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A Migratory Perspective on Genetic and Life-History Variation in Atlantic Salmon, *Salmo salar* L.

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ABSTRACT

FACULTY OF MEDICINE, HEALTH, AND LIFE SCIENCES

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A MIGRATORY PERSPECTIVE ON GENETIC AND LIFE-HISTORY VARIATION IN ATLANTIC SALMON, *SALMO SALAR* L.

By Nigel Massen

Understanding the pattern and process maintaining diversity within populations is a key goal for ecologists. Such variation may be delimited spatially, temporally, or by differences in life-history. It may lead to great variation in phenotypic traits and population genetic structure. Furthermore, where species are threatened or endangered, it is of prime importance in identifying units for management. Atlantic salmon (*Salmo salar* L.) display extreme phenotypic and behavioural within-population diversity, and, as such, are an excellent model species for evolutionary studies. Understanding their population structure is of increasing importance due to their worldwide decline in numbers, and in some cases, local extinction.

Salmon populations from the River Eden in Cumbria, UK, were sampled in the years 1999, 2000, & 2002. A radio-tracking analysis and demographic data revealed gender-specific differences in body-size depending on how long a salmon had spent at sea i.e. their sea-winter tactic. These differences are likely to stem from gender-specific costs in both foraging at sea, and the upstream migration. In addition, gender-specific patterns were observed in the distance a salmon would migrate upstream to spawn, these differences were again dependent on the sea-winter tactic employed by the individual. Females spawn, on average, at different distances upstream depending on their time at sea, no such trend occurs in males. It is hypothesised that this is due to male behaviour being shaped to compete for more mating opportunities, in contrast to female behaviour, which is shaped to seeking out better quality breeding sites.

A genetic analysis of adult salmon using 11 microsatellite loci revealed that river geography contributes to population structure. This occurs at both the between-river, and the within-river level (overall $F_{ST} = 0.01$). Individual assignment testing revealed gender-specific patterns of dispersal; furthermore, differences were found between the sexes in heterozygosity and allelic richness. Estimations of effective population size were all < 100, well below the level of 500 suggested to counter the deleterious effects of genetic drift.

A genetic analysis of juvenile salmon using 10 microsatellite loci found that in the Eden catchment, the within-river component of genetic variation was 10x higher than the between-river component (AMOVA; overall $F_{ST} = 0.048$). This suggests that managing salmon on a river-by-river basis may not always be appropriate. There is an isolation-by-distance (IBD) relationship between sampled populations in the Eden at distances up 60km. This relationship was further resolved by incorporating migratory distance into the model. Above 60km it is likely that genetic drift/gene flow equilibrium, a prerequisite for IBD, has not had sufficient time to form since disturbances in last ice age.

Both genetic analyses highlighted differentiation of the River Caldew, a branch of the Eden catchment recently recolonised by salmon. Although more differentiated than other river regions, it had none of the differences in genetic diversity that would be expected of a very recently recolonised population.

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List of abbreviations

1SW	One sea-winter
Â	Allelic richness
ANOVA	Analysis of variance
AMOVA	Analysis of molecular variance
ART	Alternative reproductive tactic
CEH	Centre for Ecology and Hydrology
D2S	Distance-to-sea
D_{CE}	Genetic distance
DNA	Deoxyribonucleic acid
EA	Environment Agency
FCA	Factorial correspondence analysis
ESL	Estimated spawning location
F_{ST}	Wright's within-population differentiation
GIS	Geographical information systems
GLM	General linear model
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
IBD	Isolation by distance
MSW	Multi sea-winter
N_e	Effective population size
NERC	Natural Environment Research Council
NGR	National grid reference
OSR	Operational sex ratio
R	Relatedness
S2S	Site-to-site
SWT	Sea-winter tactic

There was only one catch and that was Catch-22, which specified that a concern for one's own safety in the face of dangers that were real and immediate was a process of a rational mind. Orr was crazy and could be grounded. All he had to do was ask; and as soon as he did, he would no longer be crazy and would have to fly more missions. Orr would be crazy to fly more missions and sane if he didn't, but if he was sane he had to fly them. If he flew them he was crazy and didn't have to; but if he didn't want to he was sane and had to. Yossarian was moved very deeply by the absolute simplicity of this clause of Catch-22 and let out a respectful whistle.

"That's some catch, that Catch-22", he observed.

"It's the best there is," Doc Daneeka agreed.

Joseph Heller
Catch-22, 1961.

1 Introduction

Molecular markers offer an unprecedented view of the world's biota, allowing researchers to study intraspecific diversity over a great range of geographical and temporal scales. More than simply describing differences *between* populations, molecular tools are increasingly being used to describe the distribution of genetic diversity *within* them. Where individuals within a population display phenotypic or behavioural diversity, such as life-history variation, contrasting migratory patterns, or sex-biased dispersal, then it is crucial to understand what effects this has on the distribution of genetic variation. Investigating such biological phenomena will further our knowledge of general theory and, where species are at risk or endangered, provide practical information for conservation and management strategy.

Atlantic salmon (*Salmo salar* L.) is an extremely useful model species for studies of within-species diversity. In this chapter I will summarise the biological characteristics of Atlantic salmon, focusing on life-history, and the factors thought to lead to its variability. A brief history of the use of molecular markers, particularly in fisheries, will put into context both recent research, and the present study. I will briefly review the key parameters and statistics used to quantify genetic diversity and test for genetic population structure. I will then assess our current knowledge of Atlantic salmon population genetic structure, and identify areas where we can move beyond simply describing genetic variation, to testing models incorporating life-history.

1.1 Atlantic salmon biology

1.1.1 Distribution and colonisation

The natural range of Atlantic salmon covers all rivers flowing into the North Atlantic Ocean and Baltic Sea. Within Europe salmon can be found from the Douro River of northern Portugal, to the Kara River in the west of Siberia. In North America, Atlantic salmon are found from the Connecticut River to the Hudson Strait and Ungava Bay in the north (Altukhov *et al.*, 2000; Nilsson *et al.*, 2001). In the central north, Atlantic salmon inhabit the rivers of northern Norway, Iceland and southern Greenland (Zelinsky, 1985). Within river systems, various life stages of Atlantic salmon can be found from estuary to small headwater streams, their range being limited by large natural falls and, more recently, by man made-made obstacles (e.g. dams on the River Pitlochry, Scotland, UK).

1.1.1.1 Landlocked salmon

Regions on both sides of the Atlantic have land-locked, non-anadromous salmon. These populations spawn in rivers and descend into lakes to feed until maturity; in all respects other than the location of their open-water life phase, they are the same as anadromous salmon. It has been claimed that such populations justify sub-specific status (Wilder, 1947) though it is now clear they are more likely to arise independently from local populations.

1.1.2 Phylogeny

The family Salmonidae is comprised of three sub-families; Salmoninae, Coregoninae and Thymallinae (Kendall & Behnke, 1984; Norden, 1961; Stearley & Smith, 1993). All salmonids belong to the sub-family Salmoninae, which has been the subject of numerous systematic studies over the last century. A wide range of approaches, including morphology (Behnke, 1980; Burnham-Curtis & Smith, 1994; Cavender, 1980; Gorshkov *et al.*, 1979; Holcik *et al.*, 1988; Kolyushev, 1971; Norden, 1961; Sanford, 1990; Shaposhnikova, 1968; Shaposhnikova, 1971; Shaposhnikova, 1975; Smith & Stearley, 1989; Stearley, 1990; Stearley & Smith, 1993; Vladykov, 1962; Vladykov, 1963; Williams, 1987; Wilson & Li, 1999), karyology (Booke, 1968; Hartley, 1987; Phillips *et al.*, 1989a; Phillips *et al.*, 1989b), allozymes (Allendorf & Seeb, 2000; Johnson, 1980; Tsuyuki & Roberts, 1966; Utter *et al.*, 1973), and sequence data (Grewe *et al.*, 1990; Partti-Pellinen *et al.*, 1991; Phillips & Pleyte, 1991; Skurikhina *et al.*, 1986; Thomas *et al.*, 1986), have yet to produce a consensus on the status of all problem taxa within the family. This is complicated by the high levels of morphological plasticity seen within many of the genera e.g. *Salvelinus* (Sandlund *et al.*, 1992), leading to a large number of synonymous species and sub-species being described within this genus. Hybridisation and introgression are common between many of the salmonids e.g. trout, *S. trutta* and Atlantic salmon, *S. salar* (Hurrell &

Price, 1991; Jansson *et al.*, 1991; see also Utter & Allendorf, 1994). Hybridisation can lead to inconsistencies between uni-parentally inherited characters (i.e. mtDNA loci) and classic Mendelian characters (Phillips & Oakley, 1997) causing problems for population genetics and phylogeny reconstruction.

Hybridisation also introduces taxonomic difficulties for Atlantic salmon as they fail to fulfil the criteria required of a biological species (Mayr, 1969; Mayr, 1982); namely, being reproductively isolated. Under a strict biological species concept both trout and Atlantic salmon cannot be regarded as distinct species.

1.1.3 Life cycle

Atlantic salmon are anadromous fish; their reproductive and nursery phase occurring in freshwater, complemented with a rapid growth phase in the open ocean, before a return to freshwater to spawn. Anadromy is a trait recognised in 18 fish families and as many as 120 species, evolving independently in several groups (Myers, 1949).

Figure 1.1 Atlantic salmon parr from the River Eden.

The first life stage of the salmon begins when the egg hatches into an alevin (~2cm long) within the gravel; this usually takes place in April or early May in the UK. The alevin is sustained by its yolk sac, which after four to five weeks is exhausted and the alevin emerges from the gravel. At the time of the 'swim-up' the alevin develops skin pigmentation, fills its swim bladder with air, and begins to feed; the young fish is now known as a fry. Fry spend some time establishing feeding territories on the 'nursery area', their survival depending on the availability of food and space (Kalleberg, 1958) such that a carrying capacity is quickly reached. This early dispersal is generally no more than 700m from the redd (Beall *et al.*, 1994; Gustafson-Greenwood & Moring, 1990). Allan & Ritter (1975) refer to fry which have dispersed from the redd as parr, however, fry are more commonly referred to as parr only at the end of their first year. At this time they are 6.5-7cm in length and have developed parr markings; these are vertical blueish bands along the body (Figure 1.1), they also have red dots along the lateral line.

While in freshwater, parr undergo a number of morphological changes which prepare their body for life at sea (Hoar, 1976). These include a change in the length/weight ratio, darkening of the pectoral and caudal fins, a layer of subcutaneous guanin being laid down, and the parr marking disappearing. The time at which parr undergo smoltification is largely dependant on the location of the river; in the Hampshire Avon, 90% of smolts are yearlings (Mills, 1989); in contrast, smolts of up to seven years of age have been recorded in northern Norway (Dahl, 1910). As well as undergoing physical changes, the smolts begin their smolt migration i.e. the descent to the sea. This is mostly achieved by passive downstream migration (Thorpe & Morgan, 1978), normally tail first, although active swimming is required through lakes.

Maturation in salmon occurs at sea, with adults spending up to four winters feeding in the Atlantic Ocean and neighbouring sea areas.

1.1.4 Adult migration

'I have never expressed a doubt that salmon return on the whole to their native rivers, and indeed I see evidence that such return is, in some cases at least, practically perfect'. This statement by Huntsman (1939) put to rest the debate over whether salmon retuned to their natal rivers. This return to a natal river, or 'home-stream', is the characteristic dominating salmon life-history, ecology and evolution (reviewed in Banks, 1969; Stabell, 1984).

The return migration is separated into two distinct phases: open ocean migration, and upstream migration. In open ocean, it is thought that salmon rely on the sun to orientate themselves, a mechanism known from other fish species (Hasler & Wisby, 1958). Use of celestial features has also been suggested (Neave, 1964), as has the ability to detect the earths magnetic current (Stasko *et al.*, 1973). Once in freshwater a number of mechanisms lead salmon to return to their natal areas. In his extensive review, Stabell (1984) summarised as follows: 1) less than 3% of salmon stray to non-native watersheads; 2) homing is related to the presence of reproductively isolated populations; 3) salmon are able to detect pheromones from fish odours as they ascend the river – the pheromone hypothesis; 4) olfaction plays the central role in migration, both upstream and in coastal waters; 5) there are population specific odours which salmon can detect; and 6) salmon imprint on their native river as they descend before going to sea, this imprinting occurs in a sequential fashion, and this series of olfactory cues is what guides them back upstream as adults.

Although the case for natal philopatry is clear at the catchment/watershed scale, what remains less clear is the extent to which salmon migrate to natal spawning areas within their home catchment area. Limited direct evidence from radio-tracking suggests that salmon return to natal tributaries (Youngson *et al.*, 1994). However, the vast majority of evidence for natal homing is indirect evidence, based on fine-scale population differentiation rather than on observation of adults. There is a clear need for studies to combine both direct, and indirect, methods as many questions remain unanswered and cannot be approached comprehensively with either approach alone. For example, how accurately do salmon home to their natal rivers? How is this affected by the distance migrated? Does migration upriver differ between sexes? Or between life-histories? These questions are the key themes of this thesis.

1.1.4.1 Spawning

Once in their home-stream, spawning salmon acquire reddish blotches and black spots on the body. Full-grown males undergo strong modification to the shape of the head, especially the jaws; a strong hook or kype develops from conjunctive tissue on the lower jaw, and fills the hollow between the extended premaxillary bones (Vladykov, 1963). Males then compete with other full grown males and precocious parr, to fertilise eggs laid in redds on the spawning ground.

1.1.5 Life history strategies in Atlantic salmon

Atlantic salmon exhibit a high degree of behavioural variation in life-history strategy (Mills, 1989). Every stage of the salmon life-cycle has shown the ability to vary both within, and between, different populations throughout their range. The most important and, arguably, the most intriguing aspect of this variation, is the amount of time a salmon spends at sea before returning to freshwater. This 'sea-wintering' variation is associated with difference in size and behaviour on return to freshwater (Fleming & Reynolds, 2004).

Within the UK smolts usually run downstream in spring (for exceptions see e.g. DAFS, 1972; Mills, 1971), once at sea they are generally aged with reference to the number of winters they have spent at sea. A fish descending from freshwater in spring and spending one winter at sea before returning to freshwater some time the following year, is therefore termed a one sea-winter (1SW) fish. Fish spending more than one winter at sea are termed multi sea-winter fish. Fish returning to freshwater to spawn after only 1SW are commonly termed grilse (in the UK) in contrast to 'adult' salmon. Within the angling community grilse are often defined by weight, being any adult weighing less than 3.5kg; however, late running grilse may weigh up to 5 kg (Youngson & Hay, 1996).

The 1SW/MSW division is further complicated by variation in run timing between 1SW and MSW fish. Within the UK (and much of Europe), MSW salmon have historically returned early in the year, and therefore show no summer scale growth in the year of the spawning migration (Gardner, 1976); these fish are known as spring fish, or 'springers'. The exact date where it is practical to set a 1SW/MSW classification is likely to be somewhat river specific due to both salmon biology and the opinion of the researcher in question. Throughout much of the UK any fish returning to freshwater before 1st June is taken to be a spring fish (1st May also used, Gough *et al.*, 1992). However, this date classification, though popular, is somewhat unsatisfactory as it fails to reflect that large numbers of MSW *do* return in the autumn.

The factors that determine whether a salmon will return to freshwater as a 1SW or MSW fish are far from clear. This area of investigation has received a considerable degree of attention for many years, yet no clear answers have been found (Gardner, 1976); and, although a plethora of hypotheses exist, none can be applied to all, or even a majority of rivers/populations. Both environmental and genetic factors clearly play a role in the status of 1SW fish, and a number of studies exist supporting the importance of both factors.

1.1.5.1 Genetic theory

There is clear evidence of 1SW fish breeding with 1SW fish (Piggins, 1974) in some populations. A long term study on the status of 1SW fish within populations (Elson, 1973) has shown that 1SW X 1SW pairings produced 61% 1SW, while older salmon pairings only 39% 1SW (from both early and late runs). However, early running MSW fish produced 60% 1SW fish. So the results are far from clear. Late running MSW fish produced only 28% 1SW fish and 72% MSW fish. Elson suggests that early running MSW fish may breed with earlier running 1SW's, thereby accounting for the high levels of 1SW fish in the early run MSW fish. There is however, no apparent reason why MSW fish could not also breed with later running 1SW fish since both are spawning at roughly the same time.

The general conclusions drawn from both Piggins and Elson's data are that: 1) there clearly is a genetic element to the time of return; 2) 1SW parents will produce some MSW offspring in addition to 1SW; and 3) under some circumstances MSW fish may produce more 1SW than MSW offspring.

1.1.5.2 Environmental theory

A number of studies have found that the fry of MSW salmon become mostly 1SW fish (95%) when transplanted and released in river that produces mostly 1SW fish (White & Huntsman, 1938). This suggests that the salmon fry adopted the life-history strategy of local salmon, rather than those of their parents in their true home river. This by implication is due to response to local environmental factors in the stream where the fry were transplanted. In addition to the mark recapture experiments described above, is the common observation that rivers producing MSW salmon are different (usually larger) to those producing mostly 1SW. No studies exist which compare river environmental conditions to salmon production, indeed, there is no consensus on which conditions are critical to the production of spring salmon.

It is clear that 1SW fish are present in all components of salmon populations under study. What is not clear is how the proportions of 1SW and MSW fish change over time; what migratory differences might exist between the two life-histories? How do they contribute to population structure?

1.1.6 The decline in salmon populations

There is compelling evidence of a range-wide decline in salmon populations (ICES, 2000); the total global catch of salmon in 1975 was 11,632 tonnes, falling to 8,135 in 1985 (Mills, 1989). Furthermore, in some rivers (e.g. River Eden, Cumbria) there has been a proportional decline in the numbers of spring salmon within the total salmon population (Gough *et al.*, 1992; Small, 1995), i.e. there is a change in the 1SW:MSW relationship.

The abundance of MSW fish can be viewed from two perspectives. In the first instance the decline of MSW salmon catches can be considered within a trend of declining numbers of all salmon caught: both 1SW and MSW. This allows investigation of the absolute numbers of MSW salmon. The second viewpoint from which to assess MSW salmon populations is their relative abundance in the total salmon population.

Declines of MSW fish have been documented across their European range. Long term reduction in catches of spring fish have been observed in Scotland (Williamson, 1988), the overall five-year mean spring catch dropped from 88,000 in 1952, to 28,000 in 1983 (Shearer, 1986). This is coupled with a shift towards an increased proportion of 1SW's in the population (Williamson, 1988). In the Loire-Allier and Adour-Gaves-Nive basins of France there has been an increase of 1 & 2SW fish coupled with a decline in 3 & 4SW fish, in the Austrias in Spain spring fish have declined from 36% to 3% of the annual catch (Ventura, 1988). In North America, a decline in the proportion of 1SW salmon in the annual catch has been seen in the rivers Miramichi, Restigouche, and Matamek of Canada (Porter *et al.*, 1986); in the Miramichi, 90% of MSW fish are being selectively removed by exploitation, causing the spawning stock to be almost exclusively 1SW fish (Saunders, 1969). Fisheries statistics from these rivers show the 1SW:MSW ratio to be 2:1, declining to just 28:1 among the population surviving to spawn (Saunders, 1969).

Older, i.e. MSW fish, are under a greater degree of river exploitation than are 1SW's (Gee & Milner, 1980). This seems to be largely due to exploitation by rod fishing on those rivers studied. On the Severn (UK), 3 & 4SW fish are exploited heavily, whilst 1SW are under exploited (Churchward & Hickley, 1991). On the Frome (UK), the exploitation rate of MSW fish was calculated at 32%, whilst 1SW exploitation was only 11% (Beaumont *et al.*, 1991). A similar pattern has also been seen in the Elorn River in France, where the rod exploitation rate of MSW fish was 30% compared to less than 5% in 1SW's (Prouzet, 1990).

There is therefore, a general argument that the change in the proportion of MSW salmon is due to a selective pressure for fewer years at sea due to two reasons: 1) fewer years at sea results in a lower total risk of predation (although predation is highest in the first few months at sea Hansen & Quinn, 1998); and 2) exploitation of migrating adults from angling and netting is heavily MSW-biased.

1.1.6.1 Current Conservation Efforts

In 1983, the North Atlantic Salmon Conservation Organisation (NASCO) was formed with the objective of coordinating and cooperating the conservation, restoration, enhancement, and management of Atlantic Salmon stocks by all nations in the North Atlantic area (Windsor & Hutchinson, 1988). This international working group sets regulatory measures, directs scientific research, promotes exchange of information on laws, catch and conservation programmes.

The Atlantic Salmon Trust was set up in 1967 (under the original name of the Atlantic Salmon Research Trust), to conserve salmon within the UK, and to contribute to international efforts. Together with its North American counterpart, the Atlantic Salmon Federation (formerly the International Atlantic Salmon Foundation), and the Restoration of the Atlantic Salmon in North America, they were successful in attempts to control the Greenland fishery, seen as one of the main causes of overexploitation of salmon stocks. This was achieved through the Committee for Atlantic Salmon Emergency (CASE), and resulted in the 'Pelly Bill' (Bill 2191), giving the US President the power to restrict imports of fish produce from countries 'conducting fishing operations in a manner or under circumstances which diminishes the effectiveness of an international fishery conservation programme' (Mills, 1989). This proposal was approved by what is now the North Atlantic Fisheries Organisation (NAFO).

In Europe, Atlantic salmon are listed in Annex II and V of the European Habitats Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (92/43/EEC), which theoretically provides them a high level of protection. Within the UK, Atlantic salmon were identified as a Species of Conservation Concern by the UK Steering Group for Biodiversity (Locke & Robinson, 2003). At a local scale within England and Wales, regional Environment Agency (EA) offices interact with local river boards to assess the need for, and implement, measures to protect and supplement salmon stocks. My project is in co-operation with the EA, and the results will contribute to their management plans.

1.2 Molecular markers; history and application

1.2.1 History of microsatellite analysis

In a 'points of view' chapter in Carvalho & Pitchers *Molecular Genetics in Fisheries,* Wright & Bentzen took the 'radical' point of view that the future of genetic analysis in fisheries would be microsatellites. Nearly a decade later, microsatellite analysis has come of age, and is the principal method used to investigate the population genetics of a diverse range of taxa. Microsatellites have a number of key properties that currently make them the most suitable marker for population studies: 1) highly polymorphic; 2) small amounts of DNA required for PCR amplification allows non-destructive sampling; 3) cross-species utility of some microsatellites; 4) found in large numbers throughout the genome; and 5) Mendelian inheritance. Fisheries management moves towards an increasingly fine-scale view of populations, with the characterisation of discrete genetic components (Allegrussi *et al.*, 1998) being of prime importance for understanding evolutionary mechanisms, as well as the conservation and management of natural populations.

1.2.2 What are microsatellites?

The widespread occurrence of microsatellites was demonstrated in the 1980's (Hamada *et al.*, 1982); they have since been located in the genome of every organism analysed (Hancock, 1999). Microsatellites full potential as Mendelian markers became accessible following the widespread use of PCR (Saiki *et al.*, 1988), whereby oligonucleotide primers are used to amplify microsatellites to be quickly, and accurately, genotyped by gel electrophoresis (Karp *et al.*, 1998).

Complex genomes are generally regarded as having three tandemly-repeated components (Armour *et al.*, 1999): 1) large repeat sequences (>5Mb in length) where the repeat may make up several percent of the entire genome; known as major satellites, these are often associated with centromeres; 2) minisatellites are repeat units (>10bp in length) that may be present at thousands of loci per genome; and 3) *microsatellites.* Microsatellites are short (<8 bp in length) DNA motifs, arranged in di-, tri- or tetra-nucleotide tandem repeats, they are also referred to as Simple Sequence Repeats (SSR's Tautz, 1989), Variable Number Tandem Repeats (VNTR's) and Short Tandem Repeats

(STR's Edwards *et al.*, 1991), although the term microsatellite generally now encapsulates all of these. Microsatellites are highly polymorphic (Tautz, 1989; Weber & May, 1989), and have a mutation rate estimated at 10^{-4} per generation (Weber & Wong, 1993).

1.2.3 Evolution of microsatellites

Widespread use of microsatellites in many areas of biology has prompted increasing debate over the means by which they evolve (Eisen, 1999). An extensive range of statistics is available to analyse population structure from microsatellite data (e.g. population differentiation), though these measures are highly dependent on the theoretical model of evolution considered (Nadir *et al.*, 1996).

The main mechanism thought to generate microsatellites is slip-strand mis-pairing (SSM Fresco & Alberts, 1960). This is an error during replication when DNA polymerase slips, causing the template and the replicated strand to be misaligned, if they realign imperfectly, this will result a mutation. A second mechanism may also provide microsatellites: unequal crossing over (UCO), an imperfect alignment between recombining chromosomes leading to changes in microsatellite length. Extensive *in vivo,* and *in vitro,* work suggests that SSM is the dominant force in microsatellite instability (Sia *et al.*, 1997).

Two models of microsatellite evolution dominate current theory: the Stepwise Mutation Model (SMM Kimura & Ohta, 1978), and the Infinite Alleles Model (IAM Kimura & Crow, 1964). Under the SMM, microsatellite alleles mutate by loss or gain of a single repeat, such that different allele sizes could arise on more than one occasion (homoplasy). In contrast, the IAM predicts that alleles mutate in multiples of the tandem repeat, and that each allele is produced only once, this means that individuals sharing alleles are identical by descent (IBD), a prediction that cannot be made under the SMM.

There is another theoretical model rarely cited in the literature, the *K*-alleles model (KAM), a model intermediate between the IAM and SMM (Crow & Kimura, 1970).

The KAM predicts exactly *K* possible allelic states, and represents an IAM where *K* is infinity. The KAM effectively places an upper bound on microsatellite size.

1.2.4 Distribution and functionality of microsatellites

Microsatellites are usually regarded as having a random or almost random distribution across the genome (Schlötterer, 2000; Schlötterer & Wiehe, 1999), which may result from an unbiased single-step random-walk processes (Bell, 1996). They are generally thought to be functionless, and therefore completely neutral – as reflected in evolutionary models describing their behaviour. This property is seen as their key benefit to use in population genetics. However, in recent years evidence has been accumulating which suggests that this may not always be the case, and that microsatellites may be distributed in a non-random fashion (reviewed in Li *et al.*, 2002). This is likely to be more common in disease causing triplet microsatellites (Nadir *et al.*, 1996) associated with coding regions. A number of studies indicate that selective pressure against the presence of microsatellites in coding-regions may be stronger for different classes (i.e. di-, tri, or tetra-), and may vary with repeat length and putative function (Li *et al.*, 2002).

1.3 Definitions of some key statistical terms for molecular markers

1.3.1 Analysing population structure

Almost all species exhibit some level of sub-structure in their total genetic variation. This is typically due to non-random mating in the way individuals are spatially distributed (Hartl & Clark, 1997), but may also be related to other factors such as sexual selection (Gross, 1991), or due to temporal isolation (Hendry *et al.*, 1995).

Knowledge of how genetic variation is partitioned among populations has important implications for understanding evolutionary biology and ecology. Applications include analysing levels of gene flow, estimations of effective population size, and the detection of range expansions or population bottlenecks. Furthermore, information such as this is rapidly becoming integral to conservation management (Nielsen, 1998).

1.3.1.1 F-statistics

The fixation index (Wright, 1921), and the statistical measures associated with it, allow the quantification of genetic structuring within populations. This index is equal to the reduction of heterozygosity observed in one level of a population hierarchy, relative to a different, more inclusive level of that hierarchy. The statistic F_{ST} denotes the amount of variation due to structuring between sub-populations relative to the population as a whole, e.g. differences in the reduction of heterozygosity between rivers in a catchment where the catchment is the population overall. It is arguably the most informative single value about population structure, and as such, is widely compared between studies.

F_{ST} has a theoretical minimum of 0 and a maximum of 1, where 1 would be complete fixation of an allele in the population studied. Typically F_{ST} values are less than 1% of the total genetic variation, as most genetic variation in the population is within the individuals making up the sub-populations (Hartl & Clark, 1997). Quoted F_{ST} values are usually Weir & Cockerham's (Weir & Cockerham, 1984) *theta* (θ_{ST}), a variance based method of estimating F_{ST}. Regarded as a correlation between alleles within a sub-population, this method makes no assumptions concerning the number of samples, number of populations, or heterozygote frequencies.

A second measure of population differentiation R_{ST}, takes into account the high mutation rate of microsatellites and their evolution under a stepwise mutation model (Slatkin, 1995). However, simulations suggest that whilst R_{ST} may well better describe population demographic parameters in large populations analysed at many loci (i.e. n_s=100, n_l=20), F_{ST} is less biased where these conditions are not met due to the high variance of R_{ST} based estimators (Gaggiotti *et al.*, 1999).

1.3.1.2 Analysis of molecular variance (AMOVA)

Genetic structure in a population can be analysed in an analysis of variance framework (originally defined by Cockerham, 1969). This method is implemented in the software ARLEQUIN 2.001 (Schneider *et al.*, 2000) following the treatment of Excoffier *et al.*, (1992). This is an analysis of variance of gene frequencies which takes into account the number of mutations between genotypes. AMOVA is an hierarchical analysis which separates and tests the significance of levels of structuring of the genetic diversity present in a population. AMOVA differs from ANOVA in that it does not require the assumption of a normal population, as significance is determined by permutation.

1.4 Population Structure in Atlantic Salmon

Salmon have long been considered to exhibit 'local peculiarities' (Belding & Kitson, 1934; White & Huntsman, 1938), and have a well-documented propensity to form locally adapted populations (Taylor, 1991). Salmon have always been excellent candidates for the study of adaptive variation due to their high degree of population structure; indeed, Wright (1931) considered that natural selection would be most effective in species with extensive population sub-division throughout varied environments. There is considerable evidence for natural selection in Atlantic salmon; numerous morphological and physiological traits have been studied (reviewed in Taylor, 1991).

This local adaptation is largely a result of their homing fidelity, and is to be expected in any homing species e.g. the American shad, *Alosa sapidissima* (Waters *et al.*, 2000). This philopatry leads to reduced gene-flow between spawning populations, and an increased selective pressure to adapt to an environment found consistently in generation after generation. The presence of locally adapted populations within drainage systems has given rise to the concept of a salmon 'stock': a group of salmon that spawns in a particular region, at a particular time of year, and does not interbreed to any extent with other groups (Ihssen *et al.*, 1981). It follows that the existence of such stocks can be established using molecular data, since greater genetic variation would be expected between stocks than within them (Waples, 1998).

When considering genetic variation in a species, it is important to determine the independence of the marker being analysed. This is always a factor to consider when analysing allozyme data (Verspoor & Jordan, 1989), where the marker may itself be under selection. Instead, research has increasingly made use of neutral genetic markers (such as microsatellites) to study population structure.

1.4.1 Genetic variation in salmon populations

1.4.1.1 Range wide variation

A number of studies have indicated significant genetic variation between North American and European populations (e.g. King *et al.*, 2001). Divergence at transferrin loci (Tf) between populations on different sides of the Atlantic have led to the proposition of *S. salar* L. being split into sub-species of populations from North America and Europe; *S. salar americanus* and *S. salar europaeus* respectively (Payne *et al.*, 1971). This raised the question of whether variation at a single locus is sufficient to re-arrange sub-specific taxonomy (Behnke, 1972). Numerous studies since have confirmed the divergence of North American and European populations using chromosome number (Hartley & Horne, 1984; Morán *et al.*, 1993), and chromosome arm number (reviewed in Hartley, 1987). Studies at numerous protein loci confirm varying degrees of continental divergence of populations (Bourke *et al.*, 1997; Verspoor *et al.*, 1991; Verspoor & Jordan, 1989; Verspoor & McCarthy, 1997), as does RFLP variation in mtDNA (Bermingham *et al.*, 1991; King *et al.*, 2000), and the mini-satellite locus Ssa-A45/1 (Taggart *et al.*, 1995). Genetic variation at microsatellite loci has further confirmed divergence of these populations (King *et al.*, 2001; McConnell *et al.*, 1995); 266 alleles were found at 12 microsatellite loci from populations from Europe and North America, significant isolation by distance was found within, and between, the two populations. The North American populations were found to have less neutral genetic differentiation, fewer alleles, and less heterozygosity than their European counterparts (King *et al.*, 2001). The authors conclude that this is likely due to the more extensive history of glaciation in North America than in Europe.

Some degree of differentiation has been found between salmon from the North Atlantic and those from the Baltic Sea (Ståhl, 1987). The Baltic Sea is the largest brackish water environment in the world, and underwent freshwater and saltwater phases as the ice retreated at the end of the last ice age (~10,000 years ago). Salmon in the Baltic show low levels of variation compared to those in the Atlantic (Gross & Nilsson, 1999; Nilsson *et al.*, 2001; Ståhl, 1987), indicating some population bottleneck within the Baltic. Tracking the origin of salmon populations in the Baltic is highly problematic; present day populations are likely have originated from glacial refugia east of the Baltic, although some gene flow with Atlantic populations cannot be ruled out (Nilsson *et al.*, 2001). Based on variation at the Tf2 allele, Payne et al. (1971) split British salmon into 'Boreal' populations in the extreme south east, and 'Celtic' races throughout the rest of the country, few comparable studies exist to support or dispute this division. A study confirming this split, utilising microsatellite markers, of all UK salmon populations, river-by-river, seems a profitable short-term goal for molecular ecologists in this country.

1.4.1.2 Within river variation

At fine geographic scales, levels of allozyme variation are limited (Jordan *et al.*, 1992; Ståhl, 1987); however, the development of mini- and later microsatellite analyses, has catalysed growth in the number of such studies being carried out. The use of such highly polymorphic molecular markers has allowed researchers an unprecedented view of within-catchment population structure in Atlantic salmon. These studies have revealed surprising amounts of fine-scale population structure (e.g. Galvin *et al.*, 1996; McCubbing & Hartley, 1995; McElligott & Cross, 1991), including differences between tributaries. However, these studies typically lack the resolution to quantify the proportion of genetic variation distributed between rivers, to that within rivers (though see Garant *et al.*, 2000). Failing to take into account within river genetic variation, may have led to an overestimation of the significance of between river differentiation, this is a serious consideration where rivers may be managed on the basis of 'river stocks'.

1.5 Study site: The River Eden

The River Eden, Cumbria, drains an area of *ca.* 2372km^2, rising to 708m above sea level on limestone fells above Mallerstang Common, with a source 120km from the Solway Firth where it joins the Irish Sea (Figure 1.2). The Eden catchment is comprised of five main tributaries; the rivers Caldew, Eden, Lowther, Eamont, and Irthing. The Lowther is heavily abstracted to provide public water supply for north west England. Three other large salmon rivers flow into the Solway Firth: the Rivers Annan, Border Esk, and Nith.

Mean daily flow though the study period was 66.1m^{3}sec^{-1} in the lower reaches of the Eden. The average Atlantic salmon catch in the River Eden is 3107 ± 464 fish, based on Environment Agency (EA) rod, net, and trap data for 1999 – 2003. Eden salmon typically spend three years in freshwater before their oceanic phase. 24% of fish will spend one year at sea and 76% two or more years at sea (EA catch data from 1993 - 2003), before their spawning migration, when they return to the Eden over a nine month period (March - November).

1.5.1 Conservation status

The River Eden is a candidate Special Area of Conservation (cSAC Locke & Robinson, 2003), containing species listed in the habitats directive (1.1.6.1). Proposed as an SAC in 1997, it was awarded cSAC status in 1998. Full confirmation of SAC status will make the Eden a Site of Community Importance. In 1997 it was designated a Site of Special Scientific Interest (SSSI), under Section 28 of the Wildlife and Countryside Act 1981.

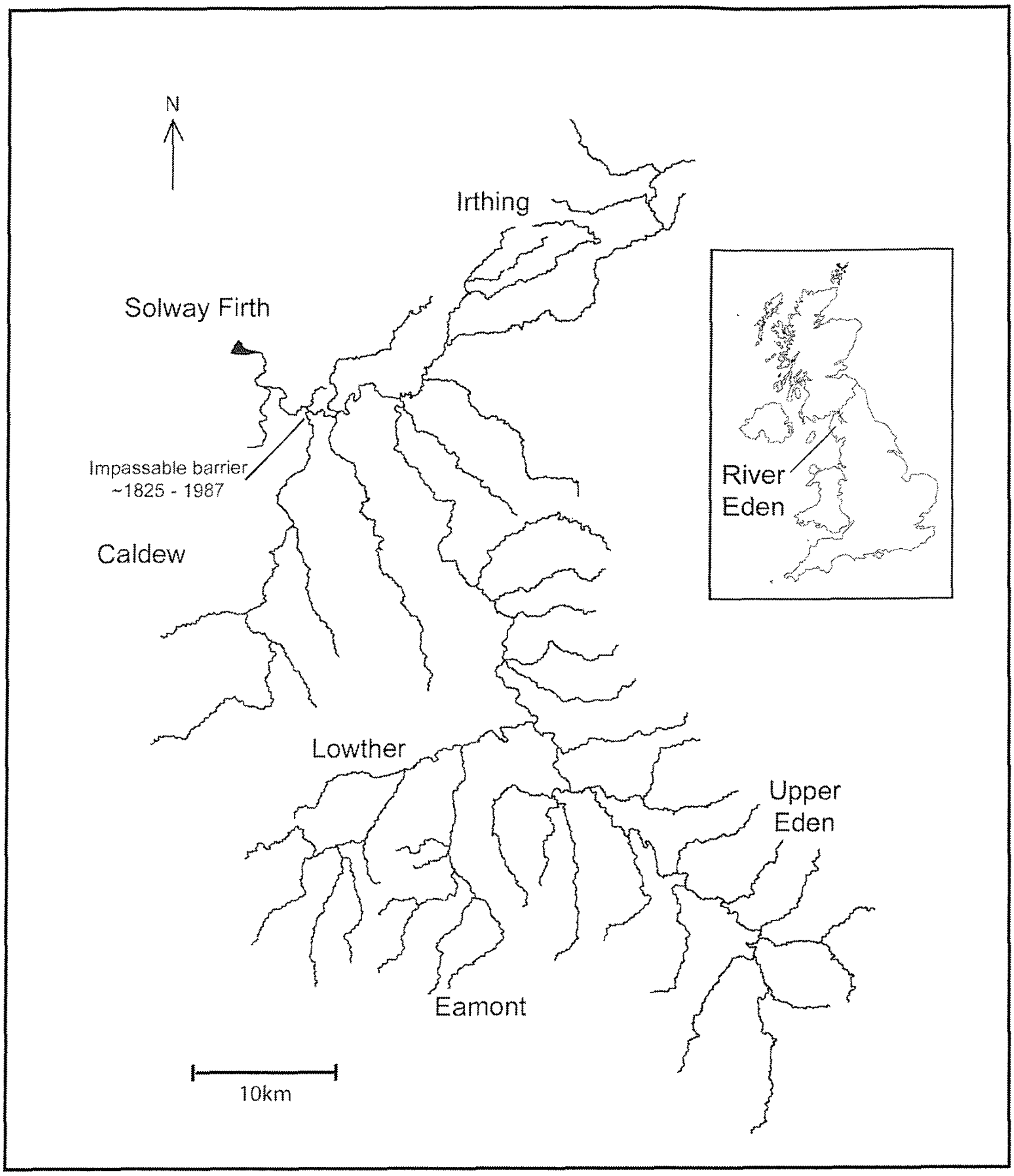

Figure 1.2 The River Eden catchment area.

1.6 Thesis outline

In this thesis, I will investigate the interaction between demographic characteristics and population genetics Atlantic salmon. I will determine what role migration takes in salmon population structure, how it is determined by life-history, and the resulting consequences in population genetic structure.

1.6.1 Objectives

- In Chapter Two, the effects of migratory timing and sea-winter tactic on body size in returning adult salmon will be investigated. What is the nature of the interaction between behaviour and phenotype, and what is its role in shaping salmon populations?
- In Chapter Three, I focus on the distance migrated. How does this vary between fish of differing sea-winter tactic? Between the sexes? I discuss the role of breeding site quality in determining the distance upstream a salmon will migrate.
- In Chapter Four, the patterns observed in migratory distance are examined using microsatellite markers. What role does geography, migration, life-history, and sex play in the distribution of genetic variability in spawning adults?
- In Chapter 5, I use microsatellites to determine the population structure present among, and between, populations of juvenile salmon in the Eden catchment. How is genetic variation partitioned within, and between, river regions?
- In Chapter 6, I test for isolation by distance between populations of juvenile salmon. How can isolation by distance models take into account migratory distance? What additional information can we gain? At what scale is isolation by distance maintained?
- In Chapter 7, the general discussion, I draw together information on aspects of body size, migration distance, adult and juvenile population genetics to assess what common patterns are found. In particular, what use can this information be for management? At what scale are management units meaningful? What is the significance of these findings?

