loci. Separation of molecules in an electrical field is involved in many techniques, so this type of work is sometimes referred to as ‘electrophoretic study’. Early molecular studies started in the 1960s with the examination of protein variability, proteins being the primary products of most structural genes. Many of the proteins studied are enzymes and variability at a gene locus is due to the presence of several alternative alleles in a population, so these enzyme variants are often referred to as allozymes. One of the problems of protein studies is that fresh or freshly frozen tissue is required. Generally it is necessary to kill the trout to get sufficient tissue from several different tissue types. In the 1980s attention turned to the direct examination of the genetic material itself, DNA. Initial DNA studies involved DNA present in the mitochondria (mtDNA), because its smaller size made it more amenable to analysis with the techniques available at the time.

The development of techniques based on the Polymerase Chain Reaction (PCR) in the early 1990s enabled selected sections of DNA to be isolated and copied and facilitated examination of nuclear genes. For PCR, ethanol preserved tissue can be used and much smaller amounts of a single tissue are required, making biopsy sampling feasible. Adipose and other fin clips are now routinely used. Dried scales and museum specimens, even from more than 100 years ago, can also be used, although the DNA can be partly degraded and special techniques are required.

Nuclear DNA studies involve both functional genes, potentially subject to natural selection, and non-functional genes, which are not subject to direct natural selection and are primarily influenced by random genetic changes (genetic drift). However, non-functional genes can be influenced by selection on adjacent functional genes on the chromosome (hitch-hiking selection). Functional gene studies can now be undertaken on hundreds or even thousands of genes in salmonid fishes. Studies of non-functional genes usually involve regions known as minisatellites or microsatellites. These comprise short DNA sequences repeated tens or hundreds of times in tandem, with different alleles at a gene locus differing in the number of repeats. In many cases, the aim is not to study the genes themselves but to use them as indicators or markers to characterise the overall genetic make-up of an individual or population. Genes that can be studied by molecular techniques are often referred to as ‘molecular markers’. Different molecular markers are appropriate for different types of study.

Molecular marker studies are particularly valuable in detecting reproductive discontinuities, or genetic population structuring. They can be used to determine the existence of partially or completely reproductively isolated populations within or among lakes and rivers. Where interbreeding between two populations is absent or at a very low level, gene exchange (gene flow) will also be absent or limited. In this situation the two populations will diverge genetically from each other under the influence of natural selection and genetic drift. Depending on the length of isolation, the extent of differences in environmental conditions, and the effective number of breeding individuals in each population (effective population size N_e), populations may end up with completely different alleles, as is frequently the case with brown trout. In other cases populations share many alleles, but at significantly different frequencies. However, different allele frequencies can result in completely different composite genotypes, and thus phenotypes, in two populations.

The unique properties of mtDNA – normally maternal inheritance, no recombination, haploid, high rate of evolution (but see Ballard and Whitlock, 2004, for exceptions) – make it particularly useful for tracing ancestral lineages and patterns of colonisation. This marker has been widely applied to study population and species evolution in a geographical context (Avise, 2000).

Microsatellites are highly variably with 30 or more alleles commonly found at a single gene locus in a brown trout population. This high variability means millions of different composite genotype combinations are possible when several microsatellite loci are considered simultaneously. This makes microsatellite DNA profiling particularly suitable for identification of an individual's parentage in wild and experimental populations and for assigning individuals to their stock of origin (Ferguson *et al.* 1995; Hansen *et al.*, 2001c). However, the same high variability means that microsatellites are of limited value for population studies aimed at identifying management or conservation units, except in specific circumstances. If enough loci are examined, significant differences will be found between any two samples.

1.2.2 Quantitative traits

Studies of quantitative traits still largely involve examination of individual phenotypes. An animal's phenotype is the product of both its genotype and the environment in which it develops. Many aspects of body morphology, behaviour, survival, growth, physiology, migration pattern, mating, spawning, temperature and pH tolerance, resistance to disease and parasites and most other traits of interest in brown trout management, are phenotypic quantitative traits (for example Blanc *et al.*, 1982, 1994; Blanc, 2005; Largiadèr and Scholl, 1996; Mezzera *et al.*, 1997; Vandeputte *et al.*, 2002; Hallerman, 2003; Aparicio *et al.*, 2005). Many of these traits vary among populations (Table 1.1). They probably have an important influence on the fitness of each population, under the specific environmental circumstances it experiences.

Quantitative traits are the product of multiple genes (5-20+ gene loci) as well as being influenced by the environment. The proportion of overall variance of the phenotype that is due to genetic variance is referred to as the broad-sense heritability (h^2_b). Most genes responsible for quantitative traits cannot be studied directly at present, although with current developments in molecular methodology this is rapidly changing. For example, some current salmonid molecular studies are examining the Major Histocompatability (MH or MHC) genes, which are important in disease resistance as well as in mate choice (for example, Campos *et al.*, 2006; Coughlan *et al.*, 2006; Rajakaruna *et al.*, 2006). Genetic scans involving hundreds of gene markers can be used to find markers that are physically linked to quantitative trait loci (for example, Campbell and Bernatchez, 2004). Recent advances in DNA microarray technology allow the examination of thousands of functional genes and can detect differences in gene expression linked to quantitative traits and stock differences (for example, Roberge *et al.* 2006). Such studies are likely to be commonplace in the near future.

Until recently, quantitative traits have been mainly examined through experimental study. One of the best ways of doing this is to undertake 'common garden' experiments. Different families and groups of salmonids are reared from egg to adult in a communal environment. DNA profiling or other genetic markers are used to identify parentage of individuals (Ferguson *et al.* 1995; Palm and Ryman, 1999; McGinnity *et al.* 2003). Since all individuals experience the same environmental conditions throughout their lives, any differences in quantitative traits such as survival or maturity must be due to genetic differences. There is potential for maternal effects but this can be taken into account by producing reciprocal hybrids. There are also natural communal situations where two or more brown trout forms coexist in the same water.

Table 1.1 Phenotypic traits that vary within and among brown trout populations. Many of these characters are quantitative traits and population-specific expression is likely to contribute to the fitness of individual populations under the specific environmental circumstances that they experience. Based on: Blanc *et al*. (1982, 1994); Blanc (2005); Cawdery and Ferguson (1988); Skaala and Jørstad (1988); Elliott (1994); Largiadèr and Scholl (1996); Mezzera *et al*. (1997); Palm and Ryman (1999); Vandeputte *et al*. (2002); Aparicio *et al*. (2005); Monet *et al*. (2006).

Quantitative trait
Survival rates at various life history stages
Longevity (maximum age)
Growth rates at various life history stages
Growth potential (maximum size)
Feeding preferences / changes with age
Food conversion efficiency
Body shape
Head size and shape
Fin size, shape and number of fin rays
Number of gill rakers
Colour, pattern and size of spots
Dorsal fin margin colour
Number and shape of parr marks
Presence of pre-opercular mark
Fat content of body
Swimming rate and sustainability
Extent and timing of juvenile movement
Age and timing of juvenile migration to lake
Age and timing of smolt migration to sea
Length of time spent in lake or sea
Ability to adjust physiologically to marine conditions
Extent and pattern of migration at sea
Age of maturity (male and female)
Age and timing of migration from lake to river
Age and timing of migration from sea to river
Development of secondary sexual characters
Mating behaviour
Time of spawning
Place of spawning (inlet, outlet, lake, main river, tributary)
Fecundity
Egg size
Survival after breeding and repeat spawning in succeeding years
Embryo developmental rate and hatching time
Alevin developmental rate
Orientation of fry to water currents
Upstream / downstream migration of parr
Temperature tolerance at different life history stages
pH tolerance at different life history stages
Tolerance of low oxygen at different life history stages
Stress tolerance at different life history stages
Disease and parasite resistance at different life history stages
Agonistic behaviour
Behaviour in the presence of predators

1.2.3 Comparison of molecular and quantitative studies

Differentiation between populations based on neutral molecular markers is generally summarised in the statistic F_{ST} (or analogues such as G_{ST}, Θ_{ST}), which theoretically ranges from 0, signifying no genetic differentiation, to 1.
Quantitative gene differentiation can likewise be summarised as Q_{ST}. Studies on numerous organisms have shown that F_{ST} and Q_{ST} are highly correlated ($r > 0.75$) although Q_{ST} is generally larger than F_{ST} (Merilä and Crnokrak, 2001; Moran, 2002; McKay and Latta, 2002). This would be expected since natural selection will result in more rapid genetic change between populations compared to genetic drift of neutral alleles, except where populations have a very low effective population size ($N_e << 100$) or selection is very weak (<1%). Thus divergence based on neutral genetic markers is likely to considerably underestimate divergence in quantitative genetic variation. It should not be assumed that because there is no significant molecular divergence, or because the level of divergence is low, that important adaptive differences do not exist between populations. On the other hand, if molecular genetic variation exists between populations then there are almost certainly quantitative genetic differences as well, many of which are likely to be adaptive.

1.3 Extent of genetic diversity among brown trout populations in England and Wales

Current brown trout genetic diversity in England and Wales is the result of:

- Postglacial colonisation of each river system by one or more of the six lineages, with hybridisation between lineages in some waters but not in others.
- Natural selection resulting in adaptation to local environmental conditions over the 14,000 years since colonisation.
- Genetic drift resulting in random genetic changes especially in small isolated populations.

Extensive molecular genetic studies of brown trout populations in north west Europe have been carried out over the past 25 years. However, there are relatively few studies of brown trout populations in England and Wales compared to the work that has been undertaken in Ireland, Scotland, France, Spain and Scandinavia. With a few exceptions (Hauser *et al.*, 1991; Marshall *et al.*, 1992; Bembo *et al.*, 1994) studies involving England and Wales are currently available only as unpublished theses and reports (Fleming, 1983; Marshall, 1989; Hauser, 1990; Hall, 1992, 1995; Griffiths *et al.*, 2004; McKeown, 2005). By placing these limited studies from England and Wales in the wider context of studies from elsewhere in Britain and Ireland and throughout north west Europe it is possible to estimate the extent of genetic variation within and among brown trout populations in this region. These comparisons suggest that similar genetic diversity currently exists in England and Wales to that reported from elsewhere in north west Europe. There is no support for the contentions of some commentators (for example, Parton, 1997; Purdom 2002, 2003) that extensive stocking has resulted in the widespread replacement of native brown trout with trout of farm origin. Given the evidence (see section 2) that much stocking activity has had little or no genetic impact, this is not surprising.

McKeown (2005) found the same six mtDNA lineages in samples from England and Wales as in Ireland and Scotland. There was no difference in genetic diversity in samples from eastern England, south east England, south west England, north west England and Wales from each other or in comparison to regions in Ireland and Scotland. As elsewhere in the range (for example, Antunes *et al.*, 2001), brown trout in England and Wales show geographically mosaic patterns of lineages with genetic variation unrelated to geographical distance in most cases, except for in some sea trout populations (Ferguson, 2006). Several genetically distinct stocks occur within some river systems in England and Wales (Fleming, 1983; Hall, 1992). Hall (1995) found significant genetic differences between early and late components of the adult sea trout run in the river Dee (Wales).

Variation in quantitative traits such as body morphology, survival, growth, physiology, migration patterns, mating, spawning, temperature and pH tolerance, resistance to disease and parasites, and behaviour of brown trout also provide indirect evidence of genetic diversity within and among populations (Marcil *et al.*, 2006; Monet *et al.*, 2006). Although brown trout are phenotypically plastic, that is they can produce multiple phenotypes from a common genotype depending on environmental conditions, part of the phenotypic variability has a genetic basis. The extent of plasticity is also genetically determined. While background body colouration is largely influenced by environmental conditions, many characteristics have high heritability, including fine spotting pattern (Skaala and Jørstad, 1988; Skaala *et al.*, 1992), presence of red and black spots (Blanc *et al.*, 1982, 1994), body stripes (Largiadèr and Scholl, 1996), number and shape of parr marks (Mezzera *et al.*, 1997), dorsal fin margin colour, number of opercular spots, presence of pre-opercular mark and diameter of black spots (Aparicio *et al.*, 2005). In most cases, there is not direct evidence that such phenotypic traits in brown trout and other salmonids are locally adaptive and contribute to fitness of populations. Proof of a selective advantage is extremely difficult to obtain (Taylor, 1991; García de Leániz *et al.* 2007).

Genetic diversity within populations is summarized by heterozygosity, or by number of alleles since rare alleles have little impact on heterozygosity. The relationship between heterozygosity at molecular marker loci and genome wide heterozygosity within populations is generally weak (Reed and Frankham, 2001, 2003; Coltman and Slate, 2003; DeWoody and DeWoody, 2005), although this may be due to the use of inappropriate statistics (Aparicio, *et al.*, 2006).

Ferox, which are long-lived, piscivorous brown trout have been shown to be genetically distinct and reproductively isolated from co-occurring brown trout in a number of lakes in Ireland and Scotland (Ferguson and Taggart, 1991; Ferguson, 2004; McKeown, 2005; Duguid *et al.*, 2006). Although not investigated to date, ferox populations in Wales and Cumbria are also likely to be genetically distinct and share a common ancestry with ferox in Ireland and Scotland.

In many river systems there are waterfalls and other impassable barriers to upstream movement. Populations living in these isolated upstream sections are among the most genetically distinct brown trout populations because there is no natural gene flow into them. Upstream sections often have more extreme environmental conditions resulting in greater genetic differentiation due to natural selection. In addition, effective population sizes in these isolated populations are sometimes low and so genetic drift contributes to further genetic differentiation.

2 Genetic changes due to introductions of farm-reared brown trout

2.1 Why stock farm-reared brown trout?

Due to anthropogenic factors such as pollution, habitat degradation, introduced diseases and parasites and over-fishing, stocks of brown trout have declined in many water systems. In Switzerland, fisheries management (including stocking practices), proliferative kidney disease (PKD) and deterioration in habitat quality have been identified as the main causes of brown trout decline in recent years (Burkhardt-Holm *et al*., 2005; Borsuk *et al*., 2006). Stocking with farm-reared brown trout has been the most prevalent method of attempting to mitigate real or perceived population decline, although the benefits of stocking are generally overestimated (see section 4). In spite of, or perhaps because of, widespread stocking, many populations have continued to decline.

Farm-reared brown trout are stocked in many European countries where native populations are present. Stocking levels in each country have generally amounted to at least one million farm-reared brown trout each year (Sweden: Jonssonn *et al.*, 1999; Finland: Kahilainen and Lehtonen, 2001). In Norway over three million brown trout were released annually (L'Abée-Lund, 1991) and over four million brown trout have been stocked each year in Spanish rivers, amounting to some 4540 trout km^{-1} year^{-1} (Almodóvar *et al*., 2006). The number of farm-reared brown trout used for supplemental stocking in England and Wales is difficult to estimate because available figures include all brown trout production (Dunn, 2005). However, it would appear to be of the order of two million brown trout per year.

2.2 Extent of genetic changes in native populations following introgression with farm-reared trout

Genetic changes in wild brown trout populations due to introgression with farm-reared strains of brown trout have been studied in many European countries. The main studies on genetic changes in native population as a result of stocking are summarised in Table 2.1.

Studying genetic changes as a result of supplemental stocking requires genetic markers that are diagnostic, or partially diagnostic, for the farm-reared and native stocks concerned. In the first such study, Taggart and Ferguson (1986) made use of the differential occurrence of LDH-C1* alleles in farm-reared and native stocks to determine the proportion of stocked brown trout in angling catches and to detect introgression of farm genes in the Lough Erne (Northern Ireland) native brown trout population.

The same LDH-C1* markers have been widely applied in Spain and Mediterranean drainages of France to detect genetic impacts of stocking farm-reared trout. Farm strains of brown trout in Spain are of northern European origin, most likely to be from Germany (García-Marín *et al.*, 1991), Scotland or Switzerland (Almodóvar *et al.*, 2006). They are fixed, or show a very high frequency, for the LDH-C1*90 allele, which is absent in native Iberian populations (García-Marín *et al.*, 1991; Morán *et al.*, 1991). This locus is relatively easy to screen for in large numbers of specimens, either using allozymes or, more recently, DNA. The latter enables screening in fin biopsies and archived scales (McMeel *et al.*, 2001). Other nuclear allozyme coding loci have been used in addition to LDH-C1*90 (for example, Sanz *et al.*, 2002), as have mtDNA markers (Hansen *et al.*, 1995; McMeel and Ferguson, 1997) and microsatellites (Poteaux *et al.*, 1999; Hansen *et al.*, 2000a, 2001a, 2001b). Aparicio *et al.* (2005) have shown that dorsal fin margin colour, number of opercular spots, presence of pre-opercular mark, diameter of black spots and other morphological features could be used to differentiate among native, farm-reared and hybrid trout in Mediterranean drainages of Spain, allowing a rough field assessment of the proportion of introgression in populations without the need for expensive genetic laboratory facilities.

