HABITAT-SPECIFIC GENETIC AND PHENOTYPIC DIFFERENTIATION IN JUVENILE COHO SALMON

by

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ABSTRACT

Juvenile coho (*Oncorhynchus kisutch*) will spawn and have their offspring reared in very different habitat types in coastal British Columbian streams. For instance, some utilize main channel river areas whereas others take advantage of off-channel habitats. However, during winter, juveniles produced in the main channel areas are believed to move into off-channel habitats for reasons of protection. These main channel juveniles tend to be much larger at the start of winter than the off-channel resident juveniles. It is possible that these two size morphs reflect genetic differentiation that would indicate either the fine-scale population structure or heritable growth-related differences between individuals using different habitats. On the other hand, the variation may simply reflect phenotypic plasticity. There is little understanding of the correspondence between neutral molecular genetic variation and adaptive variation, yet patterns of molecular variation are most often used to develop management plans directed towards conserving genetic diversity in salmon populations. This study describes variation for both microsatellites (neutral genetic markers) and growth, presumably an adaptive trait, in closely located populations of coho salmon.

The genetic basis of the observed size variation was explored by studying juvenile coho from the Mamquam River. After 6 months rearing in a common environment, no size differences were observed between juveniles originating from different habitats. More variation for size was observed between individuals sampled from within habitat types than was observed among habitats. This result suggested that the size variation is a result of phenotypic plasticity associated with environmental differences in rearing habitat. It does not exclude the possibility that variation for growth among individuals is associated with adaptive differences on a microhabitat scale. Microsatellite analysis revealed fine-scale population structure within the Mamquam River resulting from either founder effects associated with channel colonisation, the existence of separate races, or sampling bias. Heritability for growth over the six-month rearing period and size were estimated by a novel technique that uses molecular marker similarity to infer relatedness. Positive co-variances were observed between relatedness and phenotypic similarity for growth rate and initial size indicating heritability for these traits.

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Chapter 1 GENERAL INTRODUCTION

Over the last century and particularly in the past 20 years, coho salmon (*Oncorhynchus kisutch*) populations have shown dramatic declines in abundance throughout the southern half of their range in North America (Northcote and Atagi 1997). In British Columbia, the 1998 commercial and sport fishery was closed for the first time in response to record low levels of escapement. Similarly, several populations in California, Oregon and Washington are now considered extinct or endangered (Nehlson et al. 1991, Moyle 1994). Both biotic and abiotic factors have been blamed for the decline. These factors include overfishing; destruction of freshwater habitat by development, forestry, dams, and pollution; genetic deterioration through misguided stock enhancement efforts; and climatic conditions resulting in lower ocean productivity and decreased marine survival (Walters 1993, Fraser et al. 1992, Beamish and Bouillon 1993). Coho have tremendous cultural, economic and biological importance and concern for their preservation has motivated research to document patterns of biodiversity within the species. Understanding the structure of genetic diversity would allow more intelligent decisions to be made with regard to both sustainable exploitation and conservation of the species.

Coho, like all species of Pacific salmon, show a remarkably high rate of natal site fidelity. This spawning behaviour often results in reproductive isolation between populations and creates the potential for local adaptation. Indeed, phenotypic variation for behavioural, morphological and ecological traits is common and often observed on very small scales (Taylor 1991, Carvalho 1993). When the covariance for these traits among related individuals or populations is determined, the genetic component can be quantified (Falconer and Mackay 1996). This information is not widely available for coho and typically descriptions of population structure are based on patterns of neutral biochemical variation using protein isozymes and more recently using DNA-based techniques such as microsatellites. However, the relationship between molecular variation and phenotypic diversity in salmon is not well understood (Hedgecock et al. 1994). For one thing, phenotypic traits are often quantitative traits under the control of several to many interacting genes and at the same time modified by environmental variation. Separating genetic components from environmental effects is difficult and not always meaningful. Secondly, the type of information gained from molecular markers is selectively neutral which may not reveal adaptive differences between populations. Certainly, many populations showing striking phenotypic differences cannot be differentiated by molecular markers (Healey 1991). Molecular similarity thus does not mean populations are genetically identical for traits under selection (Lewontin 1989).

One goal of studies describing genetic and phenotypic variation within and among salmon populations is to elucidate unique units for conservation and stock management (Small et al. 1998, Beacham et al. 1998). Traditionally, in salmon management, local populations have been viewed as unique, persistent, and locally adapted stocks (Ricker 1972). However, more recently the idea of the Evolutionary Significant Unit has become prominent (Waples 1995). ESUs are defined according to geographic units showing unique evolutionary and ecological

characteristics shared among populations within them. For example, genetic differentiation between upper and lower Fraser River coho stocks reflects patterns of re-colonisation of the Fraser by coho from different glacial refugia (Small et al. 1998, McPhail 1997). The genetic differentiation is concordant with phenotypic differences in morphology and behaviour observed between the regions that are, in turn, attributed to different selection pressures (Taylor and McPhail 1985a, b). However, for conservation and management it is also important to understand the impact of shorter-term micro-evolutionary processes over more restricted geographic scales. Most specifically of interest is the distribution of genetic variation for adaptive traits within localised populations. This variation operates on temporal and spatial scales that are potentially affected by selection arising from factors such as environmental degradation or stock enhancement.

In this study, patterns of genetic and phenotypic diversity were investigated in populations of coho salmon that are spawned in close spatial proximity. The specific purpose was to describe genetic variation for groups occupying different habitats on the Mamquam River. The Mamquam is a coastal river located in the Lower Mainland of British Columbia. In an attempt to help restore and enhance populations of chum (*O. keta*) and coho salmon, the Canadian Department of Fisheries and Oceans (DFO) constructed several artificial spawning channels in the Mamquam watershed. These channels also provide overwinter refugia for juvenile coho. The winter period is often considered crucial for mortality of juvenile coho because of limited habitat and other factors. Juveniles experience little or no growth during this period, yet size is positively correlated with survival. By December, great variation in size is apparent in overwintering fry from the Mamquam River (Figure 1). These phenotypes appeared to be associated with specific habitats. Larger fry (averaging approximately 8 cm) did not appear in off-channel (artificial spawning channels) habitat until winter and probably migrated there from

main channel habitat. Smaller fry (averaging approximately 5 cm) appeared to be summer residents of the channel remaining there after emergence and throughout their summer residency. The goal of my study was to determine whether or not the variation in observed phenotypes had a genetic basis or simply resulted from phenotypic plasticity in response to environmental variation in habitat.