2 Sex and life-history variation in migration timing and body size

2.1 Introduction

In indeterminately growing species, classical life-history theory predicts a correlation between an individual's age and body size (Roff, 1992; Stearns, 1992), though this might be more appropriately described as a correlation between survival and body size (Sibley & Calow, 1986). There is great variation in this correlation, with species displaying strong intra-sexual variation and sexual dimorphism (e.g. salmonids, Fleming, 1996; Fleming, 1998; Fleming & Reynolds, 2004). Body size is a key delineate of several aspects of reproductive success (Wootton, 1990), making understanding the life-history choices shaping body size a consistent theme in ecology (Krebs & Davies, 1997). The purpose of this chapter is to investigate the body size outcomes of life-history choices in salmon sea-wintering behaviour.

2.1.1 Alternative reproductive tactics

Taborsky (1999) identified three characteristics that lead to the widespread occurrence of alternative reproductive tactics (ART's) in fish species: 1) indeterminate growth; 2) external fertilisation – enabling multiple paternity; and 3) parental investment. Of these, Taborsky highlighted indeterminate growth as by far the most important due to the resulting intrasexual variation in body size. Individuals of a species with indeterminate growth are required to make decisions to allocate energy to either growth or reproduction; often through a choice among ART's. This creates a trade-off between current fecundity, and the potential to increase future fecundity by further growth (Katsukawa *et al.*, 2002). Furthermore, costs associated with an increased period of growth must be considered. There is a predation risk associated with a longer period of foraging/growth (Mittelbach & Chesson, 1984), and there may be the possibility of spawning more than once (iteroparity), as opposed to waiting for a single breeding opportunity (semelparity), albeit with a larger body size.

Under the conditional model of life history strategy, individuals maximise their lifetime fitness by making choices among ART's based on some aspect of their status at a given time, the 'switchpoint' (Gross, 1996). In salmonids, individual status decisions are often based on growth or a threshold body size (Jonsson, 1985; Nordeng, 1983; Northcote, 1992; Rochet, 2000; Thorpe *et al.*, 1998), though this is body size relative to peers (Baum *et al.*, 2004). Adult Atlantic salmon (*Salmo salar* L.) display extreme variation in body size on their return to freshwater (Groot & Margolis, 1991; Scott & Crossman, 1973). One sea-winter salmon (1SW) return to freshwater in the year following their downstream migration as smolts, whereas multi sea-winter (MSW) salmon remain in the ocean over two or more winters. These two sea-winter tactics (SWT's) result in adult phenotypes differing in body size, with associated differences in reproductive behaviour (Fleming, 1998). In males, the larger MSW fish tend to dominate spawning grounds and compete for access to females, while the smaller 1SW's behave as satellite males (Belding & Kitson, 1934; reviewed in Fleming & Reynolds, 2004). A third SWT, employed only by males, is precocious mating by parr (Jones, 1959), through delaying the migration to the sea (Letcher *et al.*, 2002) and competing with adult males. As anadromous salmon enter freshwater, their return migrations are highly structured (e.g.Webb & Hawkins, 1989), with fish migrating from spring, through to the beginning of winter. The time of return to freshwater has been linked to migratory distance (Saunders, 1967), though there is evidence that fish may well spend significant amounts of time in the lower river before moving upstream (Hawkins & Smith, 1986).

2.1.2 Spawning behaviour

Adult body size is a key determinant of reproductive success in salmonids, giving females increased fecundity (Blair *et al.*, 1993; Einum *et al.*, 2004) and the potential to dig more nests (Fleming et al, 1996), as well as a number of other benefits relevant to breeding competition, such as nest guarding ability, deeper nest depths, and aggressive advantage (reviewed in Fleming & Reynolds, 2004). Increased body size in males is positively correlated with access to mates, allowing larger fish not only increased access to spawning, but the capacity to monopolise the spawning ground (Fleming, 1996; Fleming, 1998; Fleming *et al.*, 1996). This is in part due to the development of larger secondary sexual characters such as the kype (Fleming & Gross, 1994; Tchernavin, 1944), thereby increasing competitive ability.

2.1.3 Objectives

The objectives of this chapter are to investigate how adult body size varies with SWT. In particular, are MSW fish always bigger than 1SW fish? Therefore, is it useful to describe fish as either 1SW or MSW? Alternatively, is size simply a function of months spent at sea, with no distinction between sea-winter tactics? Are earlier returning fish within each of these SWT's smaller than late returning ones? What effect does sex have on these relationships? I tested these questions using catch data from Atlantic salmon returning to the River Eden to spawn in 1999, 2000, and 2002.

2.2 Materials and Methods

2.2.1 Sampling strategy

Sampling of adult Atlantic salmon took place on their return migration to the River Eden in 1999, 2000 & 2002. Fish were rod, trap, and net (68%, 28% & 6% of total sample size respectively) caught by anglers in the lower reaches of the catchment before examination by Environment Agency (EA) staff. Whilst emphasis was placed on the unbiased sampling of both 1SW and MSW fish, there remains a possibility of bias towards MSW fish in the catch effort due to the nature of angling. Weight and fork length (measured from the snout to the fork of the tail) were measured (to the nearest *g* and *mm* respectively). Sex was identified where possible (in some cases sexual characteristics were not sufficiently developed to confidently assign sex; these individuals were excluded from the analysis). To investigate age structure, scale samples were taken at time of capture such that fish age could be calculated from scale readings conducted by the EA.

2.2.2 Data analysis

I used an analysis of variance (ANOVA) approach to test for biological factors affecting adult fork length on salmon return to freshwater. Specifically, a three-way factorial ANOVA was used to test the effects of sex, sea-winter tactic, and date of return (and interactions among them) on fork length using a general linear model (GLM). These analyses were performed using the software MINITAB 14 (2000). Due to loss of orthogonality in the model, two procedures were carried out to avoid interpretive bias that might result from higher order interactions (Grafen & Hails, 2002): 1) ANOVA tables were constructed with the factors correctly adjusted for equal or higher order terms; and 2) to avoid the possibility that the order of the factors in the analysis was affecting the interpretation, the model was fitted with these explanatory variables in different orders (RESPONSE = DATE|SEX|SWT, RESPONSE = SEX|SWT|DATE, RESPONSE = SWT|DATE|SEX). Tukey's test was implemented in Minitab for the *post hoc* analysis of significant effects with adjustment for multiple comparisons.

2.3 Results

2.3.1 Date of return migration

190 adult Atlantic salmon were caught, tagged, sexed, and aged on their return to the Eden during 1999, 2000, and 2002 (n = 43, 77, & 70 respectively). Salmon returned to freshwater from March to November (Figure 2.1). Until the end of June in each year they were almost exclusively MSW fish (Figure 2.2). Through July and August the proportion of MSW fish decreased in both males ($r^2 = 90.0$, $F_{1,8} = 62.83$, $P < 0.001$) and females ($r^2 = 75.0$, $F_{1,8} = 20.97$, $P = 0.003$). Males, and to a far greater extent females, experienced a second run of MSW fish in September and October.

Table 2.1 Mean, minimum, and maximum fork lengths; standard deviation (SD), and number of one sea-winter (1SW) and multi sea-winter (MSW) Atlantic salmon returning to the River Eden in 1999, 2000 & 2002.

Fork length		*n*	Average (mm)	SD	min	max
All fish		190	747.82	81.89	540	1000
Male	overall	109	740.55	82.22	540	1000
	1SW	55	690.36	63.33	540	820
	MSW	54	791.67	66.44	680	1000
Female	overall	81	757.59	80.92	590	980
	1SW	22	674.09	53.15	590	820
	MSW	59	788.73	66.17	650	980

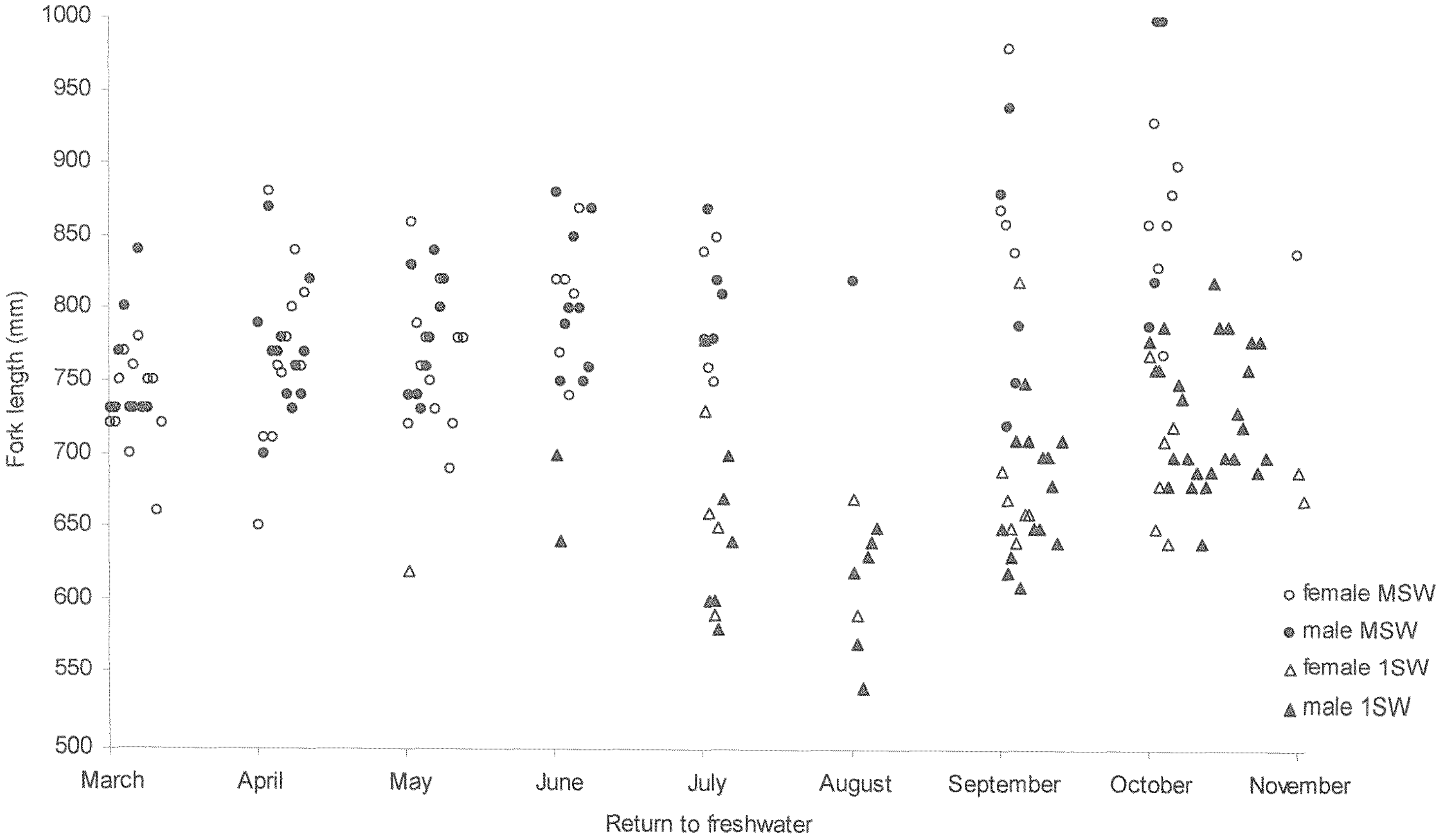

Figure 2.1 Body size (fork length, mm) of adult Atlantic salmon from the River Eden by their monthly return to freshwater (March – November). Individuals are grouped by their gender and sea-winter tactic

Figure 2.2 Proportion of multi sea-winter (MSW) Atlantic salmon from the total River Eden return each month for males and females.

Table 2.2 Analysis of variance for fork length using correctly adjusted mean squares. Date is a covariate; factors are: sex (2 levels: male & female), and sea-winter tactic (SWT, 2 levels: 1SW & MSW). Significant main effects and interactions at $P < 0.05$ are marked in bold.

Source of variation	Sum of squares	d.f.	Mean squares	*F*-ratio	*P*
Date	78742	1	65659	18.61	**<0.001**
Sex	43513	1	1176	0.33	0.564
SWT	117926	1	552462	156.59	**<0.001**
Sex X date	51099	1	42265	11.98	**<0.001**
SWT X date	3544	1	1762	0.50	0.481
Sex X SWT	21183	1	18987	5.38	**0.021**
Sex X SWT X date	9842	1	9842	2.79	0.097
Error		182	3528		

2.3.2 Fork length

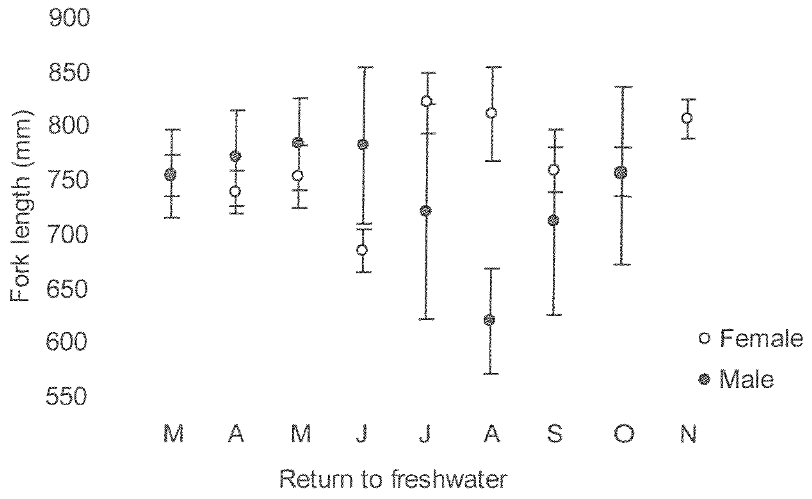

Figure 2.3 The interaction between gender and return to freshwater on body size (fork length, mm) of adult Atlantic salmon from the River Eden. Return to freshwater is classified by month of return (March – November). With standard error bars.

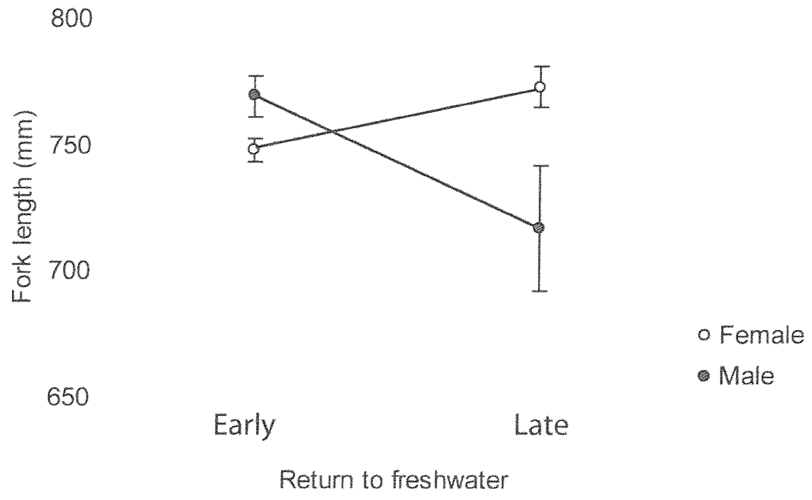

Figure 2.4 The interaction between gender and return to freshwater on body size (fork length, mm) of adult Atlantic salmon from the River Eden. Return to freshwater is classified by the early/late classification (separated by 1st June). With standard error bars.

An ANOVA showed that significant differences in fork lengths of returning adult salmon were partly attributable to the SWT adopted by the individual fish, and the month in which they returned to freshwater (Table 2.2). Salmon sampled from the River Eden ranged in length from 54cm, to almost 1m (Figure 2.1). Mean fork length was 75cm (± 8.18cm; Table 2.1). Among SWT's, female 1SW fish exhibited noticeably lower variability in fork length (mean 67.40cm, ± 5.32; Table 2.2).

Table 2.3 Pairwise analysis of means from the significant main effect and interaction using Tukey's test for multiple comparisons. t values above diagonal, *P* values below.

		Male		Female	
		1SW	MSW	1SW	MSW
Male	1SW	*	5.381	-3.181	-5.865
	MSW	<0.001	*	3.098	-1.161
Female	1SW	0.009	0.012	*	3.887
	MSW	<0.001	NS	<0.001	*

The observed variation in fork length over time is gender dependent ($F_{1,\,182} = 11.98$, $P < 0.001$; Table 2.2). Overall, returning female salmon were larger than males ($t = -3.386$, $P < 0.001$); this reflects the fact that a greater proportion of fish in the female catch were MSW's. Returning males were larger than females early in the year, but the situation reverses in July when the sexes diverge, with males much smaller than females in July and August (Figure 2.3). This difference is reduced by September, and by October the sexes are similar in fork length. Exaggerating this interaction by categorising fish as either early or late running clarifies and highlights the differing trajectories of male and female body size over the course of the year. However it masks the fact that the greatest level of divergence between males and females was in high summer (July/August; Figure 2.4).

The factors of sex and SWT interact to account for a significant amount of variation in fork length ($F_{1,\,182} = 5.38$, $P = 0.021$; Table 2.3; Figure 2.5). SWT is also significant as a main effect ($F_{1,\,182} = 156.59$, $P < 0.001$), with MSW salmon bigger than 1SW's overall (t = 6.619, $P < 0.001$). Pairwise comparisons indicate that the difference in fork lengths between male and female 1SW fish are not apparent in MSW fish (Table 2.3).

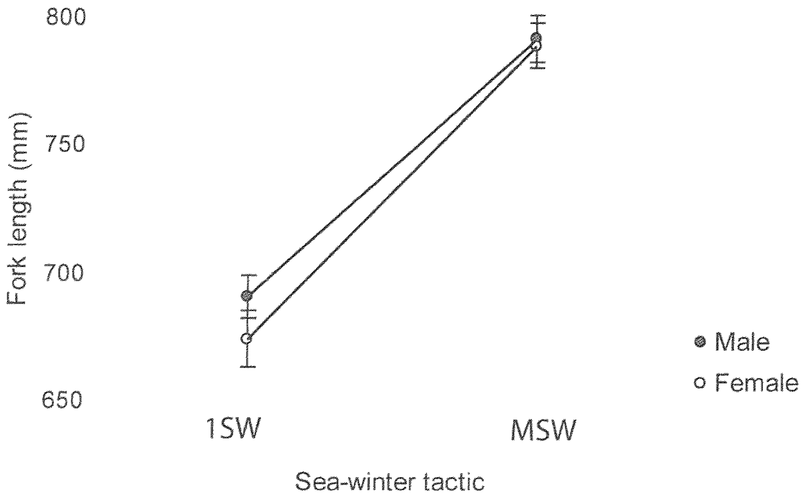

Figure 2.5 Effect of the interaction between sea-winter tactic (SWT: 1SW or MSW) and gender, on body size (fork length, mm) of adult Atlantic salmon from the River Eden. With standard error bars.

2.4 Discussion

These data reveal the complex effects of life-history strategy on body size. I demonstrate that alternative sea-winter tactics, and the date at which a salmon returns to fresh water, are key determinates of body size in Atlantic salmon. Crucially, the effect of both these factors is dependent on gender. Thus, variation in adult salmon body size is the result of a complex interaction between gender and life-history.

2.4.1 Date of return to freshwater

Consistent with previous findings (Jonsson *et al.*, 1991b; Power, 1981), there was a large degree of variation in body size of salmon on their return to freshwater. A general characterisation of the salmon return over time would be that MSW fish return early, and that 1SW fish return from June onwards, along with a small number of MSW fish returning at the very end of the year. However, in addition, I found that differences in body size over time are gender dependent. As the proportion of MSW returnees declines in July – September, so the body size of males sharply declines; in contrast, females are bigger than males in each of these three months. Explaining the divergence of males and females in body size across this period is difficult due to the low returns in August (male: $n = 1$ MSW & 6 1SW; female: $n = 0$ MSW & 2 MSW), which skew the average fork lengths. Previous work has linked the timing of return to freshwater with sufficient water flows for upstream migration (e.g. Jonsson *et al.*, 1991b; Jonsson *et al.*, 1990), though it may vary in its effect (Erkinaro *et al.*, 1999; Lilja & Romakkaniemi, 2003). Water flow potentially acts as a measure of whether migration is physically possible and, as Banks (1969) suggests, a stimulus to enter freshwater whilst in the ocean.

Both the total number of fish sampled, and the proportion of MSW fish, was lower in July, August, and September, when water flows in the Eden are at their lowest; 9.31, 8.59, & 8.678 m^3s^{-1} respectively (hydrometric flow data based on mean daily flows from 1999 – 2002; Environment Agency, unpublished data). However, this seems only to affect MSW fish as large numbers of 1SW fish return in September when water-flow is very low and has been low for some months. Salmon return timing is clearly determined by more than just environmental conditions (e.g. water flow, though temperature (Jensen et al., 1986); tide (Erkinaro *et al.*, 1999); and light (Hellawell *et al.*, 1974) have also been implicated) as these alone do not provide a satisfactory explanation for the broader question of why *any* salmon return to freshwater early in the year. Whilst there is evidence that those returning earliest migrate furthest upstream (e.g. Webb, 1992) to spawn in the most productive juvenile habitats (Baum *et al.*, 2004; Garant *et al.*, 2003), there is evidence of some salmon spending several months (Hawkins & Smith, 1986), and sometimes more than a year (Robitaille *et al.*, 1986; Shearer, 1992) in freshwater before completing their upstream migrations; during such time they would encounter huge variation in water flow. The expected cost in lost feeding opportunity at sea, and therefore greater body size, must be offset by some advantage of returning to freshwater earlier than physically necessary to spawn. The majority of MSW fish return before water flows drop in July-September, suggesting that while it may be possible to return at the end of the year, as a proportion did in the Eden, there must be high costs associated in doing so.

One potential pressure on an early return to freshwater is to locate and secure suitable spawning grounds and/or mates. It is likely that fish returning earlier in the year trade-off the potential increase in body size from remaining at sea, to gain access to higher spawning areas (e.g. Laughton & Smith, 1992; Webb, 1992), where they can secure better territories and breed earlier in the spawning season. Where there is competition among females for nest sites, prior residency is a key factor in the outcome of physical encounters (Foote, 1990). Furthermore, it is known from other salmonids that there is substantial nest destruction by later spawning females (e.g. McPhee & Quinn, 1998). This situation has more recently been recognised in Atlantic salmon (Fleming, 1996), though the effect of this is likely to decrease with body size, as larger females are able to dig deeper redds (Crisp & Carling, 1989; Gross, 1985). The potential cost of nest destruction may be further offset by the greater juvenile growth achieved by the earliest emerging fry, who, although suffering greater predation, out-compete con-specifics due to their greater body size (Brännäs, 1985; Metcalfe & Thorpe, 1992). Male salmon are under no pressure to find and hold redds. Presumably though, the earlier they return, the more likely they are to find a location in which to spawn competitively, as population density increases through the spawning season. In particular, 1SW males range more widely in search of females than MSW males (e.g. Webb & Hawkins, 1989). This is likely to be a combination of their late return and their inability to compete directly with larger males; however, the later they return, the larger they are. This raises the possibility that for some males, returning very late may make it difficult to seek out multiple females, though the extra size gained from a late return may offset this due to the enhanced competitive ability in the limited mating opportunities that are found.

2.4.1.1 Delimiting salmon populations by date?

I examined the utility of date categories for classifying salmon runs (e.g. early/late). Whilst month-by-month average body lengths by sex are most informative they may be impractical for management. The early/late classification commonly used in UK rivers and elsewhere (usually June 15th separate early/late) is useful but perhaps over-simplified; it fails to indicate differences between the sexes during the middle of the year. It does indicate the main differences in the body-size over time relationship in males and females – an increase in female body size and a decrease in male body size.

2.4.2 Body size variation between sea-winter tactics

Whilst there is a significant difference in body size between 1SW and MSW fish, this distinction is not clear-cut. Some late running 1SW fish were as large as early running MSW fish. An early running MSW fish entering the river in March will have had only four months longer at sea than a late running 1SW entering the previous November. That same MSW fish could be as much as nine sea months younger than a late running MSW fish of the same year. Given that situation, we might expect a greater difference in size between the fish separated by greater sea age. This was not found to be the case, 1SW fish were generally smaller than MSW regardless of their date of return. This confirms that SWT's are discrete units (as opposed to, say, measuring salmon sea age absolutely in months giving a continuum of sizes over the course of the year), which are useful for describing and testing hypotheses of salmon populations.

The interaction between the effect of gender and sea-winter tactic indicates that there is sexual dimorphism present in the body size of 1SW but not MSW fish. This is likely to be due to key differences in the manner of reproductive investment between the sexes, as well as differing allocation of resources between 1SW and MSW fish. Overall we might expect females to be smaller than males of any given age as a consequence of their greater investment in gametes - females invest nearly 5 times (up to 27%) more of their body weight in gametes than males (Fleming, 1998). However, there was no sexual dimorphism in MSW fish; only 1SW males and females were different in size. Holtby & Healey (1990) suggested that Coho salmon (*Oncorhynchus kisutch*) may show sex-specific foraging strategies and risk-taking where there are sex-specific reproductive size constraints. In the case of Atlantic salmon, MSW females may be foraging more effectively whilst feeding in the open ocean, since their risk of predation falls after the first few months at sea (Hansen & Quinn, 1998). Furthermore, since the opportunity for iteroparity decreases with size, MSW may well take greater risks whilst feeding to achieve the maximum benefit from a single reproductive season. In contrast, 1SW females may take fewer risks in the ocean, achieve proportionately smaller body size, and yet stand a higher chance of iteroparity. It is likely that the extent to which salmon re-spawn, currently very poorly understood, is a key factor in life-history decisions.

MSW females may allocate proportionately more resources to growth than 1SW females in order to benefit from factors unrelated to fecundity. Chinook (*Oncorhynchus tshawytscha*) and sockeye (*Oncorhynchus nerka*) salmon females have been shown to sacrifice fecundity for body size in some circumstances (Healey, 1986; Healey & Heard, 1984). In particular, MSW females, being less likely to be iteroparous, may invest more heavily in body size so as to compete more effectively against other females in terms of nest depth and nest defence.

2.4.3 Conclusions

I present results demonstrating gender-specific responses to sea-wintering tactics in Atlantic salmon. These patterns are related to sex-specific costs and benefits of foraging and migratory timing. Conformation of these mechanisms will require more extensive data sets utilising size, age and sex data, coupled with more accurate estimates of energy allocation e.g. in secondary sexual characters and egg production. Though constant disruption may exist within individual life-history factors, overall these mechanisms undoubtedly maintain an evolutionary stable strategy (ESS), which, as demonstrated, will need to account for the key differences in body size responses of males and females to life-history decisions.

3 Variation in spawning migration distances

3.1 Introduction

No greater competition between individuals is seen than when males and females come together to breed (Darwin, 1871; Kvarnemo, 1994; Reynolds, 1996). The classical view of breeding system biology is that of one sex (usually the males), competing for access to the limiting sex by defending territories or resources (Clutton-Brock, 1989; Davies, 1991; Emlen & Oring, 1977). More recently, attention has focused on the dynamic role of females in mate choice and the resulting ability to maximise their reproductive success (Ahnesjo *et al.*, 1993). Environmental heterogeneity will frequently lead to variability in the quality of breeding habitat. This requires individuals to make decisions about breeding location that will directly influence their reproductive success (Clutton-Brock, 1989; Hendry *et al.*, 2001).

3.1.1 Salmon breeding behaviour

Atlantic salmon undertake their characteristic upstream migration following one or more years spent feeding at sea (Mills, 1989; Stabell, 1984). Individual salmon move upriver to spawning areas known as redds, whereupon males compete for mating opportunities with females. The larger MSW males tend to dominate spawning grounds and compete for access to mates, while the smaller 1SW males behave as satellites (Belding & Kitson, 1934; reviewed in Fleming & Reynolds, 2004). Since males compete for females sited on their redds, breeding location is the choice of females. Redd location has important repercussions for the survival of offspring. Since there is relatively little movement of fry from their spawning areas (Gustafson-Greenwood & Moring, 1990; McCormick *et al.*, 1998; Raddum & Fjellheim, 1995) and parr tend to hold local fixed territories (Juanes *et al.*, 2000), the quality of juvenile rearing habitats is chosen by the spawning female salmon (Fleming, 1996; Webb & Hawkins, 1989). Therefore, the selection of the spawning site and its associated role as a juvenile foraging habitat is a key factor in reproductive success.

3.1.2 Variation in redd quality

Within catchment areas such as the Eden, there are gradients in the quality of habitat for juvenile salmonids. Differences in the organic productivity of headwater streams (Minshall *et al.*, 1992) over main river bodies, are likely to lead to increased growth in juvenile offspring of fish spawning in these areas. Naiman et al (1987), found that as stream size increased, there was a decrease in total carbon inputs, with most carbon being stored in 1st – 3rd order streams. Furthermore, numbers of invertebrates eaten by juvenile salmonids are largest in streams (Keeley & Grant, 2001). The increased reproductive success associated with spawning in small streams is due to the increased growth potential of an individual fish's progeny. A combination of these factors result in offspring of both 1SW and MSW fish from streams being larger than offspring resulting from spawnings in the main river body (Garant *et al.*, 2003). This leads to an overall relationship between distance upstream and juvenile growth (Baum *et al.*, 2004). A strong selective pressure for an increased migration upstream to spawn develops, even given that access by adult salmon to increasingly shallow water may be limited by physical parameters (Jonsson, 1991).

If the choice of breeding location is adaptive, Hendry et al. (2001) identify three patterns which should be appreciable in natural populations. First, individuals choice of breeding location should reflect a balance between intrinsic habitat quality against the cost of competition (Conradt *et al.*, 1999), the risk of predation (Candolin & Voigt, 1998), and the cost of the migration required to reach that breeding location (Bohlin *et al.*, 2001). Second, the condition of the individual may influence choice of breeding location since different phenotypes (i.e. ART's) may be habitat specific in their advantage (Kvarnemo, 1995). Third, individual condition, competition, and habitat quality should have a detectable influence on reproductive success (Huhta *et al.*, 1998). I will focus on how individual condition, as a choice between alternative reproductive tactics (either 1SW or MSW), affects decisions about breeding location taken over a catchment-wide scale.

3.1.3 Objectives

The behaviour of females has largely been overlooked in studies of reproductive behaviour in salmonids, yet intersexual dynamics are likely to shape both current reproductive activity, and the evolution of future reproductive behaviours (Henson & Warner, 1997). Incorporating information on both sexes is a key goal in better understanding complex reproductive behaviours and their associated effect on fitness. The objective of this chapter was to investigate what effect life-history choices in Atlantic salmon had on their spawning location as determined by upstream migratory distance. In particular, I will determine what differences might exist between the sexes in the way they choose breeding locations. I tested the hypothesis that sex and choice of reproductive tactic in individual salmon will determine where they spawn within the catchment area.

3.2 Materials & Methods

3.2.1 Sampling strategy

Sampling of adult Atlantic salmon took place on their return migration to the River Eden in 1999, 2000, & 2002. Fish were caught by rod, trap and netting in the lower reaches of the Eden before being examined by Environment Agency (EA) staff. Fish were anaesthetised (2-Phenoxyethanol, $0.1 \mathrm{ml.l}^{-1}$) before the insertion of a radio tag (HS Electronics, UK, model SAL3 in 1999; Biotrack, UK, model TW3 in 2000/2002) into the stomach via the oesophagus. Radio tags were individually identifiable by a combination of output frequency and pulse interval. Fish were also tagged externally at the base of the dorsal fin with spaghetti tags to allow visual identification. The movements of radio-tagged salmon were recorded by the EA using standard procedures through a series of fixed stations (29 locations) and 3-element Yagi antennae with portable receivers (AOR8000, Lotek SRX_400). During the spawning season mobile recording took place throughout the catchment approximately every 10 days. The furthest upstream location was taken as the Estimated Spawning Location (ESL) for all fish recorded as active during the spawning season when in known spawning areas. This ESL was recorded as a six-figure National Grid Reference (NGR). Weight and fork length were measured (to the nearest *g* and *mm* respectively) and sex identified where possible (in some cases sexual characteristics were not sufficiently developed to confidently assign sex, these individuals were excluded from the analysis). Scale samples were taken at time of capture such that fish age could be calculated from scale readings conducted by the EA.

3.2.2 Data Analysis

3.2.2.1 Mapping

The NERC Centre for Ecology and Hydrology (CEH), Dorset, generated site information for each ESL using their river network mapping data (Dawson *et al.*, 2002; Moore *et al.*, 1994). For each supplied NGR, the CEH measured (Km) the distance from the ESL to the mouth of the Eden. NGR's were converted to *XY* co-ordinates from within the river network such that sites could be mapped using the geographic information system (GIS) software ARC-VIEW 3.2 (ERSI).

3.2.2.2 ANOVA

A three way factorial ANOVA was used to test factors affecting spawning site selection using the general linear model (GLM). These analyses were performed using the software MINITAB 14 (2000). Due to loss of orthogonality in the model, two procedures were carried out to ensure unbiased interpretation of the results due to the potential influence of higher order interactions (Grafen & Hails, 2002). 1) ANOVA tables were constructed from sequential sums of squares so as to correctly adjust factors for equal or higher order terms. 2) To avoid the possibility that the order of the factors in the analysis was affecting the interpretation, the model was fitted with these explanatory variables in different orders (RESPONSE = DATE|SEX|SWT, RESPONSE = SEX|SWT|DATE, RESPONSE = SWT|DATE|SEX).

3.2.2.3 Sex ratio

The operational sex ratio (OSR) was calculated as the proportion of sexually active males to females (Emlen & Oring, 1977) by assuming all returning males to be sexually mature. This contrasts with the sex ratio at birth, which takes no account of situations where an individual's survival to maturity may be sex-biased. Deviations from a 1:1 sex ratio were tested by χ^2 in 1SW and MSW fish. The OSR calculated did not include mature parr, which assumably, if sampled, would increase the proportion of males.

3.3 Results

190 adult Atlantic salmon were caught, tagged, aged and radio-tracked on their return to the River Eden during 1999, 2000, and 2002 (Table 3.1).

Table 3.1 Summary statistics for adult salmon returning to the River eden in the years 1999, 2000, and 2002. Mean, min, and max refer to the distance upstream (Km) to which salmon were radio-tracked within the catchment to their estimated spawning location. ± standard deviation.

	Male		Female	
	1SW	MSW	1SW	MSW
n	55	54	22	59
Mean	49.45 ± 4.15	50.24 ± 4.29	35.80 ± 5.16	64.18 ± 3.89
Min	2.20	9.35	7.73	1.52
Max	105.04	104.23	106.76	107.33

3.3.1 Sex ratio

The sex ratio in 1SW fish was male biased (n = 77, $\chi^2 = 14.14$, d.f. 1, $P < 0.001$), while there was no significant difference from 1:1 in the sex ratio of MSW fish (n = 113, $\chi^2 = 0.22$, d.f. 1, P = ns). The percentage of males in the returning fish increased from 43% in March, to 70% in October (Figure 3.1). In November there were no males at all in any year (1999, 2000 & 2002) and only three females in one year (1999). It is likely that these three females in November were not typical and unduly influence the dataset. When these three females are removed there was an increase in the monthly percentage of males over the course of the year ($r^2 = 67.1$, $F_{1,7} = 12.22$, $P = 0.013$).

3.3.2 Distribution of spawning sites

Mapped ESL's (Figure 3.2 & Figure 3.3) indicate that Atlantic salmon spawned along the length of the main body of the River Eden. Furthermore, returning adults were present in the Rivers Eamont & Lowther, and in the River Caldew. The distance that salmon migrated upstream to their ESL's varied from 1.5 km to 107.3 km. There is a significant interaction (Table 3.2) between the sea-winter tactic chosen, and the sex of the individual making that choice ($F_{1,189} = 6321.4$, $P = 0.009$), in determining how far upstream a salmon will spawn from the sea, such that the effect of tactic upon spawning site selection is gender dependent. The main effect of tactic accounted for a significant amount of the variation in the location of the ESL ($F_{1,189} = 4337.5$, $P = 0.03$).

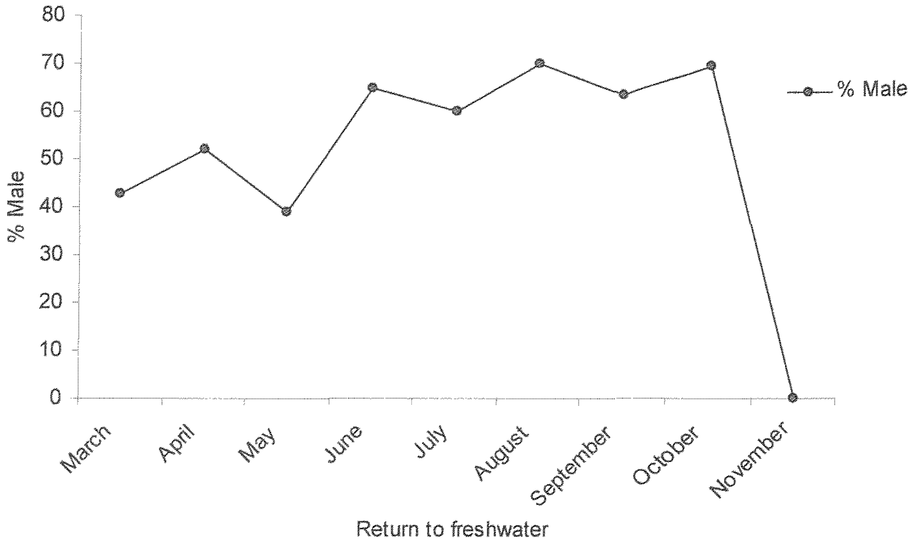

Figure 3.1 Proportion of male salmon in the total sample of return fish by month of their return to freshwater.

There was no difference in the mean distance migrated to the spawning site in male 1SW and MSW fish. In contrast, female MSW fish spawned further up the catchment than their 1SW counterparts. Furthermore, MSW females mean spawning distance was higher upstream than both 1SW and MSW males, whilst 1SW females mean spawning distance was further downstream than either group of male salmon (Figure 3.4). Therefore, the effect of sea-winter tactic on the selection of spawning sites acts only on female Atlantic salmon.