To reliably determine genetic changes due to the stocking it is necessary to have information on the genetic composition of the native population prior to stocking and/or genetic profiles of the farm strain(s) used for stocking. Hansen (2002) used genetic baseline data obtained from scale samples that had been collected prior to the onset of stocking. Lahnsteiner and Jagsch (2005) compared nineteenth century and current brown trout populations in Austria based on mtDNA variation in DNA obtained from gills of preserved museum specimens. Where baseline data are incomplete or absent, various statistical procedures can be used to estimate the genetic impacts of stocking (for example, Hansen *et al.*, 2001b).

From the studies in Table 2.1, a number of general conclusions can be reached.

The extent of genetic change (introgression) in native populations due to supplemental stocking with farm-reared brown trout is, in most cases, much less than might be anticipated given the scale of stocking and the fact that it has been carried out for over 100 years in some cases. In some situations, in spite of extensive stocking history, there is no detectable introgression of farm genes or it is very low (<5%). A minority of populations show extensive introgression (50-80%) and a few appear to be 100% of farm origin. In the latter cases it is not possible to determine if the population was effectively extinct before stocking took place or if the native population was replaced by the stocked trout. The majority of populations show less than 25% farm gene introgression. In spite of extensive stocking, these populations still retain at least 75% of their native genetic make-up. In such situations natural selection is likely to reduce the farm-gene influence once stocking ceases. In addition, restorative measures can be applied to reduce and perhaps eventually eliminate the farm gene component (see section 5.6).

The magnitude of genetic introgression as a result of farm-reared brown trout supplemental stocking is highly variable, unpredictable and shows no obvious relationship to the magnitude of stocking. However, although the number of trout stocked may be known, the proportion of the native population formed by these fish is generally unknown. It is difficult to compare stocking levels among populations. Even so, where relative numbers of stocked and native brown trout have been estimated, the genetic impact is much less than expected from equivalent survival levels. Hansen (2002) found 6% introgression in a population where the expected genetic contribution by farm-reared brown trout was 64%, based on the number of stocked trout and assuming equal survival and reproduction of wild and farmed trout.

There are many reasons for this reduced genetic impact of stocked trout (see section 3). There are some indications that introgression increases with a longer period of stocking (for example, Martínez *et al.* 1993). Araguas *et al*. (2004) found an increase in introgression of 1% per year in brown trout populations in the eastern Pyrenees (Spain). Izquierdo *et al*. (2006) found introgression of farm alleles in brown trout populations where 10 years previously Morán *et al*. (1991) found only pure native individuals even though stocking had been undertaken prior to this study.

Stocking of farm-reared brown trout results in reduced genetic variability among populations. It has a homogenisation effect. Stocking can result in an increase, decrease or no change in detectable genetic variation within populations. As farm-reared brown trout are generally genetically distinct from the populations into which they are stocked, an increase in genetic variability can occur as a result of interbreeding where a substantial native population exists. Where the stocked fish make up a substantial proportion of the fish in a population and where that farm strain has a reduced level of genetic variability, stocking can result in a decrease in genetic variability.

Movement of stocked fish can result in introgression in areas not directly affected by stocking. García-Marín *et al*. (1999) and Araguas *et al*. (2004) found introgression in sanctuary areas, designed to protect the native trout from genetic influences of stocking, as a result of movement from a stocked area. Several studies have indicated that stocked trout move more than native trout. It should not be assumed that the genetic impact of stocking will be confined to the area of the river where stocking takes place. In Belgium, Van Houdt *et al.* (2005) found that although intensive stocking had genetically homogenised the downstream sections of rivers, physical migration barriers had preserved the native upstream brown trout populations.

Stocked farm-reared brown trout cause greater introgression in the freshwater brown trout in a river compared to the anadromous component. Thus, in the Karup River in Denmark, Hansen *et al.* (2000a) found 46% farm trout introgression in the freshwater trout component compared to <7% in the sea trout component. Comparison of nuclear genes and mtDNA indicated a greater influence of male farm trout in the freshwater component, in keeping with the greater tendency of male trout to remain in freshwater. Ruzzante *et al.* (2004) found that, although trout stocked into rivers were present as pre-spawners in the sea (Limfjord), virtually no sea trout of farm origin were found among the spawning individuals.This suggests farm trout that became anadromous experienced high mortality at sea. Studies on other salmonids also indicate that introgression from farm strains is lower in anadromous populations compared to freshwater ones (Utter, 2001). Stocked trout, even if able to smoltify, may be poorly adapted to the marine environment. Svärdson and Fagerström (1982) found major genetically based differences in marine migration patterns among sea trout originating from different Swedish rivers and it is likely that these differences are adaptive. Given that anadromy is a threshold quantitative trait (Hallerman, 2003; Ferguson, 2006), stocking with farm trout is likely to increase the freshwater component in a river and reduce the sea trout run. Thus although introgression from farm trout is much less in anadromous populations it does not mean that stocking does not have an adverse impact on such populations.

Almodóvar *et al.* (2006) found a significant negative correlation between the introgression rate and both the maximum annual discharge and irregularity of discharge. They also found that introgression was positively correlated with conductivity, bicarbonates and pH. That is, introgression tends to be highest in heavily stocked fertile waters with low and even flow. Introgression is also higher for lake populations than for river populations in some situations. In north west Spain, Martínez *et al.* (1993) found little introgression in river populations but substantial introgression in lake populations. However, this does not appear to be a universal finding as studies in Denmark (Hansen *et al.*, 1993) and Norway (Heggenes *et al.*, 2002, 2005) found low levels of introgression in lake populations in spite of stocking for several decades. In Northern Ireland, Ferguson and Taggart (1986) found that introgression levels varied considerably (19-91%) among different afferent rivers for Lower Lough Erne.

Higher levels of farm gene introgression appear to have taken place in Mediterranean drainages of Spain, France and Italy compared to populations in the Atlantic region. This is perhaps surprising given that virtually all brown trout used for stocking are derived from the north Atlantic area and thus are genetically more similar to other Atlantic populations than to those in the Mediterranean area. There are several possible explanations for this phenomenon. It may be that Mediterranean populations had reduced population sizes prior to stocking taking place, so the proportion of stocked trout was greater in these populations. Atlantic brown trout may be competitively superior to Mediterranean and impacts may be due to competitive exclusion of native trout followed by breeding of Atlantic trout with Atlantic trout. In that respect, stocking of Atlantic trout may be more similar in its effects to introduction of a non-native species than to genetic changes occurring as a result of hybridisation, as appears to be case in the Atlantic region. It has been suggested that the presence of sea trout populations in many Atlantic rivers, but not in the Mediterranean reduces the impact of stocking in the former (Almodóvar *et al.*, 2006).

The two types of genetic marker, nuclear and mtDNA, have indicated different levels of introgression in some studies (Poteaux *et al.*, 2001; Sanz *et al.* 2006), with higher levels sometimes indicated by the maternally inherited mtDNA. While this may be due to the markers not being totally diagnostic, in some cases it is likely to be a result of sex-biased gene flow. Atlantic salmon farm females have a higher reproductive success in the wild than males (Fleming *et al.*, 2000).

Several studies (Skaala *et al.*, 1996; Poteaux *et al.*, 1999; Almodóvar *et al.*, 2001, Hansen, 2002) have shown that when stocking of farm-reared brown trout ceases the level of farm gene introgression decreases over subsequent years. This would be expected from the lower fitness of farm x native hybrids (see section 3.3.11), that is, natural selection occurs against farm genes. The decrease in frequency of farm alleles could also be due to genetic drift (Sanz *et al.*, 2006). However, if drift is involved it would be expected to increase the frequency in some instances, which has not been reported.

These findings for brown trout (negligible or low introgression in many populations to complete replacement in a few populations) are mirrored by many studies on introgression of other farm-reared salmonids on wild stocks (for example, LeClair *et al.*, 1999; Englbrecht *et al.*, 2002; Small *et al.*, 2004; Piller *et al.*, 2005). This suggests that similar factors influence the survival and reproduction of all farm-reared salmonids in the wild.

Table 2.1 Summary of the main studies of introgression by farm trout genes as a result of stocking farm-reared brown trout into native brown trout populations. Studies are ordered alphabetically, by country.

Reference(s)	Location of study	Main conclusion(s)
Weiss *et al*. (2001)	Austria – Danube headwaters	44% introgression from Atlantic farm strains
Lahnsteiner and Jagsch (2005)	Austria	Genetic differences among nineteenth century wild, current wild and current hatchery populations
Van Houdt *et al*. (2005)	Belgium – Scheldt and Meuse rivers	Intensive stocking had homogenised the downstream sections but physical migration barriers had preserved native upstream populations
Hansen *et al*. (1993)	Denmark	Stocking of farm trout directly into a lake had little or no genetic effect on the native population
Hansen *et al*. (1995)	Denmark – Karup River	Genetic contribution of hatchery trout much less than expected from number of stocked fish
Hansen *et al*. (2000a)	Denmark – Karup river	46% farm-trout genes (direct and/or offspring) in freshwater trout component but <7% farm trout genes in anadromous trout
Hansen *et al*. (2001a)	Denmark	Introgression from farm trout had occurred in only two out of five rivers potentially influenced by stocking
Hansen *et al*. (2001b)	Denmark	Genetic contribution by farm trout too small to indicate that stocking had contributed to the rehabilitation of the population
Fritzner *et al*. (2001)	Denmark - Funen	Variable levels of introgression from farm-reared brown trout
Hansen (2002)	Denmark	Low genetic contribution from farm-reared trout in one river but high in another
Ruzzante *et al*. (2001)	Denmark – Limfjord tributaries	Introgression from 0 (1 tributary) to 32% (mean 23%)
Ruzzante *et al*. (2004)	Denmark – Limfjord (sea) tributaries	Although trout stocked into rivers were present as pre-spawners in the Limfjord, virtually no sea trout of farm origin were found among the spawning individuals, suggesting that farm trout that became anadromous experienced high mortality at sea
McMeel and Ferguson (1997); Duguid and Ferguson	England – River Dove system	Up to 51% introgression in stocked areas (mean 22%)

(1999)		
Guyomard, 1989	France	Introgression rates up to 80% in stocked rivers
Barbat-Leterrier *et al.* (1989)	France – three Mediterranean rivers	Introgression rates from 0-40%. Apparent random mating of stocked and native trout
Poteaux *et al.* (1998)	France – Orb River system, Mediterranean drainage	Differences in introgression among tributaries of a river system. Evidence of selection against hybrids with reduction in level of introgression after six years without stocking
Berrebi *et al.* (2000)	France - Pyrenees: 13 localities in Mediterranean drainages	Introgression levels of farm trout genes from 0-77%.
Poteaux *et al.* (2001)	France – Mediterranean rivers Sorgue and Orb	Different molecular markers indicated different levels of introgression
Aurelle *et al.* (2002)	France – Pyrennes: Atlantic drainages	With a few exceptions, populations had little introgression (5%-8%) from farm-reared trout
Riffel *et al.* (1995)	Germany – Danube headwater	40% introgression from Atlantic farm strains
Apostolidis *et al.* (1996, 1997)	Greece	Extensive introgression (around 75%) from non-native trout
Marzano *et al.* (2003)	Italy – Apennine populations	Stocking had resulted in reduction in genetic variability and extensive introgression
Caputo *et al.*(2004); Lucentini *et al.* (2006);Splendiani *et al*. (2006)	Italy	Extensive stocking had resulted in introgression in many but not all populations
Taggart and Ferguson (1986)	N. Ireland – Erne system	Hatchery genetic contribution (direct + offspring) varied from 19% to 91% among rivers with 21.5% overall in angler-caught trout from lake – introgression from hatchery strain had occurred
Skaala *et al.* (1996)	Norway – river with sea trout and freshwater trout	Stocked F_1 hatchery-reared non-native brown trout spawners found to spawn among themselves and with wild trout. Number of hybrid and introduced trout offspring was much lower than expected given relative numbers of introduced and wild spawners - wild trout showed nearly three times higher survival than hybrids of wild and introduced trout

Borgstrøm *et al*. (2002)	Norway – sea trout river	Survival of second generation hatchery reared 0+ trout to age 3+ was significantly lower than wild parr despite hatchery fish being larger at stocking
Heggenes *et al*. (2002)	Norway - Lake Møsvatn	Although stocking for around 40 years with 3,500 summer 0+ and 700 summer 1+ annually, <3% of trout sampled from the system originated directly or indirectly from stocked fish
Heggenes *et al*. (2005)	Norway - Lake Tinnsjø	Although stocked with some 50,000 summer 0+ annually (comparable magnitude to natural recruitment) for >30 years, minimal introgression to native trout
Antunes *et al*. (2001)	Portugal – 16 samples, 8 rivers	Zero to low introgression in nearly all populations
Delling *et al*. (2000)	Slovenia – *Salmo marmoratus*, Soca River	Stocking with non-native trout since 1906 had resulted in up to 90% introgression
Jug *et al*. (2005)	Slovenia – *S. marmoratus*	Stocking resulted in 50% of *S. marmoratus* populations studied being introgressed with non-native genes
Morán *et al*. (1991, 1995)	Spain – north	Little evidence of introgression in spite of extensive stocking
García-Marín *et al*. (1991)	Spain	Hatchery strains genetically homogenous and distinct from native populations
Martínez *et al*. (1993)	Spain – north west	Little introgression in river populations but substantial introgression in lake populations – in latter, the longer the period of stocking, the greater the introgression
Arias *et al*. (1995)	Spain – north west: 44 localities of four river systems, most stocked over the previous 30 years	Only eight samples showed individuals of stocked origin - overall proportion of stocked individuals was 4%
García-Marín *et al*. (1998)	Spain – north east	Much greater genetic impact in unfished areas than fished ones due to greater susceptibility to angling of hatchery trout
García-Marín *et al*. (1999)	Spain – eastern Pyrenees	5% introgression at one site, which had not been directly stocked. Extent of introgression variable and independent of stocking effort
Machordom *et al*. (1999)	Spain – central	Introgression rates of 2-29.4%. Extent of introgression not in concordance with the respective stocking effort

Almodóvar *et al*. (2001)	Spain – Duoro river system: 16 streams	25% of sites showed farm gene introgression ranging from 1-19% (mean of 3%). No introgression at sites previously stocked but where stocking ceased ten years prior to study
Sanz *et al*. (2002)	Spain – west: 36 localities	Introgression rates ranged from 0% (8 samples) to 36.3% (mean 10.9%)
Araguas *et al*. (2004)	Spain – eastern Pyrenees	Allele frequencies changed not just in the stocked areas but in adjacent protected areas where stocking is prohibited. 28% decrease in genetic differentiation among populations due to stocking
Aparicio *et al*. (2005)	Spain – Mediterranean drainages: 23 populations	Eight pure Mediterranean populations – rest showed introgression rates of 3-68% (mean 12.4%).
Madeira *et al*. (2005)	Spain - Cantabrian and Mediterranean sea drainages: 20 localities from 11 river systems	Introgression very variable among populations (2.5%-65%). Higher level of introgression in Mediterranean populations (mean 30.6%) compared to the Cantabrian populations (9.7%)
Sanz *et al*. (2006)	Spain	Allozyme and mtDNA data indicated different levels of introgression possibly due to sex-biased gene flow
Ayllon *et al*. (2006)	Spain – River Navia	No farm trout alleles detected in 2002 and 2003 from previous introduction of 133000 farm juveniles from 1985 to 1992
Almodóvar *et al*. (2006)	Spain – review of published and unpublished data on 307 population samples from 73 river systems	50% of populations analysed show introgression - mean 13.4%. Farm alleles varied from 0% in 154 populations to 100% in 2 populations. Extent of introgression varied significantly among different regions. North Atlantic rivers showed lowest introgression (mean 5%) with little or no presence of farm alleles in 87% of 114 populations in spite of greatest magnitude of stocking (expressed as trout km^{-1} year^{-1}). 53% of Mediterranean populations were highly introgressed, with a mean of 20% farm alleles.
Ryman (1981)	Sweden	Reduction in population differentiation as a result of stocking
Largiadèr and Scholl (1995)	Switzerland – Adriatic and Danubian drainages	Very high introgression of Atlantic alleles into Adriatic populations as a result of stocking
Largiadèr and Scholl (1996); Mezzera and Largiadèr (2001a)	Switzerland – Doubs river, Rhône system	Low to high introgression among sites - differences in local habitat conditions can affect the degree of introgression – non-random mating of hatchery and native trout
Hauser *et al*. (1991)	Wales	Sea trout offspring stocked above water-falls stayed in freshwater and hybridised with native trout

3 Why has stocking had less genetic impact than expected?

3.1 Origins of farm strains of brown trout

As with wild brown trout populations in England and Wales, only limited information is available on the genetic composition of farm-reared brown trout strains used for supplemental stocking. Brown trout culture started in the middle of the nineteenth century. Early records (for example, Smiley, 1884) indicate that brown trout eyed-eggs were imported into England from German farms. The first brown trout farms in Britain were set up by Armistead in 1868, initially in Cumbria and then at Solway (near Dumfries, Scotland) and by Maitland in 1873 at Howietoun (near Stirling, Scotland). Both farms were based substantially on Loch Leven (Scotland) broodstock but broodstocks from other populations, including sea trout, were also incorporated (Maitland 1887; Armistead, 1895). These farm strains were widely known as 'Leven' strains in spite of their mixed ancestry. Many current farm strains of brown trout are derived, directly or indirectly, from these Howietoun and Solway strains. When new farm strains have been set up this has generally been done using brown trout from existing farms rather than obtaining broodstock from the wild. Just as farm-reared brown trout do less well in the wild compared to native trout (see below), so wild trout do less well than farm-reared trout under culture conditions (Krieg *et al.*, 1992). In addition, disease status is easier to control when using trout from existing farms rather than from the wild.