Three approaches were used to address this question. First, fish were removed from their habitats and reared in a common laboratory environment under controlled conditions for the 6-month period from summer emergence to winter. This 'common garden' experiment allowed the genetic component of juvenile growth rate to be studied by controlling the environmental component of variance for this part of their life. Secondly, molecular variation was described using microsatellite DNA markers. Microsatellites are hypervariable nuclear loci containing simple sequence repeats of 1 to 6 base pairs. Sub-populations from within the Mamquam River were assayed as well as populations from the Cheakamus and Vedder Rivers to allow comparison with nearby populations isolated by distance. Using the microsatellite variation to infer relatedness among individuals, these data sets were combined in order to estimate the heritability (h^2) of size-related traits. Estimating heritability from inferred relatedness based on biochemical variation is a novel technique developed by Ritland (1996) and others (Lynch 1988, Queller and Goodnight 1989). Since both quantitative and molecular approaches will be used, the results allow a comparison between molecular variation and variation for quantitative traits in the population studied. This association is not understood (Mitchell-Olds 1995, Cheverund 1988). However, in the field of conservation biology it is often assumed that measures of molecular variation, such as heterozygosity, are directly related to population viability by indicating future adaptive potential and low levels of inbreeding (Hedrick and Miller 1992).

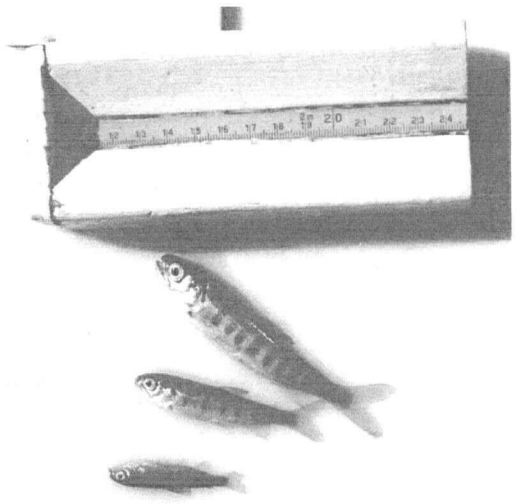


Figure 1. Photograph demonstrating size variation among similar aged overwintering fry from the Mamquam River.

1.1 Background Literature Review

Pacific Salmon are an important resource. The commercial fishery in 1997 landed over CDN $90 million wholesale (Catch Statistics Unit, Canadian Department of Fisheries and Oceans). In addition, recreational fisheries in North America make a significant contribution to the economy particularly with the coho and chinook sport fisheries. Salmon also hold enormous cultural importance for the indigenous peoples of British Columbia. Most importantly, they are a functional component of coastal and interior freshwater ecosystems. Decaying carcasses from annual spawning runs increase the productivity of streams and provide a substantial protein source for wildlife such as bears and eagles.

Coho salmon (*Oncorhynchus kisutch*) are one of seven Pacific salmon (*Oncorhynchus spp.)* species native to North America. Other species include sockeye (*O. nerka*), pink (*O. gorbuscha*), chum (*O. keta*), and chinook (*O. tshawytscha*). Recently, steelhead and cutthroat

trout have been re-classified in the salmon genus. Masu (*O. masou*) and Amago (*O. masu ishikawai*) salmon are native to Asia. The life cycle of Pacific salmon is complex. Most species are anadromous; spawning in freshwater in rivers or lakes, migrating to the ocean as smolts, maturing whilst undergoing complex ocean migrations in north Pacific Ocean feeding grounds, and then returning to their native stream for spawning and subsequent death. They are able to exploit a wide variety of habitats including streams, lakes, marshes, estuaries, and inshore and offshore marine habitat. Species can be distinguished from one another by their morphology and life history characteristics. For instance, sockeye rear predominately in nursery lakes whereas coho and chinook rear in streams. In addition, there is significant variation for morphological, behavioural, and ecological traits among populations of the same species.

1.1.1 Ecology and Life History of Coho Salmon

The ecology and life history of coho has been reviewed in Sandercock (1991). Coho is an endemic species throughout the North Pacific Basin. Populations have also been introduced, with varying degrees of success, to other cold temperate regions. The most notable introduction was to the Great Lakes, where a significant sport fishery is now maintained predominately via hatchery introductions (Sandercock 1991). In British Columbia, coho is the most geographically widespread of all Pacific salmon species spawning in at least 970 of 1500 known spawning streams (Slaney et al. 1996). Like other species of Pacific Salmon, coho are anadromous, returning to their native stream to spawn. Their homing ability is remarkable. Spawners recognize their native stream through olfaction, but the mechanism of how they navigate there after long ocean migrations is not well understood. In tagging experiments of chum salmon, a related species, rates of straying between streams were estimated to be lower than 1% (Tallman and Healey 1994).

The life cycle begins with spawning. Most adult coho spawn in the autumn although there is a large amount of variation in the timing of spawning between populations. Populations in more northern latitudes generally spawn earlier, but there is also considerable variation between the first and last spawner within many populations. Many spawners are opportunistic, holding in rivers for extended periods of time and delaying spawning until environmental conditions are favourable. Other species of Pacific salmon, such as sockeye or chinook, tend to spawn within a narrower time period (Sandercock 1991). Timing of spawning is correlated with stream flow and temperature. Coho show less restrictive requirements than related salmon species for spawning habitat. Populations have been observed spawning over a variety of locations and habitats, which may explain their widespread distribution throughout British Columbia and the Pacific Northwest.

By the time they reach sexual maturity, adults reach a size of approximately sixty cm and weigh around four kg. During freshwater migration to spawning streams, coho can leap up to two meters to cross barriers (Sandercock 1991). On reaching their spawning area, females stake out territories within the stream (~11-12m^2) in order to dig nests, called redds. One dominant male usually courts a female. When she is ready to mate he swims along side her and they simultaneously release eggs and milt. Afterward, she swims upstream to dig up gravel to cover up the nest and then build a new nest. The fecundity of females varies by population generally showing a north-south cline with more fecund females in northern locations. The number of eggs per female can range from 2000-5000. Within two weeks of spawning both female and male adults die. Spawning runs provide an important source of protein to the ecosystem. Decaying carcasses also increase the primary productivity of the stream and likely the recruitment of new cohorts by providing a winter food source for juveniles (Bilby et al. 1998).