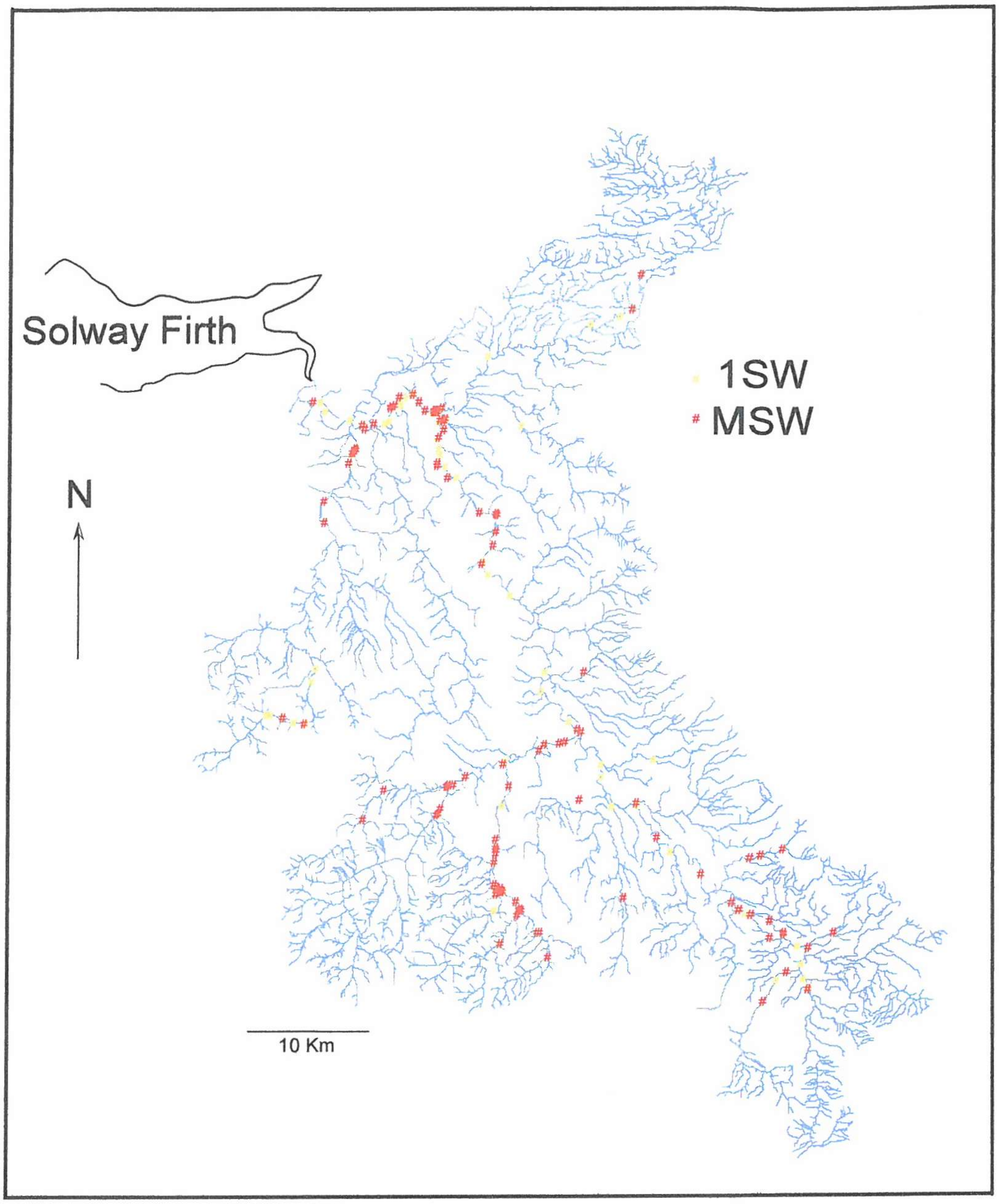

Figure 3.2 Estimated spawning locations (ESL's) of radio tracked 1SW and MSW Atlantic salmon in the River Eden in 1999, 2000, & 2002. © Crown copyright, GD272191

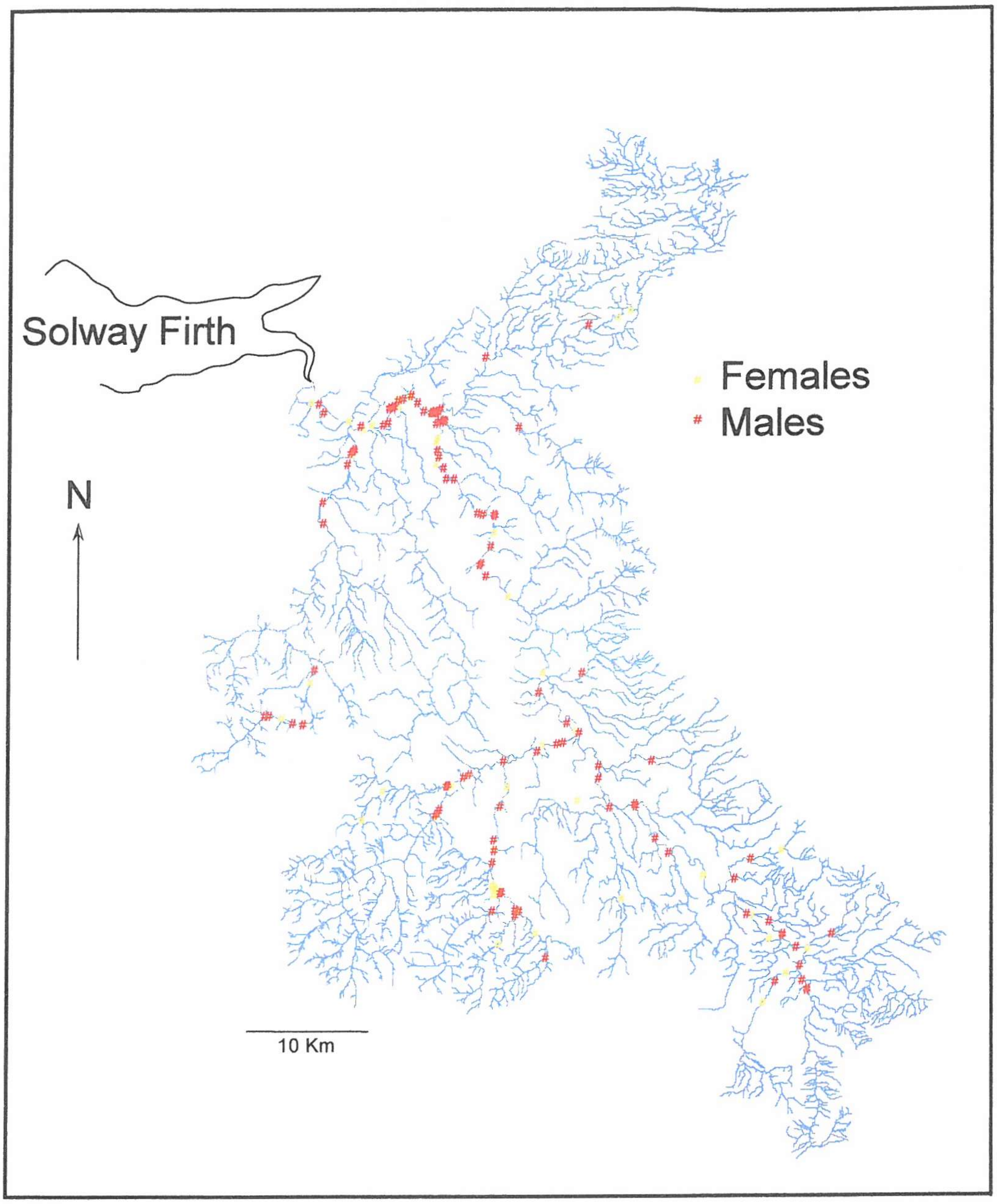

Figure 3.3 Estimated spawning locations (ESL's) of radio tracked Male and Female Atlantic salmon in the River Eden in 1999, 2000, & 2002. © Crown copyright, GD272191

Table 3.2 Analysis of variance for distance spawned upstream from the sea using correctly adjusted mean squares. Date (covariate); Sex: Two levels (male & female) and Tactic: Two levels (1SW & MSW). Significant main effects and interactions at $P < 0.05$ are marked in bold.

Source of variation	Sum of squares	d.f.	Mean squares	*F*	*P*
Date	477.2	1	3.0	0.00	0.955
Sex	18.2	1	717.4	0.79	0.376
Tactic	1635.5	1	4337.5	4.76	**0.030**
Sex X date	68.3	1	217.8	0.24	0.626
Tactic X date	334.6	1	297.9	0.33	0.568
Sex X tactic	613.0	1	6321.4	6.93	**0.009**
Sex X tactic X date	52.0	1	52.0	0.06	0.811
Error		182	165909.1		

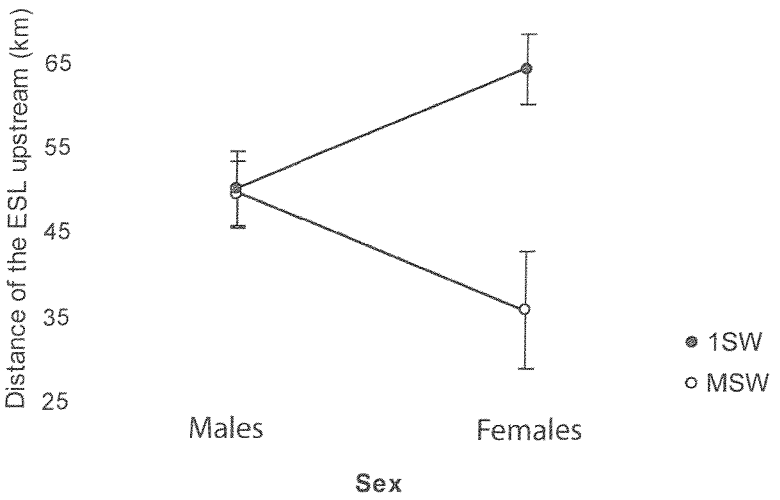

Figure 3.4 The interaction between sex and tactic on the distance spawned upstream from the sea (km). With standard error bars.

3.4 Discussion

There is a gender-differentiated effect of sea-winter tactic on the distance an Atlantic salmon will migrate upstream to spawn in the Eden catchment. While tactic does not influence male spawning site selection (no difference in mean distance migrated upstream between 1SW and MSW males), it is the key factor explaining the distance migrated upstream by females (Figure 3.4). Furthermore, female MSW fish respond in an opposing fashion to 1SW females in their selection of spawning sites. To the best of my knowledge this is the first study to find either tactic specific migratory patterns in Atlantic salmon, or any variation in the effect of these tactics with gender. These results provide the first evidence of sex-biased structure in Atlantic salmon migrations. I will discuss these results with respect to the differing strategies of males and females in response to competition for access to mates and to spawning grounds.

3.4.1 Date of return

When anadromous salmon return from saltwater, their return migrations are highly structured (e.g.Webb & Hawkins, 1989). Previous authors have highlighted the date that salmon return to freshwater as one of the principal factors in explaining the location of spawning sites (e.g. Hawkins & Webb, 1986; Laughton, 1989; Laughton, 1991; Laughton & Smith, 1992; Webb, 1989; Webb, 1992; Webb & Hawkins, 1989) though these focus almost exclusively on MSW fish. Saunders (1967) identified an inverse relationship between the time of return in 1SW salmon and the distance they migrated in the River Northwest Miramichi. These observations of 1SW fish also suggested that fish which smolted higher in the catchment were returning to areas higher in the catchment. Saunders (1967) suggests that the date of river entry is related to spawning site distance in, what Webb (1989) refers to as, a serial fashion, i.e. those fish returning first have more time available to ascend the river. This may appropriately model salmon movement in a simple river system with a single main stem available for spawning where there is constant gradient and water flow. However, as Webb (1989) points out, progressively more complex river systems are likely to have multiple, overlapping ascents by salmon. In these more complex, and more realistic, scenarios, differing water flow conditions between tributaries will necessitate salmon waiting in holding pools until local conditions allow upstream migration. Laughton & Smith

(1992) identify a 'more or less precise' relationship between the time of river entry and the spawning location in the Rivers Dee and Spey. However, they dispute the idea that this is simply a function of the time required to complete migration (e.g. Struthers, 1984), since some early running 1SW fish spawned as high as MSW fish. Laughton & Smith (1992) draw no comparisons between 1SW and MSW fish, although, they note that the relationship between distance migrated and date of river entry holds within each age class (i.e. 1SW and MSW).

My results disagree with the general findings of much of the previous work on salmon spawning site choice; I find that date of river entry does not explain a significant proportion of the variance in migration distance once sex and sea-winter tactic are accounted for. There are two explanations for the disparity in results. Firstly, previous authors have not taken into account sea-winter tactic or sex of the salmon studied, and, since these two factors are themselves closely linked to date of salmon entry (see previous chapter), this complexity might not have been fully accounted for. Secondly, it is likely that there is great variability between river systems in the factors determining the selection of spawning sites (Jonsson, 1991). In particular, rivers in the extreme north of the salmon's range may well experience a more influential effect of date of river entry due to extreme environmental conditions relating to water flow (e.g Kallio-Nyberg & Romakkaniemi, 1998; Karlsson & Karlström, 1994), although this has not always been found to be the case (Lilja & Romakkaniemi, 2003).

3.4.1.1 The effect of sex

The approach that males and females take towards reproduction, are radically different in Atlantic salmon. Females invest nearly 5 times as much of their body weight (up to 27%) in gametes as do males (Fleming, 1998), a factor shaped by natural selection. Males concentrate on factors of sexual selection, developing secondary sexual characters and fighting for mating opportunities (Darwin, 1871; Hendry & Berg, 1999; Mills, 1989). This fits to the general sexual selective paradigm whereby female breeding traits are shaped by selection for increased offspring production and greater juvenile survival. In contrast, male breeding efforts are driven by the number of matings than can be successfully won in competition from other males (discussed in

Fleming & Reynolds, 2004). The cost of this male competition is high, with fewer males surviving spawning than females (reviewed in Fleming, 1996).

The differing patterns of distance migrated to spawn can be explained by a model whereby female salmon are under selective pressure to penetrate further upstream to reach more productive spawning grounds. In contrast males are under such high pressure to increase their number of mating opportunities that the extra cost to migrate higher (to better females i.e. females on more productive spawning grounds) is traded off against energetic cost of competing with other males. It is possible that the small number of 1SW females that do spawn in the Eden are not moving far upstream due to intense competition from bigger females (both in terms of physical aggression and number of available spawning sites). Smaller males (i.e. 1SW's) are likely to act as satellite males (Fleming, 1998), but small females cannot sneak matings, they need to prepare redds and attract competing males; they are tied to obtaining a redd. Furthermore, if MSW males are not preferentially migrating to certain areas of the river to spawn, then the competition is evenly spread for smaller (i.e. 1SW) males resulting in the same average migration distance for the two tactics. In contrast, 1SW females are always going to have to find unoccupied (i.e. low quality) redds late in the season. Females migrating early in the year are at risk of nest destruction from the digging of later running larger females (Fleming, 1996; McPhee & Quinn, 1998). The risk of nest damage is likely to be negatively correlated with body length such that smaller females may be more likely to suffer nest damage (Fleming, 1998). This may be a further factor in the late return of 1SW females.

3.4.1.1.1 Sex-biased dispersal

Differences in male and female spawning distances may be used to test for sex-biased dispersal. If males are dispersing to a greater degree within the catchment then this should be detectable using molecular markers, as should greater structure between 1SW and MSW females. Sex-bias in dispersal is a balance between Local Mate Competition (LMC), and Local Resource Competition (LRC Perrin & Mazalov, 2000). Sex-biased dispersal is predicted to be male biased among polygamous and promiscuous species (Dobson, 1982) where LMC is high for males. Furthermore, where prior ownership of a territory is crucial, such as female salmon on redds, LRC is high, and that sex gains little from dispersal.

3.4.2 Heritability of spawning location

These results offer no insights into the degree of philopatry in Atlantic salmon; there was no temporal aspect to the analysis as the spawning locations of the parents of the salmon sampled are unknown. If a relatively high degree of site fidelity is assumed, as suggested by previous work (e.g. Stabell, 1984; Youngson *et al.*, 1994) then there must be a heritable component to migratory distance in females that does not seem to prevail in males. Heritable components have been identified for other aspects of salmonid biology. Garant et al.'s (2003) work on salmon from the Sainte-Marguerite River suggests that there is a higher heritability component to body size in smaller streams than in the main river body, and furthermore, heritability values for body size are greater in MSW fish than grilse. A correlation between habitat quality and heritability has previously been documented for traits in birds (Larsson *et al.*, 1997; Merila, 1997; Qvarnstrom, 1999) and mammals (Réale *et al.*, 1999).

3.4.2.1 Biased sex ratio

There is a clear male bias in the sex ratio in River Eden salmon. This is partly due to differences in the proportions of males and females returning within each SWT: MSW fish return in equal sex ratio, 1SW fish are predominantly male (71%). However, this snapshot view of the sex ratio, based on absolute numbers of fish, is likely to drastically underestimate the true operational sex ratio at any given time (reviewed in Fleming & Reynolds, 2004). In particular, males remain sexually active for a longer period of time than females, and can mate a greater number of times, at greater frequency (Fleming & Reynolds, 2004; Groot & Margolis, 1991). This leads to a substantial male bias in OSR's which increase as the breeding season progresses (Hendry *et al.*, 1995; Quinn *et al.*, 1996). The excess of males further intensifies the competitive pressure on males attempting to mate (Kokko & Monaghan, 2001), placing the emphasis for male mating effort to be on gaining access to as many females as possible. Under such conditions selective pressures to mate with females high in the catchment where juvenile production is greatest may well be outweighed by the energy cost required to do so, energy better expended acquiring multiple mating opportunities lower down the catchment.

3.4.3 Mature Parr

An important factor, which cannot be fully accounted for in this study, is the effect of mature male parr on the mating models described. It is clear that mature parr make a considerable contribution to total progeny (e.g. Taggart *et al.*, 2001), and they must therefore add considerably to competition between males. Furthermore, the presence of mature parr should act to substantially increase the male-bias in the OSR from that calculated here.

3.4.4 Conclusions

Adult migratory patterns differ significantly in the distance a salmon will swim upstream to spawn. These differences are under the influence of sea-winter tactic and sex. Life-history factors related to time spent at sea strongly affect female spawning site choice, a situation not found in males. This is likely to be a direct result of differing approaches to breeding competition between the sexes.

4 Genetic Variation in Populations of Adult Salmon Migrating Upstream to Spawn

4.1 Introduction

From relatedness of juvenile individuals within short stretches of river (Fontaine & Dodson, 1999), to range-wide analyses spanning the Atlantic Ocean (King *et al.*, 2001), salmonids display a propensity for genetic population structure at all levels examined. Numerous studies have investigated population structure between catchments (e.g. McElligott & Cross, 1991), and within catchments (e.g. Garant *et al.*, 2000). However, these studies largely focus on juvenile fish; few have investigated populations composed of returning adult salmon (but see e.g. Hendry *et al.*, 2002). Sampling adult fish enables aspects of migratory behaviour relating to dispersal and life-history analyses to be taken into account, since sampling is of individuals who have reached adulthood. These phenomena are impossible to account for using juvenile fish alone.

4.1.1 Adult salmon

It is comparatively easy to sample salmon fry and parr from their natal areas; a sampled riffle area can be defined as a sub-population, numerous riffle areas can be sampled within a single river, and this in turn constitutes a river population. Similarly, several river populations can be sampled in this fashion, thus allowing the investigator a powerful hierarchical structure to analyse (e.g. AMOVA; 5.2.4). When sampling adult fish on their return migration upriver to spawn, defining populations is more problematic. Ideally, adult fish would be observed on redds, with samples taken from them at the time of spawning. In this way, diversity between, and among, local breeding populations could be compared. However, aside from the practical difficulties of sampling on such a scale, adult salmon are protected from interference during spawning (Environment Agency guidelines). It is therefore necessary to estimate individual spawning locations (ESL's; see Chapter Three) to place them in geographically defined populations. However, due to limits on sample size, individuals are widely spread throughout the catchment, with individuals rarely sampled in sufficient density at any one location to make up a riffle type sub-population (as defined above for juveniles). However, fish can be assigned at the river scale from their ESL data, and then, from that data, to distance categories within those rivers. In this way it is possible to characterise the effect of migratory distance on population structure. Furthermore, since fish are all aged (see 2.2.1), it is possible to determine the effect that life-history, in particular sea-winter tactics (see Chapters One & Two), has on population sub-division.

4.1.2 Estimating population viability: effective population size

Effective population size (N_e) has emerged as a key component of most theoretical predictions in conservation biology (Frankham *et al.*, 2002). Typically defined as: '[T]he number of individuals that would give rise to the calculated loss of heterozygosity, inbreeding, or variance in allele frequencies if they behaved in the manner of an idealised population' (Frankham *et al.*, 2002), the loss of genetic diversity associated with a reduction in N_e can be critical in endangered populations, where inbreeding effects may lead to population, or even species extinction (Soule & Mills, 1998). Estimations of N_e can be made directly where there is detailed information of breeding structure and population size (Caballero, 1994); however, recent advances in molecular methods have made it possible to estimate N_e, using markers such as microsatellites, from allele frequency data (see e.g. Shrimpton & Heath, 2003).

4.1.3 Sex-biased dispersal

Under a pattern of sex-biased dispersal, one sex is more philopatric than the other. The mechanisms responsible for this are discussed in 3.4.1.1.1.

Recent studies have utilised molecular markers, such as microsatellites, to test for differing dispersal between the sexes (reviewed in Goudet *et al.*, 2002; Prugnolle & de Meeus, 2002). These indirect methods can be applied on a relatively large scale, often without the need for intensive field observations (Neigel, 1997). There are several approaches to this end: 1) testing for relatedness between individuals (Knight *et al.*, 1999; Surridge *et al.*, 1999). Individuals of the more philopatric sex should have higher relatedness, being more related at any given site than the dispersing sex, which mix between sites to a greater extent; 2) the dispersing sex should have lower between-sample site F_{ST} values due to the increased gene flow with neighbouring sub-populations (Mossman & Waser, 1999). This method is expected to perform similarly to a relatedness approach since the two measures are connected; and 3) individuals from the more philopatric sex should be assigned with a greater degree of accuracy to their source population than the dispersing sex (Favre *et al.*, 1997). Individual-based assignment methods are now recognised as a powerful tools for studying dispersal and population structure (reviewed in Berry *et al.*, 2004). Individuals are assigned within confidence limits to candidate populations based on their multilocus genotype.

Investigations into sex-biased dispersal using molecular markers, like those using more traditional mark-recapture methods, have mostly been limited to birds, where dispersal is typically female biased (e.g. Piertney *et al.*, 2000), and to mammals, where male biased dispersal is prevalent (e.g. Devillard *et al.*, 2004). However, molecular markers have been used to characterise sex-biased dispersal in other taxa, e.g. amphibians (Lampert *et al.*, 2003), reptiles (Casale *et al.*, 2002), and insects (Sundstrom *et al.*, 2003). Of the few studies of sex-bias in fish species, only four have found significant within-population sex-bias in dispersal. One of these is in the great white shark, *Carcharodon carcharias* (Pardini *et al.*, 2001), two in Brook trout, *Salvelinus fontinalis* (Fraser *et al.*, 2004; Hutchings & Gerber, 2002), and one in brown trout, *Salmo trutta* (Bekkevold *et al.*, 2004). In all cases the dispersal was male biased, as predicted for this polygamous mating system (see Chapter Three, Perrin & Mazalov, 2000).

4.1.4 Objectives

In this chapter, I will assess population genetic structure in adult Atlantic salmon returning to the Eden Catchment. The influence of geography, migration and life-history will all be taken into account.

In Chapter Three, evidence from radio-tracking indicated that there is a sex-bias in the migratory patterns of adult salmon in the Eden catchment (Figure 3.4). I will test for sex-biased dispersal between river regions, and over migratory distance using assignment testing.

Effective population size will be estimated from molecular data and compared to census size data. This will be a key parameter in the future management of Atlantic salmon in the Eden catchment.

4.2 Materials and methods

4.2.1 Sampling strategy

Adult Atlantic salmon were sampled on their return to the Eden in the years 1999, 2000, & 2002 (see details of sampling in 2.2.1). At the time of capture, either a small number of scales (1999 only), or a section of the adipose fin was removed from each salmon by Environment Agency staff. These tissue samples were transferred to labelled microtubes of 95% ethanol. Data from the measurement of fish taken on capture, their estimated spawning location (ESL's; see Chapter Three), and age (see Chapter Two), were collated and added to a database of genotype information. Fish were classified by river region, i.e. the river of their ESL, and into distance bins reflecting the distance migrated upriver. These bins were short (1 – 35km), medium (36 – 70km), and long (71 – 107km) migratory distance.

4.2.2 DNA extraction

A rapid DNA extraction was carried out using a Chelex based protocol (Beacham & Dempson, 1998; Estoup *et al.*, 1996). Chelating resin based procedures were first used for forensic material and museum specimens (Eellegren, 1994; Walsh *et al.*, 1991). They have found favour among molecular ecologists due to their low cost and simple, rapid protocol (Beacham & Dempson, 1998; Estoup *et al.*, 1996), especially compared to more expensive and laborious phenol-chloroform methods.

Labelled 1.5ml microtubes were prepared, each containing 300μl of Chelex Buffer A (5% Chelex [Sigma], 100mM NaCl, 50mM Tris, 1% Triton X-100, 10mM EDTA) and 5μl proteinase K (10mg/ml on ice). The buffer was stirred continuously to ensure the Chelex beads were in suspension. Wide-bore 1000μl pipette tips were used when pipetting the buffer solution to avoid tip clogging. Adipose fin clips were removed from 95% ethanol and blotted dry on tissue paper before being placed into their respective microtubes.

Sample tubes were then placed in an incubation oven where they were rotated at 55°C for 4 - 6 hours. Tubes were then spun at 14,000rpm for 4 minutes, the supernatant was transferred to new labelled 1.5ml microtubes to which 300μl of Chelex Buffer B (5%

Chelex, 10mM Tris, 1mM EDTA) was added. Samples were then stored at -20°C. Aliquots (1µl) of these extractions were used for PCR.

4.2.3 PCR

All fish were screened at 11 microsatellite loci (Table 4.1). These were amplified in three sets using multiplex PCR kits (Qiagen: sets described in Table 4.1). Each set contained primers labelled with a mix of dyes, such that their alleles were not overlapping (Figure 4.2). Primers were forward labelled with TET (green), HEX (yellow), or FAM (blue) dye, all Applied Biosystems. By multiplexing primers in this way, one PCR amplified up to four loci, saving time and materials. Furthermore, I found that multiplexing at the PCR stage gave more consistent results than adding together differently dyed products post-PCR.

Each 10µl PCR consisted of 1µl of DNA extract, 5µl Qiagen multiplex mastermix, 1µl Qiagen Q-Solution, 1µl primer mix (All forward and reverse primers in each set stored in Tris-EDTA (T.E.) buffer at 100pmol/µl) and 2µl ddH_2O. Reactions were carried out in tape-sealed 96-well Thermofast low-profile plates (Abgene). The amplification protocol (carried out on a Hybaid Thermocycler) consisted of an initial activation step of 15 minutes (95°C), followed by 30 cycles of denaturing for 30 seconds (94°C), annealing for 90 seconds (58°C), and extension for 60 seconds (72°C). A final extension of 30 minutes (72°C) completed the PCR. Reactions were held at 4°C once cycling was complete.

4.2.4 Automated genotyping

For analysis, PCR products were diluted approximately 1/10 with ddH_2O (depending on strength of PCR), 1µl of this dilution was combined with 1.2µl of loading mixture (0.4µl GENESCAN-500 ROX standard [Applied Biosystems], 0.5µl deionised formamide, 0.3µl blue dextran/EDTA loading buffer) on a 96 well microplate, and the samples denatured for 2 minutes at 96°C in a Hybaid Thermocycler. Samples were transferred onto ice and kept as such while 0.9µl of the denatured samples were loaded into a 64+4 well RapidLoad membrane comb-dipping tray (Gel Company) ready for loading. Samples were subject to electrophoresis on an Applied-Biosystems Prism 377 sequencer. Gel data files were generated using GENESCAN 3.1.2 software (Applied-

Biosystems), which allows individual lanes to be tracked and all fragments to be compared to internal size standards. Scoring and output of allele sizes was performed using GENOTYPER 2.1 (Applied-Biosystems). Every run included two control individuals with known genotypes allowing comparisons with all new data to correct for inconsistencies between runs.

4.2.5 Data analysis

Data from individuals sampled in different years were pooled due to low sample sizes. Pooling of samples did not allow a full investigation of the temporal component of salmon population structure (Garant *et al.*, 2000), though temporal stability was assumed. Pooling sample years made the analysis of regional, distance, sea-winter tactic, and sex groups possible by generating workable sample sizes.

All raw microsatellite data generated in GENOTYPER was analysed using MSA (Dieringer & Schlotterer, 2002) to obtain descriptive statistics (N, H_E, H_O, etc). Genotype frequencies were tested for departures from Hardy-Weinberg expectations and linkage disequilibrium at all populations at all loci using ARLEQUIN 2.001 (Schneider *et al.*, 2000). The Hardy-Weinberg test utilised the Markov chain randomisation test of Guo & Thompson (1992) to estimate exact P values for each locus-population combination. P values were Bonferroni corrected for multiple comparisons (Rice, 1989).

Since the number of alleles observed in a sample is highly dependent on sample size, allelic richness ($\hat{A}$) was calculated for each population using FSTAT 2.9.3.2 (Goudet, 2001). This method uses the rarefaction index of Hurlbert (1971) to correct for the effects of differing sample sizes (ElMousadik & Petit, 1996; Petit *et al.*, 1998). Allelic richness is less restrictive than methods based on heterozygosity, and is sensitive to events such as recent bottlenecks or population admixture (see e.g. Comps *et al.*, 2001); it is possible for substantial amounts of allelic diversity to be lost before any decrease in heterozygosity is detected (Allendorf, 1985; Waples, 1990).

4.2.5.1 Null alleles

Where significant deficiencies from Hardy-Weinberg expectations (HWE) are observed these may be due to violation of the assumptions of the equilibrium. Factors such as non-random mating, overlapping generations, substantial migration and natural selection may all lead to deviations from HWE (Hartl & Clark, 1997). However, deviations from HWE may be artefacts due to the presence of null alleles, leading us to overestimate the proportion of homozygotes (Pemberton *et al.*, 1995). Null alleles occur when a mutation in flanking sequences stop primers from binding (Callen *et al.*, 1993), leading to heterozygotes being scored as homozygotes. Null alleles are implicated where deviations from HWE occur at a locus over several populations while not occurring at other loci across those populations. The frequency of a null allele (r) can be estimated from the apparent deficiency of heterozygotes they induce (Equation 1; Brookfield, 1996).

Equation 1 Estimating the frequency of a null allele (Brookfield, 1996).

$$r = \frac{H_E - H_O}{1 + H_E}$$

4.2.5.2 Population differentiation

To estimate differentiation between river regions, pairwise F_{ST} values were calculated (Weir & Cockerham, 1984); the statistical significance of the F_{ST} estimates was obtained by permutation of genotypes among populations in ARLEQUIN 2.001. AMOVA (Excoffier *et al.*, 1992), implemented in ARLEQUIN 2.001, allowed the quantification and testing of genetic differentiation at three hierarchical levels: among river regions, among distance classes within river regions, and within sample sites.

4.2.5.2.1 Assignment testing

4.2.5.2.1.1 To river regions

The accuracy with which individual fish could be assigned to their river of sampling was tested using Rannala & Mountain's (1997) Bayesian assignment method in the software GENECLASS 1.0.02 (Baudouin *et al.*, 2004). This approach has demonstrated the ability to resolve population structure not identified by other methods (Castric & Bernatchez, 2004), and has been used forensically to determine a fish's river of origin for law enforcement purposes (Primmer *et al.*, 2000). GENECLASS does not assume genetic equilibrium and is therefore, robust to violations of HWE. The software estimates the likelihood that an individual is from its sampled river (correctly assigned), from another sampled river (mis-assigned), or not from any of the rivers sampled (un-assigned). Likelihood estimates were generated using 50,000 replicates by the simulation method of Cornuet et al (1999). Rivers were excluded as a source when an individual's multilocus genotype fell outside the 95% likelihood for that river. Assignment testing was carried out for each sex separately.

4.2.5.2.1.2 To distance categories

In a similar method to that described for river regions, individual fish were assigned to distance categories (see 4.2.1). This was carried out for the sexes separately.

4.2.5.2.2 Factorial correspondence analysis (FCA)

FCA (Benzécri, 1973) is an ordination method allowing the graphical representation of variance in a given dataset. FCA maximises the correspondence between allele scores and individual scores, enabling individuals to be plotted as dots in a hyperspace, their coordinates determined by their genotype relative to the overall variability in genotypes. Vectors from this cloud of data points are used to create a set of factorial axes. These account for differing amounts of the variability in this hyperspace and can be plotted against each other to visualise patterns. By convention the first axis makes the greatest contribution to the variation. This procedure was carried out in the software GENETIX 4.04 (Belkhir *et al.*, 2001).

4.2.5.3 Differences between the sexes

To test for potential intra-sexual differences in population genetic parameters, several measures were calculated for the sexes separately: H_E, allelic richness, and F_{ST}, were calculated as described above. Furthermore, relatedness values (R) were calculated for each sex using RELATEDNESS 5.0 (Queller & Goodnight, 1989).

4.2.5.4 Effective population size (N_e)

N_e is typically defined as the size of the 'ideal' population that will result in the same amount of genetic drift as in the actual population being considered (Wright, 1969). An ideal population is expected to loose heterozygosity at a rate of $\Delta H = -1/2N$ per generation, where N is size of the adult population (Wright, 1969). So for a population of 100 individuals, it is expected that the population would lose 1 = (2 x 100), or 0.5%

of it's heterozygosity per generation. However, the assumptions of an ideal population, namely: non-overlapping generations, random mating within sub-populations, constant population size, equal sex ratio, and equal reproductive success in all individuals (Frankham *et al.*, 2002; Hartl & Clark, 1997), are frequently violated in nature, particularly by salmonids. Estimations of N_e are therefore, generally expected to be considerably lower than the population census size (see e.g. Shrimpton & Heath, 2003).

A number of methods were used to estimate the effective population size of a generation of Atlantic salmon from the River Eden. These were implemented in the software NE-ESTIMATOR 1.3 (Peel *et al.*, 2004), which calculates N_e by two single point estimations; one based on linkage disequilibrium (Bartley *et al.*, 1992), implementing Burrow's D^* (Campton, 1987) - a correlation of linkage disequilibrium across loci. The other single point method is based on heterozygote excess (Luikart & Cornuet, 1999; Pudovkin *et al.*, 1996). When the number of breeders is small, male and female allele frequencies will differ due to binomial sampling error; this will lead to an excess of heterozygotes relative to Hardy-Weinberg expectations. This difference in heterozygosity is dependent upon, and can be used to estimate, the number of breeders (N_{eb}), from which N_e can be calculated. NE-ESTIMATOR also estimates N_e by the temporal method of Waples (1989). This approach requires two samples of individuals separated by at least one generation to estimate the harmonic mean effective population size by examining the change in allele frequencies between generations. Furthermore, NE-ESTIMATOR acts as an interface to the software TM3 (Beaumont, 2003) and MLNE (Wang, 2001; Wang & Whitlock, 2003), both of which calculate maximum likelihood estimates of N_e from temporal differences in allele frequencies.

To provide data-sets of cohorts for the calculation of N_e, all fish were sorted into a 0+ year class, that is, the year they emerged as fry. This was achieved by subtracting fish age from the year of sampling. Temporal methods of estimating N_e require samples of individuals separated by at least one generation. I defined a generation as four years, this was based on the average age of salmon sampled during the study (Figure 4.1; range 2 – 6 years, mean age 3.92 ± 0.05). N_e values quoted for temporal methods (moments based, TM3, & MLNE) are therefore estimates N_e of that generation. However, the mean age of returning adult salmon may not reflect the true mean age of mating fish due to the presence of precocious parr. To simulate the reduced mean generation time, N_e was also calculated over a three year generation span.

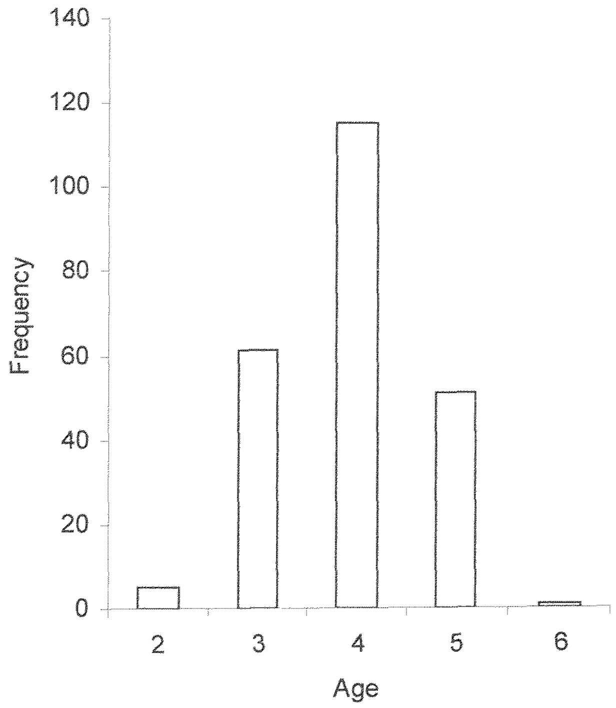

Figure 4.1 Histogram of adult Atlantic salmon ages on their return to the Eden Catchment ($n = 233$).

Table 4.1 Locus name, primer sequence, allele size range from previous studies, number of alleles observed in previous studies, optimal annealing temperature, observed heterozygosity, repeat motif, and the original author of microsatellite loci screened in adult Atlantic salmon from the River Eden.

	Locus	Primer sequence (5' – 3')$^{\Delta}$	Allele size bp	*N*a	T_a °C	H_O	Repeat motif$^{¥}$	Authors
Set 1	Ssa85	f: AGG TGG GTC CTC CAA GCT AC r: ACC CGC TCC TCA CTT AAT C	110 - 138	12	58	0.3 - 0.8	$(GT)_{14}$	(O'Reilly *et al.*, 1996)
	Ssa171	f: TTA TTA TCC AAA GGG GTC AAA A r: GAG GTC GCT GGG GTT TAC TAT	233 - 267	29	58	0.6 – 0.9	$(TGTA)_{14}(TG)_7$	(O'Reilly *et al.*, 1996)
	Ssosl311	f: TAG ATA ATG GAG GAA CTG CAT TCT r: CAT GCT TCA TAA GAA AAA GAT TGT	166 -	23	55	0.7 – 0.9	$(TG)_{38}$	(Slettan *et al.*, 1995)
	Ssosl438	f: GAC AAC ACA CAA CCA AGG CAC r: TTA TGC TAG GTC TTT ATG CAT TGT	116 - 146	7	50	0.76	$(AC)_{26}AT(AC)_6$	(Slettan *et al.*, 1996)
Set 2	Ssa14	f: CCT TTT GAC AGA TTT AGG ATT TC r: AAA CCA AAC ATA CCT AAA GCC	138 - 145	3	57	0.3 - 0.5	$(TC)_{10}N_{15}(TC)_3N_2(AC)_{12}(TC)_3N_5(CA)_4$	(McConnell *et al.*, 1995)
	Sleen82	f: CAT GGA GAA TCC CAC TTT CTT A r: CAG GGA GTG ATA TGG GAC ATA A	196 - 230	15	58	0.41 - 0.62$^{\Phi}$		(cited in King *et al.*, 2001)[¶]
	Ssa202	f: CTT GGA ATA TCT AGA ATA TGG C r: TTC ATG TGT TAA TGT TGC GTG	268 - 320	18	58	0.4 – 0.8	$(CA)_3(CTCA)_{17}$	(O'Reilly *et al.*, 1996)
	Ssa197	f: GGG TTG AGT AGG GAG GCT TG r: TGG CAG GGA TTT GAC ATA AC	131 - 203	21	58	0.4 – 0.8	$(GT)_5C(TG)_4TC(TG)_3A(GTGA)$	(O'Reilly *et al.*, 1996)
Set 3	Ssa404	f: ATG CAG TGT AAG AGG GGT AAA AAC r: CTC TGC TCT CCT CTG ACT CTC	194 - 314	20	59	0.95	$GACA_{27}$	(Cairney *et al.*, 2000)
	Ssa410	f: GGA AAA TAA TCA ATG CTG CTG GTT r: CTA CAA TCT GGA CTA TCT TCT TCA	198 - 324	25	58	0.90	$(GACA)_{22}$	(Cairney *et al.*, 2000)
	Ssa418	f: CAC ACC TCA ACC TGG ACA CT r: GAC ATC AAC AAC CTC AAG ACT G	328 - 570+	22	64	1.00	$GATA_{59}$	(Cairney *et al.*, 2000)

$^{\Delta}$ f, and r, are forward and reverse primers respectively.
$^{¥}$ of the original clone, N is any nucleotide.

$^{\Phi}$ H_E
[¶] Sleen82 (GenBank accession numbers U86703 and U86706).

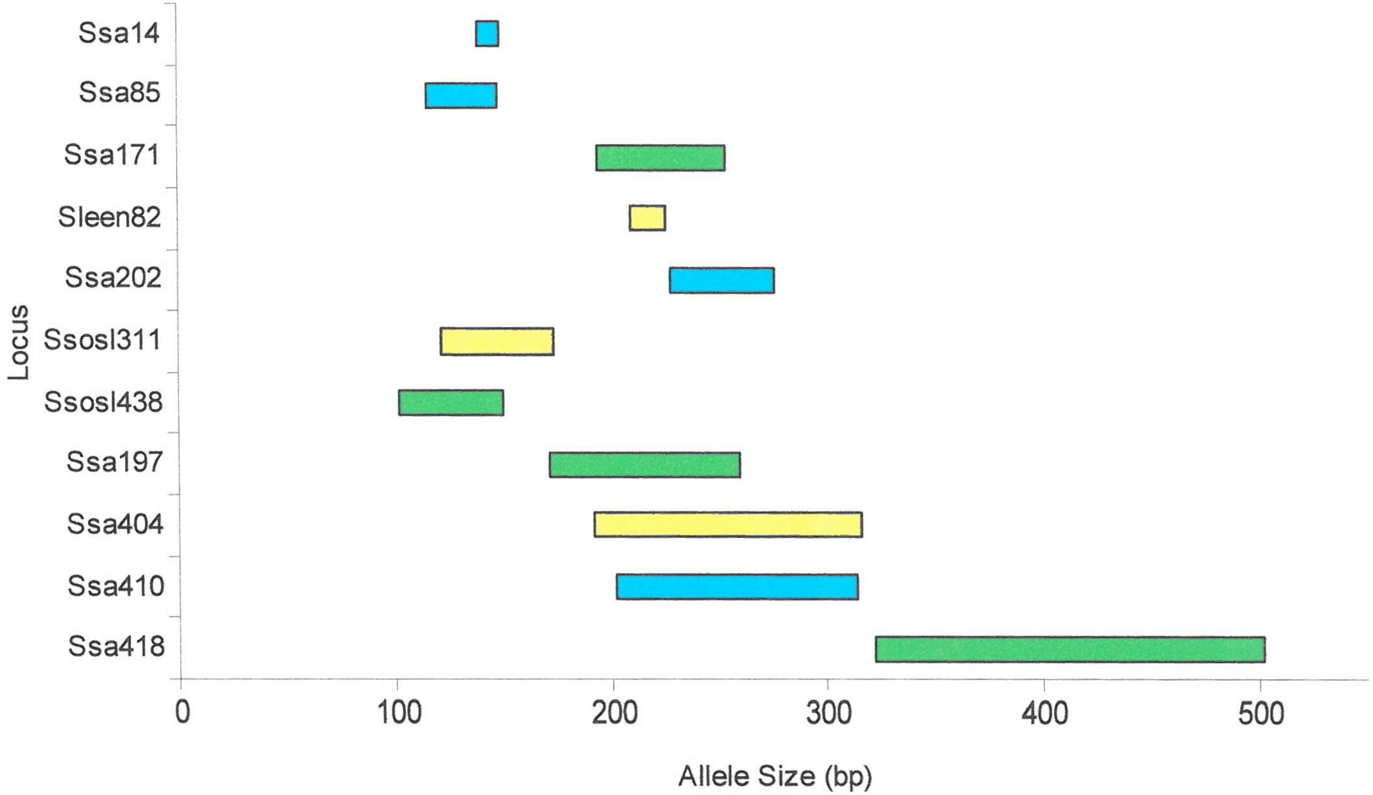

Figure 4.2 Allele size ranges observed in this study for each of 11 microsatellite loci screened in adult Atlantic salmon from the River Eden. The three colours represent the dye used to label each primer: blue is FAM; yellow is HEX; and green is TET (Applied Biosytems).