The common origin of many farm strains has resulted in them being remarkably similar genetically, even though there has often been no interchange of trout in recent years. McMeel and Ferguson (1997) found that brown trout from two farms in England were genetically very similar to each other and both possessed a mtDNA haplotype at moderate frequency that had otherwise only been seen in the main brown trout farms in Northern Ireland (Movanagher) and the Republic of Ireland (Rosscrea). Duguid (2002) did not find this haplotype in 44 population samples from Scotland. In a study of some 60 wild populations from Britain and Ireland, McKeown (2005) found this haplotype only at very low frequency (one or two individuals) in the Tyne and in a tributary of the Thames. Whether its presence in these rivers is the result of previous stocking with farm-reared brown trout or shared wild ancestry is unknown. It is also absent in the current Howietoun strain. It would thus seem most likely that the origin of this 'farm haplotype' is from outside Britain and Ireland. Taggart and Ferguson (1986) noted that the Movanagher stock was originally based on brown trout from English and Danish farms. Genetic similarity has been found also among trout farms in Spain (Almodóvar *et al.*, 2006), France (Krieg and Guyomard, 1985) and Italy (Caputo *et al.* 2004). Until recently all farm strains used in these countries were of northern European origin, most likely from Denmark (Caputo *et al.* 2004), Germany (García-Marín *et al.*, 1991), Scotland or Switzerland (Almodóvar *et al.*, 2006).

3.2 Genetic and phenotypic differences between farm-reared and wild brown trout

The environmental and other conditions under which brown trout are reared on farms are very different from natural conditions. Farm conditions differ from natural conditions in the following ways:

- Physical conditions are simpler in the quantity and type of cover, type of substrate, and water depth.
- Current velocities are generally lower than in the wild;
- There are differences in chemistry, temperature and turbidity of water.
- The quantity, method and timing of delivery and nutritional composition of food is different, with an abundance of artificial food being delivered to the surface of the water on farms.
- Fish are kept at greater densities than in the wild;
- Predators and competitors are absent.
- There is generally treatment for diseases and parasites.

Given these very different conditions, it is not surprising that farm-reared brown trout differ from wild brown trout. Three main types of difference are involved:

- Farm-reared brown trout differ genetically due to founder effects (original broodstock taken from the wild) and subsequent domestication in culture, involving artificial selection, relaxed natural selection and genetic drift.
- Farm-reared brown trout differ phenotypically in behaviour, physiology and morphology due to the farm-rearing environment being very different from that in the wild, as well as through gene-environment interactions.
- Learning differences. Learning opportunities are different under culture conditions, especially in relation to feeding and anti-predator behaviour.

3.2.1 Founder effects

Given the extensive genetic differentiation among wild populations, a farm strain derived from a non-native stock will be genetically different from the wild population into which it is stocked irrespective of any further changes in culture. Since most farm-reared brown trout strains derive from a mixture of a small number of wild populations, they will be non-native in almost all stocking situations. Even when derived from the same river as a wild stock, the farm strain may differ because a small number of broodstock was used, or the broodstock was taken from one component of the wild stock. For example, trout may have been taken that were running or spawning at a particular time, traits with high heritability in salmonids (Sakamoto *et al.*, 1999; Fleming and Petersson, 2001).

3.2.2 Domestication

After the farm strain is established, genetic changes can occur in the culture environment. Such domestication occurs due to deliberate artificial selection for perceived advantageous traits such as high growth rate, early adult return or early and synchronous maturity. There is generally much greater survival under farm conditions. Genotypes survive that would not do so in the wild. In other words, there is a relaxation of natural selection on unfavourable genotypes for natural conditions. Relaxed selection as a result of greater survival under farm conditions, and low N_e, can result in deleterious alleles, normally kept at low frequency in the wild, rising to high frequencies or even fixation (Lynch and O'Hely, 2001).

Similarly phenotypes that would be poorly adapted for natural conditions may be inadvertently selected for under farm conditions. Farm conditions change post-release survival and reproduction, so natural selection will affect farm-reared trout differently from equivalent age wild fish. Even environmentally produced phenotypic differences can result in differential selection once the fish are released into the natural environment (Kostow, 2004; Reisenbichler *et al.*, 2004; Goodman, 2005). Thus altered selection can start in the first generation after a hatchery strain is founded.

The process and effects of domestication are well known for many mammal species. Given the high fecundity of brown trout and other salmonids relative to domesticated mammals, much higher levels of artificial and natural selection can apply and thus the process of domestication is much more rapid. Substantial changes can occur in a few generations.

Under farm conditions matings are forced, but not at random. There is no opportunity for mate choice, competition and other aspects of the natural reproduction process (Petersson *et al.*, 1996; McLean *et al.* 2005). Genetic, phenotypic and learning changes can occur even if brown trout are farm-reared for only a short period of time after hatching. Much of the higher survival occurs in the few weeks after start-feeding, which may represent a critical phase of the inadvertent domestication selection under farm conditions (Glover *et al.*, 2004).

While farm brown trout have not been subjected to the same programme of deliberate selection for faster growth that has occurred in the Atlantic salmon farming industry, farmers have exerted some selection, often breeding from the largest or 'best' individuals. Selection has also taken place for spawning time with earlier and synchronous spawning being favoured to fit with human schedules. Selection was undertaken in the early days of brown trout farming as clearly documented in the books by Maitland (1887) and Armistead (1895). In addition to faster growth, selection in salmonids can result in changes in many aspects of behaviour. Inadvertent selection can occur as a result of hatchery procedures, for example due to different feeding levels. A sea ranching programme for brown trout was found to select for faster-growing individuals (Petersson and Järvi, 2000). Glover *et al.* (2004) reared groups of brown trout full-sibs, obtained from wild broodstock, under hatchery conditions for 35 days with different levels of food availability and found that different families survived best under different food availability.

Roberge *et al.* (2006) found that the progeny of farm salmon, after five to seven generations of domestication, had significant changes in gene expression compared to the progeny of wild fish. The transcription profiles of 3557 genes showed some 20% difference for 1.7% of the expressed genes at the juvenile stage. Overall 16% of the genes in two farm strains showed significant transcription differences with parallel changes occurring in all cases indicating directional selection.

Since some farm strains of brown trout have been in culture for more than 30 generations there has been time for considerable domestication to occur, probably as much as in the more intensely selected Atlantic salmon that has only been in culture for less than 10 generations. Given that more information is available for Atlantic salmon than brown trout, comparisons of farm and wild Atlantic salmon stocks are relevant for studying the genetic impacts of stocking farm-reared brown trout (see for example, McGinnity *et al.*, 1997, 2003; Fleming *et al.*, 2000; Hindar *et al.*, 2006; Jonsson and Jonsson, 2006; Ferguson *et al.*, 2007). Rainbow trout and some other *Oncorhynchus* species, and *Salvelinus* species, have a similar history of farm-rearing, so information from these species is also relevant. The genetic impacts of supplemental stocking farm-reared fish may even be greater for brown trout than for other salmonid species, because brown trout has among the highest inter-population genetic differentiation of all salmonid species (Ryman, 1983). That is, information from other species may give a conservative indication of impacts of stocking farm-reared brown trout. In this review information on the genetic impacts of stocking is drawn from brown trout studies where possible but where this is not available information is taken from relevant studies involving other salmonid species.

3.2.3 Genetic Drift

As well as changes due to deliberate and inadvertent selection, random changes can occur in farm-reared brown trout strains, as a result of genetic drift and by inbreeding. Both lead to a loss of genetic variation, especially in those cases where farm strains have involved low numbers of broodstock and unequal sex ratio in the founding or subsequent generations. While some farm strains of brown trout have been shown to have reduced variability (Ryman and Ståhl, 1980; Vuorinen, 1984), other strains have elevated levels as a result of mixed origins (Van Houdt *et al.*, 2005). Genetic variation in Finnish farm strains of brown trout has been found to decline with time with, on average (Aho *et al.*, 2006). Loss of genetic variability can result in reduced hatchability and survival, reduced growth rate, loss of disease resistance, developmental abnormalities and changes in other characteristics likely to impact on survival in the wild (Skaala *et al.*, 1990; Kincaid, 1995).
In a survey of 19 brown trout farms in the USA, Kincaid (1995) found that, for most farms, broodstock number was mainly in the range 101-500 although one was <25 and three were >1000 (overall mean 476). In 12 of the farms the sex ratio was different from 1.0. Due to differences in sex ratio and unequal offspring survival, the actual N_e would be considerably less than these broodstock numbers (N_b). We do not know whether these values are typical of brown trout farms in England and Wales.

In experimental studies with brown trout Dannewitz *et al.* (2004) found that N_e ranged from 12-59% of N_b, and Page *et al.* (2005) found N_e values of 9-41% of N_b for lake char. Thus typically N_e may be, on average, about one third of N_b, which would result in inbreeding of >1% per generation in many of these farm strains. Mass-strip spawning (milt pooling) can further reduce N_e due to sperm from a few males dominating egg fertilization. In a mass-strip spawning of 2000 adult rainbow trout, Bartley *et al.* (1992) estimated an N_e of 89.

3.3 Factors reducing genetic impact of stocking

Having considered the changes that occur during brown trout culture, we can identify many reasons why supplemental stocking of farm-reared brown trout has had less impact on wild populations, either in terms of increasing population numbers or in producing genetic changes, than might be anticipated from the scale of stocking. These involve both genetic and non-genetic factors. They are summarised in Table 3.1.

3.3.1 Stocking level

The low level of introgression seen in some cases may simply be because the number of fish stocked is low relative to the wild population. Little genetic change would be expected even if the farm-reared fish had equivalent survival and reproduction to the wild ones. In the late nineteenth and early twentieth centuries, stocking often took place into waters with plentiful trout already, perhaps in a misguided attempt to increase growth rate or in pursuit of the Victorian 'new blood' philosophy. Since female trout produce some 2000 eggs per kg, in larger water systems natural recruitment can be of the order of many millions. Even today stocking often consists of only a few thousand fry or parr and amounts to much less than 1% of natural recruitment in some waters.

3.3.2 Reduced survival of farm-reared brown trout

To produce genetic change in a population, stocked trout must survive and reproduce. Many studies have shown reduced survival of farm-reared brown trout relative to both native wild and feral wild brown trout. This is typical of the situation for other farm-reared salmonids (see reviews by Lasenby and Kerr, 2001; Weber and Fausch, 2003; Thorpe, 2004). Cresswell *et al.* (1982) found that less than 1% of stocked legal-size brown trout contributed to the catch in the season after stocking. Skaala *et al.* (1996) introduced first generation hatchery-reared non-native brown trout spawners into a river with native anadromous and freshwater trout and sampled the subsequent generation offspring at 0+, 1+ and 2+ stages. The wild trout offspring showed nearly three times higher survival than the introduced trout. However, as with many such experiments, it is impossible to differentiate the effects of hatchery rearing from those of non-native origin (see discussion in Brannon *et al.*, 2004a). Hesthagen *et al.* (1999) found that farm-reared brown trout had significantly shorter life spans than native fish. Borgstrøm *et al.* (2002) released second generation farm-reared 0+ brown trout into a sea trout stream in Norway and found the survival of the stocked parr to age 3+ was significantly lower than for the wild parr in spite of the former being larger at introduction. Baer (2004) found that only 12-19% of stocked yearling brown trout were recaptured after six months compared to 40-70% of 1+ and up to 100% of older wild trout.

In experiments with farm salmon under natural river conditions McGinnity *et al.* (2003) found that a non-native farm strain of Atlantic salmon showed 32% survival relative to native wild salmon from fertilised egg to the smolt stage, 7% survival from smolt to adult return and potential egg deposition, and 2% of the survival of the wild salmon in the overall life cycle. Reisenbichler *et al.* (2003) note that steelhead trout show 80% reduction in survival from egg to adult under natural conditions after 6-10 generations of hatchery rearing. Reduced survival of farm-reared brown trout can be the result of many different factors with, in most situations, a complex combination of factors being involved. Irrespective of the reasons, reduced survival clearly lowers the potential introgression of farm-reared brown trout into wild populations.

3.3.3 Stocking technique

Farm-reared brown trout generally have to be transported from the farm to the site of release. Johnsen and Hesthagen (1990) found that the closer the fish farm to the water being stocked, the higher the trout recapture rate. Loading and transport stress due to handling and crowding and the associated increase in cortisol levels can result in fish being more vulnerable to changes in physical and chemical factors, as well as altering aggression, territoriality and learning ability (Jonssonn *et al.*, 1999). The recovery process can take days or weeks (Pickering *et al.*, 1982; Schreck *et al.*, 1997). Different chemical and physical conditions in the water they are stocked into can also result in stress. Acclimatization to the new conditions prior to release increases survival (Jonssonn *et al.*, 1999). The hatchery strain used, health status, the age and size of fish stocked, the time of year that stocking is undertaken, the frequency of stocking, rate of stocking, site of stocking and method of stocking (clumped, dispersal, or trickle) have all been shown to influence the survival of farm-reared brown trout (Lasenby and Kerr, 2001 and references therein).

3.3.4 Environmental conditions

Madeira *et al.* (2005) proposed that different environmental conditions could explain why introgression rates are higher in Mediterranean drainages compared to Cantabrian (Atlantic) drainages in Spain. They note that Cantabrian rivers are short with high gradients whereas Mediterranean rivers have lower slopes and are seasonal rivers with low water level and warm temperature in summer. In addition, they note that brown trout population numbers are much higher in Cantabrian than Mediterranean rivers and thus stocked brown trout face less competition in the latter as well as making up a higher proportion of the brown trout population. As mentioned in section 2.2, Almodóvar *et al.* (1996) found a significant negative correlation between the introgression rate and both the maximum annual discharge and irregularity of discharge. This is in keeping with the finding by Martínez *et al.* (1993) of little introgression in river populations but substantial introgression in lake populations. Almodóvar *et al.* (2006) also found that river productivity appeared to be important. There is greater introgression in more productive rivers (as measured by conductivity, bicarbonates and pH), possibly due to the farm trout facing less competition from wild-trout. It was less likely that these rivers were already at their carrying capacity prior to stocking. Williams *et al.* (1997) found that farm-reared rainbow trout had poor survival in the lower part of a river where increased water flows and velocities and a steep gradient existed. No introgression had occurred in that part of the river whereas extensive introgression had occurred in the upper part of the river.