Precocious males, called 'jacks', are an additional and interesting factor in the reproductive life history of salmon. Jacks are sexually mature undersized males that return to spawn the same year they smolt. They are much smaller than either adult females or males and do not show adult colouring. Jacks gain access to females by sneaking in to deposit their milt alongside the dominant male. Gross (1985) suggested that this behaviour is an Evolutionarily Stable Strategy (ESS). Jacks avoid the risk of mortality an extra year of ocean life brings at the cost of reduced access to females. However, there is some debate about the evolutionary mechanism behind reproductive strategies (Healey and Prince 1995). There is great variation in the proportion of jacks in different coho populations. The proportion of jacks returning from hatchery populations has been associated with factors such as the timing and size of juveniles at smoltification.

Over the winter, eggs incubate in gravel. Survival of eggs is dependent on a number of factors including physical characteristics of the stream such as flow, temperature, silt load, and concentration of dissolved oxygen. Late spawners may destroy the nests of early spawners and predation is also a significant cause of mortality. Eggs that survive the incubation period hatch in spring. The timing of hatching is correlated with stream temperature. The hatched eggs, called alevin, still have yokes attached. Once the yoke is absorbed the juvenile coho emerge from the gravel as fry. For the next year or more fry are resident in fresh water before migrating to the ocean as a smolt. The proportion of juveniles spending more than one year in freshwater depends on the population. In latitudes that are more northerly there tends to be a higher proportion of two-year old and even older smolts. This pattern likely reflects the shorter periods favourable for growth in northern climates.

After emergence, coho fry may migrate to different parts of the watershed in order to find suitable rearing habitat. Some fry move into lakes and use them as nurseries while others use stream habitats (Swain and Holtby 1989). During this period of their life they are vulnerable to

predation so habitat that provides cover becomes an important feature. Coho juveniles use logs and boulders for cover as well as structural features of the stream such as overhanging roots that form undercuts. In general, a more structurally complex stream habitat seems to support a higher density of fry. Their diet consists largely of drifting invertebrates, but older fry will eat juvenile salmonids including alevin of their own species. They are diurnal feeders and territorial behaviour is common. In slow-moving reaches of the stream or in lakes schooling behaviour is often exhibited. Within these schools relative social status may be important. Hierarchies have been observed with dominant fish in front, prime feeding positions and sub-dominants behind (Nielson 1992). In slower reaches of the stream, fry may actively defend feeding territories by displays of aggressive behaviour (Hartman 1965).

Juveniles are not distributed randomly within freshwater habitat. Some areas of the stream provide more opportunities for feeding and cover and therefore support a higher density of fry. Habitat preferences show marked changes over time depending on the season and environmental conditions. For example, during the summer, main channel reaches provide a more productive habitat for coho fry due to drift of invertebrates and other sources of food from upstream reaches. Water temperature in the surface-fed main channels is also higher than in side-channels that are typically groundwater fed. The higher temperatures allow for greater levels of primary production. However, in winter main channel habitats are less suitable for fry since frequent freshets can displace them downstream. Thus, coho seek overwintering refuge off the main channel in areas with slower stream velocity (Skeesick 1970, Bustard and Narver 1975). It is often suggested that one bottleneck to coho production may be the limitation of suitable overwintering habitat for juveniles in fresh water (Swales et al. 1988). In southwestern BC, some of the best winter refuge available may be the artificial side-channels originally created for enhanced chum spawning (Decker 1999). Low flows and higher temperatures in these habitats

allow the fish to conserve energy, which is crucial, considering they experience little growth over the winter (Weatherley and Gill 1995). Artificial channels replace habitat features that have historically been destroyed through logging, development, road building and other activities that impact riparian areas.

After at least a year in fresh water, juveniles migrate to the ocean following a physiological change called smolting. Changes in colouring and behaviour signal they are beginning to smolt. Factors that affect their timing of migration include day length, water temperature, and concentration of dissolved oxygen, flow conditions and food availability (Holtby et al. 1989). Smolting juveniles become less defensive of their territories and eventually move downstream in schools. Smolts are generally at least 10 cm in length and it has been suggested that this represents a threshold for marine survival (Brannon et al. 1982). On reaching estuaries, the smolts are very susceptible to predation. However, if they survive downstream migration they undergo high growth rates in oceanic conditions, estimated at 1.1mm/day for the first 6 months (Healey 1980). Their diet initially consists of invertebrates but they gradually become more piscivorous. The estimated rate of survival from smolt to adult in hatchery populations ranges from 10-40% depending on the timing of release (Bilton et al. 1980). With the exception of jacks, coho generally spend two years maturing at sea before returning to their native stream to begin the life cycle over again. Levels of mortality in the ocean-going stages of the coho lifecycle are estimated by escapement data, but the ecology and behaviour of coho during this period are largely unknown.

1.1.2 Phenotypic Variation

Considerable variation is observed for morphological, meristic, behavioural and reproductive traits among coho populations (Taylor 1991, Carvalho 1993). Life history characteristics, such as fecundity, egg size, and the timing of alevin emergence, smolting and spawning, often show

north-south clines corresponding to temperature and other environmental factors associated with latitude (Sandercock 1991). More northerly stocks tend to have a greater proportion of two-year-old smolts, and this trend has been attributed to the shorter summer growing periods. As well, the timing of emergence in northern stocks is later while the spawning runs occur as early as August. Notwithstanding these trends, quite a few exceptions exist. For instance, two-year-old smolts used to be common in Carnation Creek, situated on the southwest corner of Vancouver Island, until the watershed was logged. Since then most smolts are only one year old. Warmer stream temperatures from the cleared riparian area caused faster fry growth leading to earlier smolting (Holtby et al. 1989). Thus disturbance, such as the impact of streamside logging, can increase the year-to-year variation observed in populations by changing temperature and other physical variables of stream habitat (Scrivener 1987). Different stocks also show variation for levels of infection and resistance to parasites, disease, and fungal infection (Quinn et al. 1987). They also vary in physiological characteristics such as pH tolerance (Carvalho 1993). Moreover, while coho do not normally exhibit multiple spawning runs, a few rivers are reported to have two distinct runs (Sandercock 1991).