4.3 Results

Table 4.2 Summary statistics for 11 microsatellite loci genotyped in 156 returning adult Atlantic salmon from the River Eden. Total number of alleles for each locus (*A*), allelic size range; and, the number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness ($\hat{A}$) in each population.

			River Region			
Locus			Upper Eden	Lower Eden	Caldew	Eamont
Ssa14	6	A	5	5	3	5
139 - 149		H_O	0.310	0.379	0.333	0.486
		H_E	0.501	0.644	0.612	0.664
Ssa85	16	A	15	14	13	12
116 - 148		H_O	0.877	0.867	0.864	0.889
		H_E	0.900	0.898	0.870	0.867
Ssa171	15	A	12	11	11	13
194 - 254		H_O	0.814	0.963	0.800	0.806
		H_E	0.871	0.907	0.891	0.876
Sleen82	9	A	14	13	12	15
210 - 226		H_O	0.639	0.500	0.429	0.656
		H_E	0.736	0.588	0.674	0.686
Ssa202	13	A	9	8	5	7
228 - 276		H_O	0.900	0.828	0.900	0.829
		H_E	0.878	0.817	0.894	0.859
Ssosl311	25	A	20	17	13	18
122 - 174		H_O	0.825	0.885	0.905	0.806
		H_E	0.914	0.892	0.911	0.923
Ssosl438	15	A	13	8	9	11
102 - 150		H_O	0.600	0.690	0.591	0.730
		H_E	0.770	0.687	0.724	0.730
Ssa197	23	A	18	16	16	15
172 - 260		H_O	0.810	0.966	0.864	0.865
		H_E	0.877	0.906	0.931	0.893
Ssa404	26	A	24	18	14	20
192 - 316		H_O	0.904	0.846	0.900	0.912
		H_E	0.942	0.899	0.923	0.935
Ssa410	29	A	25	18	21	21
202 - 314		H_O	0.930	0.900	0.909	0.912
		H_E	0.948	0.929	0.959	0.934
Ssa418	37	A	32	21	18	25
322 - 502		H_O	0.923	0.857	0.842	0.871
		H_E	0.966	0.944	0.957	0.944
		$\hat{A}$	12.41	11.79	11.86	11.99

4.3.1 *Microsatellite polymorphism*

Genotypes at 11 microsatellite loci were calculated for 156 adult Atlantic salmon on their return to the River Eden in the years 1999, 2000, & 2002 (Table 4.2). No sample collection took place in 2001 due to the severe outbreaks of foot and mouth disease in Cumbria and elsewhere in the United Kingdom.

A total of 214 alleles were observed, varying from six in Ssa14, to 37 in Ssa418. Expected heterozygosity ranged from 0.50 at Ssa14 in the Upper Eden, to 0.97 at Ssa418, also in the Upper Eden. Observed levels of heterozygosity were high overall: 0.85 ± 0.1. The number of alleles per sample at any given locus ranged from three at Ssa14 in the Caldew, to 32 at Ssa418 in the Upper Eden. The average number of alleles, per locus, at each site, was 14.39 ± 6.17. There was no difference in allelic richness between regions (ANOVA; $r^2 = 0.28$, $F_{3,43} = 0.04$, $P = 0.99$).

Of 44 locus/site comparisons, six showed significant deviations from HWE (13.64%) after correcting for the number of tests (overall $\alpha = 0.0125$). There was a clear pattern to these deviations: four of them being at Ssa14 (also one each in Sleen82 & Ssosl311), suggesting the presence of a null allele at this locus. The frequency of a null allele was calculated as 0.14 ± 0.02). No significant linkage disequilibrium was observed between any loci.

4.3.2 *Population differentiation*

Overall F_{ST} was low: 0.01 ± 0.0037. Significant F_{ST} values were observed in all but one pairwise comparison between river regions (Table 4.3). This suggests detectable levels of spatial variance among salmon from different river regions in the Eden. In particular, the Caldew is most strongly differentiated from the main body of the Eden, both Upper and Lower, and from the Eamont. The Eamont and the neighbouring Upper Eden region show no significant differentiation in F_{ST} values.

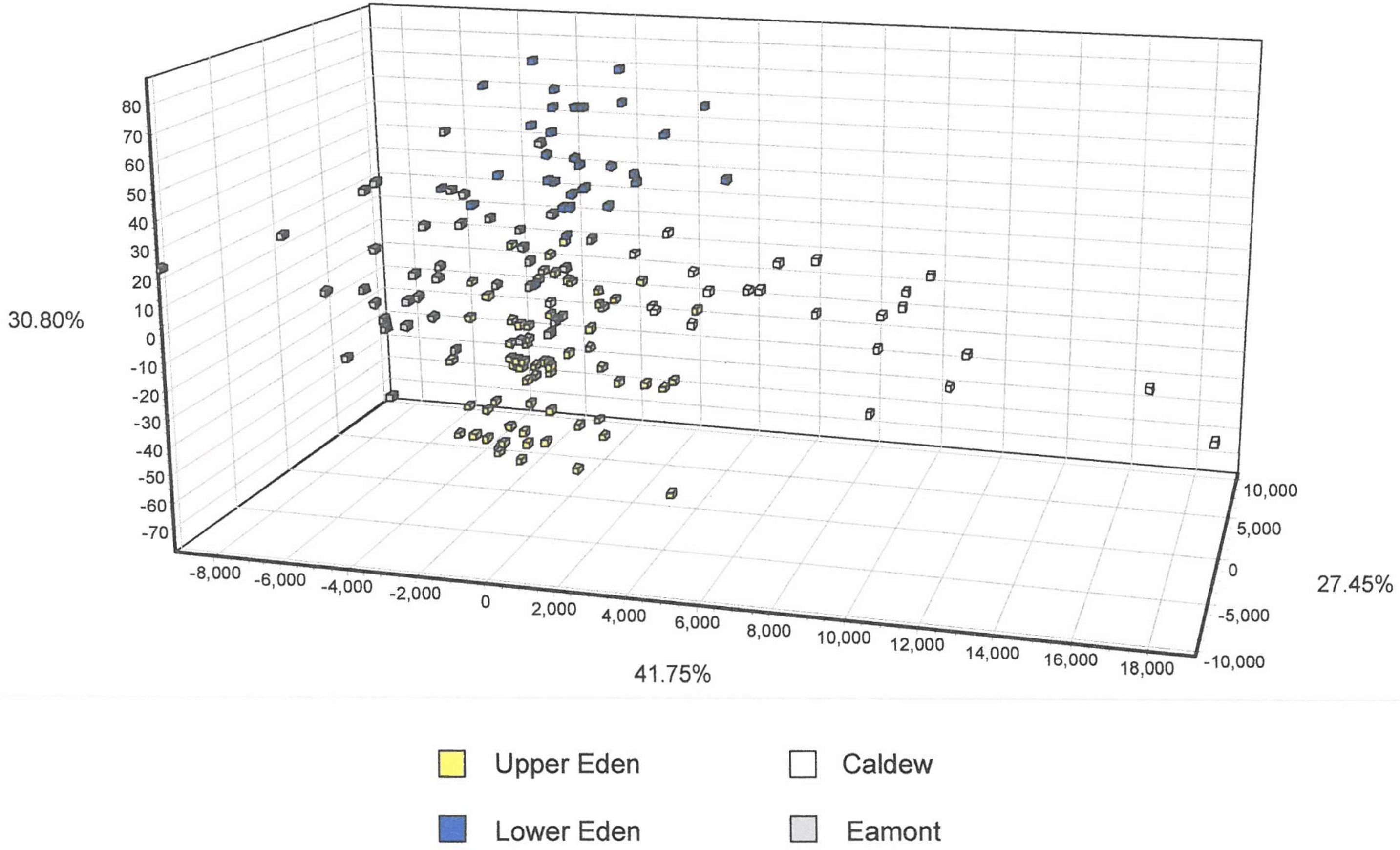

Figure 4.3 Factorial correspondence analysis showing relationships among the multilocus genotypes of individual adult Atlantic salmon from four regions of the River Eden. The three axes are the first, second and third principal factors of variability in order of the amount of variation they explain.

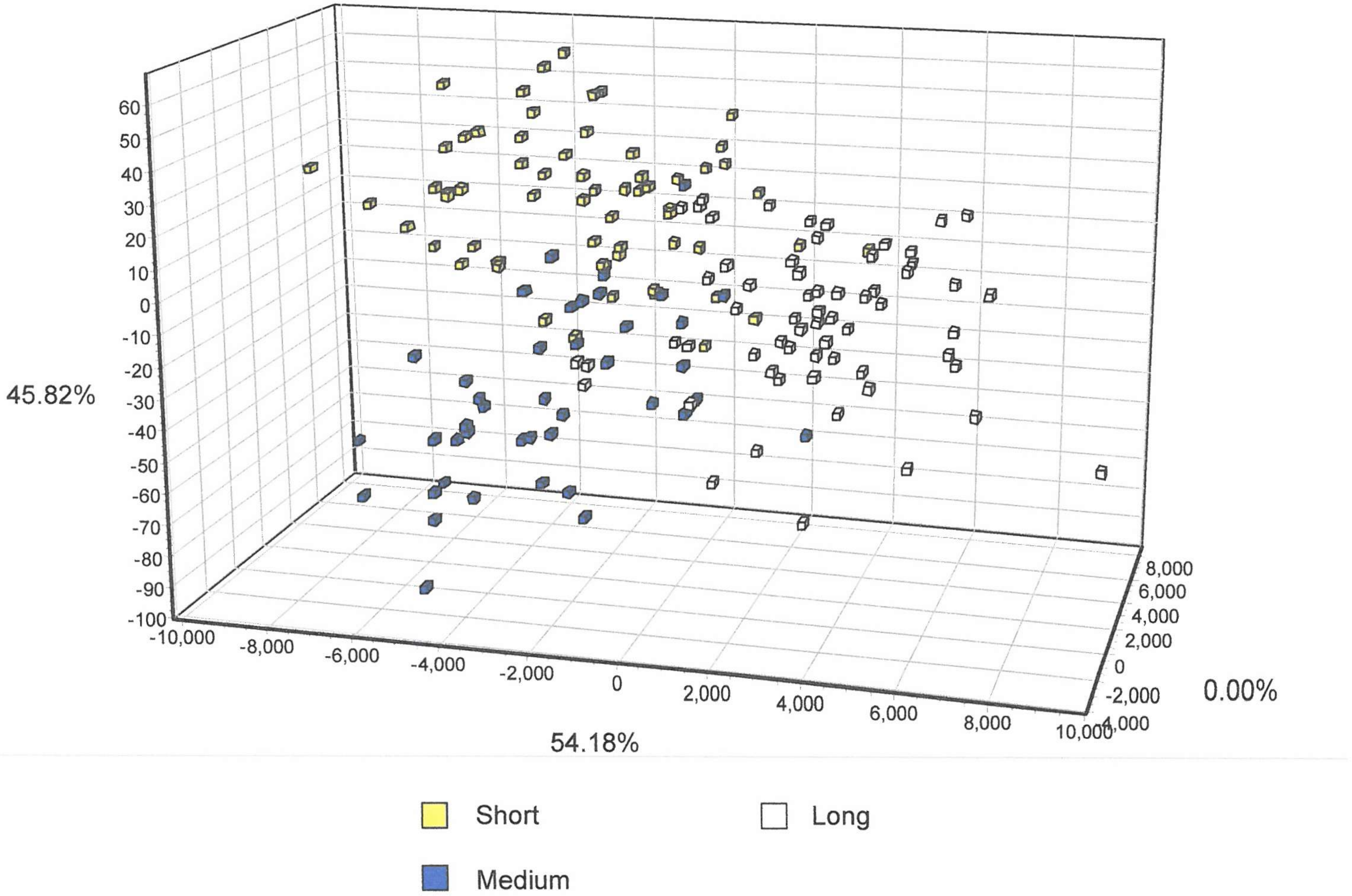

Figure 4.4 Factorial correspondence analysis showing relationships among the multilocus genotypes of individual adult Atlantic salmon from the River Eden grouped into distance categories (short: 1 – 35km; medium: 36 – 70km; and long: 71 – 107km). The three axes are the first, second and third principal factors of variability in order of the amount of variation they explain.

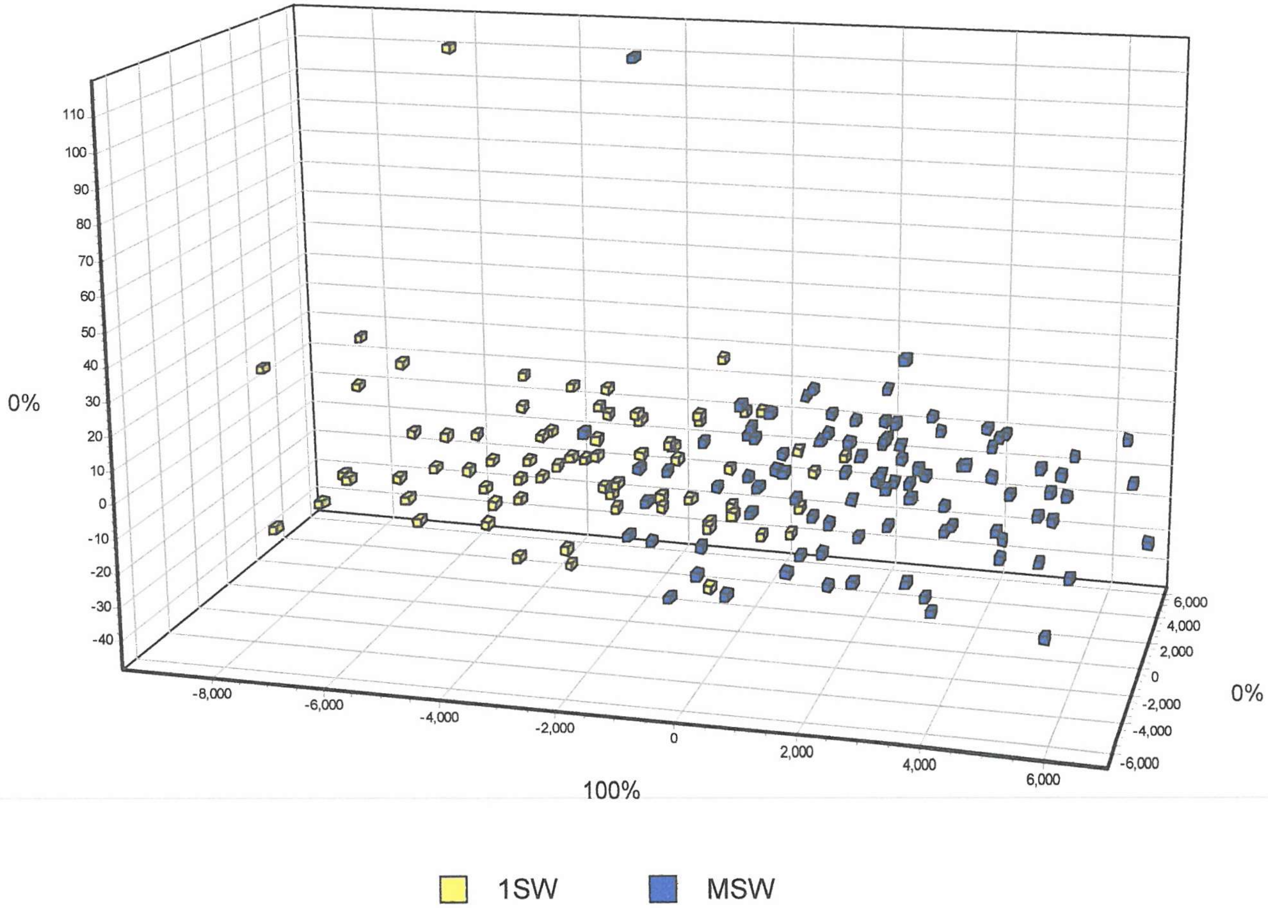

Figure 4.5 Factorial correspondence analysis showing relationships among the multilocus genotypes of individual adult Atlantic salmon from the River Eden grouped into sea-winter tactics (One sea-winter: 1SW; and multi sea-winter: MSW). The three axes are the first, second and third principal factors of variability in order of the amount of variation they explain.

Differentiation between the four rivers is graphically illustrated using plots of factorial correspondence analysis (Figure 4.3). The separation of populations is clear, yet there is still a large amount of overlap, and no populations are wholly discrete. The Caldew exhibits a greater degree of spread in data points than the other rivers, this suggests a more diverse, or more appropriately, less distinctive, population.

Table 4.3 Pairwise F_{ST} comparisons for the four River Eden regions (below diagonal). Above the diagonal are significance values obtained from 1000 permutations (values in bold are $P < 0.05$).

	Upper Eden	Lower Eden	Caldew	Eamont
Upper Eden	*	**0.001**	**0.005**	0.1556
Lower Eden	0.012	*	**0.027**	**0.0010**
Caldew	0.013	0.014	*	**0.0000**
Eamont	0.002	0.006	0.015	*

4.3.2.1 Assignment

4.3.2.1.1 To river regions

A total of 88 male (97.78%) and 64 female (96.97%) adult salmon were assigned within 95% confidence limits to at least one of the Eden river regions (Table 4.4). The proportion of fish correctly assigned was very high: 86 males (95.56%) and 62 (93.94%) females were correctly assigned to their river of sampling. Only two males (2.22%) and two females (3.03%) could not be assigned to either of the four river regions; these fish may be genuine straying individuals from other rivers. There was no significant difference in assignment accuracy between males and females ($T = 0.72$, $P = 0.26$).

4.3.2.1.2 To distance categories

A total of 88 male (94.56%) and 64 female (92.77%) adult salmon were assigned within 95% confidence limits to at least one of the upstream distance categories (Table 4.5). The proportion of fish correctly assigned was very high: 82 males (87.23%) and 60 (86.96%) females were correctly assigned to their river of sampling. Only six males

(6.38%) and five females (7.25%) could not be assigned to either of the four river regions. There was no significant difference in assignment accuracy between males and females (T = 0.17, *P* = 0.44).

Table 4.4 Numbers of returning adult salmon that were classified back to their river of sampling, to another river, or not assigned to any river, in the Eden catchment, for males and females separately. Figures in bold are for fish correctly assigned to their sampling river.

		River region				
Males		Upper Eden	Lower Eden	Caldew	Eamont	unassigned
n = 39	Upper Eden	**37**	0	0	1	1
21	Lower Eden	0	**20**	0	1	0
17	Caldew	0	0	**17**	0	0
13	Eamont	0	0	0	**12**	1
	Correctly assigned	86				
	Mean	95.56				
Females		Upper Eden	Lower Eden	Caldew	Eamont	unassigned
n = 27	Upper Eden	**25**	0	0	1	1
10	Lower Eden	0	**9**	0	1	0
9	Caldew	0	0	**9**	0	0
20	Eamont	0	0	0	**19**	1
	Correctly assigned	62				
	Mean	93.94				

Interestingly, males and females exhibit differing patterns in their assignment accuracy. Males are most accurately assigned to the medium distance category, and less likely to be assigned correctly to either short, or medium categories. The situation is reversed in females, where they are least likely to be correctly assigned to the medium distance category (Figure 4.6).

Table 4.5 Numbers of returning adult salmon that were classified back to their upstream distance category of sampling, to another distance category, or not assigned to any distance category in the Eden catchment, for males and females separately. Figures in bold are for fish correctly assigned to their sampling distance category.

Males		Short	Medium	Long	unassigned
$n = 39$	Short	**35**	2	0	3
21	Medium	1	**19**	0	0
17	Long	2	1	**28**	3
Correctly assigned		82			
Mean		87.23			

Females		Short	Medium	Long	unassigned
$n = 27$	Short	**20**	0	1	0
10	Medium	2	**16**	0	3
9	Long	1	0	**24**	2
Correctly assigned		60			
Mean		86.96			

4.3.2.2 Distance upstream

To assess how the upstream position of populations might contribute to population structure, individuals were grouped into distance classes (short, medium, and long). A graphical representation of these distance classes (Figure 4.4) highlights that upstream distance, in effect, migratory distance, does contribute to population structure. Though individuals from the short, medium, and long categories do overlap, they form clear clusters of individuals.

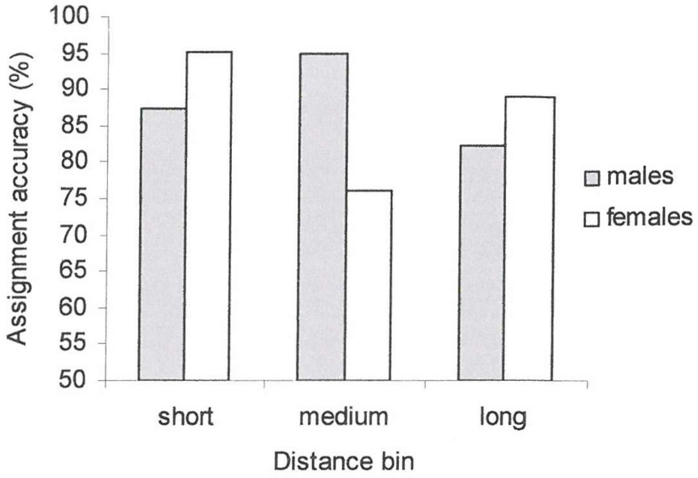

Figure 4.6 Difference in the assignment accuracy of males and female Atlantic salmon to distance categories in the Eden catchment.

Table 4.6 Hierarchical analysis of molecular variance (AMOVA) of microsatellite allele frequencies from four river regions (Lower Eden, Upper Eden, Caldew, and Eamont). Within each region, individuals are binned to three distance classes: short, medium, or long. Values in bold are $P < 0.05$

Source of variation	d.f.	Variance component	% variation	*P*
Among river regions	3	0.024	0.60	**<0.000**
Among distance bins within river regions	8	0.027	0.67	0.102
Within distance bins	300	3.925	98.72	**0.009**
Total	311	3.976		

An AMOVA compared the contributions of river regional structure to that of upstream distance to the overall genetic structure (Table 4.6). Both account for similar amounts of the total variability (0.60%, and 0.67% respectively), however, only the variation between river regions is significant.

4.3.2.3 Between tactics

Ignoring geographical population structure (either river region, or distance class) and grouping individuals by sea-winter tactic highlights the role that life history plays in shaping genetic diversity. A plot of factorial correspondence analysis vectors (Figure 4.5) shows a good deal of variability among genotypes to be explained by sea-winter grouping. 100% of this variation is explained on a single axis suggesting a strong polarisation of this variable. Though the two sea-winters overlap by at least 50%, they remain clear groups, suggesting that to at least some extent, gene flow may be restricted by life-history.

4.3.3 Differences between the sexes

Comparing parameters between the sexes (Figure 4.7) revealed a number of interesting differences between the population structure of males and females. Females appear more diverse than males, having both a higher expected heterozygosity than males (ANOVA; $F_{1,11} = 10.25$, $P = 0.009$) and a higher level of allelic richness (ANOVA; $F_{1,11} = 15.22$, $P = 0.003$). However, though males were more related than females (ANOVA; $F_{1,11} = 10.21$, $P = 0.01$), neither males nor females were significantly more inbred than the other - no difference in F_{IS} between the sexes (ANOVA; $F_{1,11} = 1.16$, $P = 0.306$). Calculating F_{ST} for the sexes separately (over river region sub-populations), indicates that while males have a higher F_{ST} value, their vastly overlapping confidence intervals make this difference non-significant.

4.3.4 Effective population size

The mean N_e based on two separate generation comparisons (1994 – 1998, and 1995 – 1999) over five different methods of estimation is 69.62 ± 18.40 (Table 4.7). However, these results also illustrate the high degree of variability in this estimation with N_e ranging from 4.6 to 172.2 depending on the method of calculation and the years used for comparison.

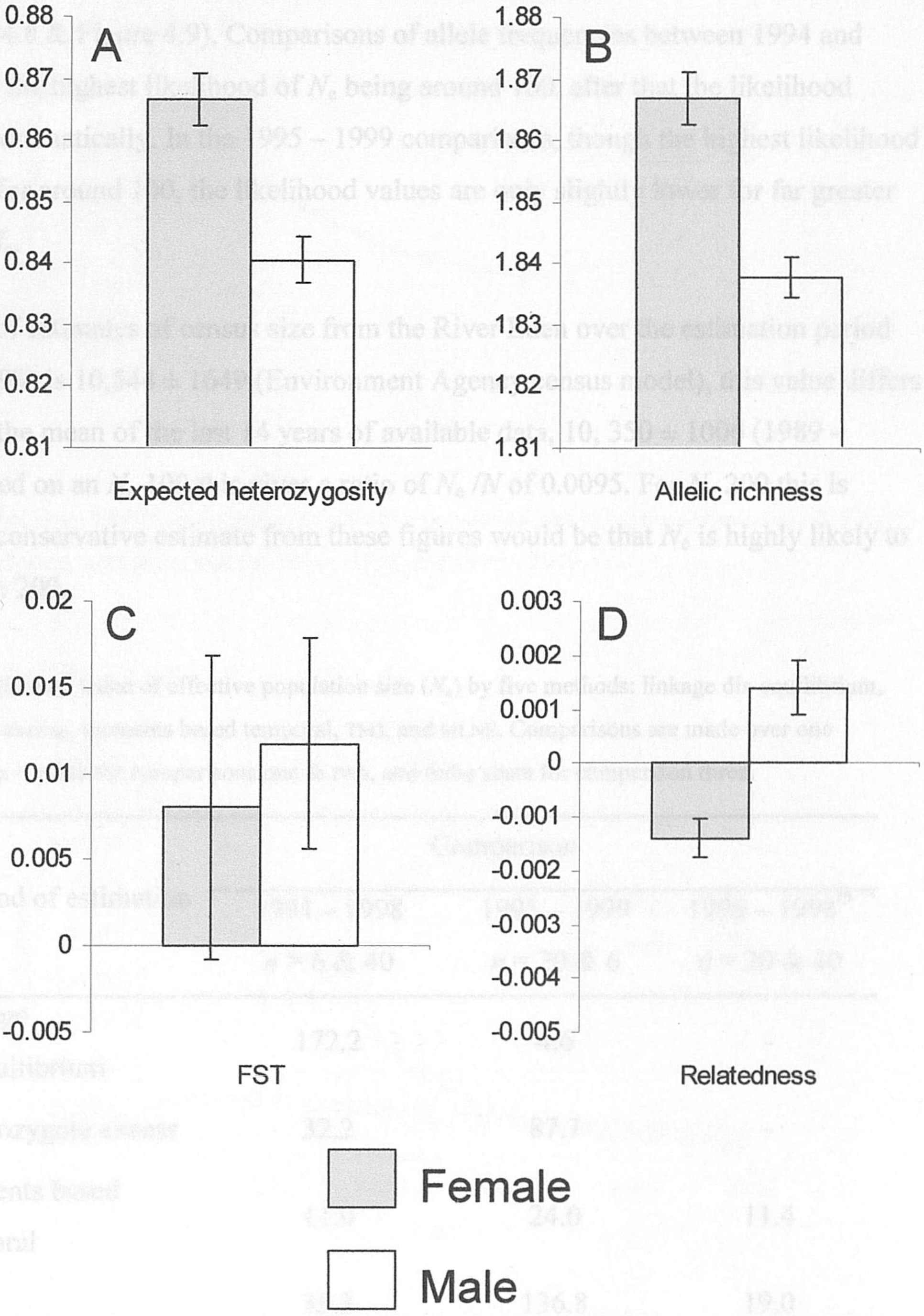

Figure 4.7 Comparisons between the sexes: A, Mean expected heterozygosity over all regions and loci with standard error bars; B, Mean allelic richness ($\hat{A}$) with standard error bars; C, F_{ST} with 95% confidence intervals estimated from 1000 bootstraps; D, Mean relatedness with standard error bars.

By plotting the output of likelihood values from the software MLNE and TM3 it is clear that the two year group comparisons give differing accounts of the likelihood value of N_e (Figure 4.8 & Figure 4.9). Comparisons of allele frequencies between 1994 and 1998 show the highest likelihood of N_e being around 100, after that the likelihood values fall dramatically. In the 1995 – 1999 comparisons, though the highest likelihood values are for around 100, the likelihood values are only slightly lower for far greater values of N_e.

The mean of estimates of census size from the River Eden over the estimation period (1994 – 1999) is 10,544 ± 1649 (Environment Agency census model), this value differs little from the mean of the last 14 years of available data, 10, 350 ± 1006 (1989 – 2003). Based on an N_e 100 this gives a ratio of N_e /N of 0.0095. For N_e 200 this is 0.0190. A conservative estimate from these figures would be that N_e is highly likely to be less than 200.

Table 4.7 Estimated value of effective population size (N_e) by five methods: linkage dis-equilibrium, heterozygote excess, moments based temporal, TM3, and MLNE. Comparisons are made over one generation i.e. 4 years for comparisons one & two, and three years for comparison three.

Method of estimation	Comparison		
	1994 – 1998 n = 6 & 40	1995 – 1999 n = 20 & 6	1995 – 1998$^{\Phi}$ n = 20 & 40
Linkage disequilibrium	172.2	4.6	-
Heterozygote excess	32.2	87.7	-
Moments based temporal	11.0	24.0	11.4
TM3	35.3	136.8	19.0
MLNE	70.1	122.3	36.4

$^{\Phi}$ Results for the linkage disequilibrium and heterozygote excess methods are the same as for 1994 – 1998 since these are point estimations based on a single years allele frequencies (1998: the larger sample size).

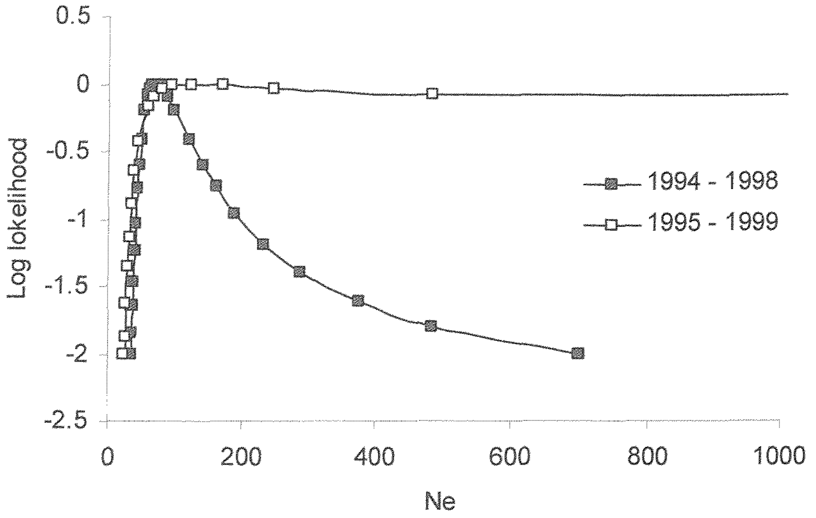

Figure 4.8 Maximum likelihood estimates of effective population size using the software MLNE. Single generation estimates were generated for 1994 – 1998, and 1995 – 1999.

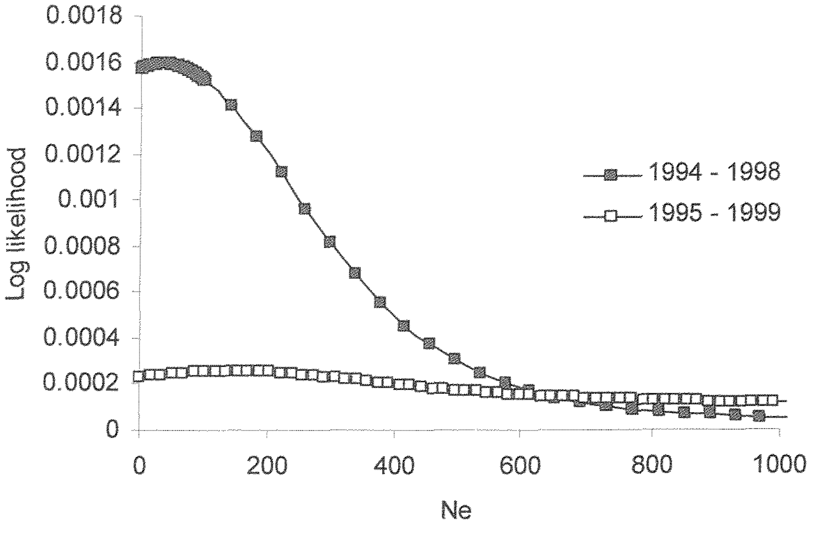

Figure 4.9 Maximum likelihood estimates of effective population size using the software TM3. Single generation estimates were generated for 1994 – 1998, and 1995 – 1999.

4.4 Discussion

4.4.1 Regional population structure in the River Eden

Pairwise F_{ST} comparisons and plots of FCA axes indicate that gene-flow is to some extent restricted between adult Atlantic salmon spawning in different geographic regions of the Eden catchment. However, catchment-wide, the strength of this differentiation, although significant, is low (F_{ST} 0.01). In their comprehensive review of salmonid biology, Hendry & Stearns (2004) list F_{ST} (some studies quote θ_{ST}, or G_{ST}) values from 32 studies on the population genetics of Atlantic salmon. Summarising their tabulated data indicates that the mean F_{ST} of studies to date is 0.06 ± 0.009. The lowest published F_{ST} is 0.001, and the highest 0.221. Taking these results as a benchmark, an F_{ST} 0.01 is lower than the average, but an order of magnitude higher than the lowest published value. Undoubtedly, there are numerous differences in the way these studies were conducted, and on the life-stages and scales over which populations were defined. This comparison does however, bring a sense of scale to the differentiation observed in this analysis.

4.4.1.1 The role of migratory distance in population structure

Plotting FCA axes with genotypes delimited by distance class (Figure 4.4), makes clear that the population is structured by the distance individuals migrate upstream. Incorporating migratory distance into the analysis in this way is crude, since the data is based on individuals and not on populations. There are no geographical defined populations within the river regions, so individuals are binned into three equal distance categories. Combining distance bins with river region geography in an AMOVA suggested that both levels of structure contribute equally to the population-wide genetic variation, though the variation attributable to distance was non-significant. This result means there is no upstream distance based structure to the population, a situation difficult to reconcile with the 3-D plot of FCA axes. Figure 4.4 shows clusters which, though broad and overlapping in distribution, demonstrate differentiation between three groups of individuals; those with shorter, medium, or longer upstream migrations.

This inconsistency could be a consequence of small sample size. When comparing four river regions, sample sizes are 66, 31, 22, & 37; these are small, but workable. A typical number of samples desirable for representing a population is ~50. When comparing distance classes alone (as in the FCA; Figure 4.4), sample sizes are 59, 39, & 61. However, when comparing distance classes *within* regions (as in the hierarchical structure of an AMOVA), sample sizes start to become unacceptably small in some cases (Table 4.8). Where sample sizes are unacceptably small, it is possible that signal may be lost in the noise (Waples, 1998).

Table 4.8 Sample sizes for adult AMOVA.

Upper Eden			Lower Eden			Caldew			Eamont		
Short	Med	Long	Short	Med	Long	Short	Med	Long	Short	Med	Long
39	16	11	3	5	23	8	11	3	6	7	24

4.4.1.2 The role of life-history in population structure

These results suggest that sea-winter tactics, a central aspect of salmon life-history (see Chapters Two & Three), play a role in shaping the population genetic structure observed in the returning adults. FCA indicates a strong division of individuals by sea age (Figure 4.5). Due to limitations on sample size, it was not possible to assess the contribution of sea-winter tactics relative to geographical population structure. To the best of my knowledge, no studies have explicitly addressed the issue of gene flow between 1SW and MSW life histories. Differing sea-winter tactics are often associated with run-timing e.g. Spring run salmon. Reproductive isolation has been found between such temporal stocks of Sockeye salmon, *Oncorhynchus nerka* (Fillarte *et al.*, 2003), though there was no consistent pattern between life-history traits.

4.4.2 Assignment of individuals

4.4.2.1 To river regions

A high degree of accuracy was obtained in assigning individual fish to their river of sampling. This supports the evidence for differentiation (F_{ST} and FCA) between regions of the Eden. Furthermore, the samples which were mis-assigned are informative with regards to population structure. In both males and females, one fish from the Upper Eden, and one from the Lower Eden were mis-assigned to the Eamont within 95%

confidence limits. Individuals may be mis-assigned to populations when the likelihood functions of the two populations overlap, a situation occurring when differentiation is low (Waser & Strobeck, 1998). Assignment error rates have been found to correlate negatively with F_{ST} (Bjornstad & Roed, 2002). Referring back to the pairwise F_{ST} results (Table 4.3), there is no significant differentiation between the Upper Eden and the Eamont, while the Lower Eden and the Eamont are differentiated by the lowest significant value of F_{ST} observed. These results suggest that the Caldew region alone is uniquely differentiated from the rest of the Eden (see Chapter Five for a more thorough investigation of the River Caldew and how it relates to the rest of the Eden).

4.4.2.2 To distance categories

Adult salmon were assigned with a high degree of accuracy to distance categories reflecting how far an individual fish had migrated upstream in the Eden. This further highlights that population differentiation is not only between river regions; it is within them, and that this within-river component of variation is acting along a migratory gradient.

4.4.2.3 Sex-biased dispersal in Atlantic salmon –genetic evidence

Whilst males and females did not differ overall in the numbers of individuals assigned to distance categories, there was a clear difference in the pattern of assignment accuracy (Figure 4.6). Whilst male and female assignment values are similar for both short and long categories, female salmon are more accurately assigned in both cases. Over medium distances there is a marked difference in assignment, females being assigned with far less accuracy than males. These results reflect the situation identified from radio-tracking, female populations are split to either short or long migrations (depending on sea-winter tactic), whereas males are no different (Figure 3.4). The assignment accuracies suggest that these short and long female populations are more clearly defined than the medium distance grouping, and therefore, more stable over generations. Female fish in the medium category are more likely to be strays from short or long populations than fish in either short or long populations are likely to be strays from the medium distance. In males, the medium distance populations are most distinct and reliably assigned to, possibly due to the non-differentiation in spawning migrations between differing sea-winter tactics. Therefore, a greater proportion of males in short and long populations are likely to be strays from the medium distance category than vice versa.

These results, combined with the radio-tracking data in the previous chapter, provide the first evidence of sex-biased dispersal in Atlantic salmon. This is not a sex-bias in the simple sense of one sex dispersing more than the other. More, it is a bias in the way the sexes *migrate*, with dispersal over a migratory gradient occurring differently in males and females. It suggests that females are more likely to home to their population of origin if it is a short or a long distance upstream. Males are more likely to home back to their population of origin if it is a medium distance upstream. It might be better described as gender dependent homing. Possible reasons for this pattern of migration are likely to reflect differing outcomes of sea-winter tactics (see 3.4).

Previous authors have found significant sex-biased dispersal in fish species (Bekkevold *et al.*, 2004; Fraser *et al.*, 2004; Hutchings & Gerber, 2002; Pardini *et al.*, 2001), and some evidence for it in *Pseudotropheus (Maylandia)* complex, (Knight *et al.*, 1999) and Trinidadian guppies *Poecilia reticulata* schooling together (Griffiths & Magurran, 1998). However, sex-biased dispersal is not universal in fishes; it has not been found in a number of other cases: Pink salmon, *Oncorhynchus gorbuscha* (Gilliam & Fraser, 2001), Killifish, *Rivulus hartii* (Thedinga *et al.*, 2000), and Galjoen, *Coracinus capensis* (Attwood & Bennett, 1994).

4.4.2.4 Unassigned fish

Fish which were not assigned to any of the sampling rivers may be strays. These could potentially be from a local source – the River Irthing; part of the Eden catchment not included in this analysis. Alternatively, they could be from the Rivers Esk, Nith, or Annan (all of which are major rivers sharing the Solway Firth), or anywhere else.