3.3.5 Physiological differences

Ruzzante *et al.* (2004) found extremely low survival of stocked farm-reared brown trout in the sea, which probably explains the much reduced genetic impact of stocking in the sea trout component compared to the freshwater component of populations (Hansen *et al.* 2000a). It may be that the farm-reared brown trout are less able to physiologically adjust to marine conditions. Sundell *et al.* (1998) found wild brown trout had significantly higher Na^+K^+-ATPase activity and lower plasma sodium levels compared to farm-reared brown trout during parr-smolt transformation. In the Baltic Sea, Saloniemi *et al.* (2004) found wild Atlantic salmon smolts had four and a half times higher survival than farm-reared smolts of the same size, although the larger size of the farm smolts partially compensated for their lower survival rate resulting in a two-fold greater survival of the wild fish overall. The better survival of wild smolts relative to farm-reared smolts was more pronounced in a low-survival year compared to a high-survival year. Carline and Machung (2001) found that the critical thermal maximum was significantly higher for wild brown trout than for a farm-reared strain and suggested that the difference is genetically based.

3.3.6 Morphological differences

Genetic and environmental factors can result in changes in external and internal morphology of farm-reared salmonids, which can alter survival, swimming ability and spawning behaviour (Swain *et al.* 1991; Gross, 1998; Hard *et al.*, 2000; von Cramon-Taubadel *et al.*, 2005; Jonsson and Jonsson, 2006; Wessel *et al.*, 2006a). Enders *et al.* (2004) found that the deeper bodies and smaller fins of farm Atlantic salmon increased their swimming costs by up to 30% in turbulent flow. Morphological characters such as fin shape and body shape are important adaptive features in the natural environment (Riddell *et al.*, 1981; Taylor, 1991). Marchetti and Nevitt (2003) found that the brain size of farm-reared rainbow trout was smaller than that of wild fish. Kihslinger *et al*. (2006) noted a similar effect in Chinook salmon with significant differences occurring in the forebrain within a single generation of hatchery rearing.

3.3.7 Behavioural differences

Rearing under farm conditions, even if only for a few months, can result in many behavioural changes including changes in aggression, reduced ability to find food, altered sheltering behaviour, reduced awareness of predators and altered mating behaviour. These differences can result from environmental or genetic differences and behaviour is among the first traits altered by domestication (Olla *et al.*, 1998). Differences in aggressive behaviour between farm-reared and wild brown trout and other salmonids have been with reported in many studies. Farm-rearing commonly results in an increase in aggression (Bachman, 1984; Johnsson *et al.*, 2001; Weber and Fausch, 2003; Wessel *et al.*, 2006b), although decreased aggression can occur in some environmental circumstances (Ruzzante, 1994; Petersson and Järvi, 2003). Einum and Fleming (1997) found that, under communal farm conditions, offspring of farm salmon were more aggressive than the offspring of two wild populations and they also left cover sooner after a simulated predator attack. Hybrids between wild and farm fish were generally intermediate, indicating a genetic basis to these differences. Selection for faster growth can result in increased growth hormone levels, which result in increased aggressive behaviour in brown trout and other salmonids (Johnsson *et al.*, 1996; Fleming *et al.*, 2002).

Competitive interaction between farm-reared and wild salmonids can be influenced by the prior residence of the wild fish before the stocked fish arrive (Weber and Fausch, 2003). Deverill *et al.* (1999) and Johnsson *et al.* (1999) found that farm-reared brown trout continued in energy expending agonistic encounters with wild trout, although they failed to displace wild fish already in residence in energetically favourable positions. Farm-reared brown trout have been shown to display reduced territory holding (Sundström *et al.*, 2003).

Stocked brown trout have been found to have reduced ability to obtain food (O'Grady 1983; Bachman, 1984; Kahilainen and Lehtonen, 2001; Sundström and Johnsson, 2001) resulting in lower survival. Jonssonn *et al.* (1999) found that stocked farm-reared brown trout remained within the area where they were stocked, which could result in competition for food and local starvation. Some stocked farm-reared brown trout may not learn to eat wild food items (Elliott, 1975). Selection for faster growth in brown trout results in higher food consumption (Sanchez *et al.*, 2001), which may alter their willingness to take risks during foraging. Teixeira and Cortes (2006) found differences in the diet of wild and stocked brown trout with stocked trout feeding almost exclusively on food items captured near the surface.

Farm-reared brown trout have been shown to have reduced awareness of predators and are more willing to forage under the risk of predation (Dellefors and Johnsson, 1995; Johnsson *et al.*, 1996; Fernö and Järvi, 1998). Alvarez and Nicieza (2003) found that domestication weakened behavioural defences in farm-reared brown trout, with F_2 farm trout and the offspring of wild trout reared under hatchery conditions being insensitive to predation risk. They were predominantly active during the day whereas wild fish switched to nocturnal activity in the presence of predators. In some cases stocked farm-reared brown trout displayed increased movements, which could result in greater exposure to predators. Sundström *et al.* (2005) found wild and farm-reared brown trout responded differently to a simulated predator attack in heart rate increase and duration. Colouration differences as a result of farm-rearing may make stocked trout more vulnerable to predation and influence the outcome of behavioural interactions (Weber and Fausch, 2003). For example, some farm-reared brown trout can have a silvery appearance for several months after stocking (Cresswell *et al.*, 1982).

3.3.8 Angling susceptibility

Stocked farm-reared brown trout have been shown to have greater susceptibility to being caught by anglers than wild trout (for example, Pedersen *et al*., 2003; Almodóvar and Nicola, 2004). This may in part be due to the preference of farm-reared fishes to take prey from the surface (Reinhardt, 2001). Irrespective of the reasons, proportionately more stocked trout are removed by anglers. Ironically García-Marín *et al*. (1999) found less introgression in heavily stocked and fished areas compared to adjoining areas set aside as non-fishing refuges to protect wild trout, due to the greater susceptibility of stocked fish to angling. Mezzera and Largiadèr (2001b) found that the greater susceptibility to angling of stocked farm trout in the Doubs River (Switzerland) substantially reduced the introgression rate in stocked populations. The legal catch size (25cm) in this case was such that many fish were being caught prior to spawning. The greater susceptibility of hybrid farm x wild brown trout, as well as farm-reared brown trout, to angling (Mezzera and Largiadèr, 2001b) indicates that this susceptibility is not just a direct effect of culture conditions but is a result of genetic changes during domestication.

3.3.9 Breeding ability

Even if stocked farm-reared brown trout survive, the ability to breed successfully is also changed by farm-rearing and domestication, which can result in differences in mating behaviour. In a comparison of wild sea trout and a sea-ranched strain derived from the same stock, Petersson and Järvi (1997) found significant differences in mating behaviour. Sea-ranched males achieved fewer spawnings than wild males, possibly as a result of courting nest-preparing females less and chasing away other males less frequently. Farm Atlantic salmon have been found to display inappropriate breeding behaviour and construct fewer and poorer quality nests in the wild compared to wild Atlantic salmon (Fleming *et al.*, 1996). Fleming *et al.* (2000) found that female farm Atlantic salmon have about one third of the breeding success of wild females and farm males had only a few percent success relative to wild males, although relative spawning success may be density-dependent (Fleming *et al.*, 1997). Skaala *et al.* (1996) introduced first generation hatchery reared non-native brown trout spawners into a river with native anadromous and freshwater trout and sampled subsequent F_1 offspring at 0+, 1+ and 2+ stages. The introduced fish were found to mate among themselves and with the wild trout although the number of hybrid and introduced trout offspring was much lower than expected given the relative numbers of introduced and wild spawners.

Brown trout introduced directly into a lake show reduced spawning capacity and do not enter spawning rivers (O'Grady, 1984; Hesthagen *et al.*, 1999) presumably because lack of prior experience means that they do not have appropriate homing behaviour to take them to spawning areas. Chilcote *et al.* (1986) found that farm-reared steelhead trout spawning under natural conditions produced only 28% of the smolts compared to wild fish. Leider *et al.* (1990) found that the potential reproductive success of naturally spawning hatchery origin steelhead trout was some 11-13% of wild trout. Berejikian and Ford (2004) reviewed six studies of the relative fitness of non-native domesticated steelhead trout in the wild and found that lifetime fitness ranged from 6% to 35% (mean 17%) of the native wild population. McLean *et al.* (2003) found that wild female steelhead produced nine times and 42 times (two separate years) the number of adult offspring per fish that farm females did, spawning in the wild. Even short periods in a hatchery can reduce the fitness under natural spawning conditions, as shown by several studies on sea-ranched Pacific salmonids (Reisenbichler and Rubin, 1999). Connor and Garcia (2006) suggest that hatchery females have difficulty locating and identifying suitable spawning habitat due to hatching and early rearing under artificial conditions and thus unlike wild salmon they did not have the opportunity to experience suitable habitat as embryos and fry and also lacked exposure to pheromones deposited in the redd during spawning.

3.3.10 Assortative mating

Many studies (for example, Largiadèr and Scholl, 1996; Poteaux *et al.*, 1999; Berrebi *et al.*, 2000; Almodóvar *et al.*, 2006) have found evidence of non-random mating, that is breeding of wild with wild, and farm with farm trout. Such assortative mating would reduce the occurrence of hybrids and the introgression of farm genes into the native population. Non-random mating could be due to mate choice or differences in time or place of spawning. There is a growing body of evidence that salmonid mating is not at random but involves specific mate choice by the female. Many salmonids can differentiate between kin and non-kin using olfactory cues, which involve major histocompatability (MH) and other genes (Rajakaruna *et al.*, 2006).

Temporal isolation may be an important mechanism for preventing or limiting interbreeding of farm-reared and wild brown trout. Time of spawning in salmonids has high heritability (Sakamoto *et al.*, 1999; Fleming and Petersson, 2001) and is readily changed by artificial and natural selection. Spawning time is a trait that is frequently altered during the domestication of farm salmonid strains (Brannon *et al.* 2004b). Stefanik and Sandheinrich (1999) compared the timing of spawning and emergence of stocked and wild populations of brown trout in nine streams in Wisconsin. They found that stocked populations of brown trout had a median date of redd formation 10 days before wild trout and that 75% of redds were constructed 12 days before wild trout. They also found that on average alevins arising from stocked fish redds, constructed on the median date, emerged 17 days earlier than did alevins of wild trout. Shields *et al.* (2005) found a significant difference in the time of spawning between stocked farm-reared brown trout and wild trout in the Upper River Avon (Wiltshire, England). The stocked fish spawned in November and the wild fish in January or later. Observations supported a similar temporal difference in spawning in other southern England chalk-streams. Hansen *et al.* (2006) found later spawning of native trout (January-February) compared to introgressed brown trout (November-

December) in the Skern River (Denmark). Differences in timing of stocked and wild brown trout would prevent or at least limit the interbreeding between stocked farm-reared and wild brown trout, as well as reducing the survival of the farm offspring if emergence occurred too early.

The earlier emergence of stocked juveniles could give them a competitive advantage over wild trout, although in some circumstances it could result in high mortality due to emergence occurring before food and environmental conditions are appropriate (Brannon, 1987; Heggberget *et al.*, 1988; Quinn *et al.,* 2000). The poor performance of the farm trout may be the result of spawning too early in the winter. Thus selection in hatcheries for earlier spawning may result in spawning at an inappropriate time in the wild with consequently poor reproductive success. Brannon *et al.*, (2004b) have argued that spawning time, and consequent emergence timing, is the trait that probably has the most significant influence on fitness in salmonids. Shields *et al.* (2005) also found evidence of a spatial difference in spawning. Wild brown trout spawned in a spring-fed tributary and farm-reared brown trout spawned in the main river, in what was probably sub-optimal spawning habitat.

3.3.11 Reduced survival of hybrid offspring

As noted above, farm-reared brown trout and other salmonids have reduced survival compared to wild fish. Many studies have shown that hybrids between native wild and farm-reared salmonids also have reduced survival relative to wild fish in almost all situations. Typically hybrids are intermediate in survival between wild and farm-reared fish, as would be expected from additive genetic variation for traits linked to survival. The exact survival of hybrids varies with direction of cross (wild♀ x farm♂ or farm ♀ x wild ♂) and generation (F_1, F_2, BC_1). For Atlantic salmon overall lifetime survival has been shown to be around 35% for F_1 hybrids and 31-89% for F_2 and BC_1 generations (McGinnity *et al.*, 1997, 2003). Lowered fitness of hybrids thus reduces the level of introgression and this selection against hybrids acts to reduce the level of introgression once stocking ceases, as has been observed in several studies (for example, Poteaux *et al.*, 1998; Almodóvar *et al.*, 2001).

Table 3.1 Main reasons for low genetic impact of farm-reared brown trout stocked into native populations

Factor in stocked farm-reared brown trout	Key reference(s)
Low proportion relative to wild trout	Madeira *et al*. (2005)
Poor survival relative to wild trout	Kelly-Quinn and Bracken (1989); Skaala *et al*. (1996); Weiss and Smutz (1999); Borgstrøm *et al*. (2002); Aarestrup *et al*. (2005)
Handling and transport stress reduce survival – elevated cortisol	Johnsen and Hesthagen (1990); Jonssonn *et al*. (1999)
Stress due to novel physical and chemical conditions reduces survival	Jonssonn *et al*. (1999)
Farm strain – differential survival of different strains	Lasenby and Kerr (2001)
Health status of stocked fish can impact on survival	Lasenby and Kerr (2001)
Method and site of stocking can alter survival – clumped, dispersed, or trickle stocking	Cortes *et al*. (1996); Hesthagen *et al*. (1999); Lasenby and Kerr (2001); Martínez *et al*. (1993)
Greater survival of stocked trout in lakes than rivers	Martínez *et al*. (1993); White *et al*. (1995)
Farm-reared trout have shorter life spans than native	Hesthagen *et al*. (1999)
Age and size at stocking – larger fish have better angling return but smaller fish adjust more quickly to natural environment	Cresswell *et al*. (1982); Lasenby and Kerr (2001); Kahilainen and Lehtonen (2001); Hyvärinen and Vehanen (2003)
Time of stocking influences survival – spring, summer or autumn	Pirhonen *et al*. (2003)
Population productivity and competition – high density of wild trout reduces stocked trout survival	Lasenby and Kerr (2001)
Physical conditions of river affect survival – flow rate, velocity, gradient, temperature, pH	Madeira *et al*. (2005); Almodóvar *et al*. (2006)
Physiological differences of farm-reared trout, especially in parr-smolt transformation	Sundell *et al*. (1998)
Poor survival of farm trout that migrate to sea	Ruzzante *et al*. (2004)
Differences in dispersal movement within river	Jørgensen and Berg (1991); Weiss and Kummer (1999); Weiss and Smutz (1999); Bohlin *et al*. (2002)
Morphological differences of farm-reared salmonids	Swain *et al*. (1991); Gross (1998)
Increased aggression of farm-reared brown trout	Weber and Fausch (2003); Johnsson *et al*. (1996); Fleming *et al*. (2002)
Competition with wild trout already resident in territories	Deverill *et al*. (1999); Johnsson *et al*. (1999)
Reduced territorial holding of stocked trout	Sundström *et al*. (2003)

Factor in stocked farm-reared brown trout	**Key reference(s)**
Lowered ability to find food, at least initially	O’Grady (1983); Bachman (1984); Kahilainen and Lehtonen (2001); Sundström and Johnsson (2001)
Low dispersal of farm-reared trout from stocking site, possibly resulting in competition for food	Jörgensen and Berg (1991); Jonssonn *et al*. (1999)
Reduced awareness of predators and more willing to forage under risk of predation, for example, during day	Dellefors and Johnsson (1995); Johnsson *et al*. (1996); Alvarez and Nicieza (2003)
Colouration differences	Cresswell *et al*. (1982); Weber and Fausch (2003)
Greater susceptibility of stocked trout and hybrids to angling	Mezzera and Largiadèr (2001b); García-Marín *et al*. (1999); Champigneulle and Cachera (2003)
Reduced spawning ability	Fleming *et al*. (1996); Skaala *et al*. (1996)
Inability to find spawning grounds – no natal site to return to	O’Grady (1983)
Temporal differences in spawning	Stefanik and Sandheinrich (1999); Shields *et al*. (2005)
Spatial differences in spawning	Shields *et al*. (2005)
Non-random mating: preferential mating of farm with farm and wild with wild	Largiadèr and Scholl (1996); Poteaux *et al*. (1999); Berrebi *et al*. (2000); Almodóvar *et al*. (2006)
Farm-reared trout spawning in sub-optimal habitat	Shields *et al*. (2005)
Low fitness of farm fish and offspring	McGinnity *et al*. (2003)
Reduced fitness of farm x wild hybrids and backcrosses	McGinnity *et al*. (2003)

4 Impacts of stocking on the fitness, characteristics and viability of wild brown trout populations

4.1 The importance of genetic diversity within and among populations

Effective management and conservation of brown trout and other salmonids requires recognition and conservation of genetic diversity within and among populations. One of the main arguments for the preservation of such genetic diversity is that it is essential for populations and species to be able to respond to both short-term and long-term environmental challenges (Lande and Shannon, 1996; Frankham *et al.*, 2004). Genetic diversity enables a species to thrive in diverse environments. Environments are constantly changing and genetic variability is necessary for organisms to continue to produce the adaptations necessary for survival, for example if the climate changes or new diseases emerge. Loss of within population variability increases the likelihood of extinction for that population. Loss of variability among populations increases the likelihood of species extinction. There are many examples from agricultural crops of the problems of genetic uniformity when a new disease strikes. The ability to improve the performance of farm strains in the future will also depend on the availability of genetic variability among wild trout populations.