Phenotypic differences among populations are often associated with habitat use. For example, Taylor and McPhail (1985a,b) were able to differentiate interior and coastal forms of coho in British Columbia through morphological and behavioural differences. Coastal forms have large median fins and a deep, robust body form in contrast to interior forms that have small-median fins and more streamlined bodies. They associated the difference with swimming performance and, indeed, laboratory experiments showed interior juveniles had greater swimming stamina, while coastal juveniles showed better 'fast-start' capability. The distinction probably represents trade-offs for adaptive variation associated with spawning and reproductive fitness (Taylor and McPhail 1985b). The spawning journey ranges from several hundred kilometres for interior BC

stocks to a few hundred meters for coastal BC coho. Thus, interior stocks may be selected for qualities imparting swimming efficiency and endurance whereas coastal forms may be selected for aggressive behaviour to defend redds and mates. When grown in a common environment, individuals from these populations continued to show the same morphological and behavioural differences suggesting the traits were inherited (Taylor and McPhail 1985b). Recent molecular genetic data verify these results with interior and coastal forms showing distinct patterns of microsatellite DNA variation (Small et al. 1998).

On a finer geographic scale, Swain and Holtby (1989) associated morphological and behavioural differences in juvenile coho with two rearing habitats located within the same watershed on Vancouver Island. Juvenile coho reared in Mesachie Lake showed fewer displays of aggressive, territorial behaviour and had more streamlined bodies than those rearing in the outlet stream of the lake. These differences likely had adaptive significance. Lake-reared juveniles school and swim for prolonged periods whereas stream-reared juveniles guard territories with short periods of burst swimming in an aggressive display to competitors. When reared in a common laboratory environment, the fish continued to show distinctions suggesting the variation is either genetic or the result of environmental variation at a very early life history stage. Similarly, Roseneau and McPhail (1987) showed inherited differences in agonistic behaviour between two closely located populations of coho within the same watershed. Morphological differences between local populations were also observed among juvenile coho living within a small watershed on northern Vancouver Island (Bailey and Irvine 1991).

On an even smaller scale, Nielson (1992) described a complex foraging phenotypic polymorphism in juvenile coho rearing in a Washington stream. The polymorphism was associated with microhabitat use. By studying physical differences between habitats within the stream, such as flow rate, cover, and invertebrate drift, as well as behavioural, feeding, growth

and morphological characteristics of the fish, Nielson (1992) identified 3 behavioural ecomorphs. 'Dominant' and 'sub-dominant' fish were found in the faster reaches of the stream. They exhibited schooling behaviour, showed the highest instantaneous rate of growth over the summer period, and fed mainly on invertebrate drift. 'Floaters', on the other hand, reared in pools. They showed territorial behaviour, lower rates of growth, and fed mainly on invertebrate fall. Neilson (1992) suggested differences between the fish resulted from the timing of emergence, relative fitness; genetics or the early dietary experience of the juveniles. Either way, this study and the others described show that phenotypic variation in salmon is linked to habitat variation on scales ranging from microhabitat to broad geographic distances.

1.1.2.1 Growth variation in juvenile salmonids

Salmon and other fish species are also known to show significant variation in growth patterns, both among and within populations (Kirkpatrick and Selender 1979, Thorpe 1987, Ferguson and Mason 1981, Ryman et al. 1978, Wood and Foote 1996). Variation for growth is significant because size is a trait that often affects the fitness and survival of an individual. For example, larger individuals may possess a status advantage when competing for resources with their smaller counterparts. Similarly, larger size can positively affect survival during crucial periods of mortality such as the overwintering period of juveniles in freshwater habitat or the process of smoltification. On the other hand, large size may be disadvantageous in situations where size-dependent predation is biased toward larger individuals.

The size of an organism is affected by both genetic and environmental factors. It is well known that certain families and populations display higher-than-average growth rates relative to other groups. This variation forms the basis of quantitative genetic theory and breeding programs used to improve agricultural stocks and crops (Falconer and MacKay 1996). However, growth is also affected by several abiotic and biotic factors including temperature, oxygen concentration,

availability of resources, metabolic rate, and intraspecific competition to name a few. In addition, the interaction between genotype and environment affects growth so that certain genotypes may respond differentially to varying conditions when compared to other genotypes (Lewontin 1974).

Patterns of growth variation are often associated with life history choices. Two famous examples are the dwarf and normal size forms of adults that exist in both whitefish (*Coregonus diepeaformis*) (Kirkpatrick and Selender 1979) and sockeye salmon (Wood and Foote 1996) populations. The kokanee form of sockeye is a lifetime freshwater resident unlike its anadromous counterpart. This difference results in reproductively isolated sympatric populations. In Atlantic salmon (*Salmo salar*), patterns of growth in underyearling parr determine the timing of smoltification (Thorpe 1997). By winter, bimodal length-frequency distributions emerge. The upper modal group smolts after one year and the lower modal after 2 years. Similarly, bimodality for size has been observed in laboratory studies of juvenile coho (Saxton et al. 1983, Clarke and Shelbourne 1986). Laboratory populations reared at 14°C to 15°C under a simulated photoperiod resulted in two distinct size groups: one containing fish under 25g, the other fish over 35g (Clarke and Shelbourne 1986). Similar to sockeye, the smaller portion of the population failed to smolt during the first summer. In studies of coho juvenile bimodality, social status was linked to growth which is, in turn, linked to variation for standard metabolic rate (SMR) (Metcalfe and Huntingford 1990) and resting metabolic rate (MR) (Yamamoto et al. 1998).