4.4.3 Effective population size

From two different cohort comparisons, and using five different methods, I have found that effective population size in the River Eden is approximately 100, with all methods producing estimates of $N_e < 200$. Even discounting the variability in these estimates, and the violations of their various assumptions, this figure is well below the minimum value of 500 suggested for salmonids by Waples (1990), and for other species by Franklin (1980).

Previous authors have estimated low figures for N_e, both in populations known to be threatened, and in some which are not. Maximum likelihood estimates of N_e in Steelhead trout (*Oncorhynchus mykiss*) from Washington State ranged from 228 – 344, overall N_e/N was 0.73 (Ardren & Kapuscinski, 2003). In Steelhead trout from British Columbia (not currently thought to be threatened), N_e was estimated by the temporal method as 92 – 560 (Heath *et al.*, 2002). Using an estimate derived from the effective number of breeders, Simon (1986) estimated N_e of a Coho salmon (*Oncorhynchus kisutch*), population at 64 – 282. In endangered Chinnok salmon (*Oncorhynchus tshawytscha*) populations from the Sacramento River, N_e was estimated from variance in male and female spawners as 21.9 – 401, (Hedrick *et al.*, 1995). From the same populations N_e was estimated as 88.5 by the temporal method (Bartley *et al.*, 1992). In Brown trout (*Salmo trutta*) populations in Swedish lakes N_e was estimated as 52 – 480 using the temporal method (Jorde & Ryman, 1996). Sampling from one of those lakes, N_e was estimated at 58 for females (mtDNA Laikre *et al.*, 1998). These examples differ in their methods; however, my estimate of N_e is certainly within the range of estimates from other salmonids.

4.4.3.1 N_e/N

In addition to raw estimates of N_e, are ratios of the effective population size to census size (Kalinowski & Waples, 2002). Theoretical studies have determined that N_e/N should be 0.5 in most populations and only in extreme cases <0.25 (Nunney, 1991; Nunney, 1996; Nunney & Campbell, 1993). However, populations examined in nature often have far lower values, in marine species such as the New Zealand snapper (*Pagrus auratus*) these have been observed as low as 0.17×10^{-5} (Hauser *et al.*, 2002). Frankham (1995) found an average N_e/N of 0.1 across a range of species. The value for N_e/N that I estimated is very low for an of N_e 100 (0.0095) and this is based on a conservative estimate of census size (Gowans, A., Personal Communication). However, it is still two orders of magnitude higher than that reported for the New Zealand snapper.

4.4.3.2 *Why is N_e lower than census size?*

Over evolutionary timescales, N_e may be limited by historic event such as bottlenecks, selective sweeps, and colonisation events (Avise, 2000), these all produce fluctuating population size. This fluctuation is reasoned to be the main cause of difference between N_e / N (Vucitech *et al.*, 1997), since previous models assumed a constant population size. Over shorter timescales in salmonids, N_e is likely to be strongly biased by the violation of three assumptions of an ideal population: 1) unequal sex ratio (see Chapter Two); 2) overlapping generations (see e.g. Felsenstein, 1971; Storz *et al.*, 2001); and 3) non-random mating, all of which will act to lower the estimated value of N_e (Frankham *et al.*, 2002).

4.4.3.3 *The consequences of low N_e*

The '50/500' rule has emerged as a general guideline in conservation genetics (Franklin, 1980; Nelson & Soulé, 1987; Soulé, 1980). Over the short-term populations with an N_e of less than 50 will be unable to withstand the deleterious effects of inbreeding (Shields, 1993). Over the longer-term populations with less than 500 will not be able to balance the variation resulting from mutation with it's loss through genetic drift.

4.4.3.4 *Sex-biased genetic diversity and relatedness?*

There seems clear evidence of a sex-bias in heterozygosity, allelic richness, and relatedness; males are overall less diverse, and more related than females. This is somewhat unusual, and to the best of my knowledge a situation not documented in the literature for any other species. The only reasonable, though inexplicable, explanation seems to be sex-linked selection. Though I fail to see how this could be detected across numerous unlinked microsatellites.

It would be interesting to compare this data with male and female smolts to assess whether there was a difference in their diversity and relatedness before going to sea.

4.4.4 *Conclusions*

Atlantic salmon exhibit a high level of population structure on their return to the Eden catchment. This population structure occurs as a function of geography; river regions, and distance categories within them, act to partition genetic variation. Furthermore, salmon life-history play a role in structuring populations, with some evidence of reduced gene-flow between sea winter tactics. Lastly, there is variation between the sexes – sex-biased dispersal. Males and females display distinctly different patterns in their upstream migrations.

5 Genetic differentiation in juvenile Atlantic salmon populations

5.1 Introduction

Evolutionary and ecological factors such as genetic drift, mutation, dispersal, competition, and landscape heterogeneity, shape the spatial distribution of genetic variation among populations. Where individuals of a species return over several generations to breed in areas that they themselves were juveniles – *philopatry*, there is a tendency for strong differentiation to occur between populations, e.g. American shad, *Alosa sapidissima* (Waters *et al.*, 2000), and Atlantic salmon (Garant *et al.*, 2000). Determining the scale at which this differentiation occurs is a key goal for molecular ecologists wishing to understand factors contributing to population structure, and to provide information for conservation and management where species are threatened or endangered.

5.1.1 Population differentiation in Atlantic salmon

The presence of locally adapted populations has given rise to the concept of a salmon stock: a group of salmon that spawns in a particular region of a river, at a particular time of year, and does not interbreed with other such groups (Ihssen *et al.*, 1981; Ricker, 1972). It follows that the existence of such stocks can be established using molecular data. Where stocks are reproductively isolated, random genetic drift will lead to quantifiable differentiation at neutral marker loci.

There is now compelling evidence of a range-wide decline in salmon populations, both in terms of total catch (e.g. ICES, 2000; WWF, 2001) and, in some rivers (e.g. River Eden, UK), a dramatic decline in the proportion of fish spending more than one year at sea (Gough *et al.*, 1992; Small, 1995). This decline has intensified the effort to characterise salmon stocks and identify appropriate units for conservation management (Moritz, 1994) by better understanding the genetic structure of populations.

5.1.1.1 Fine-scale population structure in Atlantic salmon

Recent studies using molecular markers such as allozymes, mtDNA and microsatellites have made clear that a good deal of genetic differentiation exists between salmon populations in different continents (Bermingham *et al.*, 1991), countries (King *et al.*, 2001) and within catchment areas (McElligott & Cross, 1991; Ståhl, 1987). However, the amount of genetic differentiation that exists between and within tributaries of a single river catchment is less certain. Quantifying the extent of genetic variation over such small spatial scales is also useful in examining the accuracy of natal homing, and therefore, establishing the key role of adult migration in shaping Atlantic salmon population structure.

5.1.2 Objectives

The objectives of this chapter were to investigate the population genetic structure of juvenile Atlantic salmon within a single river system. In particular, microsatellite diversity was analysed to compare the component of genetic variation between tributaries, to that within tributaries. Spatial structure was analysed to assess the relationships between river regions; are all of the river regions equal? The extent to which individuals in these regions were offspring of straying parents was investigated using individual-based assignment methods.

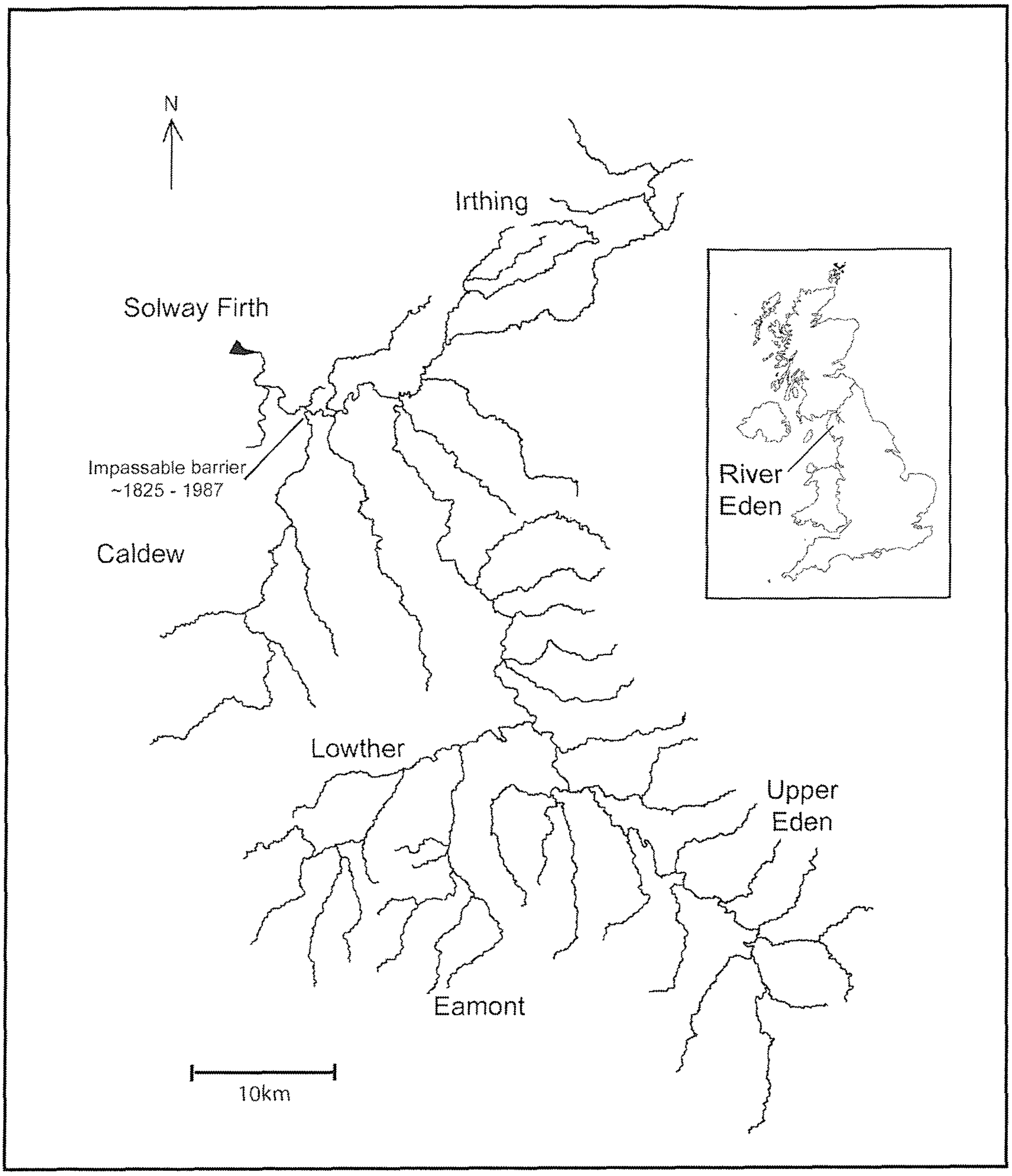

Figure 1.2 The River Eden catchment area.

Atlantic salmon fry were sampled from 34 sites from four river regions in the Eden catchment. Three of these river regions are tributaries (Upper Eden, Eamont and Caldew), while the fourth is an area in the Lower Eden before the Eamont/Eden confluence (Figure 5.1).

5.2.2 Sampling strategy

Atlantic salmon fry were non-lethally sampled by electro-fishing from sites in the Eden catchment during 2000. Sampling fry is preferable to older developmental stages that may have dispersed too far to represent the underlying population structure i.e. the genetic structure of the breeding population. One problem associated with the sampling of fry is the increased chance of over-estimating population differentiation due to sampling large numbers of related individuals (Allendorf & Phelps, 1981; Waples, 1998). Two steps were taken to avoid this: 1) sampling was carried out over a relatively large area at each site (up to 300m^2); and 2) fry (0+) were sampled late in the year (September/October) by which time individuals have dispersed and mixed within their natal areas. Sites were defined as areas of habitat suitable for juvenile salmon, typically a single riffle section. After capture, adipose fins were removed from fry and placed in 95% ethanol.

5.2.3 PCR and gel electrophoresis

DNA extraction, and amplification of microsatellite loci by PCR, was carried out as described previously (4.2.2). However, some of the primers used differed from those in Chapter Four; all fish being screened for 10 microsatellite loci amplified in two sets (Table 5.1). Sizing of microsatellite fragments by electrophoresis and allele scoring were carried out as previously described.

5.2.4 Data analysis

A number of additional analyses were carried out to those statistical treatments described in 4.2.5. To test for potential sampling of kin, relatedness values (R) were calculated for the overall population, and for each individual sample sites using RELATEDNESS 5.0 (Queller & Goodnight, 1989). An AMOVA (Excoffier *et al.*, 1992), in ARLEQUIN 2.001, allowed the quantification and testing of genetic differentiation at three hierarchical levels: among river regions, among sample sites within river regions, and within sample sites.

Differences between sites were summarised by Cavalli-Sforza & Edwards chord distance (DCE, 1967); a genetic distance measure that has a higher probability of resolving relationships among closely related populations regardless of the assumed mutational model (Angers & Bernatchez, 1998; Takezaki & Nei, 1996). D_{CE} makes no assumptions about mutation rates and assumes genetic drift to be the principal cause of population differentiation.

Pairwise matrices of D_{CE} values were estimated using the GENDIST subroutine of the PHYLIP suite (Felsenstein, 1993) from 5000 bootstrap replicates created in SEQBOOT. From these distance matrices, trees were constructed using the Neighbour-Joining (NJ) algorithm, NEIGHBOUR. The PHYLIP subroutine, CONSENSE, was used to generate an NJ consensus tree with bootstrap values from the replicated allele frequencies. The resulting tree was visualised in TREEVIEW (Page, 1996).

Table 5.1 Locus name, primer sequence, allele size range from previous studies, number of alleles observed in previous studies, optimal annealing temperature, observed heterozygosity, repeat motif, and the original author of microsatellite loci screened in juvenile Atlantic salmon from the River Eden.

	Locus	Primer sequence (5' – 3')[Δ]	Allele size bp	*N*a	T_a °C	H_O	Repeat motif[¥]	Authors
Set 1	Ssa85	AGG TGG GTC CTC CAA GCT AC ACC CGC TCC TCA CTT AAT C	110 - 138	12	58	0.3 - 0.8	$(GT)_{14}$	O'Reilly *et al.*, 1996)
	Ssa171	TTA TTA TCC AAA GGG GTC AAA A GAG GTC GCT GGG GTT TAC TAT	233 - 267	29	58	0.6 – 0.9	$(TGTA)_{14}(TG)_7$	O'Reilly *et al.*, 1996)
	Ssosl311	TAG ATA ATG GAG GAA CTG CAT TCT CAT GCT TCA TAA GAA AAA GAT TGT	166 -	23	55	0.7 – 0.9	$(TG)_{38}$	Slettan *et al.*, 1995)
	Ssosl438	GAC AAC ACA CAA CCA AGG CAC TTA TGC TAG GTC TTT ATG CAT TGT	116 - 146	7	50	0.76	$(AC)_{26}AT(AC)_6$	Slettan *et al.*, 1996)
	Ssosl85	TGT GGA TTT TTG TAT TAT GTT A ATA CAT TTC CTC CTC ATT CAG T	177 - 204	14	55	0.5 – 0.8	$(GT)_{22}$	Slettan *et al.*, 1995)
Set 2	Ssa14	CCT TTT GAC AGA TTT AGG ATT TC AAA CCA AAC ATA CCT AAA GCC	138 - 145	3	57	0.3 - 0.5	$(TC)_{10}N_{15}(TC)_3N_2(AC)_{12}(TC)_3N_5(CA)_4$	McConnell *et al.*, 1995)
	Sleen82	CAT GGA GAA TCC CAC TTT CTT A CAG GGA GTG ATA TGG GAC ATA A	196 - 230	15	58	0.41 - 0.62[Φ]		cited in King *et al.*, 2001)[¶]
	Ssa202	CTT GGA ATA TCT AGA ATA TGG C TTC ATG TGT TAA TGT TGC GTG	268 - 320	18	58	0.4 – 0.8	$(CA)_3(CTCA)_{17}$	O'Reilly *et al.*, 1996)
	Ssa197	GGG TTG AGT AGG GAG GCT TG TGG CAG GGA TTT GAC ATA AC	131 - 203	21	58	0.4 – 0.8	$(GT)_5C(TG)_4TC(TG)_3A(GTGA)$	O'Reilly *et al.*, 1996)
	Ssa289	CTT TAC AAA TAG ACA GAC T TCA TAC AGT CAC TAT CAT C	110 - 119	6	46	0.3 – 0.9	$(GT)_{12}$	McConnell *et al.*, 1995)

[Δ] f, and r, are forward and reverse primers respectively.

[¥] of the original clone, N is any nucleotide.

[Φ] H_E

[¶] Sleen82 (GenBank accession numbers U86703 and U86706).

5.3 Results

Table 5.2 Summary information for 34 sample sites at 10 microsatellite loci. Number of alleles, observed and expected heterozygosity.

		HRB	RRB	MIL	EAB	EWC	EBS	EGM	EKS	ETB	EBF	CBB	CCB	CCF	CHB	CLB	CLS	CMB	CSB	CSW	YHS	NRM	LGB	LBG	GRG	RIF	LWH	LKE	DAM	STH	SUB	LRG	LSH	NGD	LSA
Ssa85	A	5	14	5	12	12	10	12	10	10	10	11	12	8	8	8	10	9	8	7	8	13	13	10	12	11	8	10	9	9	11	11	12	15	9
	H_O	1.000	0.892	1.000	0.870	0.882	1.000	1.000	0.900	1.000	0.900	0.933	0.843	1.000	0.727	0.778	0.941	0.900	0.950	0.800	0.900	0.833	0.905	0.842	0.818	0.941	1.000	0.933	1.000	0.636	0.925	1.000	1.000	0.895	0.929
	H_E	0.772	0.882	0.750	0.855	0.884	0.867	0.890	0.865	0.850	0.885	0.876	0.871	0.850	0.835	0.821	0.848	0.855	0.801	0.770	0.825	0.863	0.865	0.835	0.830	0.881	0.828	0.818	0.872	0.831	0.873	0.866	0.877	0.916	0.865
Ssosl85	A	3	11	4	10	9	7	9	5	6	7	13	12	8	8	8	11	5	7	6	6	10	9	9	7	11	8	11	8	9	9	7	9	11	8
	H_O	0.778	0.622	0.500	0.952	0.824	0.625	0.800	0.700	0.800	0.800	0.667	0.824	0.800	1.000	0.889	0.706	0.700	0.800	0.778	0.800	0.833	0.571	0.895	0.773	0.889	1.000	0.933	0.929	0.727	0.650	0.550	0.813	0.842	0.929
	H_E	0.642	0.714	0.688	0.865	0.853	0.820	0.825	0.725	0.770	0.795	0.882	0.830	0.815	0.851	0.833	0.827	0.740	0.771	0.784	0.720	0.821	0.680	0.778	0.759	0.829	0.846	0.862	0.773	0.740	0.777	0.665	0.773	0.888	0.791
Ssa171	A	6	13	4	11	9	8	8	8	8	10	12	12	9	7	7	9	9	9	7	6	12	12	10	8	9	9	11	11	12	13	9	9	10	9
	H_O	1.000	0.917	0.667	0.882	0.765	1.000	0.778	0.750	0.800	1.000	1.000	0.872	0.900	0.778	1.000	0.750	0.900	0.842	1.000	1.000	0.909	0.941	0.778	0.727	1.000	0.778	0.846	0.923	0.800	0.769	1.000	0.867	0.944	0.857
	H_E	0.797	0.882	0.667	0.815	0.830	0.847	0.753	0.836	0.705	0.865	0.864	0.854	0.855	0.765	0.819	0.770	0.845	0.776	0.728	0.800	0.900	0.862	0.813	0.665	0.822	0.753	0.864	0.879	0.854	0.869	0.832	0.860	0.870	0.849
Ssosl438	A	3	14	5	9	12	8	8	6	5	8	10	12	7	7	5	9	4	9	3	4	9	8	4	11	7	5	16	6	6	8	9	11	10	7
	H_O	0.556	0.737	0.750	0.870	0.765	0.571	0.900	0.600	0.700	0.800	0.882	0.706	1.000	0.727	0.778	0.765	0.500	0.650	0.400	0.800	0.611	0.714	0.526	0.909	0.706	1.000	0.929	0.643	0.591	0.825	0.600	0.813	0.737	0.643
	H_E	0.475	0.868	0.781	0.767	0.808	0.806	0.745	0.600	0.665	0.770	0.780	0.814	0.755	0.727	0.667	0.804	0.615	0.721	0.405	0.640	0.716	0.719	0.633	0.829	0.728	0.747	0.921	0.712	0.733	0.756	0.761	0.809	0.794	0.786
Ssa14	A	4	3	3	4	4	4	2	4	2	3	6	4	2	4	4	4	3	4	4	3	4	3	4	4	3	3	4	3	4	4	3	2	4	2
	H_O	0.889	0.474	0.500	0.565	0.353	0.300	0.300	0.700	0.200	0.700	0.529	0.640	0.700	0.455	0.333	0.938	0.700	0.650	0.900	0.900	0.194	0.619	0.316	0.591	0.556	0.333	0.500	0.286	0.545	0.650	0.263	0.438	0.100	0.214
	H_E	0.716	0.407	0.625	0.698	0.683	0.575	0.255	0.655	0.320	0.485	0.754	0.566	0.455	0.682	0.451	0.627	0.635	0.516	0.705	0.565	0.605	0.441	0.600	0.679	0.606	0.438	0.676	0.635	0.654	0.708	0.450	0.404	0.615	0.293
Ssa197	A	7	13	3	13	13	9	11	10	10	8	11	18	9	10	11	11	8	12	8	7	15	13	14	8	9	5	8	10	15	16	15	10	11	10
	H_O	1.000	0.921	0.667	0.826	0.824	1.000	0.900	1.000	0.900	0.800	0.933	0.854	1.000	0.900	0.667	0.938	0.900	0.850	0.900	1.000	0.886	0.842	0.944	0.864	0.941	0.778	0.933	0.923	0.955	0.769	0.889	0.867	0.800	1.000
	H_E	0.790	0.872	0.500	0.871	0.867	0.852	0.885	0.860	0.880	0.850	0.867	0.853	0.864	0.885	0.883	0.871	0.815	0.875	0.795	0.760	0.861	0.860	0.877	0.745	0.822	0.698	0.816	0.846	0.894	0.798	0.897	0.822	0.816	0.839
Ssa202	A	6	13	4	11	11	8	8	7	10	6	9	12	9	9	7	12	8	11	6	5	10	10	9	9	11	6	9	9	9	10	9	9	9	10
	H_O	1.000	0.842	1.000	0.933	1.000	1.000	1.000	0.875	0.800	0.889	0.929	0.915	0.875	0.900	0.750	1.000	0.900	0.684	0.900	0.500	0.912	0.737	0.842	0.762	0.857	0.800	1.000	0.917	0.938	0.875	0.938	1.000	0.950	0.929
	H_E	0.747	0.869	0.722	0.889	0.844	0.840	0.828	0.844	0.790	0.759	0.824	0.888	0.859	0.855	0.813	0.884	0.825	0.855	0.730	0.750	0.857	0.857	0.805	0.798	0.867	0.800	0.837	0.847	0.842	0.820	0.861	0.858	0.868	0.865
Ssa289	A	3	4	2	4	4	4	4	3	4	4	4	4	5	4	3	4	3	4	3	2	4	4	4	5	4	4	4	3	5	4	4	4	4	4
	H_O	0.444	0.684	0.333	0.700	0.647	0.700	0.700	0.333	0.556	0.800	0.688	0.688	0.778	0.400	0.667	0.500	0.700	0.737	0.700	0.778	0.618	0.632	0.842	0.636	0.824	0.667	0.786	0.615	0.909	0.811	0.579	0.786	0.600	0.643
	H_E	0.364	0.592	0.278	0.579	0.604	0.650	0.610	0.586	0.593	0.655	0.674	0.692	0.710	0.645	0.568	0.671	0.585	0.633	0.485	0.500	0.591	0.641	0.630	0.631	0.659	0.685	0.681	0.618	0.732	0.669	0.609	0.717	0.639	0.538
Ssosl25	A	4	12	4	9	7	7	9	6	6	5	10	12	9	7	6	4	6	10	6	5	7	8	6	7	7	6	6	5	8	10	8	8	5	7
	H_O	0.889	0.895	1.000	0.955	0.765	0.800	0.800	1.000	0.600	0.900	0.706	0.816	1.000	0.800	0.750	0.625	0.600	1.000	0.900	0.700	0.800	0.750	0.842	0.818	0.889	0.667	0.667	0.500	0.636	0.750	0.556	0.750	1.000	0.714
	H_E	0.722	0.812	0.688	0.800	0.777	0.760	0.835	0.720	0.490	0.715	0.746	0.823	0.835	0.700	0.773	0.576	0.600	0.734	0.725	0.570	0.728	0.721	0.691	0.731	0.711	0.741	0.600	0.508	0.617	0.698	0.696	0.586	0.769	0.663
Sleen82	A	2	7	5	6	6	3	4	5	7	4	6	8	3	5	4	6	5	6	6	5	5	6	7	6	5	2	4	3	6	7	5	6	6	4
	H_O	0.889	0.632	0.500	0.864	0.750	0.400	0.800	0.800	0.889	0.700	0.813	0.792	0.900	0.556	0.500	0.765	0.800	0.850	0.889	0.900	0.686	0.619	0.737	0.591	0.611	0.222	0.600	0.667	0.773	0.500	0.579	0.688	0.650	0.786
	H_E	0.494	0.660	0.750	0.768	0.658	0.445	0.570	0.715	0.728	0.650	0.756	0.663	0.640	0.623	0.597	0.727	0.690	0.647	0.642	0.715	0.651	0.585	0.781	0.538	0.586	0.346	0.651	0.542	0.636	0.670	0.557	0.598	0.692	0.594
	H_E	0.652	0.756	0.645	0.791	0.781	0.746	0.720	0.741	0.679	0.743	0.802	0.785	0.764	0.757	0.722	0.760	0.721	0.733	0.677	0.684	0.760	0.723	0.744	0.721	0.751	0.688	0.773	0.723	0.753	0.764	0.719	0.730	0.787	0.708

Table 5.3 Pairwise F_{ST} values below diagonal. Figures in bold are significant after Bonferroni correction ($\alpha = 0.015$). Pairwise genetic distance measures (D_{CE}) above diagonal.

	HRB	RRB	MIL	EAB	EWC	EBS	EGM	EKS	ETB	EBF	CBB	CCB	CCF	CHB	CLB	CLS	CMB	CSB	CSW	YHS	NRM	LGB	LBG	GRG	RIF	LWH	LKE	DAM	STH	SUB	LRG	LSH	NGD	LSA
HRB	*	0.5308	0.5379	0.4625	0.5245	0.4764	0.5722	0.4971	0.5723	0.4986	0.5703	0.4989	0.5588	0.5546	0.5883	0.5068	0.5703	0.5505	0.5258	0.5817	0.5186	0.5200	0.4591	0.5789	0.5449	0.5428	0.4941	0.5566	0.5140	0.4441	0.5015	0.5491	0.5278	0.5490
RRB	**0.1193**	*	0.5136	0.3570	0.3396	0.4292	0.3539	0.3725	0.4093	0.3324	0.4175	0.3294	0.3824	0.3802	0.4394	0.3856	0.4027	0.3841	0.5041	0.5469	0.3821	0.3287	0.3875	0.4270	0.3598	0.3848	0.4158	0.3810	0.3494	0.3789	0.3504	0.3362	0.4192	0.3225
MIL	0.0649	0.0554	*	0.4525	0.4677	0.5374	0.5508	0.4806	0.5300	0.5407	0.5474	0.4642	0.5650	0.5251	0.5630	0.5092	0.5849	0.5140	0.5415	0.5120	0.4944	0.5261	0.4669	0.5304	0.5267	0.5217	0.4863	0.5190	0.5172	0.4251	0.4887	0.5547	0.5017	0.5589
EAB	**0.0604**	**0.0313**	0.0015	*	0.3356	0.3945	0.4148	0.3394	0.3874	0.3647	0.3822	0.3045	0.3996	0.3720	0.4352	0.3384	0.3976	0.3882	0.4115	0.4807	0.3471	0.3599	0.3125	0.3932	0.3486	0.3694	0.3434	0.3854	0.3150	0.2583	0.3188	0.3951	0.3569	0.3596
EWC	**0.0872**	**0.0447**	-0.0049	0.0051	*	0.4072	0.3644	0.3718	0.3923	0.3690	0.4187	0.3426	0.4084	0.3562	0.4146	0.3658	0.4244	0.4214	0.4487	0.4613	0.3325	0.3416	0.3586	0.3981	0.3361	0.4045	0.3794	0.3978	0.3236	0.3223	0.3425	0.4052	0.3578	0.4053
EBS	**0.0727**	**0.0280**	0.0390	0.0096	0.0024	*	0.4533	0.4121	0.4506	0.4095	0.4502	0.3920	0.4303	0.4317	0.4860	0.4471	0.4413	0.4323	0.4967	0.4981	0.3982	0.4255	0.3770	0.4764	0.4106	0.4347	0.3972	0.4632	0.4126	0.3705	0.4009	0.4427	0.4009	0.4670
EGM	**0.1350**	0.0133	0.0779	**0.0486**	**0.0358**	**0.0386**	*	0.3908	0.4643	0.3809	0.4177	0.3794	0.4215	0.4354	0.4638	0.4159	0.4558	0.4001	0.5216	0.5643	0.4174	0.3718	0.4135	0.4269	0.3486	0.3935	0.4764	0.4373	0.3821	0.4132	0.3791	0.3785	0.4184	0.4061
EKS	**0.0924**	0.0181	0.0487	0.0199	0.0124	0.0238	0.0079	*	0.3808	0.3642	0.4241	0.3407	0.4225	0.3706	0.4026	0.3667	0.3921	0.3885	0.4668	0.5188	0.3732	0.3714	0.3599	0.4045	0.3486	0.3893	0.4222	0.3919	0.3524	0.3300	0.3649	0.3788	0.3702	0.3948
ETB	**0.1409**	**0.0972**	0.0592	**0.0480**	**0.0308**	**0.0755**	**0.1018**	**0.0455**	*	0.4589	0.4799	0.4135	0.4646	0.3947	0.4014	0.4552	0.4108	0.4751	0.4899	0.5260	0.3965	0.4436	0.4004	0.4366	0.4051	0.4356	0.4379	0.3905	0.3601	0.3889	0.4211	0.4141	0.4404	0.4430
EBF	**0.0833**	0.0163	0.0419	0.0096	0.0211	0.0017	0.0065	-0.0041	**0.0760**	*	0.4243	0.3405	0.3943	0.4045	0.4544	0.3709	0.4193	0.3885	0.4965	0.5367	0.3899	0.3274	0.3703	0.4430	0.3642	0.3830	0.4364	0.4087	0.3905	0.3821	0.3522	0.3403	0.3935	0.3673
CBB	**0.1141**	**0.0458**	0.0381	**0.0162**	**0.0237**	0.0296	**0.0306**	0.0199	**0.0852**	0.0202	*	0.3755	0.4744	0.4270	0.4642	0.3611	0.4440	0.3998	0.4969	0.5192	0.3905	0.4161	0.4065	0.4451	0.4162	0.4488	0.4603	0.4244	0.3576	0.3829	0.3903	0.4054	0.3945	0.4598
CCB	**0.1021**	**0.0360**	0.0216	**0.0167**	**0.0317**	**0.0238**	**0.0250**	**0.0296**	**0.0975**	0.0098	**0.0289**	*	0.3527	0.3724	0.4398	0.3141	0.4017	0.3136	0.4780	0.5001	0.3417	0.3154	0.3319	0.4120	0.3453	0.3859	0.3723	0.4107	0.3422	0.3180	0.3264	0.3610	0.3645	0.3860
CCF	**0.0918**	0.0208	0.0503	**0.0192**	0.0267	0.0020	**0.0235**	**0.0265**	**0.1019**	0.0070	**0.0333**	0.0064	*	0.3661	0.4227	0.4178	0.4005	0.4172	0.4393	0.5167	0.3659	0.3547	0.3919	0.4792	0.3980	0.4191	0.4415	0.4865	0.4129	0.3970	0.3801	0.4034	0.4319	0.4299
CHB	**0.1019**	**0.0318**	0.0330	0.0111	0.0138	0.0338	**0.0413**	0.0022	0.0345	0.0076	0.0290	**0.0295**	0.0155	*	0.3208	0.3888	0.3814	0.4308	0.4805	0.5145	0.3273	0.4281	0.4057	0.4335	0.3810	0.4241	0.4163	0.4145	0.3436	0.3862	0.3730	0.3922	0.3998	0.4105
CLB	**0.1243**	**0.0645**	0.0426	**0.0344**	0.0203	**0.0778**	**0.0793**	0.0354	0.0155	**0.0491**	**0.0475**	**0.0655**	**0.0484**	0.0032	*	0.4833	0.3720	0.4545	0.4214	0.4530	0.3462	0.4711	0.4625	0.4596	0.4246	0.4625	0.4759	0.4344	0.4024	0.4139	0.4543	0.4392	0.4336	0.4664
CLS	**0.0907**	**0.0504**	0.0289	**0.0151**	**0.0304**	0.0211	**0.0511**	0.0241	**0.0960**	0.0166	0.0175	**0.0233**	**0.0252**	0.0232	**0.0721**	*	0.4215	0.3388	0.4666	0.5226	0.3826	0.3433	0.3438	0.4442	0.3897	0.4379	0.4019	0.4514	0.3571	0.3508	0.3435	0.3953	0.3822	0.3820
CMB	**0.1158**	**0.0405**	0.0656	**0.0350**	**0.0358**	**0.0304**	**0.0393**	-0.0032	**0.0571**	0.0263	**0.0401**	**0.0460**	**0.0276**	0.0069	0.0175	**0.0395**	*	0.4246	0.4250	0.4883	0.3624	0.4009	0.4218	0.4290	0.3692	0.4437	0.4596	0.3750	0.3688	0.4261	0.4017	0.4027	0.3951	0.4096
CSB	**0.1191**	**0.0511**	0.0472	**0.0427**	**0.0654**	**0.0492**	**0.0379**	**0.0287**	**0.1200**	**0.0234**	**0.0386**	**0.0319**	**0.0357**	**0.0357**	**0.0628**	**0.0288**	**0.0446**	*	0.4700	0.5419	0.4003	0.3571	0.3769	0.4206	0.3609	0.4496	0.4529	0.4317	0.3992	0.3884	0.3803	0.4188	0.3759	0.4185
CSW	**0.0966**	**0.1041**	0.0681	**0.0615**	**0.0615**	**0.0752**	**0.1111**	**0.0712**	**0.1072**	**0.0846**	**0.0815**	**0.0958**	**0.0625**	**0.0711**	**0.0402**	**0.0751**	**0.0538**	**0.0902**	*	0.4479	0.4173	0.4474	0.4513	0.5308	0.4670	0.5393	0.4895	0.5095	0.4594	0.4262	0.4611	0.5264	0.4469	0.5112
YHS	**0.1155**	**0.1004**	0.0309	**0.0566**	0.0170	**0.0708**	**0.1234**	**0.0820**	**0.0579**	**0.0790**	**0.0585**	**0.0593**	**0.0883**	**0.0566**	**0.0364**	**0.0721**	**0.0530**	**0.1019**	**0.0541**	*	0.4228	0.5494	0.5232	0.5376	0.5185	0.5700	0.5529	0.5266	0.5207	0.4696	0.5301	0.5651	0.4912	0.5817
NRM	**0.1094**	**0.0692**	0.0304	**0.0268**	**0.0380**	**0.0438**	**0.0601**	**0.0338**	**0.0605**	**0.0456**	**0.0344**	**0.0466**	**0.0373**	0.0160	0.0089	**0.0546**	**0.0302**	**0.0659**	**0.0702**	0.0267	*	0.3909	0.3700	0.4171	0.3870	0.4088	0.4049	0.3998	0.3385	0.3296	0.3561	0.3909	0.3523	0.4425
LGB	**0.1165**	**0.0394**	**0.0671**	**0.0470**	**0.0380**	**0.0484**	**0.0250**	**0.0311**	**0.1101**	0.0131	**0.0435**	**0.0305**	0.0134	**0.0499**	**0.0895**	**0.0290**	**0.0444**	**0.0407**	**0.0887**	**0.1178**	**0.0677**	*	0.3285	0.3960	0.3308	0.3624	0.4037	0.3924	0.3416	0.3269	0.2869	0.3146	0.3218	0.3288
LBG	**0.0667**	**0.0517**	0.0096	0.0123	**0.0289**	0.0146	**0.0373**	0.0255	**0.0788**	0.0240	**0.0351**	**0.0323**	0.0102	**0.0355**	**0.0604**	**0.0249**	**0.0485**	**0.0436**	**0.0754**	**0.0696**	**0.0513**	**0.0214**	*	0.3952	0.3603	0.3995	0.3852	0.4296	0.3267	0.2877	0.2926	0.3960	0.3658	0.4107
GRG	**0.1408**	**0.0696**	0.0441	**0.0434**	**0.0496**	**0.0748**	**0.0649**	**0.0549**	**0.0664**	**0.0630**	**0.0712**	**0.0594**	**0.0667**	**0.0537**	**0.0573**	**0.0838**	**0.0668**	**0.0758**	**0.1326**	**0.0707**	**0.0668**	**0.0712**	**0.0548**	*	0.2865	0.3598	0.4401	0.3841	0.3926	0.3864	0.3790	0.4020	0.3382	0.4282
RIF	**0.1112**	**0.0136**	0.0481	**0.0213**	0.0086	**0.0333**	0.0050	-0.0020	**0.0508**	0.0016	**0.0257**	**0.0209**	**0.0227**	0.0145	**0.0442**	**0.0304**	0.0110	**0.0315**	**0.0831**	**0.0800**	**0.0402**	**0.0233**	**0.0252**	0.0168	*	0.3281	0.4029	0.3415	0.3443	0.3328	0.3114	0.3407	0.3061	0.3701
LWH	**0.1329**	0.0085	0.0655	**0.0362**	0.0311	0.0465	-0.0055	0.0173	**0.0703**	0.0041	**0.0444**	0.0109	0.0169	0.0286	0.0675	**0.0528**	**0.0420**	**0.0492**	**0.1259**	**0.1205**	**0.0483**	0.0098	0.0235	0.0131	-0.0060	*	0.4443	0.4175	0.3443	0.3473	0.3427	0.3514	0.4057	0.3848
LKE	**0.0855**	**0.0730**	-0.0105	0.0163	**0.0225**	0.0047	**0.0796**	**0.0446**	**0.0585**	**0.0449**	**0.0477**	**0.0510**	**0.0501**	**0.0376**	**0.0512**	**0.0451**	**0.0609**	**0.0809**	**0.0849**	**0.0511**	**0.0590**	**0.0711**	**0.0374**	**0.0696**	**0.0432**	**0.0653**	*	0.3951	0.3865	0.3139	0.4038	0.4254	0.3940	0.4429
DAM	**0.1140**	**0.0511**	0.0436	**0.0266**	0.0251	0.0270	**0.0577**	0.0163	**0.0328**	**0.0324**	**0.0335**	**0.0548**	**0.0715**	0.0290	0.0315	**0.0565**	0.0246	**0.0681**	**0.0841**	**0.0379**	**0.0449**	**0.0620**	**0.0575**	**0.0518**	0.0088	**0.0532**	**0.0346**	*	0.3622	0.3558	0.4018	0.3648	0.3896	0.4129
STH	**0.1142**	**0.0420**	0.0468	**0.0337**	0.0131	**0.0431**	**0.0485**	0.0205	0.0233	**0.0454**	**0.0323**	**0.0506**	**0.0536**	0.0162	0.0273	**0.0390**	0.0278	**0.0766**	**0.0855**	**0.0669**	**0.0424**	**0.0564**	**0.0448**	**0.0522**	0.0175	**0.0341**	**0.0475**	0.0192	*	0.2699	0.3181	0.3484	0.3725	0.3570
SUB	**0.0737**	**0.0749**	-0.0172	0.0129	**0.0187**	0.0127	**0.0613**	**0.0319**	**0.0614**	**0.0486**	**0.0302**	**0.0430**	**0.0486**	**0.0395**	**0.0403**	**0.0369**	**0.0607**	**0.0746**	**0.0672**	0.0221	**0.0541**	**0.0448**	**0.0230**	**0.0680**	**0.0291**	**0.0457**	0.0117	**0.0289**	**0.0271**	*	0.3075	0.3618	0.3300	0.3864
LRG	**0.0928**	**0.0305**	0.0442	**0.0280**	**0.0308**	0.0289	0.0315	0.0290	**0.0946**	0.0126	**0.0433**	**0.0239**	0.0150	0.0317	**0.0816**	0.0198	**0.0476**	**0.0444**	**0.0881**	**0.1087**	**0.0530**	-0.0007	0.0086	**0.0680**	0.0176	0.0183	**0.0558**	**0.0601**	**0.0501**	**0.0345**	*	0.3279	0.3581	0.3562
LSH	**0.1292**	**0.0264**	**0.0809**	**0.0449**	**0.0498**	**0.0405**	0.0141	0.0121	**0.0843**	0.0049	**0.0444**	**0.0287**	**0.0421**	0.0226	**0.0747**	**0.0423**	**0.0296**	**0.0492**	**0.1231**	**0.1088**	**0.0662**	**0.0311**	**0.0529**	**0.0615**	0.0038	-0.0003	**0.0634**	**0.0376**	**0.0363**	**0.0579**	**0.0270**	*	0.3919	0.3076
NGD	**0.1032**	**0.0490**	0.0153	**0.0167**	0.0238	0.0181	0.0256	0.0124	**0.0700**	0.0182	0.0160	**0.0273**	0.0242	0.0226	0.0291	**0.0338**	0.0298	**0.0345**	**0.0722**	**0.0377**	**0.0311**	**0.0299**	**0.0252**	**0.0347**	0.0048	0.0177	**0.0367**	0.0298	**0.0398**	**0.0266**	**0.0293**	**0.0396**	*	0.3989
LSA	**0.1115**	**0.0185**	0.0585	**0.0342**	**0.0545**	**0.0287**	**0.0320**	0.0276	**0.1037**	0.0181	**0.0625**	**0.0375**	**0.0378**	**0.0343**	**0.0798**	**0.0351**	**0.0428**	**0.0460**	**0.1042**	**0.1081**	**0.0805**	**0.0442**	**0.0543**	**0.0735**	0.0152	0.0167	**0.0644**	**0.0509**	**0.0425**	**0.0680**	**0.0289**	0.0070	**0.0526**	*

5.3.1 *Microsatellite polymorphism*

Genotypes at 10 microsatellite loci were obtained for 592 juvenile Atlantic salmon fry sampled from 34 populations from the River Eden in 2000 (Table 5.2). All loci were polymorphic in each of the sample populations. A total of 173 alleles were observed, varying from six at Ssa14 to 24 at Ssa85, Ssosl438 & SSa197. Expected heterozygosity ranged from 0.26 to 0.92. Overall observed heterozygosity was 0.77 ± 0.18. The number of alleles per sample per locus ranged from 2-18, with a mean of 7.44 ± 3.21. Of 340 locus/site combinations, only 10 showed deviations from HWE (2.95%) after correcting for the number of tests (overall $\alpha = 0.0015$). Seven of these were in Ssa14 (also one each in Ssosl85, Ssosl438 & Sleen82), suggesting, as observed from adult populations, that there is a null allele at locus Ssa14. The frequency of a null allele at Ssa14 was estimated as 0.083 ± 0.037. Relatedness was low overall ($R = 0.0074$; the lowest site was EBF, $R = -0.0002$; the highest GRG, $R = 0.229$) suggesting that the sampling of siblings was rare.