Genetic diversity contributes to the fitness of populations, an important consideration for an exploited species. For a population to be exploited there must be more individuals produced than are required for the replacement of the population. Loss of genetic diversity leads to lowered abundance, lowered recruitment and greater uniformity in life history characteristics. Greater genetic diversity promotes abundance by enabling a population to exploit more efficiently the full range of habitats and resources available in a water system. Genetic diversity improves year to year stability in numbers, since survival varies less for a population able to cope with a wide range of environmental conditions. Failure to recognise that the abundance of a species depends on genetic diversity within and among populations has led to poor management, based on the erroneous assumption that abundance could be increased by supplemental stocking with a few generic domesticated farm strains.

Genetic diversity results in phenotypic diversity including variable morphology, growth rates, longevity, feeding behaviour, age of return from the sea or lake, run-timing and so on. This provides a diversity of angling opportunity and experience. The economic value of brown trout for angling is not just determined by the abundance of individuals but also by the diversity of types that are available for exploitation (Youngson *et al.*, 2003).

Finally, genetic diversity is an integral component of biodiversity and there is a legal obligation on the UK government as a signatory to the Rio Convention to protect it. In

North America, intraspecific diversity of salmonids is increasingly being protected through the description of Evolutionarily Significant Units (ESUs) (Waples 1991), which are the distinct population segments recognised under the US Endangered Species Act, through Management Units, or Conservation Units (Ford, 2004). Although these concepts are not without inherent problems (Crandall *et al*., 2000; Ford 2004) they provide a framework for legislation and conservation that avoids semantic species arguments. While the designation of species or other taxa may be appropriate in some specific circumstances (Ferguson, 2004; Duguid *et al*., 2006), the geographically mosaic pattern of mixed lineages in many brown trout populations (Antunes *et al*., 2001; McKeown, 2005) dictates that effective conservation of brown trout genetic diversity can only be based on a 'bottom-up' approach. That means the conservation of genetic differences that occur within and among populations (Laikre, 1999; Antunes *et al*., 2001; Youngson *et al*., 2003).

4.2 Is supplemental stocking of any value?

In spite of the large amount of money spent on supplemental stocking, there have been few scientific attempts to assess its effectiveness in properly controlled experiments that differentiate between the effects of stocking and other variables (Morita *et al*., 2006a). Supplemental stocking has often been undertaken without well-defined management goals and so it has been difficult to evaluate its success (Cowx, 1994). A number of recent studies have questioned the widespread assumption that stocking leads to population increases. The greatest stocking of salmonids involves Pacific salmon where some 5 billion fry are released each year. In Japan, stocking has been credited with the major increase in salmon catches during the last quarter of the 20th Century. However, Morita *et al*. (2006b) show that recent increases in Japanese pink salmon catches can be largely explained by climatic variation, with little contribution from increased stocking.

Although domesticated farm-reared brown trout have poor survival in the wild (see section 3.3), some individuals do survive and breed successfully. This has been erroneously taken as an indication that stocking is beneficial. However, the fact that some stocked fish survive does not mean the total number of fish in the water is increased. The stocked fish may survive and breed at the expense of an equal, or even greater, number of the wild fish. Even though a proportion of the angling catch consists of stocked fish or their offspring, the total catch could be reduced as a result of negative interactions between the stocked and wild fish. It is surprising how many studies ignore this possibility and equate survival of stocked fish with success of supplemental stocking.

Often supplemental stocking is undertaken without adequate consideration of why there are too few brown trout present to start with. Stocking that increases a population beyond its habitat carrying capacity is wasted effort at best and can be counter-productive as a result of increased density-dependent mortality. In other words, if reduced numbers are due to reduced habitat and food, then adding more fish will result in increased competition and lower survival overall, perhaps giving fewer fish at the end of the day than if stocking had not been carried out. In a communal experiment under natural conditions involving farm, wild and hybrid Atlantic salmon (McGinnity *et al*. 2003), 57% of the wild parr were displaced from the river by the farm offspring and hybrid fish, which were faster growing and larger. Later survival in the sea of both farm and hybrids was poor relative to wild fish and the overall adult return was only some 45% of what it would have been had only the wild fish been present. However, the adult return contained farm and hybrid fish, which in the absence of other information could have been taken to indicate that 'stocking' of the farm salmon was successful. In an experiment in the River Imsa (Norway) involving the release of 22 farm and 17 wild mature Atlantic salmon, Fleming *et al.* (2000) found that the smolt production for wild females was 31% below that expected in the absence of farm females, based on data for smolt output in that river over the previous 18 years. Chilcote (2003) found that a spawning population comprised of equal numbers of hatchery and wild steelhead rainbow trout would produce 63% fewer juvenile recruits per spawner than one comprised entirely of wild fish. He concludes: "For natural populations, removal rather than addition of hatchery fish may be the most effective strategy to improve productivity and resilience."

Often stocking is undertaken in parallel with environmental and other improvements. Then, if the total number of fish increases it is generally not possible to determine which of the actions resulted in the increase. An increasing number of examples demonstrate that environmental improvements give much greater, and longer-term, returns than stocking. Fjellheim *et al.* (2003) evaluated a stocking programme in the River Teigdalselva (western Norway), where regulation for hydroelectric purposes had caused a decline in sea trout. Absence of suitable habitat, especially during winter, caused a high mortality particularly among farm-reared trout. The stocking programme was stopped and habitat improvement work carried out on suitable stretches of the river. Trout densities increased. Control areas of the river showed no sign of decline after the stocking programme was stopped, suggesting that the carrying capacity of the river was maintained by natural recruitment and that previous stocking had not resulted in any increase in fish. The study indicates that restoring fish habitat is a better method of increasing trout densities than supplemental stocking in rivers where habitat is limiting. Oosterhout *et al.* (2005) examined stocking and habitat improvement in the restoration of Oregon coast coho salmon. They found that although stocking could provide a short-term increase it led to longer term decline as a result of interbreeding with wild fish. Only habitat restoration provided a long term gain.

4.3 Ecological impacts of stocking

Stocked farm-reared brown trout can impact on wild brown trout populations both through ecological and genetic effects. Ecological impacts arise through competition, introduction of diseases and parasites and increased predation. These can reduce survival in the wild population (for example, Einum and Fleming, 2001). Genetic changes can result indirectly from these ecological impacts, due to lowered effective population size and potentially increased genetic drift and inbreeding.

4.3.1 Competition

Brown trout compete for food and space in rivers and probably also in lakes. Body size and territoriality are often good predictors of competitive ability in rivers. Stocked trout are often larger than the equivalent wild cohort, as a result of selection for faster growth and earlier hatching, perhaps together with favourable conditions for growth (such as diet and temperature) in the farm. This larger size, along with the more aggressive behaviour typical of domesticated fish, means stocked fish can competitively displace wild fish (reviewed by Weber and Fausch, 2003). Interactions between stocked farm-reared and wild fish can affect both mortality and growth of the wild fish (for example, Einum and Fleming, 1997; Weber and Fausch, 2003). Hybrids can also be larger and more aggressive than wild trout and have a similar detrimental competitive impact. Poorer later survival of the farm and hybrid fish means they do not compensate for the displaced wild fish, so the overall number of adults and subsequent juvenile recruitment (fitness) are reduced (McGinnity *et al.,* 1997, 2003; Fleming *et al.,* 2000; Ferguson *et al*., 2007). In supplemental stocking, farm-reared brown trout are stocked on top of the wild production. If the habitat is already at carrying capacity the stocking will inevitably result in increased competition and increased density-dependent mortality. The outcome can be that less trout survive overall than would have survived without stocking.

Stocked farm-reared brown trout can also compete with wild fish for mates and for spawning grounds. Superimposition of redds is common among salmonids, especially when the density of spawners is high. Late-spawning individuals may dig up the eggs of early-spawning fish, thereby lowering the latter's reproductive success. Thus, even when farm-reared trout have low spawning success, they could reduce the success of wild fish, although the tendency to earlier spawning of farm trout limits this effect.

4.3.2 Introduction of diseases and parasites

This has been little studied in brown trout relative to other salmonids. Work on other species indicates that introduced diseases and parasites may be problematic for wild brown trout. Problems associated with *Gyrodactylus salaris* and marine salmon lice (*Lepeophtheirus salmonis, Caligus* sp.) are well documented for brown trout and Atlantic salmon. Less well studied are various bacterial, viral and other parasitic diseases that can cause significant mortality on fish farms. These are almost certain to cause increased mortality in the wild, particularly under conditions of environmental stress. It is virtually impossible to study the effects of many diseases in the wild compared to an enclosed farm situation with normally high survival. In Switzerland, proliferative kidney disease (PKD) has been identified as one of the main causes of brown trout decline in recent years (Burkhardt-Holm, 2005). PKD is endemic in many UK trout farms where it results in losses of some £2.5M per year, and there is evidence that the impact of the disease is worsening (Feist, 2004). Diseases originating from fish farms could indirectly be an important mechanism of evolutionary change in wild salmonid populations. They have, for example, been shown to result in changes in MH class II alpha genes in Atlantic salmon (deEyto *et al.*, 2007).

4.3.3 Increased predation

Introduction of farm-reared salmonids can increase predation on wild fish through the attraction of predators. Nickelson (2003) found that productivity of wild coho salmon in 12 Oregon coastal rivers and two lake basins was negatively correlated with the number of farm-reared coho salmon smolts released in each area. On the basis of indirect evidence, it was argued that this negative correlation was due to predators being attracted to concentrations of farm-reared juveniles in the coastal estuaries, resulting in increased mortality of the wild smolts.

4.4 Direct genetic impacts of stocking

Direct genetic changes result from the interbreeding of farm-reared and wild brown trout and the backcrossing of hybrids to the wild population in subsequent generations, resulting in a change in the genetic make-up of the wild population. There are four main types of genetic change that can occur.

- A change in the level of genetic variability within the wild population due to a change in effective population size. Reduced effective population size results in increased genetic drift and inbreeding. Gene flow from farm to wild brown trout populations will result in the effective population size being determined by the effective population size of the farm strain (Tufto & Hindar, 2003).
- A change in the frequency and type of alleles present in the wild population. This results in a change in the composite genotypes and phenotypes produced and thus changes in the life history and other characteristics of the population.
- A reduction in the genetic variability among wild populations.
- Extinction results in the loss of all genetic variability in that native population.

These changes (except extinction) could potentially be negative, positive or neutral with respect to the productivity, fitness, population characteristics, short-term sustainability and long-term evolutionary potential of wild populations.
In an extensive review of available studies on the genetic effects of farm-reared fish on wild populations, Hindar *et al.* (1991) found that where genetic effects of introductions on performance traits of the wild population had been determined, they were always negative. Although extensive research on stocked or escaped salmonids has been undertaken since then, that basic conclusion remains valid.

Reduction in effective population size as a result of the ecological effects outlined above, and loss of genetic diversity as a result of introgression can result in inbreeding depression and loss of fitness. Introgression can also result in outbreeding depression. The interaction of farm-reared brown trout with wild fish may also change selection pressures on natural populations, through differential impacts on particular size, life history, geographical or temporal components of the wild stock. Many of the changes are cumulative over generations as stocking with farm-reared brown trout is generally carried out on a continuing basis. Fitness may continue to fall until the population is no longer self-perpetuating.

4.4.1 Reduction in effective population size and inbreeding

Inbreeding is universally accepted as having detrimental affects on fitness. Inbreeding occurs when genetically related individuals mate, the probability of which increases as the effective population size (N_e) decreases. Inbreeding can result in a loss of genetic diversity and inbreeding depression, which is a decrease in survival, growth rate, feed conversion efficiency, fecundity and an increase in developmental abnormalities (Kincaid, 1995). Inbreeding is proportional to $1/2N_e$ where N_e is the effective population size. Two factors result in N_e being less than the number of sexually mature breeders (N_b) in a population:

- unequal sex ratio;

- variance in individual reproductive success (some individuals leave more offspring than others).

In situations where the founding or earlier generation N_e was less than the current number, N_e will be further reduced since overall N_e is the harmonic mean of the N_e in each generation. This means that long-term N_e tends towards the lowest N_e in any generation. In small populations genetic drift, even in the absence of matings between closely related individuals, can also result in the loss of genetic variability and reduced fitness. There is very strong evidence that inbreeding depression contributes to increased risk of population extinction (Frankham, 2005).

Deleterious alleles constantly arise in populations due to mutation and it is estimated that at least 100 such alleles are present in an individual when all gene loci are considered (Lynch and Gabriel, 1990). In large populations with random breeding, deleterious alleles, normally present at low frequency, occur mainly in the heterozygous state. Since they are normally recessive this means they have no effect on the phenotype. Both inbreeding and genetic drift result in deleterious recessive alleles appearing in the homozygous state and being expressed in the phenotype. While the reduction in fitness at one locus may be small, when taken over multiple loci substantial reduction in fitness can result. Reduction in fitness can also occur because homozygotes may produce proteins that function less well than heterozygous products (this is known as heterozygote advantage or overdominance). Again, when taken over multiple loci the effect can be considerable.

Several mechanisms have evolved in salmonids to avoid or reduce inbreeding and maximise offspring genetic diversity. Multiple mating, often including mature parr, is common in brown trout (García-Vázquez *et al.*, 2001) and Atlantic salmon (Thompson *et al.*, 1998). It has been shown to increase fitness and individual genetic diversity in Atlantic salmon (Garant *et al.*, 2005). A low level of straying and consequent gene flow between populations also reduces inbreeding. Overlapping generations is a further mechanism for inbreeding avoidance. The N_e of a population with overlapping generations is approximately the N_e per year multiplied by the generation time. This way, small populations of salmonids are able to maintain more genetic variability than would be expected from estimates of the number of breeding individuals in any one year (for example, Consuegra *et al.*, 2005)

Many studies have found a positive correlation between the level of genetic variability at genetic marker loci (heterozygosity) and fitness characteristics (Thelen and Allendorf, 2001; Hansson and Westerberg, 2002; Coltman and Slate, 2003; Primmer *et al.*, 2003; Reed and Frankham, 2003, Balloux *et al.*, 2004). However, other studies have failed to find a correlation and the overall concordance between heterozygosity and fitness must be regarded as weak (Wang *et al.*, 2002). Many factors including environmental conditions, age or developmental stage, genetic background and the genetic markers and fitness aspects employed can affect these correlations (Wang *et al.*, 2002). The increase in fitness with higher heterozygosity is thought to be due to two effects:

- masking of deleterious recessive alleles in heterozygous state;
- advantage of heterozygotes over homozygotes at many loci (functional or associative overdominance).

An appropriate level of aggressive behaviour is an important fitness-related characteristic in salmonids, as aggression is an important component of obtaining and defending optimum feeding stations. Tiira *et al.* (2003, 2006) found that both brown trout and Atlantic salmon fry with a reduced level of genetic diversity had significantly lower aggressive behaviour (were subordinate) than fry with higher genetic diversity. Clearly increased genetic diversity is potentially advantageous for the fitness of a population. This correlation has led some to argue that introgression from farm-reared brown trout strains would be advantageous as in some situations it results in an increase in genetic variability (see section 4.4.3). However, this suggestion ignores the converse phenomenon to inbreeding depression, which is outbreeding depression.

4.4.2 Introgression and outbreeding depression

Outbreeding refers to interbreeding of genetically distinct types. When two highly inbred genetically distinct strains are crossed the hybrids can have increased performance, a phenomenon referred to as hybrid vigour or heterosis. This is probably due to masking deleterious alleles and heterozygote advantage. When a non-inbred wild population is crossed with a genetically distinct farm strain or non-native population, the fitness of the hybrids is generally intermediate between the two parental types. That is, the hybrids with their intermediate phenotype are less well adapted to the natural conditions than the wild fish and have a lower fitness than the wild population, a phenomenon referred to as outbreeding depression. Even when hybrid vigour is shown in the F_1 generation outbreeding depression normally occurs in subsequent generations.