1.1.3 Genetic variation

The genetic significance of phenotypic variation in species of salmon is of great interest to researchers and managers. Two approaches can be used to study patterns of genetic variation and population structure. On one hand, quantitative genetic methods can be employed to

investigate the variability and heritability (h^2) of selected traits, such as growth, morphology or behaviour. Evolutionary biologists use this information to study the relative importance that selection, mutation, migration and drift play in the adaptive differentiation (and eventual speciation) of populations (Endler 1986). Aquaculturists use this information for breeding by quantifying the inheritance and degree of genetic control of economically significant traits (Kinghorn 1993, Gjedrem 1993). On the other hand, molecular genetic data can be used to differentiate between populations according to the frequency and distribution of alleles for neutral markers (subject to drift, mutation and migration, but not selection) (Utter 1991). This information is used by fishery managers to identify reproductively isolated stocks for mixed stock fishery analysis or for cross-boundary resource issues (Beacham et al. 1996). Conservation geneticists use molecular data to identify unique populations for conservation; often called Evolutionary Significant Units (Waples 1995, Echelle 1991, Allendorf and Leary 1988, Frissel 1993, Jones et al. 1996, Meffe and Vrijenhoek 1988, Smith 1994).

In quantitative genetic studies, the underlying question is about the relative fitness and viability of phenotypes whereas in molecular genetic studies the fundamental questions are about genetic diversity and historic patterns of drift and migration. When conservation geneticists use molecular data to identify ESUs, they typically assume that neutral genetic data correspond to evolutionarily significant variation, but the association between molecular data and adaptive variation is poorly understood. Complicating the situation is the fact that the scale of genetic divergence as indicated by genetic markers is quite different than traits under selection when strong selection pressures exist. Limited gene flow can keep populations similar for genetic markers not affected by natural selection. Thus, a difference in alleles or allele frequencies indicates the opportunity for adaptive differentiation, however, genetic marker similarity does not mean the populations are similar for traits under selection (Allendorf 1995, Bentsen 1991).

Also, recent evidence suggests that some markers, such as allozymes, may themselves be under natural selection, thus maintaining similar frequencies for different populations (Karl and Avise 1992, Pogson et al. 1995). Moreover, the traits that contribute to fitness are often polygenic. Quantitative traits are affected by environmental effects on phenotypes, additive genetic variation, and non-additive genetic effects such as dominance effects, epistasis, linkage and pleiotropy (Hard 1995). Therefore, elucidating the genetic basis to local adaptation is a challenging problem.

For reasons of management and conservation, some coho populations have been described biochemically (Utter et al. 1973, Wehran 1987, Small et al. 1998 a, b). Isozymes are widely used to describe protein variation within and among populations, but with the advent of the polyermerase chain reaction (PCR), nuclear and mitochondrial DNA surveys have become increasingly common. Several kinds of information can be gleaned from electrophoretic data once basic assumptions regarding neutrality and the mutation rate of alleles are made. In the case of isozymes, alleles are protein variants expressed at a single locus. For microsatellites, several alleles may occur in a population at a single locus corresponding to the number of tandem repeats of short DNA sequences in non-coding regions in the individual. Heterozygosity is a measure of allelic diversity related to Wright's F-statistics and coefficients of inbreeding (Hartl and Clark 1993). Other indices are Nei's G_{st} or multi-locus F_{st} that provide measures of allelic diversity summed over multiple loci (Weir 1996). Measures of genetic distance relate the genetic variance among populations to geographic distance or evolutionary time (Takezaki and Nei 1996, Weir 1996). When drift and mutation are assumed to be at equilibrium, migration models such as the island or stepping stone model can be used to study evolution in sub-divided populations and derive estimates for rates of migration and gene flow (Hartl and Clark 1993, Weir 1996). Similarly, the occurrence and frequencies of shared and rare alleles also provide

information regarding gene flow and population uniqueness (Slatkin 1985). In addition, electrophoretic data can be used to study the effects of small effective population size from bottlenecks and founder events on genetic diversity and inbreeding (Waples 1994, Waples and Teel 1989).

Coho salmon show remarkably little variation for isozymes across the range of the species (Table 1). Pacific salmon in general are known to show less protein variation than other fishes, but notwithstanding this fact, coho still show less variation than related *Oncorhynchus spp.* Despite the low heterozygosity levels, it is still possible to identify population structuring using isozymes. In British Columbia, lower mainland stocks are distinct from Upper Fraser River and Vancouver Island stocks. Similarly in Washington State, Columbia River stocks are genetically distinct from coastal stocks. More recently mtDNA and nDNA data have verified these results. However, the higher degree of polymorphism in electrophoretic data derived from nuclear markers such as microsatellites allows finer resolution of population structure (Table 2). Microsatellites revealed previously unresolved levels of genetic sub-structure between populations of coho in the Fraser River. For example, Small et al. (1998) found both significantly different allele frequencies and private alleles in Adams River coho compared to other upper Fraser stocks.

Quite often the population structure revealed from electrophoretic data matches patterns of variation between populations for life history, morphology and other phenotypic traits. For example, the data of Small et al. (1998) are concordant with previous evidence of adaptive divergence between upper and lower Fraser River stocks (Taylor and McPhail 1995a,b). Similarly, the Adams River stock has long been considered unique by biologists due to the occurrence of peculiar life history traits in these populations. In general, patterns of genetic diversity across salmon populations are thought to reflect routes of post-glacial colonisation of

freshwater habitat from glacial refugia with adaptive differentiation occurring after colonisation (McPhail 1997). Upper Fraser stocks which typically show less allelic diversity than lower Fraser stocks, were probably colonised by relatively few founders. Thus, the present-day genetic structure reflects past founder events and bottlenecks. For example, the Adams River stock was probably re-colonised with founders from Columbia River refugia, not coastal refugia like other upper Fraser stocks.

Table 1. Levels of isozyme variation in Coho Salmon and related Sockeye.