Table 5.4 Hierarchical analysis of molecular variance (AMOVA) of microsatellite allele frequencies from 34 populations within 4 river regions (Lower Eden, Upper Eden, Eamont & Caldew).

Variance component	d.f.	Variance component	% total variance	*P*
Among river regions	3	0.019	0.52	0.01
Among sample locations	30	0.152	4.09	<0.0001
Within sample locations	1150	3.547	95.39	<0.0001
Total	1183	3.718		

5.3.2 *Genetic differentiation among samples*

Overall F_{ST} was 0.048 ± 0.03. Significant F_{ST} values and non-null D_{CE} values were observed in almost all (85.3%) pairwise comparisons between populations (Table 5.2).

This provides the first indication of detectable levels of spatial allelic variance between populations of juvenile salmon in the Eden. An AMOVA revealed 4.9% of total genetic variance to be attributable to spatial structure between sample sites (Table 5.4; $P < 0.0001$) within regions in the Eden. A smaller, but statistically significant, variance was attributable to differentiation among river regions within the catchment (0.52%, $P = 0.01$). The variance attributable to differences between sites within rivers is almost an order of magnitude greater than that attributable to rivers, suggesting that river structure itself is not the main barrier to gene flow. No significant differences were observed in gene diversity between the four Eden regions sampled (Lower Eden 0.74, Upper Eden: 0.78; Caldew 0.77; Eamont 0.76), nor any in allelic richness (Lower Eden 10.76, Upper Eden: 11.75; Caldew 11.49; Eamont 11.08).

Table 5.5 Pairwise F_{ST} values between eden catchment river regions. All figures are significant at $P < 0.001$ after Bonferroni correction ($\alpha = 0.00025$).

	Lower Eden	Upper Eden	Caldew	Eamont
Lower Eden	*			
Upper Eden	0.01389	*		
Caldew	0.02263	0.00917	*	
Eamont	0.01862	0.00598	0.01223	*

5.3.3 Assignment testing

A total of 578 (97.64%) juvenile salmon were assigned within 95% confidence limits to at least one of the Eden river regions (Table 5.6). The proportion of fish correctly assigned was high overall, 471 (79.56%). Both the Upper Eden and the Eamont had large number of fish assigned to the Caldew, suggesting larger amounts of straying by Caldew adults than from any of the other three regions. Only 14 (2.3%) fish could not be assigned to any Eden river region.

Table 5.6 Numbers of juvenile salmon that were classified back to their river of sampling, to another river, or not assigned to any river, in the Eden catchment. Figures in bold are for fish correctly assigned to their sampling river.

		River region					
n		Upper Eden	Lower Eden	Caldew	Eamont	unassigned	% correctly assigned
90	Upper Eden	**61**	5	15	5	4	67.78
51	Lower Eden	1	**46**	2	1	1	90.20
201	Caldew	5	2	**180**	9	5	89.55
250	Eamont	25	5	32	**184**	4	73.60
Correctly assigned		471					
	Mean	79.56					

5.3.4 Spatial analysis

Differentiation between the four river regions is graphically illustrated using plots of factorial correspondence analysis (Figure 5.2). The separation between sample sites from differing river regions is visible though overlapping. By exaggerating the viewing angle, and increasing the plot size (Figure 5.3) it is easier to see the separation between the river regions. Note that the Caldew, and the Eamont (white and grey respectively) form the strongest clusters. The upper Eden in particular, seems less distinct than other regions.

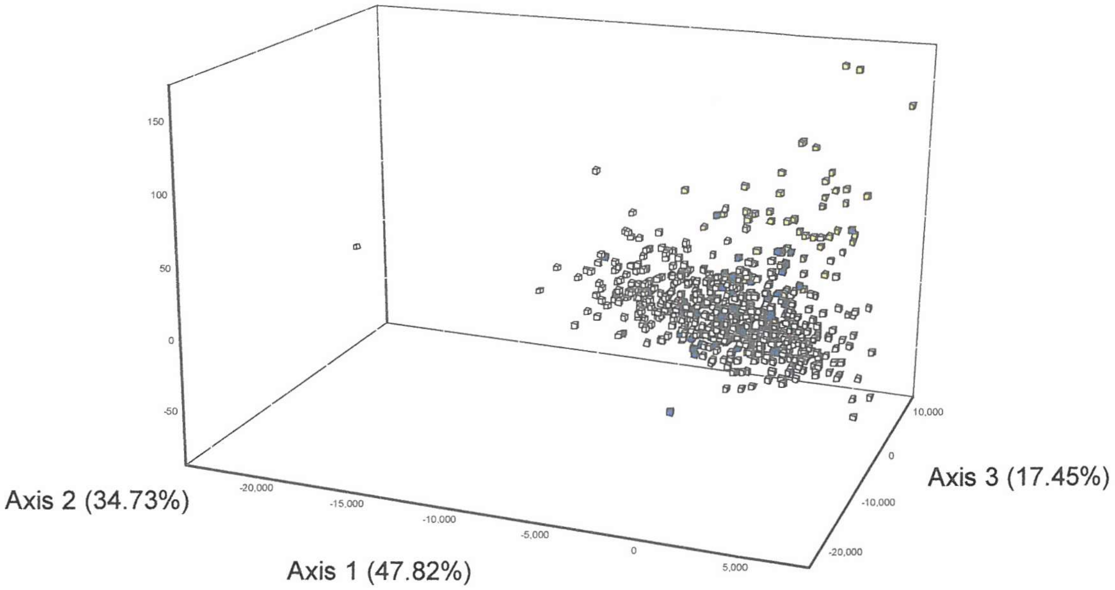

Figure 5.2 Factorial correspondence analysis showing the relationships among multilocus genotypes of individual juvenile Atlantic salmon from the Eden catchment. Individuals are grouped by their river region (Lower Eden, yellow; Upper Eden, blue; Caldew, white; and Eamont, grey).

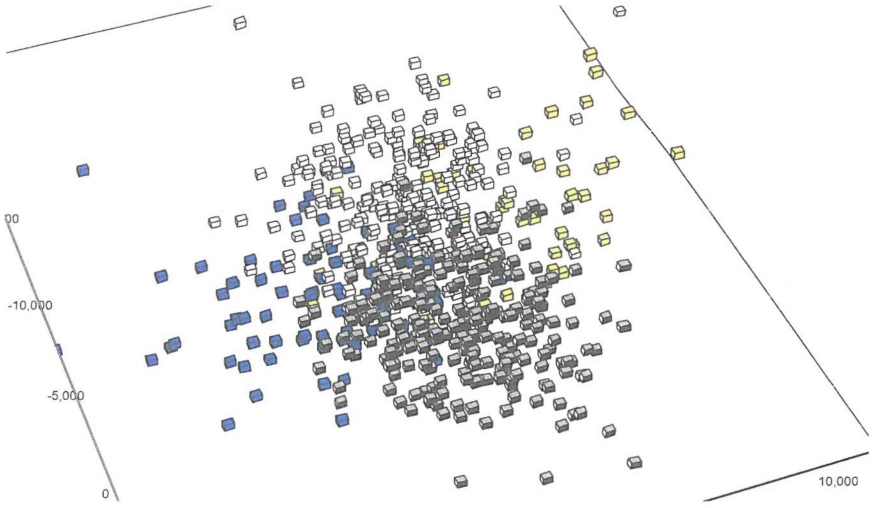

Figure 5.3 Close-up view of a factorial correspondence analysis showing the relationships among multilocus genotypes of individual juvenile Atlantic salmon from the Eden catchment. Individuals are grouped by their river region (Lower Eden, yellow; Upper Eden, blue; Caldew, white; and Eamont, grey). The viewing angle has been adjusted to offer maximum resolution of relationships.

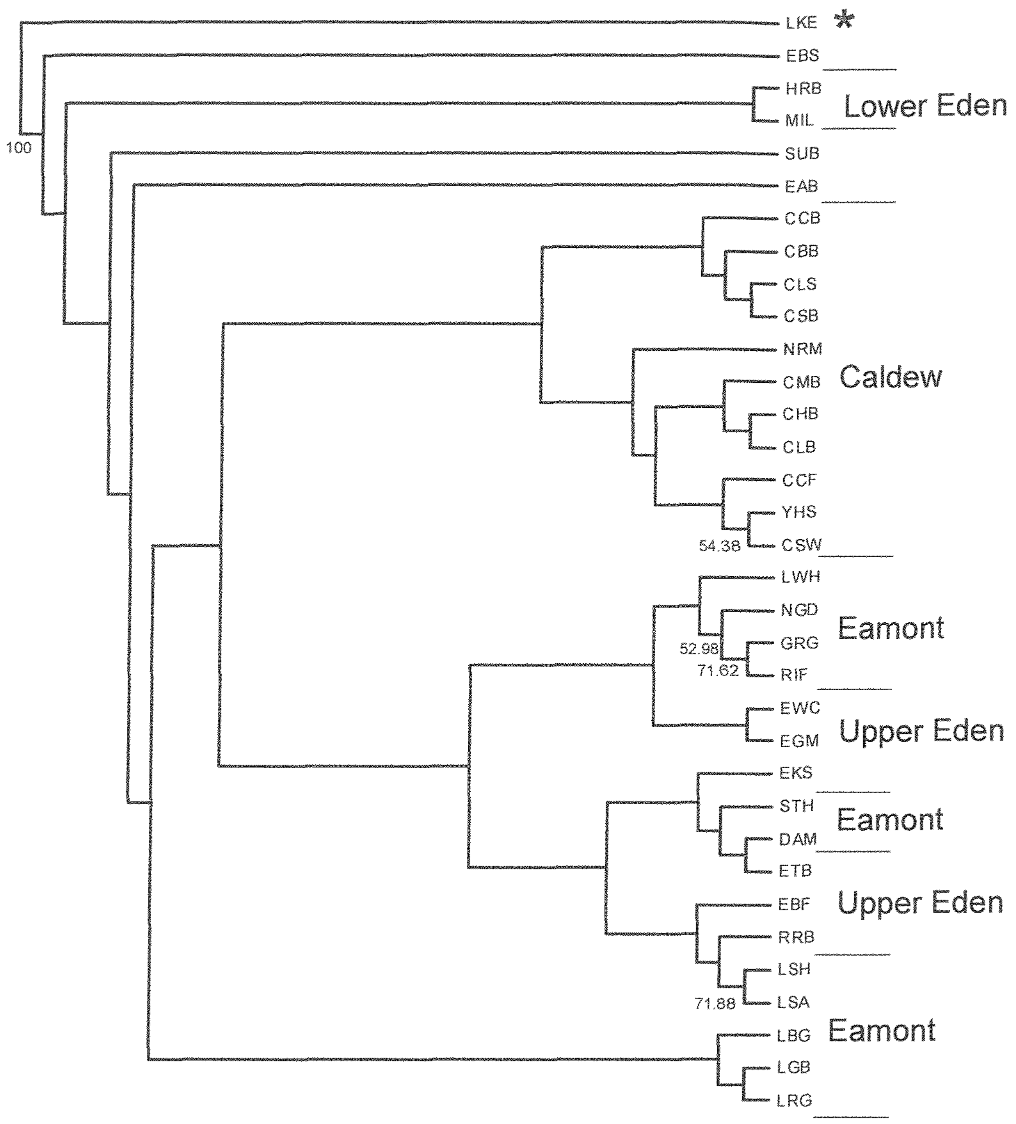

Figure 5.4 Unrooted neighbour-joining tree of genetic distances (D_{CE}). Node support values >50% shown, based on 5000 bootstrap replicates. * highlights the strong differentiation of sample site LKE from the rest of the Eamont river region.

A neighbour-joining tree illustrating structure in the D_{CE} distance matrix shows a high degree of differentiation between the Caldew, the Lower Eden (sites HRB and MIL), and a combination of the Eamont and Upper Eden regions (Figure 5.4). Site LKE

(Eamont) is strongly differentiated from all other samples (100% bootstrap value). The Eamont and Upper Eden regions appear indistinct from each other, though they tend to be mostly differentiated from the Caldew. Three sites do not fit this general relationship; LBG, LGB and LRG form a sister group to the Caldew + Eamont + Upper Eden. Sites SUB, EKS and LKE also fall outside this group. One site from the lower Eden, RRB, groups with the Caldew + Eamont + Upper Eden, some distance away from the remaining two Lower Eden populations.

5.4 Discussion

This study has further highlighted the extensive genetic diversity present in Atlantic salmon populations. Furthermore, my data indicates that the level of genetic differentiation between river regions or tributaries may be quite low relative to the population structuring between sample sites within those tributaries.

5.4.1 Differentiation between Eden river regions

Both FCA plots and a NJ tree indicate that there is regional population structure in the Eden catchment. The difference between these regions accounts for 0.52% of the genetic variation in the Eden catchment as a whole (AMOVA; $P = 0.01$) suggesting that river regions are to some extent reproductively isolated from each other. Most evidently, the River Caldew is differentiated from all other sampled regions of the Eden; this difference has a possible anthropogenic cause. The River Caldew was effectively closed to migrating salmon in the early 19th century after the construction of three impassable weirs. The first of these was only 2km upstream from the Caldew's confluence with the main Eden channel (Figure 5.1). Fish passes were constructed on all three weirs in 1987 to open the river to salmon. To assist the re-colonisation, stocking took place from 1987 until 1997 (total broodstock over 11 years was 215,419 fish; yearly mean 19,583 ± 6099, Environment Agency, unpublished data) with broodstock taken from the lower reaches of the Caldew, i.e. below the weirs. The Caldew therefore underwent a founding event in 1987, with the fish that were native to the lowest reaches of the Caldew undergoing an assisted range expansion with, presumably, an increase in their population sizes. What is extremely interesting, and somewhat inexplicable considering its recent re-colonisation, is the lack of difference in the Caldew in terms of allelic richness and gene diversity.

5.4.2 Differentiation within regions

Previous work has identified differentiation between tributaries but has not adequately quantified the variance attributable to within tributaries in comparison. McElligott & Cross (1991) found 3.4% of variation between tributaries and 4.3% between catchments in a study of protein variation in Southern Irish rivers. However, they had no site replicates to assess variation within tributaries compared to that between tributaries.

Similarly, Galvin et al. (1996) found 3.4% of minisatellite genetic diversity in the River Shannon, Ireland, to be among tributaries. However, only one site was sampled per tributary. Other studies have identified 5% of minisatellite genetic diversity between tributaries (McCubbing & Hartley, 1995) and 3.6% of allozyme variation between samples within drainages (Ståhl, 1987). Whilst there is clearly between-catchment differentiation in genetic diversity, making general comparisons from these studies is difficult due to differing units; the definitions of tributary, river, catchment and drainage are not always comparable between studies. Much previous work has found genetic variation between such units (e.g. tributary, drainage, and river) explained by accurate homing, producing stable, reproductively isolated stocks, or races, within rivers (e.g. McElligott & Cross, 1991). One study comparing between and within tributary differences (Garant *et al.*, 2000) sampled fish from 7 sites, some less than 5km apart, in the Sainte-Marguerite river, Canada. They found 0.9% of microsatellite genetic diversity attributable to between sample site differences, yet they found no significant differentiation among rivers. My results are largely in agreement with this – tributary/regional structure within the Eden is not the dominant barrier to gene-flow. Differences between sites within regions explains 10 times more variance than the differences among regions averaged over sites? This suggests that whilst homing to catchments is strong; homing within catchments is weaker and associated with differences among sites within tributaries, rather than among tributaries.

Assignment testing found that up to 20% could not be assigned to their natal rivers. This suggests that there is substantial straying between river regions by returning adults. Much of this straying seems to be of Caldew fish into the Eamont and the Upper Eden. The recent re-colonisation of the Caldew may cause this. It is possible that progeny of colonising (straying) adults are less likely to return to their natal waters. A potential heritable component to straying behaviour? Previous estimates from wild populations have been much lower, with straying rates estimated from 0 – 3.1% (Stabell, 1984), though these were estimated directly from catch – recapture experiments.

5.4.3 Conclusions

I contend that while there clearly exists a good deal of structure within catchments (e.g. McCubbing & Hartley, 1995; McElligott & Cross, 1991) the tributary/region/river may not always be the most useful unit by which that variation is described. Factors shaping variation within tributaries account for substantially more of the observed structure than those between tributaries, and as many as 20% of individuals may stray among river regions. This suggests that within-catchment natal homing to regions of the Eden may be concluded with less fidelity than has been suggested for other river systems.

6 A New Model of Genetic Isolation by Distance for Migratory Species

The Atlantic salmon is a species spending up to half its life in the open ocean, with the potential to forage over thousands of kilometres (Mills, 1989; Mills, 2000). Whilst in the ocean, salmon are largely panmictic, sharing feeding grounds with con-specifics of differing geographic origin and age. However, the Atlantic salmon is fundamentally a species with relatively limited dispersal due to a well-documented tendency to return to natal spawning grounds with high fidelity (Stabell, 1984). This highly significant aspect of salmonid behaviour, the homing migration, is the central influence on their population structure, yet this defining trait has, as yet, not been fully incorporated into models describing genetic variation in the species.

6.1.1 Isolation by distance

Wright's (1943) classical model of population structure predicts that populations close to each other will be more genetically similar to one another than will populations further apart. This model is the island model of isolation-by-distance (IBD). The concept was redeveloped as a stepping-stone model of population structure (Kimura & Weiss, 1964), under whose criteria gene flow is more likely to occur between neighbouring populations (Kimura, 1953; Kimura & Weiss, 1964; Malécot, 1955). The stepping stone model more accurately reflects the finite dispersal capacity of many organisms, whereas the island model assumes panmixia (Wright, 1943), i.e. where there is equal (but limited) migration between populations regardless of how far apart they are. Furthermore, the stepping stone model can be formulated as either a one-, two-, or three-dimensional array. Under these scenarios a one-dimensional habitat could be populations along a river bank or mountain ridge; the two-dimensional covers most landscape habitats; and three-dimensional an oceanic system (Kimura & Weiss, 1964). The stepping stone model has been found to accurately model large scale one-dimensional habitats e.g. populations of Australian Barramundi, *Lates calcarrifer*, (Keenan, 1994).

A critical assumption in the formation of IBD is that the two main processes affecting genetic differentiation, namely gene flow and genetic drift, are at, or near, equilibrium (Hutchison & Templeton, 1999; Slatkin, 1993). Attaining equilibrium between drift and gene flow is, in some cases, dependent on the scale over which the relationship is examined (e.g. Hellberg, 1994; Johnson & Black, 1998; Pogson *et al.*, 2001; Todd *et al.*, 1998). Where IBD has been examined over differing geographic scales, it can offer insights into population structure and the evolutionary history of a species. Pogson *et al* (2001) found that cod (*Gadus morhua*) populations, exhibited strong IBD relationships at large scales, however, they also found this over very short scales suggesting that dispersal distances and effective population size are much smaller than had previously been thought for such a highly dispering species. In several species of Pacific urchin, IBD was only found at distances >5000km (Palumbi *et al.*, 1997), while in sturgeonfish (*Acanthurus triostegus*), IBD relationships were detected within but not among, archipelagos (Planes *et al.*, 1996). Scale may reflect that in some populations, insufficient time has elapsed for drift and gene flow to reach equilibrium following some disturbance event (Hutchison & Templeton, 1999). Castric et al. (2003) found that whilst equilibrium conditions were reached in brook charr (*Salvelinus fontinalis*) over most of their range, northern-most populations decreased in IBD. They conclude that this follows the recent northwards range expansion of brook charr following the last ice age.

Previous authors have increased the relevance of geographic measures, taking into account environmental features which are formative in population structure. Fetzner & Crandall (2003) highlighted the increased accuracy of river distances over great circle distances (site to site measurements derived from latitude and longitude data taking no account of landscape e.g. river structure) when comparing populations of crayfish. Arnaud (2003) derived connectivity networks based on landscape features e.g. hedges, roads etc, finding that they gave a better fit to IBD than Euclidian distances in the land snail, *Helix aspersa*. In a seemingly three-dimensional habitat, the Atlantic Ocean, Tunnicliffe & Fowler (1996) found that a number of species found at hydrothermal vents were reliant on mid-ocean ridges for dispersal; no IBD relationship was found over straight line distances.

6.1.2 Objectives

To better understand the influence of adult migration on genetic differentiation I will determine the utility of a new geographic measure for testing IBD in migratory populations. Whilst the one-dimensional stepping-stone reflects the overall dimensionality, I want to build the effect of adult migrations into an IBD model for juvenile populations. I therefore differentiate between two facets of dispersal: 1) movement of juveniles within the river system (essentially the same as dispersal seen in non-migratory species); and 2) movement of adults on their return to the catchment. By integrating information about migratory distance I will seek to analyse juvenile distributions as a function of adult migrations.

I will address this second element of salmon dispersal in this chapter by incorporating information about the position of populations relative to the sea. In doing so, I will test whether this information offers improved resolution of spatial variation in genetic variation for the Atlantic salmon (genetic distance data from Chapter 5), and what wider utility this may have for other migratory species. I will examine the nature of IBD relationships over changing spatial scale within the Eden catchment and determine whether populations meet the assumption of drift - gene flow equilibrium.

6.2 Material & Methods

6.2.1 Distance measures

To investigate spatial structure in the observed genetic differentiation (see Chapter 5 for details about genetic distance), distance matrices were constructed from data generated by the NERC Centre for Ecology and Hydrology (CEH), Dorset, using their river network mapping data (Dawson *et al.*, 2002; Moore *et al.*, 1994). From grid references of sample sites (taken at the time of sampling), the distance from each site to each other, and the distance of each site to the sea, was accurately calculated using the geographic information system (GIS) software ARCVIEW 3.2 (ERSI). These pairwise distance measures are 'by river', and take into account the non-linear nature of river structure.

These data allowed two contrasting matrices of geographic distance to be produced, one containing pairwise site-to-site (S2S) distances, and the other containing pairwise differences in the distance-to-sea (D2S) for each population. S2S distances are constructed from by-river distances, which offer considerable advantages over standard geographic (great circle) distances, as these do not adequately model riverine habitats (Fetzner & Crandall, 2003).

The motivation to construct D2S as a geographic measure arose from the unrealistic assumptions that between site measurements (S2S) make about salmon migration. I perceive migration as a one-dimensional process; adults enter the estuary and proceed upstream. Decisions are taken between river branches and then between smaller streams as they proceed into the catchment, but limited movement takes places between branches once the decisions are made, hence the one-dimensional nature of the process (though the extent to which individual fish move between spawning site may well be sex, and SWT specific – see Chapter 3). The D2S model does not assume salmon are homing to a particular branch of the river. To more appropriately model this migration, D2S distances are simply the difference in the distance upstream between two sites, measured in km from the mouth of the Eden. In the example (Figure 6.1,Table 6.1) both measures are calculated for three populations from sites A, B, and C. Note that using

D2S, population C is the same distance from both A and B. D2S only takes into account distance between sites relative to their distance upstream.

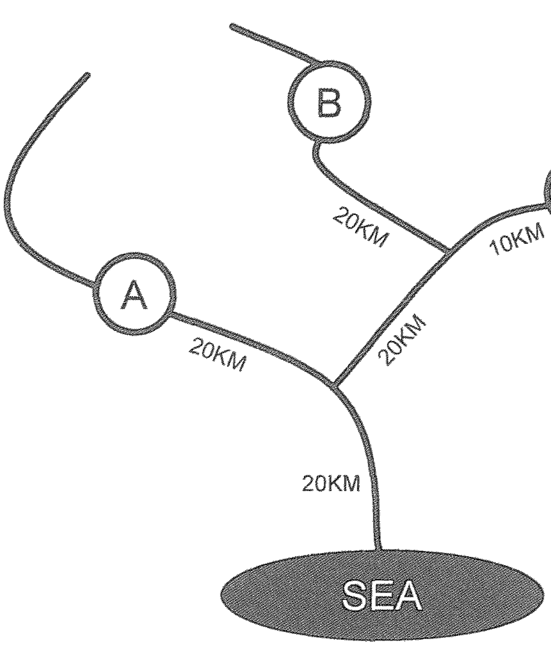

Figure 6.1 Sampling three hypothetical populations (A, B, & C) to demonstrate the difference in the two geographical between population distance measures used in this analysis: S2S, and D2S.

Table 6.1 Example pairwise comparisons for three populations (from Figure 6.1) using the S2S (below diagonal) and D2S (above diagonal) measures. All values are in km.

Population	A	B	C
A	*	20	10
B	60	*	10
C	50	30	*

6.2.2 *Testing for IBD*

To test for isolation-by-distance (IBD) using genetic distance (Cavalli-Sforza & Edwards, 1967) and geography, Mantel tests (Manly, 1991; Manly, 1997) were carried out in the software IBD (Bohonak, 2002). Both genetic distance and geographical measures were $\log_{10}$ transformed for all IBD analyses (Slatkin, 1993): $\log_{10}$(DCE), $\log_{10}$(S2S) & $\log_{10}$(D2S). Mantel testing is an appropriate way to test the significance of distance matrices as populations are treated as individual units, where alternative approaches require the assumption that each population pair is independent (Bohonak, 2002). Since I wished to compare the utility of two geographic models (S2S & D2S) for investigating IBD, the partial Mantel test was implemented in IBD. A partial Mantel test compares three matrices (e.g. DCE, S2S & D2S) and tests the significance of the relationships between two of the matrices when the effect of the other is held constant. Following the methods of Legendre & Legendre (1998), r statistics: $r_{(AB)}$ and $r_{(AC)}$ were calculated (where $_A$ is genetic distance and $_B$ & $_C$ are S2S & D2S respectively), as were partial correlations of $r_{(AB|C)}$ and $r_{(AC|B)}$, controlling for the effects of $_C$ & $_B$ respectively. The strength of IBD relationships was tested using multiple regression in MINITAB (2000).

6.2.3 *Scale dependency of IBD relationships*

To examine changes in IBD over increasing geographic scale (for both S2S and D2S) I calculated regression slopes for successively increasing geographic distances (Castric & Bernatchez, 2003). For example, the slope at 10km was derived from all pairwise comparisons up to 10km apart, the 20km slope from all pairwise comparisons up to 20km, etc.

6.2.4 *Equilibrium between gene flow and genetic drift*

6.2.4.1 *Global equilibrium*

I followed the method of Hutchison & Templeton (1999) to determine whether the processes of genetic drift and gene flow were in equilibrium. Drift will act to increase differentiation between populations, in contrast to the homogenising effect of gene flow, which will not only result in smaller genetic distances between more closely situated populations; it will reduce the variability in these distances as well. As the distance between populations increases, the effects of drift and gene flow become more random and the variability in genetic distance increases. It follows that when drift is in equilibrium with gene flow, the degree of variability in the scatter of the IBD relationship will positively correlate with geographic distance; this is Hutchison & Templeton's (1999) hypothetical relationship Type I (see Figure 6.5 & discussion). To test for such a correlation, the residuals obtained when genetic distance was regressed against geographic distance were run as a matrix against geographic distance in a Mantel test using the software IBD. This procedure was carried out for both S2S and D2S measures.

6.2.4.2 Changing equilibrium with scale

In the same way that the slopes of IBD relationships were regressed over successively greater distances (see 6.2.3), the slope of the relationship between the residuals (genetic distance and geographic distance) was calculated for different distance classes. As before, these distance bins increased in 10km amounts for both S2S and D2S.

6.3 Results

6.3.1 Isolation by distance

Variation observed in genetic distance (Table 6.2) could be explained by both geographic measures considered in this study. Mantel test correlation coefficients show that pairwise S2S distances are correlated with genetic distances ($r = 0.2562$, $P = 0.0002$), as, to a greater extent, are D2S measures ($r = 0.2759$, $P = 0.0010$). This confirms the general relationship of isolation-by-distance in salmon populations from the River Eden.

Table 6.2 Minimum, maximum, and mean average (with standard deviation) values for all pairwise comparisons of genetic distance$^{\Psi}$, site to site distances$^{\Delta}$, and distance upstream$^{\phi}$.

Measure	Minimum	Maximum	Mean
Genetic distance	0.26	0.59	0.42 ± 00.07
Site to site distance	0.10	135.48	63.66 ± 43.64
Distance to sea	0.03	83.24	30.16 ± 22.64

$^{\Psi}$ (Cavalli-Sforza & Edwards, 1967)

$^{\Delta}$pairwise river distances (km) between sites

$^{\phi}$ the difference in distance upstream between sites (km)

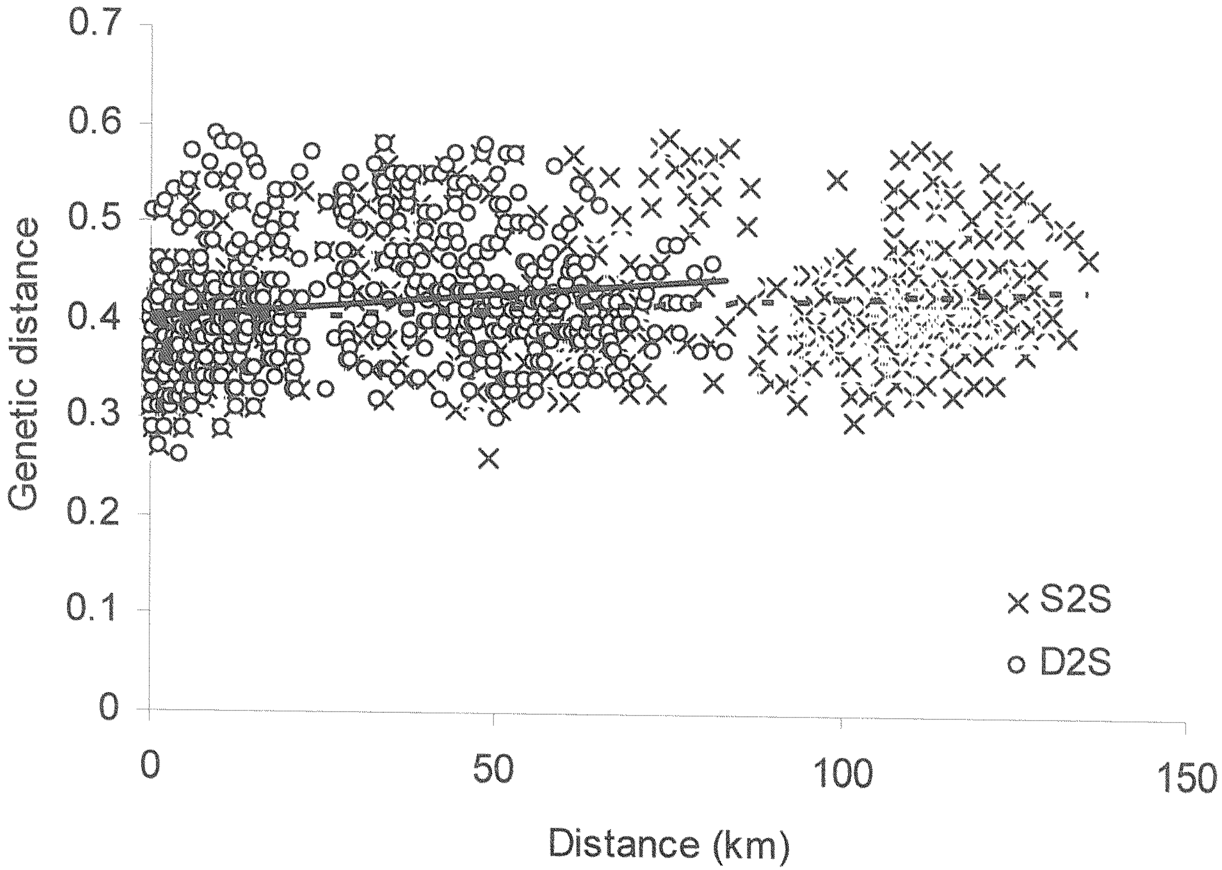

Figure 6.2 IBD relationship between all sampled pairs of juvenile salmon population from the River Eden for two geographic measures (S2S: dashed line, D2S: full line).

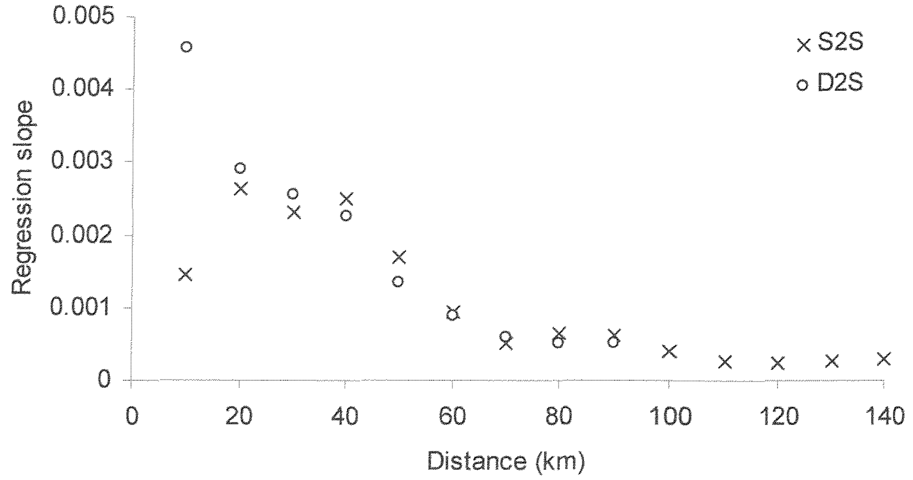

Figure 6.3 Variation in the slope of the IBD regression with increasing spatial scale. Regression slopes were calculated by successively including regressions at different distances. The value for 20km is the regression between genetic distance and geographic distance for all populations up to 20km apart; 40km, all sites up to 40km apart etc. All distance classes were computed for both measures of geographic distance: S2S &D2S.

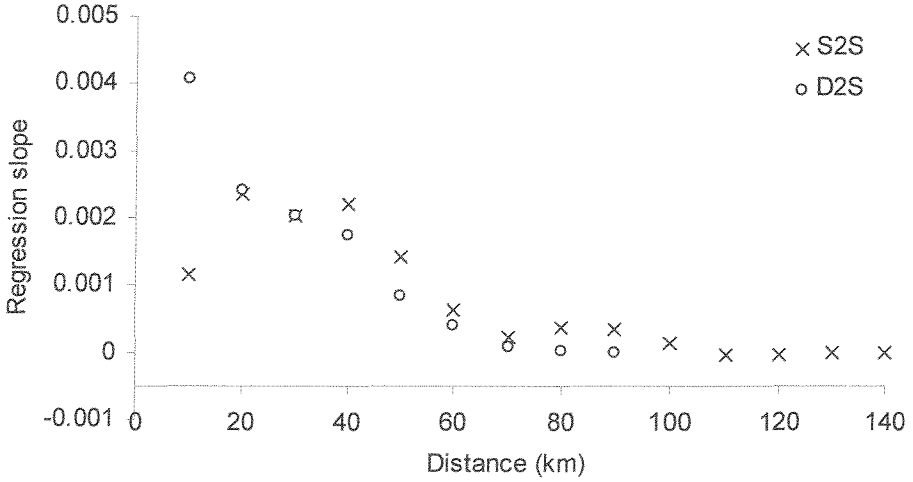

Figure 6.4 Variation in the slope of the residuals regression (from a regression of genetic distance and geographic distance) and with increasing spatial scale. Regression slopes were calculated by successively including regressions at different distances. The value for 20km is the regression between the residual (genetic distance and geographic distance) and geographic distance for all populations up to 20km apart; 40km, all sites up to 40km apart etc. All distance classes were computed for both measures of geographic distance: S2S &D2S.

Table 6.3 Slope of the regression, S.E., d.f., *F*, and it's associated significance for all pairwise comparisons of genetic and geographic distances between sites. Sites are grouped in distance categories, e.g. all sites separated by up to 10km, up to 20km etc. Values are given for two alternative measures of geographic distance: S2S and D2S.

	Distance class	Slope	S.E.	d.f.	*F*	*P*
S2S	10	0.001459	0.001826	1 115	0.638511	0.426
	20	0.002642	0.000939	1 151	7.919198	0.006
	30	0.002312	0.000629	1 165	13.49292	<0.000
	40	0.002493	0.000364	1 198	46.89644	<0.000
	50	0.001703	0.000297	1 221	32.79631	<0.000
	60	0.000929	0.000234	1 248	15.76774	<0.000
	70	0.000506	0.000155	1 310	10.68673	0.001
	80	0.000652	0.000143	1 331	20.63616	<0.000
	90	0.000630	0.000133	1 348	22.61426	<0.000
	100	0.000415	0.000112	1 377	13.76661	<0.000
	110	0.000265	8.46E-05	1 443	9.795177	0.002
	120	0.000255	6.89E-05	1 517	13.73774	<0.000
	130	0.000281	6.33E-05	1 553	19.64224	<0.000
	140	0.000289	6.21E-05	1 560	21.66425	<0.000
D2S	10	0.004573	0.001630	1 157	7.868083	0.006
	20	0.002913	0.000690	1 254	17.84137	<0.000
	30	0.002541	0.000464	1 294	29.95271	<0.000
	40	0.002259	0.000309	1 338	53.28850	<0.000
	50	0.001337	0.000195	1 424	47.17793	<0.000
	60	0.000898	0.000154	1 492	34.04454	<0.000
	70	0.000582	0.000129	1 543	20.21127	<0.000
	80	0.000524	0.000121	1 558	18.65445	<0.000
	90	0.000513	0.000120	1 560	18.25844	<0.000

Partial mantel tests revealed that the correlation between D2S and genetic distance (controlling for S2S) is significant (r = 0.1165, $P = 0.0350$) while that of S2S and genetic distance (controlling for D2S) is not (r = 0.0489, $P = 0.1960$). After considering D2S measures as an explanatory variable of genetic distance ($R^2 = r^2_C = 0.0136$), little additional information is gained by fitting S2S distances; the coefficient of multiple determination only increases to $R^2 = 0.0159$.