Outbreeding depression is to be expected when each population has different additive genetic variation for fitness related traits. That is, if the parental types are adapted to different conditions, such as farm and natural, or two different river environments.

In some situations the hybrids may show lower fitness than either of the parental populations. This can result from a breakdown of coadapted complexes of epistatic alleles (Templeton, 1986). These are groups of compatible alleles at different gene loci that are selected for their joint effect on fitness under specific local conditions. It involves the harmonious interactions of many different traits (Falconer and Mackay, 1996).

Outbreeding depression may not be evident until the F_2 or later generation, after recombination has resulted in the loss of favourable epistatic combinations of alleles (Falconer and Mackay, 1996). Both hybrid vigour and outbreeding depression can operate simultaneously, in different individuals in an introgressed population. The level of fitness change in a stocked population is related to the fitness of individual hybrids and the degree of introgression. The latter depends on the number of farm-reared brown trout and the extent to which they hybridise with wild fish. Theoretically a low level of introgression could increase fitness in a population and a higher level could diminish it, although this has not been demonstrated in practice.

While the degree of outbreeding depression seems to depend on the extent of genetic differentiation between the parents, relatively few examples are available for salmonids. Experiments need to run for at least two generations. McGinnity *et al.* (2003) found that F_1 hybrids between farm and wild salmon were intermediate between the parental types in survival and other characteristics. However, F_2 hybrids showed some 68% mortality from fertilisation to the eyed egg stage compared to 40% in the farm and 5% in the wild embryos. Backcross hybrid embryos using the same parents showed 8% mortality indicating that the high mortality in the F_2 hybrids was not the result of gamete quality.

Gilk *et al.* (2004) found reduced survival, relative to native fish, of both F_1 and F_2 hybrids of geographically separated pink salmon populations. The reduction in survival was greater in the F_2 hybrids supporting an epistatic model of outbreeding depression. Wang *et al.* (2004) found that F_1 hybrids between native and non-native pink salmon were intermediate between the parental types in development times under communal natural conditions. Development times of the backcrosses were intermediate between the hybrids and the parental types. The authors conclude that these differences in development time of the geographically separated stocks could be a possible mechanism for outbreeding depression in hybrids. Currens *et al.* (1997) found that introgression with non-native farm-reared rainbow trout reduced the ability of the wild population to combat infections of the parasite *Ceratomyxa shasta*.

4.4.3 Is introgression an advantage?

It was suggested by Moav *et al.* (1978) and Wohlfarth (1993) that introgression with farm strains that have been selected for production traits could improve the performance of wild populations. Purdom (2002, 2003) claimed, on theoretical grounds, that introgression from farm-reared brown trout would improve the fitness of wild brown trout populations. As pointed out by Reisenbichler (1997), Wolhfarth (1993) misinterpreted the data purporting to show hybrid vigour. Almost all examples of hybrid vigour come from crossing inbred strains of domesticated, agriculturally important plants (for example, maize) and animals, although this approach is more useful for obtaining strains with uniform characteristics rather than increased yield (Falconer and Mackay, 1996). Most examples of hybrid vigour refer to mid-parent heterosis (see glossary).

There are very few examples of hybrid vigour in natural populations, and thus hybrid vigour seems to be the exception rather than the rule. The Independent Scientific Advisory Board (2002) note that they found no examples where crossbreeding wild anadromous salmon with hatchery stocks had improved the survival of the wild stocks. Einum and Fleming (1997) found that hybrid wild x farm Atlantic salmon were able to dominate the offspring of both parental types in pairwise contests whereas a cross from a different wild population was intermediate between the parents in this behaviour. Glover *et al.* (2003) found higher growth in hybrids between two stocks of brown trout than in the parental stocks. There are, however, very many more examples of hybrids between farm and wild salmonids having reduced fitness relative to wild fish.

In a common garden experiment carried out under natural conditions, McGinnity *et al*. (2003) found that farm salmon showed faster growth and matured at a later age than wild salmon, as expected from the additive genetic variation for these traits and selection in the farm strains. The hybrids showed no evidence of heterosis and were intermediate in growth and age at maturity returning as two sea-winter fish rather than one sea-winter as most of the wild salmon. At first sight this might suggest that interbreeding could be advantageous giving larger salmon, which would be desirable from an angling perspective. However, wild parr were displaced from the river by the farm offspring and hybrid fish resulting in decreased wild smolt production. Later survival in the sea of both farm and hybrids was very poor relative to wild fish and the overall adult return was only some 45% of what it would have been had only the wild fish been present. Thus the potentially desirable angling characteristics were offset by substantial reduction in population fitness, which on a cumulative basis would quickly lead to population extinction. Without further additions of farm fish the increased growth and later maturity, being the result of additive gene effects, would be largely lost within two generations of backcrossing to wild salmon (Tymchuk *et al.*, 2006).

Could it be advantageous to introduce farm-reared or non-native brown trout to populations that have lost genetic variability due to small size, physical isolation or severe bottlenecks? This approach has been proposed for several endangered species thought to be suffering from inbreeding depression (for example, Hedrick, 2005). Introductions are recommended at the level of one or two individuals per generation, sufficient to increase genetic variability without the associated problems of outbreeding depression and genetic load (for example, Wang, 2004). Clearly the level of introduction in supplemental stocking of brown trout vastly exceeds this. Supplementing a very small population with individuals from other, larger populations may even increase the deleterious recessive alleles (Amos and Balmford, 2001) with consequently increased genetic load. The risk is increased when farm-reared fish are used, because relaxed selection in the hatchery environment allows accumulation of such alleles (Lynch and O'Hely, 2001). When the farm population makes a significant contribution to the wild population this can substantially reduce fitness (supplementation load) in just a few tens of generations and substantially increase the risk of extinction of the wild population (Lynch and O'Hely, 2001). These authors conclude: "…. the apparent short-term demographic advantages of a supplementation program can be quite deceiving. Long-term supplementation programs are expected to result in genetic transformations that can eventually lead to natural populations that are no longer capable of sustaining themselves."

Contrary to some statements in the literature, reduction in fitness as a result of interbreeding between farm-reared and wild brown trout does not require that there are adaptive differences among wild populations. It only requires hybrids between wild and farm-reared trout to have lower fitness than wild fish. This will be true if genetic changes have taken place in farm-reared strains that reduce their survival and reproduction in the wild. Such changes have been abundantly demonstrated for brown trout and other salmonids (section 3).

Fitness will be further reduced by local adaptive differentiation (see section 4.4.4). In a comparison of the offspring of native and non-native Atlantic salmon under communal conditions, McGinnity *et al.* (2004) found that overall lifetime success of the non-native fish, from fertilized egg to returning adult, was 35% relative to the wild fish. In this case the non-native fish were from a river some 60 km away (river estuary distance) from the river where the comparisons were undertaken. Both rivers had tributaries arising around 0.5 km apart on the same mountain. Gilk *et al.* (2004) found outbreeding depression in hybrids between spatially separated (around 1000km) pink salmon populations.

Introduction of farm-reared salmonids into feral (naturalized) populations of salmonids can also result in reduction in fitness. There are three possible reasons for this, which are not non-mutually exclusive. First, the feral fish could have become adapted to their new conditions. Since local adaptation can occur in a relatively few generations (see section 4.4.4) there has been time for this to have happened for many feral populations. Second, the feral population could have come from a source population better suited to the natural conditions than the farm strain. Third, it could be due to the negative consequences of founder and domestication effects in the farm strain, as discussed above. Miller *et al.* (2004) found that the offspring of feral rainbow trout had significantly greater survival than the offspring of farm-reared trout and hybrids. The relative survival to age 1+ compared to pure feral offspring was 0.59 and 0.37 for hybrids, and 0.21 for pure farm offspring. In North America where brown trout is an introduced species, offspring of feral brown trout showed higher survival than the offspring of two farm strains when stocked into six Michigan rivers (Wills, 2006). On average, survival of the feral brown trout was more than 100 times greater than one farm strain and more than six times higher than the other. In addition some feral brown trout survived to ages 3 and 4 while few farm trout survived past age 2.

Genetic changes due to hybridisation and introgression may change the characteristics of a population even if there are no obvious changes in fitness. Characteristics such as extent of anadromy, age and timing of adult return to rivers from the sea or lakes, age of maturity and longevity have high heritability (Jónasson *et al.*, 1997; Palm and Ryman, 1999; Fleming and Petersson, 2001: Ferguson, 2006) and diversity in such characteristics is important for angling exploitation (Youngson *et al.*, 2003). Alteration of such characteristics may have economic consequences irrespective of whether it impacts on the fitness of the population.

4.4.4 Loss of inter-population genetic heterogeneity

As most stocking involves a small number of strains, it results in genetic homogenisation of wild populations. Local adaptations and overall genetic variability can be lost (Wang *et al*. 2002), which is likely to be detrimental in allowing brown trout to continue to adapt to changing environmental conditions such as global warming and new diseases. The importance of variability among populations in respect of single locus and quantitative traits is perhaps less widely accepted than the importance of variability within populations. This is partly due to the difficulty of demonstrating that genetic variability among populations results in local adaptation compared to relative ease with which inbred individuals can be produced and the effect on fitness observed. To demonstrate local adaptation, you must show that variability in a genetically determined trait is associated with survival, reproductive success or another fitness related aspect. Although correlations have been shown in a number of cases, proof of local adaptation requires reciprocal transplantation common garden experiments, which are logistically difficult, very time-consuming and expensive.

For local adaptation to exist, populations must be sufficiently reproductively isolated to allow adaptive differences to build up without being disrupted by gene flow. Many studies have demonstrated accurate natal homing behaviour of brown trout and other salmonids - they return with high precision to the river where they were born. However, straying is also a widespread occurrence. The key question then is, does this straying result in sufficient gene flow to prevent local adaptation? We must remember that straying by itself does not mean gene flow. The stray must reproduce and its offspring survive in the new location. Hybrids between native and non-native salmonids have been shown to have much reduced survival (for example, McGinnity *et al.*, 2004). Due to this lowered fitness, effective gene flow is much less than the actual straying rate.

Numerous molecular studies have shown significant genetic differences between brown trout populations in adjacent rivers. If gene flow among populations exceeded about four migrants per generation then these differences in neutral allele frequencies could not exist (Morjan and Rieseberg, 2004). Thus effective straying rates, in terms of gene flow, are probably less than 1%. Given that strays may leave the river again and are likely to have reduced fitness (for example, McGinnity et al., 2004), this could equate to actual straying rates of at least 5%. Valid estimates of straying are lacking for brown trout populations but this level is typical of straying rates found in Atlantic salmon (for example, Potter and Russell, 1994). Quinn (2005a) notes that 95-99% of wild salmonids surviving to adulthood home to their natal site.

Differences in alleles subject to selection can be maintained at higher levels of gene flow than neutral ones and adaptive differentiation can occur when the coefficient of selection is greater than gene flow. There is good evidence that the basic requirement for local adaptation exists in brown trout, that is sufficient reproductive isolation among populations for adaptive differentiation to be produced by natural selection operating at a similar intensity and manner to that which has been demonstrated in many other organisms. To argue that local adaptation does not exist in brown trout would require an explanation of why brown trout is different from other organisms.

Brown trout populations live in waters that vary in local conditions such as river chemistry, substrate and other habitat characteristics, temperature, pH, flow regime, food type and availability, diseases and parasites, competing species and predators. It would be surprising, and contrary to accepted population genetic theory, if natural selection had not resulted in adaptive differences in many fitness-related characteristics such as body morphology, growth rate, longevity, body size, timing of out- and in-migrations, age of maturity, egg size, fecundity, time of spawning, development rate, habitat preference, aggressive behaviour, feeding behaviour, migratory behaviour and resistance to disease and parasites.

As noted above, a low level of gene flow is advantageous as it prevents loss of genetic variability in small populations, and allows the spread of favourable alleles, without preventing differentiation among populations due to local selection and drift (Morjan and Rieseberg, 2004). As the level of gene flow is important for fitness it is subject to natural selection. If high levels of gene exchange were advantageous, natural selection would have led to high levels of straying and interbreeding among populations rather than the highly accurate natal homing behaviour that is typical of brown trout and other salmonids. However, in the immediate postglacial period when new habitats were available for colonisation natural selection would have favoured straying.

It has been argued that stocking is no different from natural straying. In straying, wild trout from neighbouring populations are involved, not fish that have been domesticated for perhaps 100 years or more. As noted above, effective straying rates in brown trout, in terms of gene flow, are probably less than 1% per generation. Stocking at such a level would not be considered useful for supplementing a wild population.

Arguments that brown trout have colonised too recently (around 14,000 years ago) to have evolved adaptive differences are also invalid. Local adaptation can be based on changes at a relatively small number of gene loci and can occur within a small number of generations. For example, in salmonids, timing of spawning and emergence timing (Brannon *et al*., 2004b) and precise timing (for example, Stewart *et al*. 2002, 2006) and directional aspects of migration are important in ensuring fitness under the environmental conditions specific to individual waters.

Koskinen *et al*. (2002) showed that adaptive differences have arisen by natural selection among grayling (*Thymallus thymallus*) populations established from a common source 80-120 years ago. In spite of the low number of fish involved in founding these populations, and subsequent bottlenecks in population size, (which would promote genetic drift) the pairwise Q_{ST} values were four to ten times higher than the corresponding F_{ST} estimates. Quinn and co-workers (Quinn, 2005a) showed that Chinook salmon introduced into New Zealand have, in less than a century, acquired river-specific differences in many characteristics, including timing of adult migration and spawning, age at maturity, growth and fecundity. Using common garden experiments, they showed these differences have a genetic basis and are likely to have arisen through adaptation to different conditions in the various rivers. Brown trout were introduced into New Zealand from the late nineteenth century onwards and again there are now differences among populations in potentially fitness related traits (for example, Hayes and Hill, 2005).

Direct evidence of local adaptation in brown trout is limited. Svärdson and Fagerström (1982) used transplantation experiments to demonstrate that sea trout migration patterns in the Baltic Sea are genetically determined and present evidence for these patterns being adaptive. Different patterns of movement at sea are known for sea trout stocks from different rivers in England and Wales, which could be similarly adaptive.

4.5 Supportive breeding

The use of native broodstock taken annually from a river has been advocated as a means of potentially avoiding the genetic problems of supplemental stocking with farm-reared or non-native brown trout. This is now generally referred to as supportive breeding in the European literature (for example, Hansen *et al*. 2000b) to differentiate it from other forms of stocking. Gametes are obtained from wild native trout, crosses undertaken, eggs placed in a hatchery and subsequently young are planted out at an appropriate life history stage (fry, parr, or smolt). In North American literature such hatcheries are often referred to as conservation hatcheries (Flagg and Nash, 1999), although these can also involve permanent farm strains of native origin and the term tends not to be used consistently. Flagg and Nash (1999) define a conservation hatchery as “a rearing facility to breed and propagate a stock of fish with equivalent genetic resources to the native stock, and with the full ability to return to reproduce naturally in its habitat.” There is a growing trend in Europe and North America towards supportive breeding. From 2006 it is the only form of supplemental stocking of brown trout permitted in Denmark.

The high survival to fry, parr or smolt stage in the hatchery, relative to that in the wild is often used as argument in favour of hatchery intervention. However, when hatchery-reared juveniles are released, they typically have much lower survival thereafter than the equivalent age wild juveniles. Many of the factors already discussed for farm-reared trout are involved (chapter 3). So the potential advantage of high survival in the hatchery can be partially or completely negated by relatively poor survival after release. Hatchery-reared salmonids also have lower success in reproduction. Egg to spawning adult and preferably egg to offspring in the next generation, need to be compared. Hatchery intervention is only justifiable when overall life cycle survival is significantly higher and there is a net survival advantage (egg to egg) over naturally produced fish. Unfortunately, the information needed to make such a comparison is largely unavailable.

Hyatt *et al.* (2005) investigated the relative success of wild and hatchery-reared sockeye salmon fry in two lakes. In one lake they found that wild egg to fry was higher than hatchery egg to fry survival (11.3% versus 4.3%) and wild egg to smolt survival was higher than hatchery egg to smolt survival (5.8% versus 2.5%). In another lake the reverse situation was found (wild fry 1.5% versus 6.3% farm; wild smolt 3.6% versus 12.8% farm). In the first lake good spawning habitat was available whereas the second site had limited spawning sites. Therefore, where natural spawning or nursery habitat is inadequate, supportive breeding may be justified but not in the situation where adequate such habitat is available.