SPECIES	LOCI (N)	MEAN HETEROZYGOSITY	STOCK HETEROZYGOSITY	SOURCE
Coho Salmon	23	1.8%	Washington Coast 2.7% Columbian 0.5%	Utter et al. 1973
Coho Salmon	26	0.25 +/- 0.06%	British Columbia Lower Mainland 0.30% Upper Fraser 0.13% Vancouver Island ~0.25% Capilano Hatchery ~0.81%	Wehrhan and Powell 1987
Coho Salmon	23	0.027%	California	Bartley et al. 1992
Coho Salmon	26	1.36%	Oregon Wild Stocks	Olin 1984
Sockeye Salmon	33	4.1%	British Columbia 83 sites with H ranging from 2.3-5.6%	Wood et al. 1994

Genetic distance among salmonid populations is correlated with geographic distance. For example, sockeye in Alaska and Northern BC are more similar to each other than to either sockeye from the southern BC coast or the upper Fraser watershed (Wood et al. 1994). Similarly, chum can be differentiated into three distinct groupings, Russia, Alaska/B.C. and

coastal BC, based on minisatellite variation (Taylor et al. 1994). Similar minisatellite data for coho reveals less defined population structure (Miller et al.1996). The widespread distribution of coho in a variety of habitats may be an ecological reason for this apparent lack of structure relative to other *Oncorhynchus* species. However, the level of population structure resolved will depend on both the biochemical tool employed and the sampling intensity (Waples 1991). There is less information about coho genetic variation than other salmonids, such as sockeye or pink, probably because it is not as commercially important. However, now that coho is a large conservation concern, more genetic variation studies are being conducted (Table 2). Across salmonid species, most of the genetic variation is found within populations. This pattern, illustrated for Skeena coho salmon in Table 3, probably results from populations sharing recent shared evolutionary history and from common glacial refugia for salmonids, but could also result from high levels of gene flow.

Table 2. Heterozygosity and F_{st} values observed at three microsatellite loci in Fraser River coho, s is the standard deviation of the estimates (from Small et al. 1998).

LOCUS	HETEROZYGOSITY (H)	F_{st}
Ots 101	72-97%	0.040 (s=0.006)
Ots 3	70-82%	0.054 (s=0.009)
Ots 103	25-89%	0.059 (s=0.009)

Table 3. Hierarchical organization of genetic variation in Skeena River coho salmon. (From Wood and Holtby 1998)

SOURCE OF VARIATION	PERCENT OF TOTAL VARIATION
Geographic	3.9% Total among population variation 1.0% Among basins 1.4% Among major tributaries 1.5% Among sites
Among replicate samples/years	2.0%
Individuals within Sites	94.1%

Schluter (1995) suggests that natural selection is quite pervasive and a major factor contributing to patterns of genetic variation. One can imagine that for salmonids this is particularly true since they occupy a wide variety of habitats and have low rates of migration between populations. While many cases of phenotypic differentiation between populations are documented, fewer studies have examined the genetic basis of these differences. Given the difficulty of studying selection in the wild, even fewer studies of natural selection have been attempted in the wild than in captive populations although there are some notable exceptions (Endler 1986, Gharett and Smoker 1993). Despite the difficulty of demonstrating adaptation and adaptive pressures, even relatively low selection pressures have the potential to affect population structure and result in population differentiation through local adaptation (Wright 1978).

Over the past 20 years, the increasing commercial importance of aquaculture has focused more attention on brood stock development for salmonids and other species (Kinghorn 1985, Gjedrem 1983). Coho is one of the more commonly reared species as it tolerates hatchery conditions well. While breeding programs are well developed for other cultivated species, such as conifers or livestock, selection programs are less developed in aquaculture and quantitative genetic studies of economically significant traits are relatively recent. Traits for which heritabilities have been quantified in salmonids include size, growth rate, age at maturity, flesh colour, disease resistance and food conversion efficiency (Table 4; Withler and Beacham 1994, Silverstein and Hershberger 1994, 1995, Swift 1991). In artificial selection studies on Atlantic salmon (*Salmo salar*), gains up to 10% for size at maturity have been reported (Gjedrem 1993). Although estimated heritabilities for traits vary from study to study, some consistent trends emerge. First of all, the heritability of size (fork length or mass) decreases with age. This trend is likely due to the influence of maternal effects, such as egg size and spawning date, early in the life cycle.

Withler and Beacham (1994) reported no maternal effects after 6 months although Silverstein and Hershberger (1996) noticed effects beyond this time. Secondly, the genetic correlation between length and weight is high although length tends to be more heritable (i.e., under stronger genetic control). Thirdly, growth rate shows heritable differences associated with the sire component. Fourthly, fast juvenile growth is negatively correlated with age at maturity. There is a low genetic correlation between juvenile size and size at maturity suggesting that the selection pressures during these two life history phases are quite different. This final point is interesting because size at smoltification is positively correlated with survival during the transition from fresh to salt water (Bilton et al. 1984).

Table 4. Heritabilities of size related traits in coho salmon. Heritabilities are given for length, mass, or specific growth rate (G) for the periods shown.

GROWTH PERIOD	HERITABILITY	SOURCE
First summer	0.45 ± 0.36 (mass)	Withler and Beacham (1994)
Summer – smotification	0.11 ± 0.30 (mass)	
Saltwater phase	0.61 ± 0.32 (mass)	
14 months freshwater	0.77 ± 0.31 (mass)	Swift (1991)
	0.75 ± 0.31 (length)	
18 months freshwater	0.61 ± 0.27 (mass)	
	0.66 ± 0.31 (length)	
Saltwater phase	0.55 ± 0.35 (mass)	
	0.64 ± 0.32 (length)	
13 months freshwater	0.36 ± 0.26 (mass)	Silverstein and Hershberger (1995)
16 months	0.50 ± 0.31 (mass)	
	0 ± 0.14 (G)	
19 months	0.50 ± 0.31 (mass)	
	0.34 ± 0.24 (G)	
21 months	0.32 ± 0.25 (mass)	
	0.21 ± 0.15 (G)	
Spawn	0.26 ± 0.24 (mass)	
	0 ± 0.11 (G)	

1.1.4 Factors in the decline in coho abundance

In the Pacific Northwest, coho populations are declining at a rate faster than any other commercial salmon species (Northcote and Atagi 1997). Stocks in California, Oregon and Washington states have been listed as endangered or threatened under the U.S. Endangered Species Act (Nehlson et al. 1991, Moyle 1994). Declines have also been observed in British Columbian stocks and recently the commercial and recreational fisheries have been severely restricted or closed by the federal Department of Fisheries and Oceans. Various factors have been implicated in their decline including overfishing, habitat destruction through forestry practices and urbanisation, hydroelectric projects, marine conditions, climate change and the genetic deterioration of wild stocks through introgression with hatchery stock (Meffe 1992, Walters 1993, Beamish and Bouillon 1993). There are generally two types of strategies for improving production. The first strategy is to manage stocks by either limiting or shutting down the harvest or by enhancing the population through hatcheries. The second strategy is to manage habitat by restoring or enhancing riparian areas, restricting activities like forestry around salmon habitat, or creating wildlife reserves.