The two geographic measures considered accounted for a significant proportion of variance observed in genetic distance (Figure 6.2; $R^2_{adj} = 7.5\%$, $F_{2,558} = 23.71$, $P < 0.001$). A multiple regression highlights the significant relationship between D2S ($t_{558} = 2.77$, $P = 0.006$) and genetic distance, contrasting with a non-significant relationship between S2S and genetic distance ($t_{558} = 1.16$, $P = 0.248$). This clearly indicates that incorporating a more realistic measure of salmon dispersal into our IBD model (using D2S as a geographic measure) has greater power in explaining the genetic variation observed between sample sites.

6.3.2 IBD over varying spatial scales

The strength of the IBD regression was dependent on the scale at which the relationship was examined (Figure 6.3) for both S2S and D2S. At less than 10km the IBD relationship is non-significant using S2S measures (Table 6.3), but strongly significant at all distances thereafter. However, the strength of IBD peaks at 20km and declines steadily over greater scales. Using the D2S metric, IBD is significant at all scales examined though again the slope of IBD is steepest over distances less than 60km.

6.3.3 Equilibrium between drift and gene flow

6.3.3.1 Global

Populations of Atlantic salmon from the River Eden were found not to be at equilibrium with respect to gene flow and genetic drift. Mantel tests revealed a non-significant relationship between the residuals of the regression of genetic distance and geographic distance with distance for both S2S ($r = -0.005$, $P = 0.5159$), and D2S ($r = 0.005$, $P = 0.4670$).

6.3.3.2 Over increasing geographic distance

It is clear that over shorter scales, equilibrium is being reached between the opposing forces of gene flow and genetic drift (Figure 6.4). Using S2S measurements, equilibrium holds significantly from over 20km, up to 90km (except at 70km, and P values at 80 & 90km are at least an order of magnitude less significant than values for 60km and below). Equilibrium is most strongly maintained at a distance of 20 – 40km. Using D2S measures, equilibrium is significant at all values up to 60km. In contrast to S2S, the strength of the equilibrium is greatest at 10km and decreases steadily thereafter. This highlights that the determination of an equilibrium point between gene flow and drift is highly dependent on scale, and, critically, on the geographic model employed. In this example, S2S and D2S geographic measures offer remarkably different interpretations of the factors shaping microgeographic genetic differentiation.

Table 6.4 Slope of the regression, S.E., d.f., *F*, and it's associated significance for all pairwise comparisons of the residuals from a regression of genetic and geographic distances between sites against geographic distance. Sites are grouped in distance categories, e.g. all sites separated by up to 10km, up to 20km etc. Values are given for two alternative measures of geographic distance: S2S and D2S.

	Distance class	Slope	S.E.	d.f.	*F*	*P*
S2S	10	0.001170	0.001826	1 115	0.410	0.523
	20	0.002352	0.009390	1 151	6.280	0.013
	30	0.002023	0.000629	1 165	10.329	0.002
	40	0.002204	0.000364	1 198	36.649	<0.000
	50	0.001414	0.000297	1 121	22.605	<0.000
	60	0.000640	0.000234	1 248	7.480	0.007
	70	0.000217	0.000155	1 310	1.966	0.162
	80	0.000363	0.000143	1 331	6.386	0.012
	90	0.000341	0.000133	1 348	6.623	0.010
	100	0.000126	0.000112	1 377	1.270	0.260
	110	-0.000024	0.000008	1 443	0.082	0.774
	120	-0.000034	0.000007	1 517	0.241	0.624
	130	-0.000009	0.000006	1 553	0.018	0.892
	140	0.000000	0.000006	1 560	0.000	0.999
D2S	10	0.004060	0.001633	1 157	6.202	0.014
	20	0.002401	0.000694	1 254	12.113	0.001
	30	0.002028	0.000464	1 294	19.089	<0.000
	40	0.001746	0.000309	1 338	31.848	<0.000
	50	0.000824	0.000195	1 424	17.920	<0.000
	60	0.000386	0.000154	1 492	6.270	0.013
	70	0.000070	0.000129	1 543	0.284	0.595
	80	0.000011	0.000121	1 558	0.008	0.929
	90	0.000000	0.000120	1 560	0.000	0.999

6.4 Discussion

One central aspect of the biology of Atlantic salmon - their upstream homing migration, predisposes them to the formation of IBD relationships. This study indicates for the first time, to the best of my knowledge, that IBD forms between populations of salmon from a single catchment, over scales of less than 10km. I will discuss the finding of IBD in the context of drift-gene flow equilibrium, and the change in this with the scale of the analysis. I further discuss the utility of using a geographic measure that explicitly takes into account adult migration.

6.4.1 IBD and the its dependence on scale

6.4.1.1 IBD

The scale dependence of the IBD relationships is known from theoretical (Rousset, 1997) and empirical work (e.g. Castric & Bernatchez, 2003; Pogson *et al.*, 2001; Todd *et al.*, 1998) but is often not fully taken into account. In results strikingly similar to my own, Castric & Bernatchez (2003) found that the slope of IBD was generally strong (although exhibited great variation) below 50km in populations of *S. fontinalis* then exhibited a steady decrease in the strength of the relationship thereafter. Such a decline in IBD over larger scales has been documented for other species (Johnson & Black, 1998) identified a strong IBD effect in populations of the mangrove littorine snail, *Littoraria cingulata*, up to 300km along the north western coast of Australia. The strength of this relationship diminished over greater distances. In populations of Siberian lemmings, *Lemmus sibricus*, Ehrich & Stenseth, (2001) found that whilst there was strong genetic differentiation over microgeographic scales, patch structure better explained the pattern of differentiation than an IBD model over larger distances.

While a significant pattern of isolation-by-distance was found between populations in the Eden by both Mantel test and regression, it is clear that this relationship is strongest over very short distances and certainly weak, though significant, over 60km. Previous work has identified IBD patterns in Atlantic salmon populations over very large scales. King et al. (2001) found significant IBD both within North American and European populations and between them. However, the change in this relationship over scale was not investigated. What is clear is that IBD can develop over the full range of salmon populations at one extreme, and on a local level within a single river at the other. What are the processes leading to, and perhaps in some cases, preventing the establishment of IBD in salmon populations?

Where IBD has not been identified in species with the propensity to form such patterns (i.e. populations with limited dispersal, whose gene flow is restricted to adjacent populations), the status of equilibrium between the forces of drift and gene flow may provide answers (Hutchison & Templeton, 1999). In particular, at larger scales, historical events are likely to have more of an influence than the contemporary processes of drift and gene flow.

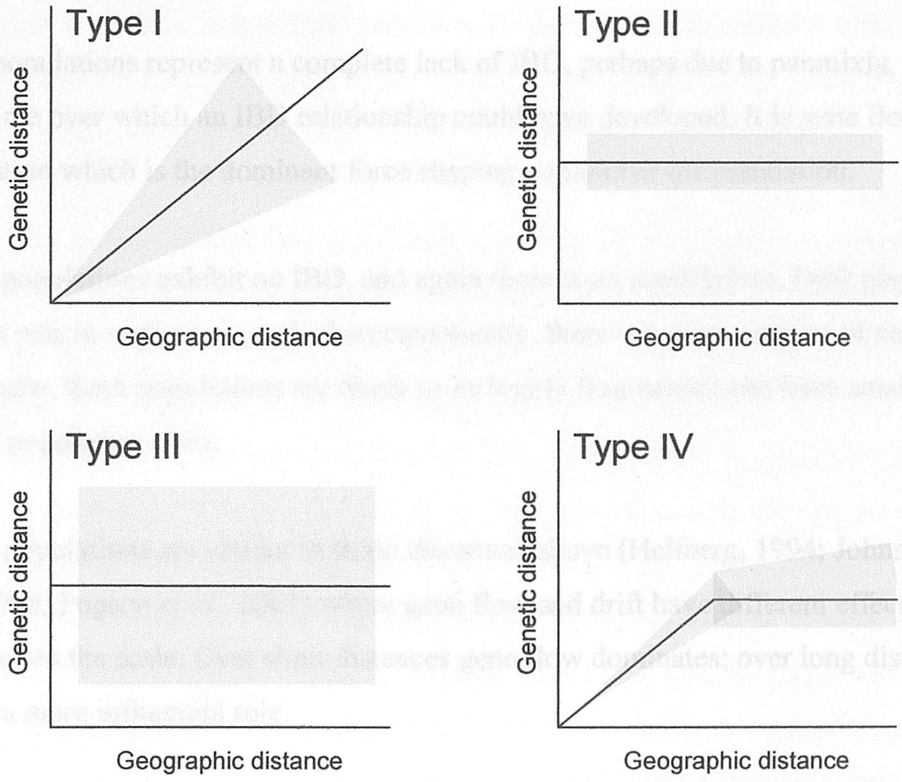

Figure 6.5 Four hypothetical relationships between genetic differentiation and geographic distance under conditions of equilibrium between drift and gene flow (Type I) and non-equilibrium scenarios (Type II – IV). Grey areas represent the degree of scatter in the IBD. The diagrams allow the investigation of the relative influences of genetic drift and gene flow (Hutchison & Templeton, 1999).

6.4.1.2 Drift-gene flow equilibrium

Hutchison & Templeton (1999) identify four types of IBD relationship to qualitatively analyse the assumption of drift-gene flow equilibrium (Figure 6.5). These four scenarios allow a comprehensive investigation of the status of drift-gene flow equilibrium, and the effects this has on IBD (e.g. Van der Strate *et al.*, 2003).

In the classic Type I scenario, the genetic isolation increases with distance. Furthermore, the degree of variability in this relationship increases with distance, reflecting the fact that drift, rather than gene flow, plays a greater role as the distance between populations increases. Populations exhibiting Type I IBD are likely to have long-term stability.

Type II populations represent a complete lack of IBD, perhaps due to panmixia, or a lack of time over which an IBD relationship could have developed. It is gene flow in this situation which is the dominant force shaping population differentiation.

Type III populations exhibit no IBD, and again there is no equilibrium. Drift plays the dominant role in such cases, and, characteristically, there is a great amount of variation in the scatter. Such populations are likely to be highly fragmented and have small effective population sizes.

Type IV populations are similar to those discussed above (Hellberg, 1994; Johnson & Black, 1998; Pogson *et al.*, 2001) where gene flow and drift have different effects depending on the scale. Over short distances gene flow dominates; over long distance drift has a more influential role.

On visual analysis alone, the pattern in IBD observed in Atlantic salmon from the Eden is similar to a Type IV situation. Clearly the effects of genetic drift are large and visible, characterised by the high degree of variability in IBD at all distances (Figure 6.2). Furthermore, the assumption of equilibrium is violated (S2S: $P = 0.519$ & D2S: $P = 0.467$) overall. However, there was a significant pattern of IBD for both the geographic measures used in this study, and, the assumption of equilibrium was upheld for distances up to around 60km. Both of these results strongly suggest that salmon populations in the Eden are accurately modelled by Hutchison & Templeton's (1999) Type IV scenario, a change in the relative influences of drift and gene flow with geographic scale. At distances of less than 60km IBD is strong, as the equilibrium between drift and gene flow breaks down from this distance onward, so the strength of the observed IBD decreases though remains detectable and significant at all scales examined.

6.4.1.2.1 Time to reach equilibrium

An equilibrium between drift and gene flow is reached with a rate that decreases with the scale at which the relationship is examined (Slatkin, 1993). Therefore, in populations where equilibrium is observed only over shorter distances relative to the study area as a whole, a lack of time necessary for populations to establish drift - gene flow equilibrium is frequently cited as the principal contributing factor (Hellberg, 1994; Johnson & Black, 1998; Pogson *et al.*, 2001; Van der Strate *et al.*, 2003).

Many species now inhabiting boreal and temperate areas in the northern hemisphere were subject to repeated cycles of range contraction followed by re-colonisation due to climate instability over the Pleistocene (McManus *et al.*, 1999; Petit *et al.*, 1999). These periods of rapid, comprehensive climate change have shaped the ecological and genetic diversity of species affected (Davis & Shaw, 2001; Hewitt, 2000; Hewitt, 2001; Hewitt, 2004; Stenseth *et al.*, 2002), though genetic responses to fluctuating climate are still poorly understood (McCarthy & Houlihan, 1997; Minns *et al.*, 1992). Towards the end of the Pleistocene, some 17,000 yr BP, ice covered substantial areas of Europe. At times, the ice cover extended over most northern areas of the UK, stretching almost to the south coast at its extreme (Svendsen *et al.*, 1999). It is during this period that many fish species in Europe would have been confined to glacial refugia (Hewitt, 1996), mostly the Iberian peninsula (Consuegra *et al.*, 2002), but also other, smaller lake refugia at glacial margins (Koljonen *et al.*, 1999; Nilsson *et al.*, 2001). The effect of this postglacial colonisation of European waters is clear in patterns of genetic diversity in salmonids (e.g. Bernatchez *et al.*, 1992; Costello *et al.*, 2003; Koskinen *et al.*, 2000; Nilsson *et al.*, 2001).

I therefore contend that the main reason for the weakening of IBD after 60km in the Eden reflects an insufficient time since the glacial instability for catchment-wide drift – gene flow to develop. This situation is likely to have been exacerbated by a population decline and fragmentation with a resulting increase in the influence of genetic drift.

6.4.1.2.2 The increasing influence of genetic drift

The effect of genetic drift is increased where populations are small and fragmented (Frankham *et al.*, 2002; Hartl & Clark, 1997). A situation likely to be occurring in declining populations of Atlantic salmon. This large effect of drift, evident from the pattern of IBD, is also concurrent with the migration of adult Atlantic salmon with precise philopatry to their natal spawning grounds. This defining trait acts to restrict gene flow, and increase the levels of isolation between populations, thereby increasing the potential influence of genetic drift.

6.4.2 Difference between geographic measures

6.4.2.1 Dimensionality in the stepping stone model – increasing power

IBD was evident between populations at distances below 10km when using D2S as the geographic measure (Table 6.3). However, when measuring S2S distances, there was no IBD up to distances of 20km. By measuring distance between salmon populations as function of upstream migration I have viewed the catchment as a one-dimensional migratory gradient, rather than a two-dimensional pattern of dispersal between sites. Crucially, measuring between sites models gene flow as occurring via migration between those sites; I sought to compare this with a model that described gene flow as being between populations of different migratory distances. By converting river geography into a one-dimensional habitat the power of detection is increased, since in area models gene flow can occur in many directions at once (Crow & Aoki, 1984). This is confirmed by the fact that IBD is detected over shorter distances using D2S as a measure of geography within an IBD model.

6.4.2.2 Incorporating homing behaviour - increasing realism

In studying IBD patterns in populations of juvenile salmon, I have sought to characterize the consequences of adult migratory behaviour on the genetic variation present within the Eden. Basing the calculation of between site differences on upstream distance, served to convert geographical distance into an effective migratory measure; D2S explained significantly more of the variation observed in genetic distance (P = 0.035). This models migratory behaviour more appropriately than between site river distances, which impose unrealistic assumptions of movement between populations, rather than focussing on the key aspect of the migration, the distance travelled upstream. Numerous studies have investigated the costs and benefits of migratory distance (see Chapter Three), highlighting its decisive role in shaping salmon populations.

Other methods to more accurately model IBD relationships in Atlantic salmon have used river network structure as a geographic matrix. Wilson (1996) found a correlation between the number of nodes (branching points in the river) through which salmon populations were separated, and genetic distance, where no correlation was found with straight line site-to-site distance. This approach replies upon a clear definition of what constitutes a node, in this case, a very small number of nodes were considered (< 6), but the approach is highly appropriate to migratory models. A similar model could be constructed using the number of suitable spawning grounds between sites as a geographic measure, and would likely offer similarly more realistic results as do node values and D2S, all of which would account for some aspect of migratory behaviour.

6.4.3 Conclusions

Using more realistic models of river populations has allowed a better understanding of the salmon I studied in the Eden. However, this method may be relevant to many species that occur in restricted habitats and have essentially, a one-dimensional dispersal route. In particular, this method seems appropriate in migratory species where dispersal distances can be transformed into a migratory gradient that accurately models movements of individuals. Such criteria may be met where wildlife corridors exist between source populations, so called linear landscape elements (e.g. bats foraging along hedge rows Verboom & Huitema, 1997).

7 Final discussion

From intrasexual variation in body size, to catchment-wide isolation-by-distance, in six chapters. At every step, I found great variation in the River Eden in terms of both phenotypic and genetic diversity. Central to this diversity is the salmon homing migration, and their complex, gender-specific, life-histories. I sought a synthesis between ecology and population genetics in order to build a more realistic approach to understanding diversity.

7.1 Summary of results

Chapter Two brought to light how the interaction between sex and life-history correlate with differences in adult salmon body size. This within-population size variation, previously largely thought to be related to date of return to freshwater, is demonstrated to be far more complex when gender and sea-winter tactics are taken into account. Moving on from this, in Chapter Three I investigated how migratory distance differs with sex and life-history. Continuing the thread of gender-differentiated differences, I found that female sea-wintering behaviour is correlated with spawning site location in a way that male behaviour is not. This is the first evidence of gender specific migratory behaviour in salmonids.

In Chapter Four, this was taken a step further with molecular markers to assess population genetic structure. Firstly, there is river regional population structure, as found elsewhere (e.g. Garant *et al.*, 2000; McConnell *et al.*, 1995; McElligott & Cross, 1991) in Atlantic salmon. However, there is also good evidence that there is substantial variation within river regions, and that the distance upstream (i.e. the adults migratory distance) has a role in structuring populations. Crucially, following on from the patterns observed in migratory distance in Chapter Three, there are differences between males and females in their assignment accuracy; evidence for sex-bias in their migrations. These adult data also offered evolutionary insights, in particular, effective population size was found to be very low ($N_e < 100$); far below the figure of 500 suggested as a minimum to maintain a sufficient level of diversity (Waples, 1990). In Chapter Five, the population genetics of juvenile salmon were investigated using a hierarchically structured sample set of riffle sites within river regions, and river regions within the catchment. This allowed the levels of differentiation between and within river regions to be addressed. As found in adult populations, clear evidence emerged that the within-river component of variation is not only important, but may be 10x greater than that between rivers. The River Caldew in particular is uniquely differentiated from the rest of the Eden catchment, perhaps as a result of its recent recolonisation. In Chapter Six I sought to further characterise the distribution of genetic diversity observed in the Eden juvenile population. Isolation-by-distance models described the pattern of variation observed. IBD was found to hold up to 60km, after which equilibrium breaks down between genetic drift and gene flow. By incorporating migration into the analysis by using the distance-to-sea (D2S) model, better resolution is gained of IBD patterns. This highlights that homing to river regions may not be as important as homing to certain distances upstream.

7.1.1 Comparisons between adult and juvenile genetics

7.1.1.1 Population structure

F_{ST} differed between adult ($F_{ST} = 0.01$), and juvenile ($F_{ST} = 0.048$) populations. This large difference is likely to be a result of the sampling structure. The juvenile figure is similar to that quoted in previous studies; it is the F_{ST} over a number of discrete sampling sites, or sub-populations. These are clearly defined sampling units. In the adult analysis, the sub-population unit is the river region, a much larger scale concept of sub-population. Recalculating F_{ST} for juveniles with the river region as the sub-population unit (as in the adults) gives $F_{ST} = 0.012$.

Both adult and juvenile analyses highlight that the river Caldew is quite differentiated from the rest of the catchment (see 4.3.2. & 5.3.2.). This is probably a result of it's very recent re-colonisation. Curiously the Caldew does not actually seem any less diverse than the rest of the Eden; this is certainly not what might have been expected. It would be highly informative to study the Caldew in greater detail, especially with regards to temporal changes. In addition, I could find no examples for salmon, or any other species, of anyone studying so recent a re-colonisation.

7.1.1.2 Null alleles

Deviations from Hardy-Weinberg expectations in both adult and juvenile populations identify the presence of a null allele in locus Ssa14. The null allele, plus the relatively low polymorphism (N_a = six) at this locus, suggest that it's utility for population genetics is limited. Many more tetra-nucleotide loci (a few of which were used in this study, i.e. Ssa404, Ssa410, and Ssa418) have been published while this study was in progress (Cairney *et al.*, 2000); there are now a vast number of microsatellite loci characterised for Atlantic salmon.

7.2 Limitations of the study

7.2.1 Sample size

Adult sample sizes were limited by the cost of radio-tags and by the size of the salmon run. This, smaller than ideal sample size has two implications: 1) bias in statistics; and 2) restricting the breadth of the analysis.

7.2.1.1 Statistical bias

Ruzzante (1998) has shown that small sample sizes in population genetics studies can bias a number of genetic distance estimators, e,g D_{CE} (Cavalli-Sforza & Edwards, 1967), used in this study to investigate IBD patterns. Furthermore, small sample sizes can lead to larger confidence intervals (Ruzzante, 1998). However, at the river region, and upstream distance categories (4.2.5.2) sample sizes were near to $n = 50$ suggested by Ruzzante (1998). Furthermore, his paper was based on only six microsatellite loci, while I used 10 or 11 to increase resolution

7.2.2 Temporal components of diversity

The limitations on sample size placed some constraints on the breadth of the analysis. To investigate sea-winter tactics and gender, it was necessary to pool samples of adult salmon from the years 1999, 2000, & 2002. This was required for both genetic and phenotypic analyses. A larger data set would have allowed the temporal component of genetic diversity to be considered, a factor that may account for significant levels of population structure in salmonids (Garant *et al.*, 2000). However, within existing statistical frameworks for AMOVA, only three hierarchical levels can be considered at any one time. Making decisions about those factors to be considered in a hierarchical analysis has a large effect on the questions asked. When river, distance upstream, sea-winter tactic, sex, and body size data are all available (as in this project), asking the right question, that is, choosing the appropriate variables, is central to the analysis. Visualisation methods (such as FCA) currently offer the best approach for investigating patterns of variation across many differing definitions of 'population'.

7.2.3 Stocking effects

The Eden, like the vast majority of salmon rivers, has undergone extensive stocking both historically, and in recent years. More recently, stocking has focused on using local fish populations, whereas in the past, salmon may have been moved the length and breadth of the country. Stocking has been, and still is, poorly documented and it's effects, success or otherwise, are poorly understood, though it is clear that stocked fish have lower sea-survival than wild fish (Jonsson, 1997; Jonsson *et al.*, 1991a).

Records for stocking in the Caldew are relatively comprehensive. Between 1987 and 1997, some 118,068 micro-tagged fry and parr were released (Environment Agency, unpublished data). Of these, only 135 were recovered (in rod, traps etc.), representing a stocking success rate of just 0.1%. However, the stocking in the Caldew (plus straying fish potentially entering from other rivers) has been enough to repopulate the river in a little over a decade.

7.2.3.1 Effect on population structure

Stocking between rivers might have a homogenising effect on population structure; conversely, stocking within a river with native fish, might well act to increase differentiation.

7.3 Recommendations for management

The key finding of my research is that populations of Atlantic salmon exhibit great phenotypic and genetic diversity over very small scales within a single river system. In particular, the conservation of life-history diversity should be a key goal in salmon management. Better knowledge of the factors determining life-history will greatly aid in this, as will quantifying the differential exploitation of 1SW and MSW fish as a potential selection pressure for fewer sea-winters.

Though many previous authors have identified significant genetic variation between rivers within a catchment, I offer the most comprehensive study thus far of variation within rivers compared to that between them. I find that previous authors may have over-estimated the importance of between-river variation by failing to consider within-river variation. In the Eden, between-site structure accounts for 10x more genetic variation in juvenile populations than between-river structure. Therefore, managing rivers as separate units may not be the most appropriate approach.

7.4 Future work

Future studies will be able further tease apart the effect that sex, and sea-winter tactics, have on population genetic structure. My results provide the first indications of great variability, yet will require bigger data-sets for a more comprehensive understanding. In particular, combining approaches such as radio-tracking with genetic analysis offer far more information than either method alone. Such varied analyses offer the opportunity to sample salmonids in ways previously highly impractical, e.g. analysis of returning adult fish.

7.4.1 Specific questions remaining to be answered

7.4.1.1 About Eden salmon

The Eden represents a unique opportunity to study a recolonisation event. The well documented re-opening of the River Caldew, and the stocking which followed it, should allow a thorough investigation of how populations develop following an invasion (assisted or otherwise). A long term study on this River could approach a variety of question related to small populations, such as how N_e relates to census size, and how other rivers might be most effectively recolonised in the future.

7.4.1.2 Atlantic salmon in general

Large aspects of Atlantic salmon biology are still poorly understood. One particular subject of great interest, both from a management, and an evolutionary point of view, is the factors leading to, and maintaining levels of precocious mating by parr. Another area still poorly understood, is to what extent salmon re-spawn; how might this vary between and within rivers. Factors such as these represent the key aspects of salmon life-history. Long-term genetic studies will be able to establish more comprehensive estimates of the heritability of differing life-history tactics.

The behaviour of adult fish within the river itself still presents many unanswered questions. Why do so many fish return early, especially given that it is known a number of these fish do not actually ascend the rivers early? How accurate is homing? Can we use radio-tagging to 'calibrate' genetic methods of estimating dispersal? More radio-tagging analyses, coupled with genetic data are likely to offer the most informative answers.

7.4.2 Methods likely to accomplish these goals

One method worth singling out is assignment testing; it is rapidly becoming one of the principal tools for assessing salmon populations. Assignment accuracy, or lack of, provides insights into dispersal, another key behaviour very difficult to study by observation alone. When data become available for all UK salmon populations, it will be feasible to assign adult salmon fish caught at sea to their native rivers, and perhaps even to particular areas within those rivers. This will allow far greater understanding of salmon behaviour at sea, something we still know relatively little about (Mills, 2000).

8 References

Ahnesjo I, Vincent A, Alatalo R, Halliday T, Sutherland WJ (1993) The Role of Females in Influencing Mating Patterns. *Behavioral Ecology* **4**, 187-189.

Allan IRH, Ritter JA (1975) Salmonid terminology. *J. Cons. Perm. Int. Explor. Mer* **37**, 293-299.

Allegrucci G, Cesaroni D, Venanzetti F, Cataudella S, Sbordoni V (1998) Length variation in mtDNA control region in hatchery stocks of European sea bass subjected to acclimation experiments. *Genetics Selection Evolution* **30**, 275-288.

Allendorf FW (1985) Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181-190.

Allendorf FW, Phelps SR (1981) Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Science* **38**, 1507-1514.

Allendorf FW, Seeb LW (2000) Concordance of genetic divergence among Sockeye salmon populations at allozyme, nuclear DNA and mitochondrial DNA markers. *Evolution* **54**, 640-651.

Altukhov YP, Salmenkova EA, Omelchenko VT (2000) *Salmonid Fishes: Population Biology, Genetics and Management* Blackwell Science, Oxford.

Angers B, Bernatchez L (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) popualtions from microsatellites. *Molecular Biology and Evolution* **15**, 143-159.

Ardren WR, Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (N-e) reveals genetic compensation in steelhead trout. *Molecular Ecology* **12**, 35-49.

Armour JAL, Alonso Alegre S, Miles S, Williams LJ, Badge RM (1999) Minisatellites and mutaton processes in tandemly repetitive DNA. In: *Microsatellites: Evolution and Applications* (eds. Goldstein DB, Schlötterer C). Oxford University Press, New York.

Arnaud JF (2003) Metapopulation genetic structure and migration pathways in the land snail Helix aspersa: influence of landscape heterogeneity. *Landscape Ecology* **18**, 333-346.

Attwood CG, Bennett BA (1994) Variation in Dispersal of Galjoen (Coracinus-Capensis) (Teleostei, Coracinidae) from a Marine Reserve. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 1247-1257.

Avise JC (2000) *Phylogeography: The History and Formation of Species* Havard University Press, Cambridge, MA.

Banks JW (1969) A review on the literature on the upstream migration of adult salmonids. *Journal of Fish Biology* **1**, 85-136.

Bartley D, Bagley M, Gall G, Bentley B (1992) Use of Linkage Disequilibrium Data to Estimate Effective Size of Hatchery and Natural Fish Populations. *Conservation Biology* **6**, 365-375.

Baudouin L, Piry S, Cornuet JM (2004) Analytical Bayesian approach for assigning individuals to populations. *Journal of Heredity* **95**, 217-224.

Baum D, Laughton R, Armstrong JD, Metcalfe NB (2004) Altitudinal variation in the relationship between growth and maturation rate in salmon parr. *Journal of Animal Ecology* **73**, 253-260.

Beacham TD, Dempson JB (1998) Population structure of Atlantic salmon from the Conne River, Newfoundland as determined from microsatellite DNA. *Journal of Fish Biology* **52**, 665-676.

Beall E, Dumas J, Claireaux D, Barrière L, Marty C (1994) Dispresal patterns and survival of Atlantic salmon (*Salmo salar* L.) juveniles in a nursery stream. *ICES Journal of Marine Science* **51**, 1-9.

Beaumont JS, Welton JS, Ladle M (1991) Comparison of rod catch data with known numbers of Atlantic salmon (Salmo salar) recorded by a resistivity fish counter in a southern chalk stream. In: *Catch Effort Sampling Strategies, Their Application in Freshwater Fisheries Management* (ed. Cowx IG). Fishing News Books, Oxford.

Beaumont MA (2003) TM3.

Behnke R (1972) The systematics of salmonid fishes of recently glaciated lakes. *Journal of the Fisheries Research Board of Canada* **29**, 639-671.

Behnke RJ (1980) A systematic review of the genus *Salvelinus*. In: *Charrs: salmonid fishes of the genus Salvelinus* (ed. Baron EK), pp. 441-480, Hague.

Bekkevold D, Hansen MM, Mensberg KLD (2004) Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout Salmo trutta. *Molecular Ecology* **13**, 1707-1712.

Belding GL, Kitson JA (1934) Spring-run and fall-run Atlantic salmon. *Transactions of the American Fisheries Society* **64**, 225-231.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpelier, France, Montpellier.

Bell GI (1996) Evolution of simple sequence repeats. *Computational Chemistry* **20**, 41-48.

Benzécri P (1973) *L'analyse Des Données, Tome 2. L'analyse Des Correspondances* Dunoud, Paris, France.

Bermingham E, Forbes SH, Friedland K, Pla C (1991) Discrimination between Atalntic salmon (*Salmo salar*) of North American and European origin using restriction analyses of mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Science* **48**, 884-893.

Bernatchez L, Guyomard R, Bonhomme F (1992) DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Molecular Ecology* **1**, 161-173.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology* **13**, 551-561.

Bickham JW, Wood CC, Patton JC (1995) Biogeographic Implications of Cytochrome-B Sequences and Allozymes in Sockeye (Oncorhynchus-Nerka). *Journal of Heredity* **86**, 140-144.

Bjornstad G, Roed KH (2002) Evaluation of factors affecting individual assignment precision using microsatellite data from horse breeds and simulated breed crosses. *Animal Genetics* **33**, 264-270.

Blair GR, Rogers DE, Quinn TP (1993) Variation in life history characteristics and morphology of sockeye salmon (*Oncorhynchus nerka*) in the Kvichak River system, Bristol Bay, Alaska. *Transactions American Fisheries Society* **122**, 550-259.

Bohlin T, Pettersson J, Degerman E (2001) Population density of migratory and resident brown trout (*Salmo trutta*) in relation to altitude: evidence for a migration cost. *Journal of Animal Ecology* **70**, 112-121.

Bohonak AJ (2002) IBD (Isolation by Distance): a program for analyses of isolation by distance. *Journal of Heredity* **93**, 153-154.

Booke HE (1968) Cytotaxonomic studies of the Coregonine fishes of the Great Lakes, USA: DNA and karyotype analysis. *Journal of the Fisheries Research Board of Canada* **25**, 1667-1687.

Bourke EA, Coughlan J, Jannson H, Galvin P, Cross TF (1997) Allozyme variation in populations of Atlantic salmon located throughout Europe: diversity that could be compromised by the introductions of reared fish. *ICES Journal of Marine Science* **54**, 974-985.

Brännäs E (1985) First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* **9**, 411-420.

Brookfield JYF (1996) A simple method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* **5**, 453-455.

Burnham-Curtis MK, Smith GR (1994) Osteological evidence of genetic divergence of lake trout *(Salvelinus namaycush*) in Lake Superior. *Copeia* **1994**, 843-850.

Caballero A (1994) Developments in the Prediction of Effective Population-Size. *Heredity* **73**, 657-679.

Cairney M, Taggart JB, Høyheim B (2000) Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. *Molecular Ecology* **9**, 2155-2234.

Callen DF, Thompson AD, Shen Y, *et al.* (1993) Incidence and origin of 'null' alleles in the (AC)*n* microsatellite markers. *American Journal of Human Genetics* **52**, 922-927.

Campton DE (1987) Natural hybridisation and introgression in fishes: methods of detection and genetic interpretations. In: *Population Genetics and Fisheries Management* (eds. Ryman N, Utter FM), pp. 161-192. University of Washington Press, Seattle, USA.

Candolin U, Voigt HR (1998) Predator-induced nest site preference: safe nests allow courtship in sticklebacks. *Animal Behaviour* **56**, 1205-1211.

Casale P, Laurent L, Gerosa G, Argano R (2002) Molecular evidence of male-biased dispersal in loggerhead turtle juveniles. *Journal of Experimental Marine Biology and Ecology* **267**, 139-145.

Castric V, Bernatchez L (2003) The rise and fall of isolation by distance in the anadromous brook charr (Salvelinus fontinalis Mitchill). *Genetics* **163**, 983-996.

Castric V, Bernatchez L (2004) Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance. *Molecular Ecology* **13**, 1299-1312.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics* **19**, 233-257.

Cavender TM (1980) Systematics of *Salvelinus* from the North Pacific Basin. In: *Charrs: salmonid fishes of the genus Salvelinus* (ed. Balon EK), pp. 295-322, Hague.

Churchward AS, Hickley P (1991) The Atlantic salmon fishery of the River Severn (UK). In: *Catch Effort Sampling Strategies, Their Application in Freshwater Fisheries Management* (ed. Cowx IG). Fishing News Books, Oxford.

Clutton-Brock TH (1989) Mammalian mating systems. *Proceedings of the Royal Society of London Series B-Biological Sciences* **236**, 339-372.

Cockerham CC (1969) Variance of gene frequencies. *Evolution* **23**, 72-84.

Comps B, Gomory D, Letouzey J, Thiebaut B, Petit RJ (2001) Diverging Trends Between Heterozygosity and Allelic Richness During Postglacial Colonization in the European Beech. *Genetics* **157**, 389-397.

Conradt L, Clutton-Brock TH, Guinness FE (1999) The relationship between habitat choice and lifetime reproductive success in female red deer. *Oecologia* **120**, 218-224.

Consuegra S, De Leaniz CG, Serdio A, *et al.* (2002) Mitochondrial DNA variation in Pleistocene and modern Atlantic salmon from the Iberian glacial refugium. *Molecular Ecology* **11**, 2037-2048.

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153**, 1989-2000.

Costello AB, Down TE, Pollard SM, Pacas CJ, Taylor EB (2003) The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: An examination of microsatellite DNA variation in bull trout, Salvelinus confluentus (Pisces : Salmonidae). *Evolution* **57**, 328-344.

Crisp DT, Carling PA (1989) Observations on siting, dimensions and structure of salmonid redds. *Journal of Fish Biology* **34**, 119-134.

Crow JF, Aoki K (1984) Group selections for a polygenic behavioural trait: estimating the degree of popualtion subdivision. *Proceedings of the National Academy of Sciences* **81**, 6073-6077.

Crow JF, Kimura M (1970) *An introduction to Population Genetics Theory* Harper and Row, New York, Evanston and London.

DAFS (1972) Directorate of Fisheries Research Report for 1972. Department of Agriculture and Fisheries for Scotland, Edinburgh: H.M.S.O.

Dahl K (1910) *The Age and Growth of Salmon and Trout in Norway as Shown by Their Scales* Salmon and Trout Association, London.

Darwin C (1871) *The Descent of Man, and Selection in Relation to Sex*, 1 edn. J. Murray, London.

Davies NB (1991) Mating systems. In: *Behavioural Ecology: an Evolutionary Approach* (eds. Krebs JR, Davies NB). Blackwell Scientific.

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. *Science* **292**, 673-679.

Dawson FH, Hornby DD, Hilton J (2002) A method for the automated extraction of environmental variables to help the classification of rivers in Britain. *Aquatic Conservation: Marine & Freshwater Ecosystems* **12**, 391-403.

Devillard S, Say L, Pontier D (2004) Molecular and behavioural analyses reveal male-biased dispersal between social groups of domestic cats. *Ecoscience* **11**, 175-180.

Dieringer D, Schlotterer C (2002) Microsatellite analyser (MSA) - a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**, 167-169.

Dobson FS (1982) Competition for mates and predominant juvenile dispersal in mammals. *Animal Behaviour* **30**, 1183-1192.

Edwards A, Civitelloi A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* **49**, 746-756.

Eellegren H (1994) Genomic DNA from museum bird feathers. In: *Ancient DNA* (eds. Herrmann B, Hummel S), pp. 211-217. Springer-Verlag, New York.

Ehrich D, Stenseth NC (2001) Genetic structure of Siberian lemmings (Lemmus sibiricus) in a continuous habitat: large patches rather than isolation by distance. *Heredity* **86**, 716-730.

Einum S, Kinnison MT, Hendry AP (2004) Evolution of Egg Size and Number. In: *Evolution Illuminated: Salmon and their Relatives* (eds. Hendry AP, Stearns SC). Oxford University Press, New York.

Eisen JA (1999) Mechanistic basis for microsatellite instability. In: *Microsatellites: Evolution and Applications* (eds. Goldstein DB, Schlötterer C). Oxford University Press, Oxford.

ElMousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree Argania spinosa (L) Skeels endemic to Morocco. *Theoretical and Applied Genetics* **92**, 832-839.

Elson PF (1973) Genetic polymorphism in Northwest Miramichi salmon, in relation to season of ascent and age at maturation and its implications for management of the stocks. *ICNAF Research Doc?* **73/76**.

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215-223.

Erkinaro J, Økland F, Moen K, Niemelä E, Rahiala M (1999) Return migration of Atlantic salmon in the River Tana: the role of environmental factors. *Journal of Fish Biology* **55**, 506-516.

ERSI ArcView 3.2. GIS and Mapping Software.

Estoup A, Langiadèr CR, Perrot E, Chourrout D (1996) Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* **5**, 295-298.

Excoffier L, Smouse PE, Quattro J (1992) Analaysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal Crocidura russula: Evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London Series B-Biological Sciences* **264**, 127-132.

Felsenstein J (1971) Inbreeding and variance effective numbers in populations with overlapping generations. *Genetics* **68**, 581-597.

Felsenstein J (1993) PHYLIP (phylogeny inference package). Ver. 3.5c, Dept. Genetics, University of Washington, Seattle, WA.

Fetzner JWJ, Crandall KA (2003) Linear habitats and the nested clade analysis: an empirical evaluation of geographic versus river distances using an Ozark crayfish (decapoda: Cambaridae). *Evolution* **57**, 2101-2118.

Fillarte EK, Etherton P, Heath DD (2003) Bimodal run distribution in a northern population of sockeye salmon (*Oncorhynchus nerka*): life history and genetic analysis on a temporal scale. *Molecular Ecology* **12**, 1793-1805.

Fleming IA (1996) Reproductive strategies of Atlantic salmon: ecology and evolution. *Reviews in Fish Biology and Fisheries* **6**, 379-416.

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Science* **55 (Suppl. 1)**, 59-76.

Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637-657.

Fleming IA, Jonsson B, Gross MR, Lamberg A (1996) An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). *Journal of Applied Ecology* **33**, 893-905.

Fleming IA, Reynolds JD (2004) Salmonid breeding systems. In: *Evolution Illustrated: Salmon and their Relatives* (eds. Hendry AP, Stearns SC). Oxford University Press, Oxford.

Fontaine PM, Dodson JJ (1999) An analysis of the distribution of juvenile Atlantic salmon (Salmo salar) in nature as a function of relatedness using microsatellites. *Molecular Ecology* **8**, 189-198.

Foote CJ (1990) An experimental comparison of male and female spawning territoriality in a Pacific salmon. *Behaviour* **115**, 283-314.

Frankham R (1995) Effective Population-Size Adult-Population Size Ratios in Wildlife - a Review. *Genetical Research* **66**, 95-107.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics* Cambridge University Press, Cambridge.

Franklin IR (1980) Evolutionary change in small populations`. In: *Conservation Biology: an Evolutionary-Ecological Perspective* (eds. Soule M, Wilcox B). Sinauer, Sunderland, MA.

Fraser DJ, Lippé C, Bernatchez L (2004) Consequences of unequal population size, assymetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). *Molecular Ecology* **13**, 67-80.

Fresco JR, Alberts BM (1960) The accomodation of noncomplimentary bases in helical polyribonucleotides and deoxyribonucleic acids. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 311-321.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology* **8**, 1513-1520.

Galvin P, Taggart G, Ferguson A, O'Farrell M, Cross T (1996) Population genetics of Atlantic salmon (*Salmo salar*) in the River Shannon in Ireland: an appraisal usingsingle locus minisatellite (VNTR) probes. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1933-1942.