Saltveit (2006) found that stocking Atlantic salmon in the River Suldalslågen (western Norway) resulted in stocked fish never exceeding 0.03% of the catch and in most years more adults were used as broodstock than returned as offspring. The broodstock were taken in spite of the fact that the natural recruitment in the river was below carrying capacity in most years. The author concludes that, even though native fish are used, the lack of positive response to stocking is possibly due to the condition of hatchery smolts especially lowered seawater tolerance, which increases mortality at sea. Stocking of 0+ parr did not lead to increased yield, which the author notes is in line with observations on other Norwegian rivers.

Before supportive breeding is undertaken, fishery managers must establish why stock levels are too low in the first place. Often it is because of barriers to migration, deterioration in habitat, or pollution. The first line of action should be to attempt to reverse these detrimental effects. In some cases, this may not be possible in the short-term and artificial stocking may be necessary for the time being to overcome a clearly identified bottleneck in production (Aprahamian *et al*. 2003). For example, in some rivers there is a shortage of spawning habitat, possibly as a result of barriers or unsuitable substrate, but adequate habitat for parr and adults. Other forms of intervention such as in-stream incubators can be used to alleviate spawning habitat bottlenecks. This aspect is not considered here, although some of the problems noted below can apply in this situation.

In supportive breeding the period in the hatchery should be as short as possible commensurate with overcoming the natural bottleneck to production. Any supportive breeding programme, as with any other stocking programme, must have well defined quantitative objectives with appropriate procedures to measure progress towards these objectives. A comprehensive management plan should be available, based on the best available scientific information, covering all aspects from broodstock selection, spawning protocols, hatchery environment, stocking out and monitoring of results and potential adverse impacts. Emphasis should be on the quality of offspring rather than their quantity. Quality should be defined by morphological, physiological, behavioural and other characteristics, as well as survival after release and subsequent reproduction. Supportive breeding must avoid adverse competition, predation and disease amplification interactions with naturally produced fish. Supportive breeding must also be undertaken within the carrying capacity of the water system.

4.5.1 Avoiding the pitfalls of supportive breeding

While supportive breeding has advantages over supplemental stocking with farm-reared domesticated brown trout, it is not without its problems. Several studies have shown that even a short period in a hatchery can result in a reduction in subsequent survival and reproductive success, both as a result of the hatchery environment altering behaviour and physiology, and genetic changes due to differential or relaxed selection (Glover *et al.*, 2004; Sundström *et al.*, 2004). High survival in the hatchery phase and lack of opportunity for selective mortality has been used to argue that selection does not occur when salmonids are reared in a hatchery but spend most of their lives in the natural environment. However, Reisenbichler *et al.* (2004) found that for steelhead, intense natural selection after release from the hatchery favoured individuals that had performed well (for example, had the fastest growth) in the hatchery. This selection resulted in genetic changes relative to naturally produced trout.

Nelson *et al.* (2005) found that hatchery reared steelhead, where the broodstock was drawn annually from the native population and released as smolts, were twice as likely to be caught by angling than wild fish. They speculate that this could have been due to differences in the early rearing environment or inadvertent selection during broodstock collection. Spawning locations also differed between farm-reared and wild fish with farm-reared trout being restricted to the lower two-thirds of the river, downstream of the farm, while wild fish spawned throughout the watershed.

In supportive breeding programmes considerable attention to broodstock choice is required. In some water systems there are multiple stocks of brown trout, which are spatially or temporally reproductively isolated. Inadvertent artificial mixing of these stocks can break down the population structure and local adaptation, leading to a loss of productivity and characteristics. Stewart *et al.* (2006) found that the timing of Atlantic salmon smolt migration in different tributaries of the River Tay (Scotland) has a genetic basis. Capture of broodstock from one stock component (for example, spawning at a particular time or place) or particular phenotype could result in an increase of this component to the detriment of others. For example, Kostow and Zhou (2006) found that hatchery produced summer steelhead resulted in a severe decline in the wild winter steelhead population as the number of hatchery steelhead caused the total number of steelhead to exceed carrying capacity thus increasing depensity dependent mortality. Before supportive breeding is undertaken, the genetic population structure within the water system, as well as the carrying capacity, should be examined.

Broodstock should be taken from the wild in each generation. Maintaining a hatchery broodstock will result in the problems of domestication already discussed for farm-reared brown trout. Some authors suggest that hatchery-reared offspring should be marked so that broodstock are only taken from wild fish. Where possible, several cohorts of mature fish should be used as broodstock, including a proportion of mature male parr. An equal number of each sex should be used, with at least 25 of each sex. Milt from several males should not be mixed together, as was the common practice in many hatcheries in the past. Mixing milt results in sperm competition and one male may fertilise all or most of the eggs (Gharrett and Shireley, 1985; Withler and Beacham, 1994; Campton, 2004, 2005) resulting in reduced N_e. A partial factorial system should be used for mating to increase the number of families and N_e. That is, the eggs and milt from each parent should be split into three or more batches. Thus, for each set of three females and males nine crosses are produced.

Once fertilisation has occurred the crosses can be mixed prior to incubation. In practice this is what happens in the wild where several males fertilise the eggs of one female and where individual males may fertilise eggs from more than one female. But mate choice and natural and sexual selection are absent in the hatchery situation (Quinn, 2005b). Campton (2005) notes that “no hatchery can accurately reproduce or mimic the total patterns of mating, reproduction and natural selection that occur under natural conditions.” Berejikian *et al.* (2005) suggest that collecting eyed-eggs from a river and rearing these for release allows natural and sexual selection to occur during spawning and helps to maintain adaptive characteristics. However, this would require that suitable spawning facilities are available in the river and that the bottleneck to natural production occurs at a later stage.

If a limited number of families is used for supportive breeding and these hatchery-reared fish contribute disproportionately to the naturally spawning fish, inbreeding and loss of genetic variability can occur as a result of lowered N_e (Ryman and Laikre 1991; Waples and Do, 1994; Ryman *et al*. 1995). However, although overall survival is high under hatchery conditions, and there is variability among families, subsequent high mortality and poor reproductive success may mean that there is not always a disproportionate contribution to the next generation. While lowering of N_e by supportive breeding is possible, in practice it will only be a problem when the hatchery-reared fish have substantially greater overall life-cycle success than the wild fish. This can occur when wild fish have very limited success as a result of environmental problems. Hansen *et al.* (2000b) found evidence of reduced effective population sizes in two out of the three Danish populations subject to supportive breeding.

As a result of environmental differences in the hatchery compared to the wild, brown trout that are hatchery-reared from native broodstock show considerable post-release mortality and poor reproductive performance. Brannon *et al.* (2004a) contend that the primary factor responsible for poor natural reproductive success of hatchery-reared native offspring is poor management, especially deliberate selection, and not an inherent problem with the culture process itself. They say: “hatchery fish are still the scapegoats for errors in fisheries management that overlook or disregard the importance of stock structure and biological requirements of anadromous salmonids.” They argue that most changes associated with hatcheries are due to non-native origin and long-term domestication, neither of which are features of conservation hatcheries in the USA that use native broodstock. Discussion of the impacts of stocking farm/hatchery-reared salmonids is confused by the failure of some authors to adequately identify the exact nature of the broodstock used and by making irrelevant comparisons. Thus there is a considerable difference between the impacts of stocking fish such as farm-reared brown trout that have been domesticated for over 100 years compared to offspring of native broodstock that are collected from the wild each generation. To compare these as simply ‘hatchery’ trout would be meaningless.

Fish conditioned to a hatchery environment show their highest mortality immediately after release when they are most vulnerable to predation. Several studies have shown that survival of hatchery-reared salmonids can be considerably increased by ‘life skills training’ prior to release. Brown and Laland (2002) have shown significantly better foraging success of Atlantic salmon on release following pre-release training involving exposure to live prey items in the presence of previously trained fish. Brown *et al.* (2003) found that exposure to live prey in a structurally enriched tank (containing plants, rocks and novel objects) also significantly enhanced foraging performance even with novel prey items. A Natural Rearing Enhancement System, (NATURES) (Maynard *et al*., 1995), is used to culture Pacific salmon in raceways that simulate the natural environment. Fish in these raceways grow accustomed to overhead cover, river structure and bottom substrate, non-intrusive food delivery, and simulated predators. However, Kostow (2004) found that acclimatization of hatchery reared steelhead juveniles in a pond prior to release resulted in smaller smolts and lower marine survival than direct hatchery releases.

To date only a few studies have considered the genetic impact of supportive breeding on wild populations. In a comparison of a sea-ranched native strain and wild brown trout planted as eggs under semi-natural conditions in the River Dalälven (Sweden), Dannewitz *et al.* (2003) found no difference in survival in the first year. They conclude that the impact of hatchery selection on the performance of native sea-ranched brown trout in the wild may not be as pronounced as some previous studies appear to indicate. Dannewitz *et al.* (2004) found that seventh generation hatchery-produced brown trout of native origin and wild-born trout were similar in performance, although in one experiment wild-born males had higher reproductive success. In the same river, Dahl *et al*. (2006) found no difference in survival and growth rates among the offspring of wild-born, hatchery and hybrid brown trout. In the case of the River Dalälven there is a high level of interbreeding between hatchery and wild-born trout (Palm *et al*., 2003) and thus hatchery selection is effectively limited to one generation.Thus it is not surprising that differences are not generally found as there is little opportunity for the hatchery and wild stocks to diverge genetically. However, Petersson and Järvi (2006) found differences in anti-predator response among wild, hatchery and hybrid brown trout from this river possibly replecting the recurrent acquisition of genetic differences within single-year classes.

Ford *et al*. (2006) evaluated the relative fitness of naturally spawned and hatchery reared coho salmon in Minter Creek (Washington, USA). They found no significant difference in the current relative fitness of natural and hatchery salmon probably again because of the high level of interbreeding between hatchery and natural fish. However, they found some 50% reduction in smolt production compared to 50 years ago although the actual number of spawners had not apparently changed. Such a reduction in smolt production could be due to changes in the freshwater habitat over this time as well as to long-term selection in the hatchery. The authors conclude that: “it seems probable that selective changes in the hatchery have contributed to reduced smolt production in Minter Creek”. They also found a long-term trend for adults to return earlier probably due to the practice of spawning earlier-running salmon in the hatchery. Hill et al. (2006) found that hatchery reared steelhead trout smolts, which were the progeny of native broodstock different from wild smolts in morphology and had a significantly lower of gill Na^+K^+-ATPase activity. Heggenes *et al.* (2006) found little apparent effect on genetic diversity in a steelhead rainbow trout population as a result of supportive breeding and attribute this to the use of substantial numbers of wild broodstock and multiple year classes in the hatchery.

A supportive breeding program for Chinook salmon in the Yakima River (Washington, USA) has been designed to minimise differences between hatchery and wild produced fish. However, Knudsen *et al*. (2006) found that first generation hatchery returns differed from equivalent wild fish in respect of size, sex ratio, age of return and timing of spawning. While it is not known whether these differences are genetic and / or environmental in nature and if they impact on fitness, it is clear that even carefully designed supportive breeding programmes do not produce fish that are identical to wild fish (Knudsen *et al*., 2006).

Table 4.1 Main impacts (not mutually exclusive) on wild populations of brown trout due to stocking of farm-reared (domesticated) brown trout.
Many of these impacts are cumulative over generations due to repeated stocking.

Displacement of wild fish. Reduction of wild parr survival and smolt production, due to competition from larger and more aggressive farm fish and hybrids, leading to reduced population size and recruitment

Attraction of predators to concentrations of stocked fish. Increased predation on wild fish and reduction in population size and recruitment

Introduction of diseases and parasites reducing wild survival and thus reducing population size and recruitment

Reduced effective population size leading to inbreeding depression and loss of genetic variability. Results in decreased survival, growth rate, feed conversion efficiency, fecundity and more developmental abnormalities

Conversion of part of wild production to hybrids, which have lower survival, thus reducing population fitness (outbreeding depression)

Higher survival of hybrids than wild trout in small, isolated population with reduced genetic variability (hybrid vigour)

Homogenisation of genetic differences among populations and loss of local adaptations

Reduced genetic variability within populations in some circumstances

Changes to life history characteristics such as extent of male parr maturity, smolt age, age of maturity, run-timing, time of spawning

Reduction in sea trout component and increase in freshwater component

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4.6 Adverse genetic impacts on indigenous trout populations

The studies reviewed above conclusively demonstrate the risks of genetically mediated impacts from stocking. It is evident from the many studies on the stocking of farm-reared brown trout that farm-wild introgression does occur in brown trout and results in genetic changes in the wild population. Studies on salmonids demonstrate that these genetic changes are almost always detrimental to the fitness and survival of individual populations (summarised in Table 4.1) and are also likely to be detrimental to the long term survival of the species. Three main conclusions arise from this review:

- Founding effects and domestication can result in genetic changes in farm-reared brown trout in a relatively few generations, and in some circumstances even within a single generation. Available data suggest that farm trout become progressively less fit for natural conditions.

- Interbreeding between farm-reared brown trout and wild fish can reduce the fitness of wild populations resulting in reduced recruitment, failure of the population to be self-sustaining and possible extinction in vulnerable populations.

- Interbreeding between farm-reared brown trout and wild fish has been demonstrated to reduce inter-population genetic variability, which on the basis of accepted population genetics and evolutionary theory is likely to reduce the ability of individual populations, and the species overall, to respond to new environmental challenges.

These conclusions are supported by many reviews for North American salmonids (for example, Meffe, 1992; Utter 1998, 2001, 2003; National Research Council, 1996; Reisenbichler and Rubin, 1999; Waples, 1999; Levin *et al.*, 2001; Independent Scientific Advisory Board, 2002; Utter and Epifanio, 2002; Brannon *et al.*, 2004a; Myers *et al.*, 2004). In the past the precautionary approach has been invoked as a reason for not stocking due to the lack of scientific evidence on potential problems. However, that supplemental stocking with farm-reared brown trout causes genetic damage and reduction in fitness is now established beyond reasonable doubt. Even supporters of hatchery intervention for defined purposes (for example, Brannon *et al.*, 2004a) acknowledge that changes due to non-native origin and long-term domestication result in supplemental stocking with farm-reared salmonids reducing the fitness of wild populations.

As there is no situation in which such stocking is biologically ‘safe’, all supplemental stocking of wild self-sustaining populations involving fertile farm-reared and non-native brown trout in England and Wales should be prohibited.

The only forms of stocking permitted should be supportive breeding (first generation offspring of native broodstock) or stocking with sterile triploid fish. It should be noted, however, that both of these forms of stocking have potential problems that need to be taken into account. Problems with supportive breeding are considered in section 4.5. While stocking with sterile fish eliminates direct effects on the genetic composition of the population, it may cause indirect genetic effects. Competition and introduced diseases, for example, may reduce the effective size of the wild population. (See review of stocking sterile salmonids by Kozfkay *et al.*, 2006).

All stocking programmes, irrespective of the type of stocking, should have clearly defined and quantifiable objectives with monitoring procedures to measure success. Prior to any stocking, a risk analysis should be undertaken and potential risks routinely monitored after stocking has taken place.

4.7 Should some self-sustaining brown trout populations be excluded from a no supplemental stocking policy due to previous history of stocking?

A large number of factors affect the survival and successful breeding of farm-reared brown trout when stocked in the wild. The extent of introgression in any population is impossible to estimate from its previous stocking history and can only be determined by genetic analyses on the specific population concerned. Some populations with a long history of stocking show little or no introgression from the farm strains. On the other hand, populations with limited previous stocking can show extensive introgression. It cannot be assumed that because a population has been stocked extensively in the past that the native genetic composition has been lost and that stocking with fertile farm-reared brown trout can continue in the future without further detriment.

While specific information on the level of introgression is very limited for England and Wales, information from studies in other European countries suggest that a majority of populations has less than 25% introgression and for many it is less than 10% (section 2.2). Unless proven otherwise, we should assume that each population has not been substantially changed in its genetic make-up due to previous stocking and should therefore be protected from further stocking. Any remaining native genetic make-up, even in a highly introgressed population, is worth conserving.