1.1.4.1 Hatchery-related Impacts

Of all the possible factors in the decline of coho stocks, the effects of hatcheries are particularly relevant to this thesis because they are blamed for genetic deterioration. Every year approximately four million hatchery-reared salmon are released into the Pacific (Hindar et al. 1991). There has long been concern about potentially negative effects that hatchery stocks have on wild stocks of salmon in spite of appearing to augment population size (e.g. Schramm and Piper 1995). Part of this concern is generated from evidence showing declines in juvenile densities in stocked streams despite enhancement efforts (Nickelson et al. 1996). Hatchery stock may contribute to the loss of wild stocks through a number of possible mechanisms. These

include displacement by intraspecific competition and introduction of disease to wild stocks. The release of hatchery stocks for harvest allows a high rate of fishing pressure to be sustained, potentially subjecting wild stocks to overharvesting (Waples 1991). Many researchers have also suggested hatchery stocks contribute to the genetic decline of wild stocks (Hindar et al. 1991, Meffe 1992, and Fleming 1984). The introgression of hatchery stocks with wild stocks may lead to the loss of locally adapted gene complexes creating outbreeding depression in wild stocks. The evidence for outbreeding depression is largely anecdotal and intuitively linked to the observations discussed above regarding the apparent degree of local adaptation in salmon populations. Since coho are more easily domesticated than other species of Pacific salmon, they make up a large proportion of hatchery and aquaculture stock. Therefore, they are potentially more vulnerable to hatchery-related genetic impacts caused by intentional or unintentional releases.

Depending on the jurisdiction, hatchery programs have been under different levels of regulation. In some areas, the founding population has been tightly controlled with efforts to seed the population from local stock. In other areas, most notably California, the origin of hatchery populations is unknown and expected to have been widespread (Bartley et al. 1992). Genetic effects, such as drift or inbreeding, associated with small founding populations in hatcheries may contribute to the potential maladaptation of hatchery stock in the wild. More worrisome is the viability of potential hybrids when hatchery fish mate with wild fish. There is no doubt that hatchery fish face different selection pressures than fish in the wild. For one thing, juvenile mortality is negligible in hatcheries whereas wild populations undergo significant mortality in the egg-to-smolt stages. Hatchery and aquaculture fish are routinely treated with antibiotics for diseases which they may subsequently transfer to wild populations. They are reared at densities much higher than wild populations and do not compete for habitat, food or mates resulting in

potential selection for docility. Indeed, morphological and behavioural differences between hatchery and wild stocks have been demonstrated (Fleming and Gross 1993, Swain et al. 1991, Taylor 1986). Understanding the genetic and ecological scale of local adaptation in salmon populations is an important indicator of how useful (or detrimental) stock enhancement is to the conservation of salmon species. Information on adaptive variation can also be used to improve stock enhancement efforts through brood stock guidelines.

Chapter 2 METHODS

2.1 Description of samples and sampling locations

Live specimens and fin clips of coho fry were collected from paired main and off-channel habitats within the Mamquam River in early summer. This river and all other sampling locations are on coastal rivers within the Lower Mainland, British Columbia (Figure 2). Salmon were sampled immediately after alevin emergence and then transferred to the West Vancouver Laboratory (DFO) where they were raised for a period of six months. These specimens were used for two purposes. The first purpose was to study variation for size among sub-populations when grown under common laboratory conditions. The second purpose was to describe genetic variation among the sub-populations using microsatellite markers. These data were combined to estimate inheritance of growth and size related traits. Fin clips were also collected from fry sampled in winter on the Mamquam, Cheakamus and Vedder Rivers. These samples were used to describe genetic variation for microsatellite markers between populations. The Mamquam and Cheakamus are located within 10km of each other in the Squamish area north of Howe Sound. The Vedder is a tributary of the Lower Fraser River, and was sampled approximately 200km from the Squamish area.