Garant D, Dodson JJ, Bernatchez L (2000) Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology* **9**, 615-628.

Garant D, Dodson JJ, Bernatchez L (2003) Differential reproductive success and heritability of alternative reproductive tactics in wild Atlantic salmon (*Salmo salar* L.). *Evolution* **57**, 1133-1141.

Gardner MLG (1976) A review of factors which may influence the sea-age and maturation of Atlanitc salmon *Salmo salar* L. *Journal of Fish Biology* **9**, 289-327.

Gee AS, Milner MJ (1980) Analysis of seventy-year catch statistics for Atlantic salmon (Salmo salar) in the River Wye and implications for management of stocks. *Journal of Applied Ecology* **17**, 41-57.

Gilliam JF, Fraser DF (2001) Movement in corridors: Enhancement by predation threat, disturbance, and habitat structure. *Ecology* **82**, 258-273.

Gorshkov SA, Dorofeyeva YA, Klyukanov VA, Kulikova NI (1979) Osteological features of the Pacific salmon *Onchorhynchus*. *Journal of Ichthyology* **19**, 1-19.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* **11**, 1103-1114.

Gough PJ, Winstone AJ, P.G. H (1992) A review of factors affecting the abundance and catch from the river Wye and elsewhere, and proposals for stock maintenance and enhancement. National Rivers Authority.

Grafen A, Hails R (2002) *Modern Statistics for the Life Sciences* Oxford University Press, New York.

Grewe PM, Billington N, Hebert PDN (1990) Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 984-991.

Griffiths SW, Magurran AE (1998) Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour* **56**, 689-693.

Groot C, Margolis L (1991) Pacific Salmon Life Histories. UBC Press, Vancouver.

Gross MR (1985) Disruptive selection for alternative life histories in salmon. *Nature* **313**, 47-48.

Gross MR (1991) Evolution of Alternative Reproductive Strategies - Frequency-Dependent Sexual Selection in Male Bluegill Sunfish. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **332**, 59-66.

Gross MR (1996) Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution* **11**, 92-98.

Gross R, Nilsson J (1999) Restriction fragment length polymorphism at the growth hormone 1 gene in Atlantic salmon (*Salmo salar* L.) and its association with weight among the offspring of a hatchery stock. *Aquaculture* **173**, 73-80.

Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361-372.

Gustafson-Greenwood KI, Moring JR (1990) Territory size and distribution of newly-emerged Atlantic salmon (*Salmo salar*). *Hydrobiologica* **206**, 125-131.

Hamada H, Petrino M, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionary diverse eukaryotic genomes. *Proceedings of the National Academy of Sciences* **79**, 646-649.

Hancock JM (1999) Microsatellites and other simple sequences: genomic context and mutational mechanisms. In: *Microsatellites: Evolution and Applications* (eds. Goldstein DB, Schlötterer C). Oxford University Press, New York.

Hansen LR, Quinn TR (1998) The marine phase of the Atlantic salmon (*Salmo salar*) life cycle, with comparisons to Pacific salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 104-118.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Sunderland, Massachusetts.

Hartley SE (1987) The chromosomes of salmonid fishes. *Biological Reviews* **62**, 197-214.

Hartley SE, Horne MT (1984) Chromosome polymorphism and constitutive heterochromatin in the Atlantic salmon, *Salmo salar*. *Chromosoma* **89**, 377-380.

Hasler AD, Wisby WJ (1958) The return of displaced large-mouth bass and green sunfish to a 'home' area. *Ecology* **39**, 289-293.

Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus). *Proceedings of the National Academy of Sciences of the United States of America* **99**, 11742-11747.

Hawkins AD, Smith GW (1986) Radio-tracking observations on Atlantic salmon ascending the Aberdeenshire Dee. In: *Scottish Fisheries Research Report*.

Hawkins AD, Webb JH (1986) Radio-tracking observations on Atlantic salmon ascending the Aberdeenshire Dee.

Healey MC (1986) Optimum size and age at maturity in Pacific salmon and effects of size-slective fisheries. In: *Salmonid Age at Maturity* (ed. Meerburg DJ), pp. 39-52. Canadian Special Publication in Fisheries and Aquatic Sciences.

Healey MC, Heard WR (1984) Inter- and intrapopulation variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. *Canadian Journal of Fisheries and Aquatic Science* **41**, 476-483.

Heath DD, Busch C, Kelly J, Atagi DY (2002) Temporal change in genetic structure and effective population size in steelhead trout (Oncorhynchus mykiss). *Molecular Ecology* **11**, 197-214.

Hedrick PW, Hedgecock D, Hamelberg S (1995) Effective Population-Size in Winter-Run Chinook Salmon. *Conservation Biology* **9**, 615-624.

Hellawell JM, Leatham H, Williams GI (1974) The upstream migratory behaviour of salmonids in the River Frome, Dorset. *Journal of Fish Biology* **6**, 729-744.

Hellberg ME (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution* **48**, 1829-1854.

Hendry AP, Berg OK (1999) Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. *Canadian Journal of Zoology* **77**, 1663-1675.

Hendry AP, Berg OK, Quinn TP (2001) Breeding location choice in salmon: causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). *OIKOS* **93**, 407-418.

Hendry AP, Leonetti FE, Quinn TP (1995) Spatial and temporal isolating mechanisms: the formation of discrete breeding aggregations of sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* **73**, 339-352.

Hendry AP, Stearns SC (2004) Evolution Illuminated: Salmon and Their Relatives. Oxford University Press, New York.

Hendry MA, Wenburg JK, Myers KW, Hendry AP (2002) Genetic and phenotypic variation through the migratory season provides evidence for multiple populations of wild steelhead in the Dean River, British Columbia. *Transactions American Fisheries Society* **131**, 418-434.

Henson SA, Warner RR (1997) Male and female alternative reproductive behaviours in fishes: a new approach using intersexual dynamics. *Annual Review of Ecology and Systematics* **28**, 571-592.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology* **10**, 537-549.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 183-195.

Hoar WS (1976) Smolt transformation: evolution, behaiviour and physiology. *Journal Fisheries Research Board of Canada* **33**, 1233-1252.

Holcik J, Hensel K, Nieslanik J, Skàcel L (1988) *The Eurasian Huchen, Hucho hucho: Largest Salmon of the World*, Dordrecht/Boston/Lancaster.

Holtby LB, Healey MC (1990) Sex-specific life history tactics and risk-taking in coho salmon. *Ecology* **71**, 678-690.

Huhta E, Jokimaki J, Rahko P (1998) Distribution and reproductive success of the Pied Flycatcher Ficedula hypoleuca in relation to forest patch size and vegetation characteristics; the effect of scale. *Ibis* **140**, 214-222.

Huntsman AG (1939) Races and homing instinct. *Salmon and Trout Magazine* **96**, 233-237.

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecological monographs* **54**, 187–211.

Hurrell RH, Price DJ (1991) Natural hybrids between Atlantic salmon, *Salmo salar* L., and trout, *Salmo trutta* L., in juvenile salmonid populations in south-west England. *Journal of Fish Biology* **39 (Supplement A)**, 335-341.

Hutchings JA, Gerber L (2002) Sex-biased dispersal in a salmonid fish. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 2487-2493.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898-1914.

ICES (2000) Extract of the report of the Advisory Committtee on Fishery Management of North Atlantic salmon stocks to the North Atlantic Salmon Conservation Organisation. International Council for the Exploitation of the Seas, Copenhagen, Denmark.

Ihssen PE, Booke HE, Casselman JM, *et al.* (1981) Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Science* **38**, 1838-1855.

Jansson H, Holmgren I, Wedin K, Anderson T (1991) High frequency of natural hybrids between Atlantic salmon, *Salmo salar* L., and brown trout, *Salmo trutta* L., in a Swedish river. *Journal of Fish Biology* **39 (Supplement A)**, 343-348.

Johnson L (1980) The Arctic charr, *Salvelinus alpinus*. In: *Charrs: salmonid fishes of the genus Salvelinus* (ed. Balon EK), pp. 15-98, Hague.

Johnson MS, Black R (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of Littoraria cingulata. *Marine Biology* **132**, 295-303.

Jones JW (1959) *The Salmon* Collins, London.

Jonsson B (1985) Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. *Transactions American Fisheries Society* **114**, 182-194.

Jonsson B (1997) A review of ecological and behavioural interactions between cultured and wild Atlantic salmon. *ICES Journal of Marine Science* **54**, 1031-1039.

Jonsson B, Jonsson N, Hansen LP (1991a) Differences in Life-History and Migratory Behavior between Wild and Hatchery-Reared Atlantic Salmon in Nature. *Aquaculture* **98**, 69-78.

Jonsson N (1991) Influence of water flow, water temperature and light on fish migrations in rivers. *Nordic Journal of Freshwater Research* **66**, 20-35.

Jonsson N, Hansen LP, Jonsson B (1991b) Variation in age, size and repeat spawning of adult Atlantic salmon in relation to river discharge. *Journal of Animal Ecology* **60**, 937-947.

Jonsson N, Jonsson B, Hansen LP (1990) Partial segregation in the timing of migration of Atlantic salmon of different ages. *Animal Behaviour* **40**, 313-321.

Jordan WC, Youngson AF, Hay DW, Ferguson A (1992) Genetic protein variation in natural populations of Atlantic salmon (*Salmo salar*) in Scotland: temporal and spatial variation. *Canadian Journal of Fisheries and Aquatic Science* **49**, 1863-1872.

Jorde PE, Ryman N (1996) Demographic genetics of brown trout (Salmo trutta) and estimation of effective population size from temporal change of allele frequencies. *Genetics* **143**, 1369-1381.

Juanes F, Letcher BH, Gries G (2000) Ecology of stream fish: insights gained from an individual- based approach to juvenile Atlantic salmon. *Ecology of Freshwater Fish* **9**, 65-73.

Kalinowski ST, Waples RS (2002) Relationship of effective to census size in fluctuating populations. *Conservation Biology* **16**, 129-136.

Kalleberg H (1958) Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta*). *Report of the INstitute of Freshwater Research* **39**, 55-98.

Kallio-Nyberg I, Romakkaniemi A (1998) Spawning migration of salmon (*Salmo salar*) in the River Tornionjoki and the Bothnian Bay on the basis of catch data from the 1920's and till 1950's. *Kala- ja riistaraportteja* **104**.

Karlsson L, Karlström Ö (1994) The Baltic salmon (*Salmo salar* L.): its history, present situation and future. *Dana* **10**, 61-85.

Karp A, Isaac P, Ingram D (1998) Molecular tools for screening biodiversity. Chapman & Hall, London.

Katsukawa Y, Katsukawa T, Matsuda H (2002) Indeterminate growth is selected by a trade-off between high fecundity and risk avoidance in stochastic environments. *Population Ecology* **44**, 265-272.

Keeley ER, Grant JWA (2001) Prey size of salmonid fishes in streams, lakes, and oceans. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1122-1132.

Keenan CP (1994) Recent Evolution of Population-Structure in Australian Barramundi, Lates-Calcarifer (Bloch) - an Example of Isolation by Distance in One-Dimension. *Australian Journal of Marine and Freshwater Research* **45**, 1123-1148.

Kendall AW, Behnke RJ (1984) Salmonidae: developmenys and relationships. In: *Ontogeny and Systematics of Fishes* (ed. Moser HG), pp. 142-149. American Society of Ichthyologist and Herpetologists Special Publication.

Kimura M (1953) "Stepping stone" model of population. *Annual Report of the National Institute of Genetics* **3**, 62-63.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics* **49**, 725-738.

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proceedings of the National Academy of Sciences* **75**, 2868-2872.

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* **49**, 561-576.

King TL, Kalinowski ST, Schill WB, Spidle AP, Lubinski BA (2001) Population structure of Atlantic salmon (*Salmo salar* L.): a range wide perspective from microsatellite DNA variation. *Molecular Ecology* **10**, 807-821.

King TL, Spidle AP, Eackles MS, Lubinski BA, Schill WB (2000) Mitochondrial DNA diversity in North American and European Atlantic salmon with emphasis on the Downeast rivers of Maine. *Journal of Fish Biology* **57**, 614-630.

Knight ME, Van Open MJH, Smith HL, *et al.* (1999) Evidnece for male-based dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology* **8**, 1521-1527.

Kokko H, Monaghan P (2001) Predicting the direction of sexual selection. *Ecology Letters* **4**, 159-165.

Koljonen ML, Jansson H, Paaver T, Vasin O, Koskiniemi J (1999) Phylogeographic lineages and differentiation pattern of Atlantic salmon (Salmo salar) in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1766-1780.

Kolyushev AI (1971) Some osteological characters of Charrs (Genus *Salvelinus*) in connection with the problem of their systematic position. *Journal of Ichthyology* **11**, 464-473.

Koskinen MT, Ranta E, Piironen J, *et al.* (2000) Genetic lineages and postglacial colonization of grayling (Thymallus thymallus, Salmonidae) in Europe, as revealed by mitochondrial DNA analyses. *Molecular Ecology* **9**, 1609-1624.

Krebs JR, Davies NB (1997) Behavioural Ecology: An Evolutionary Approach. Blackwell Science, London.

Kvarnemo C (1994) Temperature Differentially Affects Male and Female Reproductive Rates in the Sand Goby - Consequences for Operational Sex-Ratio. *Proceedings of the Royal Society of London Series B-Biological Sciences* **256**, 151-156.

Kvarnemo C (1995) Size-Assortative Nest Choice in the Absence of Competition in Males of the Sand Goby, Pomatoschistus-Minutus. *Environmental Biology of Fishes* **43**, 233-239.

Laikre L, Jorde PE, Ryman N (1998) Temporal change of mitochondrial DNA haplotype frequencies and female effective size in a brown trout (Salmo trutta) population. *Evolution* **52**, 910-915.

Lampert KP, Rand AS, Mueller UG, Ryan MJ (2003) Fine-scale genetic pattern and evidence for sex-biased dispersal in the tungara frog, Physalaemus pustulosus. *Molecular Ecology* **12**, 3325-3334.

Larsson K, Rattiste K, Lilleleht V (1997) Heritability of head size in the common gull Larus canus in relation to environmental conditions during offspring growth. *Heredity* **79**, 201-207.

Laughton R (1989) The movements of adult salmon within the River Spey.

Laughton R (1991) The movements of adult Atlantic salmon (*Salmo salar* L.) in the River Spey as determined by radio telemetry during 1988 and 1989.

Laughton R, Smith GW (1992) The relationship between the date of river entry and estimated spawning position of adult salmon (*Salmo salar* L.) in two major Scottish east coast rivers. In: *Wildlife Telemetry: Remote Monitoring and Tracking of Animals* (eds. Priede IG, Swift SM), pp. 423-433. Ellis-Horwood, New York.

Legendre P, Legendre L (1998) *Numerical Ecology*, 2nd edn. Elsevier, New York.

Letcher BH, Gries G, Juanes F (2002) Survival of stream-dwelling Atlantic salmon: effects of life history variation, season and age. *Transactions American Fisheries Society* **131**, 838-854.

Li Y, Korol A, Fahima T, Beiles A, Nevo E (2002) Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Molecular Ecology* **11**, 2453-2465.

Lilja J, Romakkaniemi A (2003) Early-season river entry of adult Atlantic salmon: its dependency on environmental factors. *Journal of Fish Biology* **62**, 41-50.

Locke E, Robinson M (2003) The River Eden cSAC Conservation Strategy. Conserving Natura Rivers, English Nature, Peterborough.

Luikart G, Cornuet JM (1999) Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* **151**, 1211-1216.

Malécot G (1955) The decrease of relationship with distance. *Cold Springs Habor Symposium in Quantitative Biology* **20**, 52-53.

Manly BFJ (1991) *Randomisation and Monte Carlo Methods in Biology*, 1st edn. Chapman & Hall, London.

Manly BFJ (1997) *Randomisation, Bootstrap and Monte Carlo Methods in Biology*, 2nd edn. Chapman & Hall, London.

Mayr E (1969) *Principles of Systematic Zoology* McGraw-Hill, New York.

Mayr E (1982) Speciation and microevolution. *Evolution* **36**, 1119-1132.

McCarthy ID, Houlihan DF (1997) The effects of temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (*Salmo salar* L.) stocks in Europe as the result of global warming. In: *Gloabl Warming: Implications for Freshwater and Marine Fish* (eds. Wood CM, McDonald DG). Cambridge University Press, Cambridge.

McConnell S, O'Reilly P, Hamilton L, Wright JM, Bentzen P (1995) Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Science* **98**, 1863-1872.

McCormick SD, Hansen LP, Quinn TP, Saunders RL (1998) Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* **55 (Suppl. 1)**, 77-92.

McCubbing DJF, Hartley SE (1995) Management options for a multistock salmon resource in a Lake District catchment. In: *Proceedings of the Institute of Fisheries Management Annual Study Course* (ed. Winfield I), pp. 55-80. Institute of Fisheries Management.

McElligott EA, Cross TF (1991) Protein variation in wild Atlantic salmon, with particular reference to southern Ireland. *Journal of Fish Biology* **39 (Suppl. A)**, 35-42.

McManus JF, Oppo DW, Cullen JL (1999) A 0.5-million-year record of millennial-scale climate variability in the North Atlantic. *Science* **283**, 971-975.

McPhee MV, Quinn TP (1998) Factors affecting the duration of nest defence and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. *Environmental Biology of Fishes* **51**, 369-375.

Merila J (1997) Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* **51**, 526-536.

Metcalfe NB, Thorpe JE (1992) Early predictors of life-history events: the link between first feeding date, dominance, and seaward migration in Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **41 (Suppl. B)**, 93-99.

Mills D (1989) *Ecology and Management of Atlantic Salmon* Chapman & Hall, London.

Mills D (2000) The Ocean Life of Atlantic salmon. Blackwell Science Ltd.

Mills DH (1971) *Salmon and trout: A Resource, its Ecology, Conservation and Management* Oliver & Boyd, Edinburgh.

Minitab (2000) Minitab 13.1. Minitab Inc.

Minns CK, Randall RG, Chadwick EMP, Moore JE, Green R (1992) Potential impact of climate change on the habitat and popualtion dynamics of juvenile Atlantic salmon (*Salmo salar*) in eastern Canada. In: *Climate Change and Northern Fish Populations, Canadian Special Publication in Fisheries and Aquatic Sciences* (ed. Beamish RJ). NRC Press, Ottawa.

Minshall GW, Petersen RC, Bott TL, *et al.* (1992) Stream Ecosystem Dynamics of the Salmon River, Idaho - an 8th- Order System. *Journal of the North American Benthological Society* **11**, 111-137.

Mittelbach GG, Chesson PL (1984) Predation Risks: Indirect Effects on Fish Populations. In: *Predation - Direct and Indirect Effects on Aquatic Communities* (eds. Kerfoot WC, Sih A). University Press of New England, Hanover, New Hampshire, USA.

Moore RV, Morris DG, Flavin RW (1994) Sub-set of UK digital 1:50,000 scale river centre-line network. NERC, Institute of Hydrology, Wallingford.

Morán P, García-Vásquez E, Verspoor E (1993) Chromosome polymorphism in the Atlantic salmon *Salmo salar*: differences between pure and mixed origin fish. *Cytobios* **76**, 113-116.

Moritz C (1994) Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution* **9**, 373-375.

Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. *Molecular Ecology* **8**, 1063-1067.

Myers GS (1949) Uses of anadromous, catadromous, and allied terms for migratory fishes. *Copeia* **1949**, 89-97.

Nadir E, Hargalit H, Gallily T, Ben-Sasson SA (1996) Microsatellite spreading in the human genome: evolutionary mechanisms and structural implications. *Proceedings of the National Academy of Sciences* **93**, 6470-6475.

Naiman RJ, Melillo JM, Lock MA, Ford TE, Reice SR (1987) Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum. *Ecology* **68**, 1139-1156.

Neave F (1964) Ocean migration of Pacific salmon. *Journal of the Fisheries Research Board of Canada* **21**, 1227-1244.

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. *Annual Review of Ecology and Systematics* **28**, 105-128.

Nelson K, Soulé ME (1987) Genetical conservation of exploited fishes. In: *Population Genetics and Fisheries Management* (eds. Ryman N, Utter FM). University of Washington Press, Seattle.

Nielsen JL (1998) Population genetics and the conservation and management of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* **55 (Suppl. 1)**, 145-152.

Nilsson J, Gross R, Asplund T, *et al.* (2001) Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonisation of the Baltic Sea area. *Molecular Ecology* **10**, 89-102.

Norden CR (1961) Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. *Journal of the Fisheries Research Board of Canada* **8**, 679-791.

Nordeng H (1983) Solution to the "Char Problem" based on Arctic char (*Salvelinus alpinus*) in Norway. *Canadian Journal of Fisheries and Aquatic Science* **40**, 1372-1387.

Northcote TG (1992) Migration and residency in stream salmonids - some ecological considerations and evolutionary consequences. *Nordic Journal of Freshwater Research* **67**, 5-17.

Nunney L (1991) The Influence of Age Structure and Fecundity on Effective Population-Size. *Proceedings of the Royal Society of London Series B-Biological Sciences* **246**, 71-76.

Nunney L (1996) The influence of variation in female fecundity on effective population size. *Biological Journal of the Linnean Society* **59**, 411-425.

Nunney L, Campbell KA (1993) Assessing Minimum Viable Population-Size - Demography Meets Population-Genetics. *Trends in Ecology & Evolution* **8**, 234-239.

O'Reilly PT, Hamilton PC, Mcconnell SK, Wright JM (1996) Rapid detection of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Science* **53**, 2292-2298.

Page RDM (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**, 357-358.

Palumbi SR, Grabowsky G, Duda T, Geyer L, Tachino N (1997) Speciation and population genetic structure in tropical Pacific Sea urchins. *Evolution* **51**, 1506-1517.

Pardini AT, Jones CS, Noble LR, *et al.* (2001) Sex-biased dispersal of great white sharks - In some respects, these sharks behave more like whales and dolphins than other fish. *Nature* **412**, 139-140.

Partti-Pellinen KA, Elo K, Palva TK, Tuunainen P, Hakumaki MOK (1991) Mitochondrial DNA variation in *Salvelinus* species found in Finland. *Journal of Fish Biology* **39**, 87-92.

Payne RH, Child AR, Forrest A (1971) Geographical variation in Atlantic salmon. *Nature* **231**, 250-252.

Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology* **4**, 249-252.

Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. *American Naturalist* **155**, 116-127.

Petit JR, Jouzel J, Raynaud D*, et al.* (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399**, 429-436.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**, 844-855.

Phillips RB, Oakley TH (1997) Phylogenetic relationships within the Salmoninae based on nuclear and mitochondrial DNA sequences. In: *Molecular Systmatics of Fishes* (eds. Kocher TD, Stepien CA). Academic Press, London, New York.

Phillips RB, Pleyte KA (1991) Nuclear DNA and salmonid phylogenetics. *Journal of Fish Biology* **39 (Supplement A)**, 259-275.

Phillips RB, Pleyte KA, Ihssen PE (1989a) Patterns of chromosomal nucleolar organiser (NOR) variation in fishes of the genus *Salvelinus*. *Copeia* **1989**, 47-53.

Phillips RB, Van Ert LM, Pleyte KA (1989b) Evolution of nucleolar organiser regions (NORs) and rDNA in fishes of the genus *Salvelinus*. *Physiology and Ecology of Japan* **Special Volume 1**, 429-447.

Piertney SB, MacColl ADC, Bacon PJ*, et al.* (2000) Matrilineal genetic structure and female-mediated gene flow in red grouse (Lagopus lagopus scoticus): An analysis using mitochondrial DNA. *Evolution* **54**, 279-289.

Piggins DJ (1974) The results of selective breeding from known grilse and salmon parents, pp. 35-39. Annual report of the Salmon Research Trust of Ireland XVIII.

Planes S, Galzin R, Bonhomme F (1996) A genetic metapopulation model for reef fishes in oceanic islands: The case of the surgeonfish, Acanthurus triostegus. *Journal of Evolutionary Biology* **9**, 103-117.

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic cod, Gadus morhua, at large and small geographic scales. *Evolution* **55**, 131-146.

Porter TR, Healey MC, O'Connell MF (1986) Implications of varying the sea-age at maturity of Atlantic salmon (*Salmo salar*) on yield to the fisheries. In: *Salmon Age at Maturity* (ed. Meerburg DJ), pp. 102-109. Canadian Special Publication of Aquatic Sciences 89.

Power G (1981) Stock characteristics and catches of Atlantic salmon (*Salmo salar*) in Quebec, and Newfoundland and Labrador in relation to environmental variables. *Canadian Journal of Fisheries and Aquatic Science* **38**, 1601-1611.

Primmer CR, Koskinen MT, Piironen J (2000) The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 1699-1704.

Prouzet P (1990) Stock charactersitics of Atlantic salmon (*Salmo salar*) in France: a review. *Aquatic Living Resources* **3**, 85-97.

Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity* **88**, 161-165.

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics* **144**, 383-387.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* **43**, 258-275.

Quinn TP, Adkison MD, Ward MB (1996) Behavioural tactics of male sockeye salmon (Oncoryhnchus nerka) under varying operational sex ratios. *Ethology* **102**, 304-322.

Qvarnstrom A (1999) Genotype-by-environment interactions in the determination of the size of a secondary sexual character in the collared flycatcher (Ficedula albicollis). *Evolution* **53**, 1564-1572.

Raddum GG, Fjellheim A (1995) Artificial Deposition of Eggs of Atlantic Salmon (Salmo-Salar L) in a Regulated Norwegian River - Hatching, Dispersal and Growth of the Fry. *Regulated Rivers-Research & Management* **10**, 169-180.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 9197-9201.

Réale D, Festa-Bianchet M, Jorgenson JT (1999) Heritability of body mass varies with age and season in wild bighorn sheep. *Heredity* **83**, 526-532.

Reynolds JD (1996) Animal breeding systems. *Trends in Ecology & Evolution* **11**, A68-A72.

Rice WR (1989) Analysing tables of statistical tests. *Evolution* **43**, 223-225.

Ricker WE (1972) Hereditary and environmental factors affecting certain salmonid populations. In: *The Stock Concept in Pacific Salmon* (eds. Simon RC, Larkin PA). University of British Columbia, Vancouver.

Robitaille JA, Coté Y, Shooner G, Hayeur G (1986) Growth and maturation patterns of Atlantic salmon, Salmo salar, in the Koksoak River, Ungava, Quebec. *Canadian Special Publications in Aquatic Science* **89**, 62-69.

Rochet M-J (2000) A comparative approach to life-history strategies and tactics among four orders of teleost fish. *ICES Journal of Marine Science* **57**, 228-239.

Roff DA (1992) *The Evolution of Life Histories* Chapman & Hall, New York.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F- statistics under isolation by distance. *Genetics* **145**, 1219-1228.

Ruzzante DE (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Science* **55**, 1-14.

Saiki RK, Gelfand DH, Stoffel S, *et al.* (1988) Primer-directed enzymatic amplification of DNA with

thermostable DNA polymerase. *Science* **239**, 487-491.

Sandlund OT, Gunnarsson K, Jónasson PM, *et al.* (1992) The Arctic charr *Salvelinus alpinus* in Thingvallavatn. *OIKOS* **64**, 305-351.

Sanford CpJ (1990) The phylogenetic relationships of salmonid fishes. *Bulletin of the Britsh Museum (Natural History), Zoology* **56**, 145-153.

Saunders RL (1967) Seasonal pattern of return of Atlantic salmon in the Northwest Miramichi River, New Brunswick. *Journal of the Fisheries Research Board of Canada* **24**, 21-32.

Saunders RL (1969) Contributions of salmon from the North-West Miramichi River, New Brunswick, to various fisheries. *Journal of the Fisheries Research Board of Canada* **26**, 269-278.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma* **109**, 365-371.

Schlötterer C, Wiehe T (1999) Microsatellites, a neutral marker to infer selective sweeps. In: *Microsatellites: Evolution and applications* (eds. Goldstein DB, Schlötterer C), pp. 238-247. Oxford University Press, Oxford.

Schneider S, Roessli D, Excoffier L (2000) Arlequin 2.001 A software for population genetics data analysis.

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. In: *Fisheries Research Board of Canada Bulletin.*

Shaposhnikova GK (1968) A comparative morphological study of taimen [Hucho Günther] and lenok [Brachymystax Günther]. *Problems of Ichthyology (English translation)* **8**, 351-370.

Shaposhnikova GK (1971) A comparative morphological description of Lake Sevan whitefishes of the genus *Coregonus*. *Journal of Ichthyology* **11**, 474-484.

Shaposhnikova GK (1975) Systematic relation of some representatives of the family Salmonidae. *Ichthyologia* **7**, 61-70.

Shearer WM (1986) An evaluation of the data available to assess Scottish salmon stocks. In: *The Status of Atlantic Salmon in Scotland* (eds. Jenkins D, Shearer WM). Institute of Terrestrial Ecology Symposium Number 15.

Shearer WM (1992) *The Atlantic Salmon: Natural History, Explotation and Future Management* Fishing News Books, Oxford.

Shields WM (1993) The natural and unnatural history of inbreeding and outbreeding. In: *The Natural History of Inbreeding and Outnreeding: Theoretical and Empirical Perspectives* (ed. Thornhill NW). University of Chicago Press, Chicago.

Shrimpton JM, Heath DD (2003) Census vs. effective population size in chinook salmon: large- and small-scale environmental perturbation effects. *Molecular Ecology* **12**, 2571-2583.

Sia EA, Jinks-Robertson S, Petes TD (1997) Genetic comtrol of microsatellite instability. *Mutation Research* **383**, 61-70.

Sibley RM, Calow P (1986) *Physiological Ecology of Animals, an Evolutionary Approach* Blackwell Scientific, Oxford, England.

Simon RC, McIntyre JD, Hemmingsen AR (1986) Family-Size and Effective Population-Size in a Hatchery Stock of Coho Salmon (Oncorhynchus-Kisutch). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 2434-2442.

Skurikhina LA, Tugarina PY, Mednikov BM (1986) Systematics of Thymallidae (*Salmoniformes*) based on DNA x DNA hybridization. *Journal of Ichthyology* **26**, 24-29.

Slatkin M (1993) Isolation by Distance in Equilibrium and Nonequilibrium Populations. *Evolution* **47**, 264-279.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frquencies. *Genetics* **139**, 457-462.

Slettan A, Olsaker I, Lie O (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311 , SSOSL444 loci. *Animal Genetics* **26**, 281-282.

Slettan A, Olsaker I, Lie O (1996) Polymorphic Atlantic salmon, *Salmo salar* L. microsatellites at the SSOSL438, SSOSL429 and SSOSL444 loci. *Animal Genetics* **27**, 57-58.

Small I (1995) River Eden 1941-94, 'spring' slamon + grilse: exploratory estimates of the trends of runs of fish into the river and exploitation rates.

Smith GR, Stearley RF (1989) Thr classification and scientific names of rainbow and cutthroat trouts. *Fisheries* **14**, 4-10.

Soulé ME (1980) Thresholds for survival: maintaining fitness and evolutionary potential. In: *Conservation Biology: An Evolutionary-Ecological Perspective* (eds. Soulé ME, Wilcox B). Sinauer, Sunderland, MA.

Soule ME, Mills LS (1998) Population genetics - No need to isolate genetics. *Science* **282**, 1658-1659.

Stabell OB (1984) Homing and olfaction in slamonids: a critical review with special reference to the Atlantic salmon. *Biological Reviews* **59**, 333-388.

Ståhl G (1987) Genetic population structure of Atlantic salmon. In: *Populations Genetics and Fishery Management* (eds. Ryman N, Utter FM), pp. 121-140. University of Washington Press, Seattle.

Stasko AB, Sutterlin AM, Rommel SA, Elson PF (1973) Migration-orientation of Atlantic salmon (*Salmo salar* L.) **4**, 119-137.

Stearley RF (1990) *Histroical biology of Oncorhynchus*, University of Michigan.

Stearley RF, Smith GR (1993) Phylogeny of the Pacific trouts and salmons (Oncorhynchus) and genera of the family Salmonidae. *Transactions of the American Fisheries Society* **122**, 122-133.

Stearns SC (1992) *The Evolution of Life Histories* Oxford University Press, London.

Stenseth NC, Mysterud A, Ottersen G, *et al.* (2002) Ecological effects of climate fluctuations. *Science* **297**, 1292-1296.

Storz JF, Ramakrishnan U, Alberts SC (2001) Determinants of Effective Populations Size for Loci with Different Modes of Inheritance. *Journal of Heredity* **92**, 497-502.

Struthers G (1984) Comparison of adult returns in the River Tay net and coble and rod fisheries from salmon smolt tagging experiments in two tributaries of the River Tay. International Council for the Exploration of the Sea.

Sundstrom L, Keller L, Chapuisat M (2003) Inbreeding and sex-biased gene flow in the ant Formica exsecta. *Evolution* **57**, 1552-1561.

Surridge AK, Ibrahim KM, Bell DJ, *et al.* (1999) Fine-scale genetic structuring in a natural population of European wild rabbits (Oryctolagus cuniculus). *Molecular Ecology* **8**, 299-307.

Svendsen JI, Astakhov VI, Bolshiyanov DY, *et al.* (1999) Maximum extent of the Eurasian ice sheets in the Barents and Kara Sea region during the Weichselian. *Boreas* **28**, 234-242.

Taborsky B (1999) Conflict or cooperation: what determines optimal solutions to competition in fish reproduction? In: *Behaviour and Conservation of Littoral Fishes* (eds. Oliveira R, Almada V, Goncalves E), pp. 301-349. ISPA, Lisbon.

Taggart JB, McLaren IS, Hay DW, Webb JH, Youngson AF (2001) Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Molecular Ecology* **10**, 1047-1060.

Taggart JB, Verspoor E, Galvin E, Morán P, Fergusson A (1995) A minisatellite marker for discriminating between European and North American Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* **52**, 2305-2311.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**, 389-399.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**, 6463-6471.

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

Tchernavin V (1944) The breeding characters of slamon in relation to their size. *Proceedings of the Zoological Society of London* **113B**, 206-232.

Thedinga JF, Wertheimer AC, Heintz RA, Maselko JM, Rice SD (2000) Effects of stock, coded-wire tagging, and transplant on straying of pink salmon (Oncorhynchus gorbuscha) in southeastern Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2076-2085.

Thomas WK, Withler RE, Beckenbach AT (1986) Mitochondrial DNA anlaysis of Pacific salmonid evolution. *Canadian Journal of Zoology* **64**, 1058-1064.

Thorpe JE, Mangel M, Metcalfe NB, Huntingford FA (1998) Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. *Evolutionary Ecology* **12**, 581-599.

Thorpe JE, Morgan RIG (1978) Parental influence on growth rate, smolting rate and survival in hatchery-reared juvenile Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **13**, 549-556.

Todd CD, Lambert WJ, Thorpe JP (1998) The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae "for" dispersal? *Journal of Experimental Marine Biology and Ecology* **228**, 1-28.

Tsuyuki H, Roberts E (1966) Inter-species relationships within the genus *Onchorynchus* based on biochemical systematics. *Journal Fisheries Research Board of Canada* **23**, 101-107.

Tunnicliffe V, Fowler CMR (1996) Influence of sea-floor spreading on the global hydrothermal vent fauna. *Nature* **379**, 531-533.

Utter FM, Allendorf FW (1994) Phylogenetic relationships among species of *Oncorhynchus*: A consensus view. *Conservation Biology* **8**, 864-867.

Utter FM, Allendorf FW, Hodgins HO (1973) Gebnetic variability and relationships in Pacific salmon and related trout based on protein variation. *Systematic Zoology* **22**, 257-270.

Van der Strate HJ, Van de Zande L, Stam WT, Haroun RJ, Olsen JL (2003) Within-island differentiation and between-island homogeneity: non-equilibrium population structure in the seaweed Cladophoropsis membranacea (Chlorophyta) in the Canary Islands. *European Journal of Phycology* **38**, 15-23.

Ventura JAM (1988) The Atlantic salmon in Austrias, Spain: analysis of catches, 1985-1986. Inventory of juvenile densities. In: *Atlantic Salmon: Planning for the Future. Proceedings of the Third International Atlantic Salmon Symposium* (eds. Mills D, Piggins D). Atlantic Salmon Trust.

Verboom B, Huitema H (1997) The importance of linear landscape elements for the pipistrelle Pipistrellus pipistrellus and the serotine bat Eptesicus serotinus. *Landscape Ecology* **12**, 117-125.

Verspoor E, Fraser NHC, Youngson AF (1991) Protein polymorphism in Atlantic salmon within a Scottish river: evidence for selection and estimates of gene flow between tributaries. *Aquaculture* **98**, 217-230.

Verspoor E, Jordan WC (1989) Genetic variation at the Me-2 locus in Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology* **35 (Suppl. A)**, 205-213.

Verspoor E, McCarthy E (1997) Genetic divergence at the NAD+ dependent malic enzyme locus in Atlantic salmon from Europe and North America. *Journal of Fish Biology* **50**, 155-163.

Vladykov VD (1962) Osteological studies on Pacific salmon of the genus *Oncorhynchus*. *Bulletin of the Fisheries Research Board of Canada* **136**, 1-172.

Vladykov VD (1963) A review of salmonid genera and thier broad geographical distribution. *Transactions of the Royal Society of Canada* **Volume 1, Series IV, Sextion III**, 459-504.

Vucitech JA, Waite TA, Nunney L (1997) Fluctuating population size and the ratio of effective to census population size (Ne /N). *Evolution* **51**, 2015-2019.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506-513.

Wang J (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research* **78**, 243-257.

Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**, 429-446.

Waples RS (1989) A Generalized-Approach for Estimating Effective Population-Size from Temporal Changes in Allele Frequency. *Genetics* **121**, 379-391.

Waples RS (1990) Conservation of Pacific Salmon II. Estimating effective population size and the rate of loss of genetic variability. *Journal of Heredity* **81**, 267-276.

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

Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology & Evolution* **13**, 43-44.

Waters JM, Epifanio JM, Gunter T, Brown BL (2000) Homing behaviour facilitates subtle genetic differentiation among river populations of *Alosa sapidissima*: microsatellites and mtDNA. *Journal of Fish Biology* **56**, 622-636.

Webb JH (1989) The movements of Atlantic salmon in the River Tay.

Webb JH (1992) The behaviour of adult salmon (*Salmo salar* L.) in the River Tay as determined by radio telemetry.

Webb JH, Hawkins AD (1989) The movements and spawning behaviour of adult salmon in the Grinock Burn, A tributary of the Aberdeenshire Dee, 1986. Department of Africulture and Fisheries for Scotland.

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* **44**, 388-396.

Weber JL, Wong C (1993) Mutation of humans short tandem repeats. *Human Molecular Genetics* **2**, 1123-1128.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

White HC, Huntsman AG (1938) Is local behaviour in salmon heritable? *Journal of the Fisheries Research Board Canada* **4**, 1-18.

Wilder DG (1947) A comparative study of the Atlantic salmon, *Salmo salar* (Linnaeus), and the Lake Salmon, (*Salmo salar sebago* Girard). *Canadian Journal of Research Sect. D.* **25**, 175-189.

Williams RRG (1987) *The phylogenetic relationships of the salmoniform fishes based on the suspensorium and its muscles*, University of Alberta.

Williamson RB (1988) Status and exploitation of Atlantic salmon in Scotland. In: *Atlantic Salmon: PLanning for the the Future. Proceedings of the Third International Atlantic Salmon Symposium* (eds. Mills D, Piggins D). Atlantic Salmon Trust.

Wilson IJ (1996) *Genetic Differentiation in Spatially Structured Populations with Particular Regard to the Atlantic salmon (Salmo salar L.)*, University of Edinburgh.

Wilson MVH, Li G (1999) Osteology and systematic position of the Eocene salmonid *Eosalmo driftwoodensis* Wilson from western North America. *Zoological Journal of the Linnean Society* **125**, 279-311.

Windsor ML, Hutchinson P (1988) International cooperation through NASCO. In: *Atlantic Salmon: planning for the Future* (eds. Mills D, Piggins D), pp. 1-11. The Atlantic Salmon Trust.

Wootton RJ (1990) *Ecology of Teleost Fishes* Chapman & Hall, London.

Wright S (1921) Systems of mating. *Genetics* **6**, 111-178.

Wright S (1931) Evolution in Mendelian Populations. *Genetics* **16**, 97-159.

Wright S (1943) Isolation by distance. *Genetics* **28**, 114-138.

Wright S (1969) *The genetical structure of populations* University of Chicago Press, Chicago.

WWF (2001) The status of wild Atlantic salmon: a river by river assessment. World Wildlife Fund.

Youngson AF, Hay DW (1996) *The Lives of Salmon* Swan Hill Press.

Youngson AF, Jordan WC, Hay DW (1994) Homing of Atlantic Salmon (Salmo-Salar L) to a Tributary Spawning Stream in a Major River Catchment. *Aquaculture* **121**, 259-267.

Zelinsky YP (1985) *Structure and Differentiation of Forms in Atlantic salmon* Nauka, Leningrad.