Although supplemental stocking with farm-reared brown trout often has little or no impact on a wild population in terms of producing genetic changes, this does not mean that stocking can continue with impunity. The more stocking is carried out, the greater the likelihood of detrimental genetic changes occurring. In addition, stocking can result in a reduction of fitness in a wild population due to the reduced fitness of hybrids between wild and farm-reared individuals. This lowering of fitness can occur without significant genetic changes being detectable in the wild population. Indeed the lower the fitness of the hybrids the greater the reduction in population fitness but the lower the genetic change, as introgression requires that the hybrids backcross with wild individuals.

4.8 Is there a safe level of introgression?

The potential benefits of supplemental stocking and the associated risk to wild populations increase simultaneously. The greater the level of stocking, and the length of time over which it is undertaken, the greater the probability of farm gene introgression and the higher the likelihood of reduction in fitness in the wild population. More stocking is then required in subsequent generations to make up for reduced natural recruitment. A very low level of stocking may have little risk, and in special situations of populations with very low effective population size may be advantageous, but it is unlikely to be of any benefit to the fishery. Thus any safe level of stocking is well below the level that would be of any management benefit.

4.9 Stocking with fertile farm-reared brown trout

In some self-sustaining wild brown trout populations it may be necessary for non-biological reasons (for example angling) for supplemental stocking with fertile farm-reared brown trout to continue, at least in the short term. If so, such stocking should be undertaken with takeable (legal) sized trout, as studies have shown that these fish are the least likely to survivie to breed. In addition these larger trout are the most likely to contribute to the angling catch without adverse competitive impacts on the wild population. Stocking should be carried out close to the start of and during the angling season in order to maximise returns. All fertile farm-reared brown trout should be physically marked (for example, with fin clips) before stocking. Anglers should be encouraged to remove all farm-reared trout caught and practice catch-and-release with wild trout. In effect this is superimposing a put-and-take fishery on top of a self-sustaining wild population.

4.10 Supportive breeding

While supportive breeding using native broodstock is successful in reducing genetic changes in a wild population, it should only be undertaken where there is a clearly identifiable bottleneck or problem involved in the natural recruitment process. Before supportive breeding is undertaken, fishery managers must first establish why stock levels are too low in the first place and attempt to reverse these effects. Often this will be because of deterioration in habitat and pollution. As in Denmark, supportive breeding should be the only form of stocking involving fertile brown trout permitted in England and Wales. Appropriate protocols must be followed to avoid potential problems of supportive breeding (see section 4.5.1)

4.11 Genetic restoration

Once stocking with fertile farm-reared brown trout ceases the level of introgression is likely to fall, as has been seen in several studies, because there is selection against farm genes. However, this is a slow process requiring many generations. In recent years interest has arisen in the active genetic restoration of introgressed populations. Such restoration can be effectively undertaken as part of a supportive breeding project.

In Lough Erne (Northern Ireland) it is possible to identify native, hybrid and farm-reared (Movanagher) brown trout using molecular genetic techniques (Taggart and Ferguson, 1986; Hynes *et al.*, 1996). Stocking with Movanagher farm-reared trout ceased in 1998 and a local hatchery was set-up using broodstock obtained from mature fish running Erne rivers. Broodstock or offspring of each cross have been screened genetically each year and only pure native Erne parr are stocked into the Erne system. Initial results suggest that the proportion of native genes is increasing in the Erne system as a result of this supportive breeding and possibly also as a result of selection against farm genes.

Crivelli *et al.* (2000) have used genetically pure populations of the marble trout (*Salmo marmoratus*) from headwater regions above impassable water-falls to carry out supporting breeding in the Soca River (Slovenia) where introgression had taken place with stocked brown trout of north west European origin. The strategy is to rehabilitate the genes of marble trout in introgressed areas until almost all non-native genes are eliminated. Stocking from a neighbourouring non-introgressed population has been used to increase native Mediterranean lineage brown trout in a population that had been substantially introgressed with farm Atlantic lineage trout (Caudron *et al.*, 2006).

Hansen *et al.* (2006) used pre-stocking microsatellite DNA data obtained from scale DNA to identify pure wild trout in the Skern River, Denmark. They suggest that such non-admixed individuals could be used for supportive breeding to restore the native population.

4.12 Prioritization of wild brown trout populations

Given that finite resources are available for habitat and genetic restoration projects it is appropriate to identify and designate key populations of special conservation merit. Such prioritization is an integral part of conservation programmes for many species including those listed under Annex II of the EC Habitats Directive. Under this approach populations of key biological significance are targeted for action. While a detailed consideration of prioritization procedures is outside the scope of this report, a few aspects will be considered briefly.

Key populations can be identified using a wide range of biological and environmental data. Quantitative traits such as run timing, spawning time and site, age of maturity, migratory and other behaviour, maximum size, feeding habits, body morphology and colouration, meristic differences, environmental tolerance, and disease and parasite resistance can be used (see Table 1.1). In addition direct genetic information from molecular marker studies can be included. Populations living under unusual environmental conditions in terms of geology, water chemistry and hydrology are also more likely to be genetically distinct. For seven Pacific salmonid species, Waples *et al.* (2001) found that both life history and genetic diversity showed a strong, positive correlation with the extent of ecological diversity experienced by a species. There was also a very strong correlation (r=0.96) between the number of major genetic and life history groups within a species. Detailed aspects of prioritising salmonid populations are dealt with by Allendorf *et al.* (1997), Laikre (1999), and Halupka *et al.* (2003).

4.13 Further studies required in England and Wales

There are only a few empirical studies on the impacts of introduced farm-reared brown trout in England and Wales, (for example, McMeel and Ferguson, 1997). There is also relatively little information on molecular genetic and quantitative genetic variability among brown trout populations in England and Wales. Further studies are needed to produce an inventory of the genetic resources represented by these populations.

It is essential that such studies involve multiple types of molecular marker in concert with investigations of quantitative trait variability. For high priority populations the extent of introgression should be determined using both mtDNA and microsatellite markers. Genetic profiles should be obtained of all the main farm strains of brown trout used for stocking in England and Wales. Genetic impacts are easier to assess if there is information on the genetic composition of both the wild population prior to stocking, from archived scales, and the farm-reared brown trout strains that have been used for stocking in recent decades. However, when such baseline data are incomplete, there are statistical methods that can be used to estimate them. Data on previous stocking of wild populations should be collated. Where it is not already being recorded, records must be kept on any future stocking of fertile farm-reared brown trout. They should include the source of trout, number, age and stocking locations, together with an estimate of wild fish density in the river. Similarly full records should be kept of all supportive breeding projects.

Such genetics work involves extensive technical and analytical procedures and studies on particular trout populations form very suitable projects for postgraduate students. From the Environment Agency's point of view, this is a cost-effective method of having the work undertaken.

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Glossary

Adaptation – genetic changes occurring as a result of natural selection that increases the overall fitness of a population under specific environmental conditions.

Allele – alternative forms of a gene. Brown trout are diploid. That is, there are pairs of chromosomes, 40 pairs in this case, with one member of a pair coming from each parent. The genetic material is present in duplicate. A gene locus consists of DNA at a certain point on both chromosomes of the pair, and each individual has two alleles. Alleles generally differ by one or more bases in the several thousand bases that make up an allele. Thus for an allele of 1000 bases in length there are potentially 4^{1000} (10^{600}) different alleles.

Allozymes – forms of an enzyme with slightly different structure (often differing by single amino acids) but the same function. Allozymes are coded for by multiple alleles at a polymorphic gene locus.

Anadromous – a migratory life cycle where spawning occurs in freshwater and the juveniles migrate to sea to feed for a period of time, generally until sexual maturity, before returning to freshwater.

Artificial selection – the intentional picking of individuals to breed from, based on desirable phenotypic characteristics.

Backcross – the mating of a hybrid with one of the parental types.

BC_1 – first generation backcross. The offspring of a cross between a hybrid and one of the parental types.

Bottleneck – one or more generations of substantially reduced effective population size in a population.

Broad-sense heritability (h^2_b) – the extent to which phenotypic variation is determined by genetic variation.

Brown trout – common name for all life history types within the *Salmo trutta* species complex throughout its range. Includes sea trout as well as river and lake forms of freshwater trout. Specific names are often given to local variants.

Broodstock – adult fish used to propagate a stock or strain.

Coadaptation / coadapted gene complexes – favourable combinations of alleles maintained within a population due to natural selection.

Diploid – an individual with two sets of chromosomes, one set of which is derived from each parent.

DNA sequencing – laboratory technique to determine the complete linear sequence of the four different bases that comprise a piece of DNA.

Domestication – genetic changes due to deliberate and accidental selection, and genetic drift, in artificial culture.

Effective population size / number (N_e) – the effective number of individuals as far as genetic transmission to the next generation is concerned. Takes account of differences in sex ratio and differential fitness of individuals in a population. N_e is smaller than the total census population size (N_c) or the number of breeders (N_b).

Electrophoresis – a technique used to separate different proteins or DNA fragments in an electrical field, based on differential electrical charges or sizes.

Epistasis – interactions between alleles at different gene loci.

F_1, F_2 – first generation, second generation.

Farm-reared brown trout – refers to brown trout that have resulted from artificial reproduction and closed culture for more than one generation. A permanent broodstock line is maintained on the farm (distinct from hatchery reared brown trout).

Feral wild brown trout – wild brown trout that have resulted from stocking of farm-reared trout in a previous generation.

Fitness – the contribution of an individual to future generations. Fitness involves both overall life cycle survival and reproductive success, including mating and offspring survival to reproductive age. Fitness of a population is the mean fitness of its individuals. (Sometimes referred to as Darwinian fitness to differentiate from colloquial use of the term fitness.).

Freshwater trout – brown trout that remain in freshwater throughout their lives. Many freshwater trout undergo migrations within rivers or between rivers and associated lakes. In some cases the entire life history can be spent in lakes.

F_{ST} – A measure of genetic differentiation between two populations based on neutral or near-neutral gene loci as examined by molecular genetic techniques. Values range from 0, indicating no differentiation, to 1 when no alleles are shared.

Gene – heritable characters are controlled by fundamental units called genes, which comprise linear sequences of the four types of base (adenine, guanine, cytosine and thymine) that make up DNA. A gene is that part of the DNA that is responsible for a specific function and that occupies a specific position or gene locus on the chromosomes, which are the physical carriers of the genetic material. Gene locus is also used to mean a specific section of the DNA that has no known function.

Genetic diversity – genetic variation within and among populations of a species.

Genetic drift – random changes in allele frequencies as a result of chance effects. Genetic drift increases as N_e decreases.

Gene flow – the introduction of alleles from one population into another as a result of migration (straying) followed by successful breeding and offspring survival.

Genetic (population) structure – the organisation of a species into populations among which there is limited or no gene flow.

Genotype – the genotype is the specific allele composition of an individual organism at a specific gene locus. The genotype is always a doublet (for example, AB, 100/90, A^1A^2) and refers to the allelic composition of a gene locus in one individual organism of the population.

Glacial refuge – an area where brown trout could survive during an ice age.

Hatchery brown trout – refers to brown trout where broodstock are taken from the wild each generation and the offspring are reared under culture conditions until the fry or later stage, before release. Used in the same sense as farm-reared brown trout by some authors.

Heritability – see broad sense heritability and narrow sense heritability.

Heterozygous – when the two alleles at a gene locus are different (for example, AB).

Heterozygosity – the proportion of individuals that are heterozygous at a particular gene locus.

Homozygous – when the two alleles at a gene locus are identical (for example, AA).

Hybridisation – the successful interbreeding of individuals from two genetically different populations, strains or species. Offspring are referred to as hybrids.

Hybrid vigour (heterosis) – increase in performance or fitness following mating (hybridisation) between individuals from two genetically distinct populations, strains or species, i.e. the reverse of inbreeding depression. **Mid-parent heterosis** is where the the hybrid is better than the mean of the two parents. **Better-parent heterosis** is where the hybrid exceeds the better parent, and is sometimes referred to as useful heterosis in animal breeding.

Inbreeding – the mating of related individuals.

Inbreeding depression – reduction in fitness of a population as a result of inbreeding.

Introgression – strictly, the introduction of genes (alleles) from one population (or strain) into another by means of hybridisation and repeated backcrossing of hybrids to parental types. Here it is used more loosely, to mean the proportion of farm genotypes in a wild population, including farm x farm offspring as well as all hybrids.

Lineage – individuals with common ancestry.

Local adaptation – genetic changes occurring as a result of natural selection that result in a population having higher fitness (better adapted) in the localised environment where it occurs.

Locus – see gene.

Metapopulation – a group of adjacent sub-populations linked by extensive gene flow, generally with extinction and recolonisation of individual sub-populations.

Microsatellites / minisatellites – short nuclear DNA sequences (of no known function) repeated tens or hundreds of times in tandem with different alleles at a gene locus differing in the number of repeats. High variability (large number of alleles) enables use for DNA fingerprinting or profiling. Most studies now involve the smaller and technically more straightforward microsatellites.

Mitochondrial DNA (mtDNA) – DNA present in the mitochondria of a cell rather than the nucleus. Normally inherited only though the female line via the cytoplasm of the egg. Only single type (haploid) and genetic composition of individual is referred to as the haplotype. Consists only of functional genes.

Molecular markers – genes that can be used as indicators or markers to characterise the overall genetic make-up of the individual or population.

Monomorphic – only a single allele present in the population at a specific gene locus.

Narrow-sense heritability (h^2_n) – the proportion of the overall phenotypic variance due to additive genetic variance.

Native wild brown trout – wild brown trout that have resulted from natural colonization of the water system.

Natural selection – differential perpetuation of alleles due to differential fitness of individuals as a result of their genetic make-up, as expressed in the phenotype. The genetic characteristics of individuals leaving more offspring will increase in the next generation while those of individuals leaving fewer offspring will decrease.

Naturalized wild brown trout – same as feral wild brown trout.

Neutral allele – an allele that has no effect on the fitness of its carriers, not subject to natural selection.

Non-native brown trout – brown trout that are the result of translocation of wild fish without hatchery rearing beyond the eyed egg stage.

Outbreeding depression – reduction in fitness following mating (hybridisation) between individuals from two genetically distinct populations or strains.

Phenotype – the overall structure of an organism (including form, shape, colour, behaviour and physiology) resulting from expression of the genotype modified by environmental influences.

Polymerase Chain Reaction (PCR) – a laboratory technique involving a thermostable enzyme that enables selected selections of DNA to be isolated and amplified.

Polymorphic – two or more alleles present in the population at a specific gene locus.

Population – a group of individuals among whom interbreeding occurs freely. Each individual has the potential to interbreed with any other individual (of the opposite sex) in the same population.

Put and take stocking – stocking into a water with little or no natural spawning, for example artificial reservoirs and dams. The aim is usually to produce a viable angling fishery.

Q_{ST} – a measure of genetic differentiation between two populations in their quantitative trait genes. Values range from 0, indicating no differentiation, to 1 when no alleles are shared.

Quantitative traits – phenotypic characters that are the product of multiple genes (5-20+ gene loci) as well as being influenced by the environment (see also heritability).

Recessive allele – an allele that only alters the phenotype when it is homozygous.

Recruitment – the input of young fish to a population each year.

Restoration stocking – stocking to re-establish a self-sustaining population in a water where the previous population had become effectively extinct.

Sea trout – anadromous brown trout where reproduction occurs in rivers and the fish migrate to the sea for feeding.

Steelhead – the anadromous (sea trout) form of rainbow trout.

Stocking – artificial release of brown trout that have been reared in a farm (hatchery) for a period of time.

Straying – movement of an individual from the population where it was born to another population. Can result in gene flow if the individual breeds and its offspring survive. Gene flow is typically much lower than the actual straying rate.

Supplemental stocking – stocking with trout of farm or non-native origin to supplement a wild population with the aim of increasing the number of trout available for angling or conservation:

- To increase directly the number of fish available where the natural production is insufficient to meet angling demand (harvest supplementation).
- To restore a depleted population where the reason for the decline has been identified and rectified. This requires that the stocked fish contribute to offspring recruitment in subsequent generations without diminishing the recruitment of the existing wild population (population supplementation).

Supportive breeding – stocking with hatchery-reared offspring of native trout, with the aim of increasing the number of trout, generally where there is an identifiable bottleneck to wild production, such as a lack of spawning grounds but suitable habitat for later stages. Can also be used to increase pure native trout, and remove farm genes, in introgressed populations.

Triploid – an individual with three sets of chromosomes, which can result from either natural or artificial processes. Usually sterile.

Wild brown trout – brown trout that have resulted from natural reproduction in the wild.

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