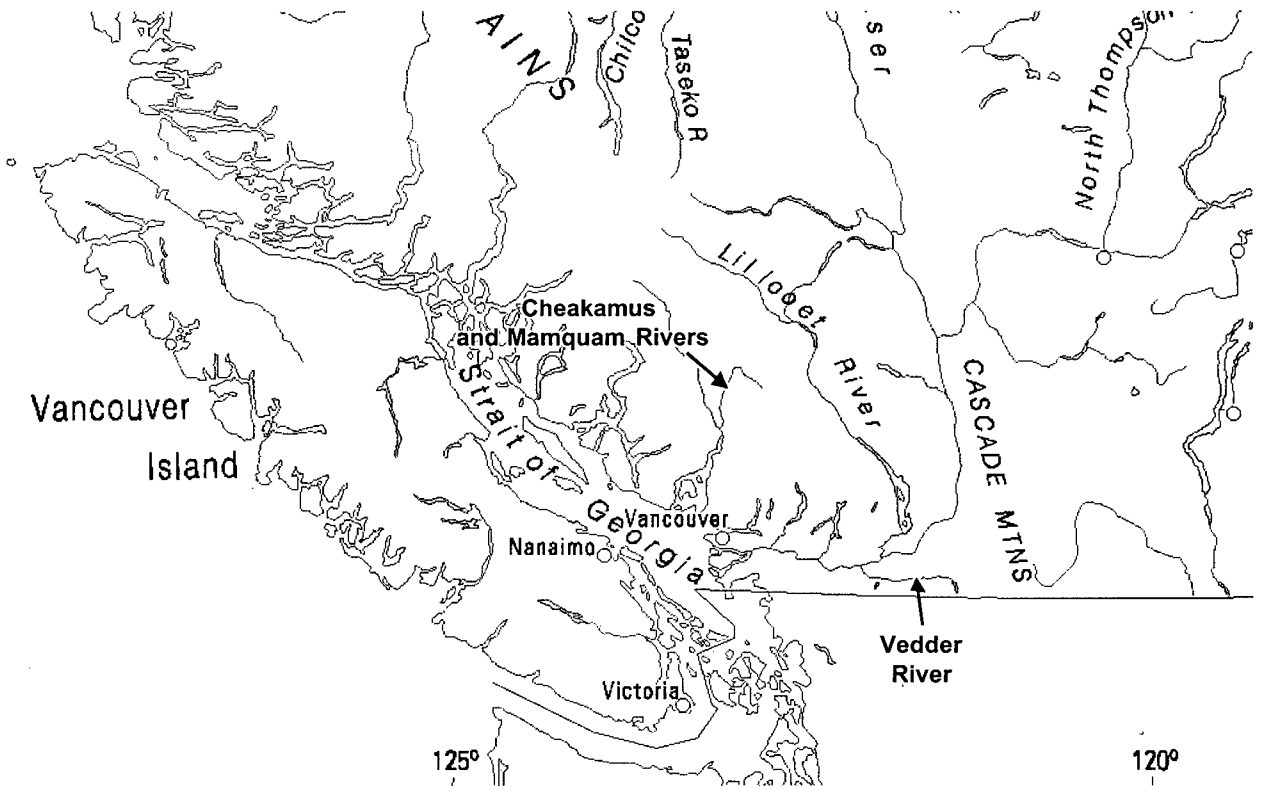


Figure 2. Locations of the Mamquam, Cheakamus and Vedder Rivers.

2.1.1 Artificially-created side channel habitat

The side-channel fish studied in this experiment were collected from artificially created habitats that were built by the DFO in order to enhance salmon productivity by providing additional spawning and nursery habitat (Sheng et al. 1990). In most cases, side channels were built to extend existing groundwater-fed areas. They range in length from about 300m to 1000m with width ranging from 5 to 6 meters. These features and others are described in Table 5. Channels are excavated to below the water table, which allows groundwater to feed them. This feature ensures a somewhat constant flow throughout the year and results in less variability in water temperature, from year to year and season to season, than what is expected in adjacent surface-fed main channels. Although initially built to enhance chum spawning, the observation that coho

utilise the channels for juvenile nursery and adult spawning habitat prompted increased investment into their construction. A typical layout of a side channel is displayed in Figure 3. The density of juveniles in the channels appears related to the availability of cover so features that add cover, such as logs or riprap armouring along the banks, have been added to the initial construction design.

Table 5. Physical characteristics for re-activated groundwater-fed channels in British Columbia. (Reproduced from Sheng et al. 1990)

CHARACTERISTIC	RANGE
Length	300-1000 m
Width	5-6 m
Depth	20-49 cm
Surface Velocity	5-15 cm/s
Discharge	0.085-0.14 m^3/s
Summer water temperature	8-13 °C
Winter water temperature	3-7 °C

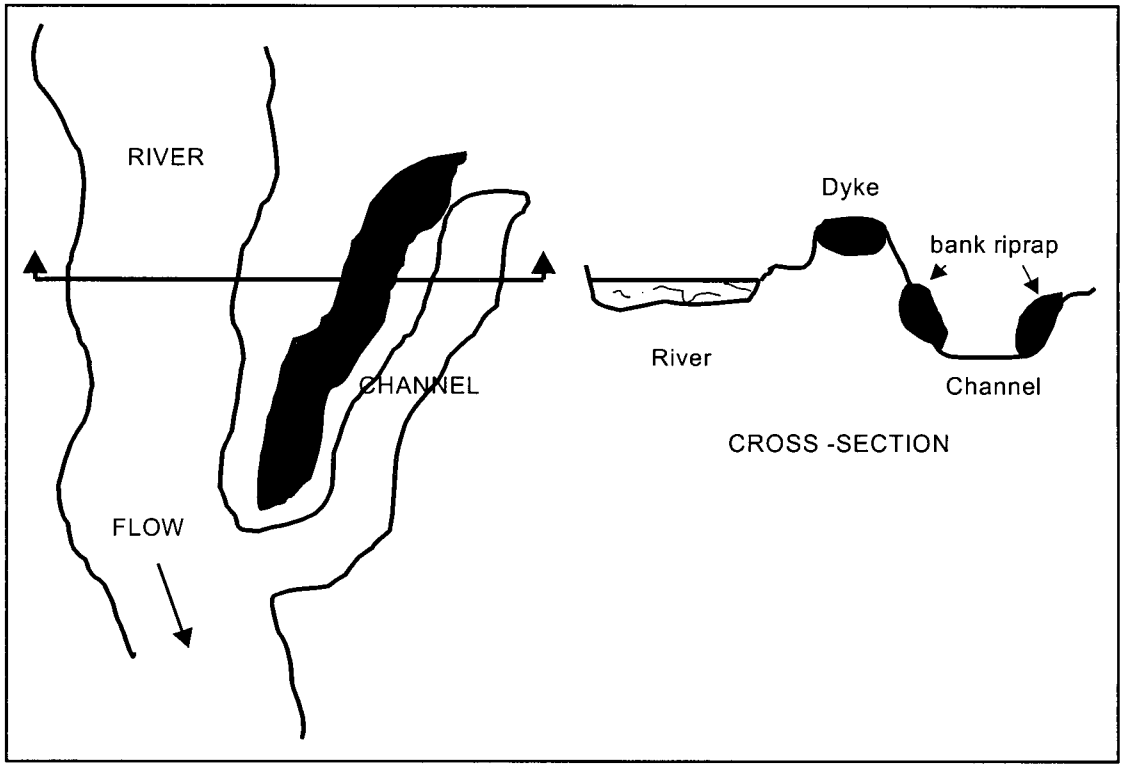


Figure 3. Typical layout for a groundwater fed channel. (Reproduced from Sheng et al. 1990)

2.1.2 Live-specimen collection

For the live samples, specimens were caught from mid-June to early-July from 4 locations in the Mamquam River. Two sites were located in main-channel habitat and the other two in off-channel habitat (Figure 4). In late spring, during routine visits to the site, the river and channel habitat were monitored to observe evidence of emerging fry. Harsh conditions over the previous winter resulting in colder than normal river temperatures probably delayed emergence that year. The first fry to emerge were in the lower Mamquam River near the confluence with the Squamish River. A sample of these fry were collected, then additional fish were collected over a three week period corresponding to their emergence at the remaining sites. The fry were caught using a pole seine net over an area stretching over a 10 to 40 m length of the river. The total effort required to collect a sample depended on the abundance and distribution of schools. Although an attempt was made to be consistent between sites, site-specific variation in collection

effort is evident (Table 6). In addition, a portion of the specimens collected in the Mamquam Channel were dug up from 3 redds because low temperatures in this channel resulted in even later emergence of fry than the other sites.

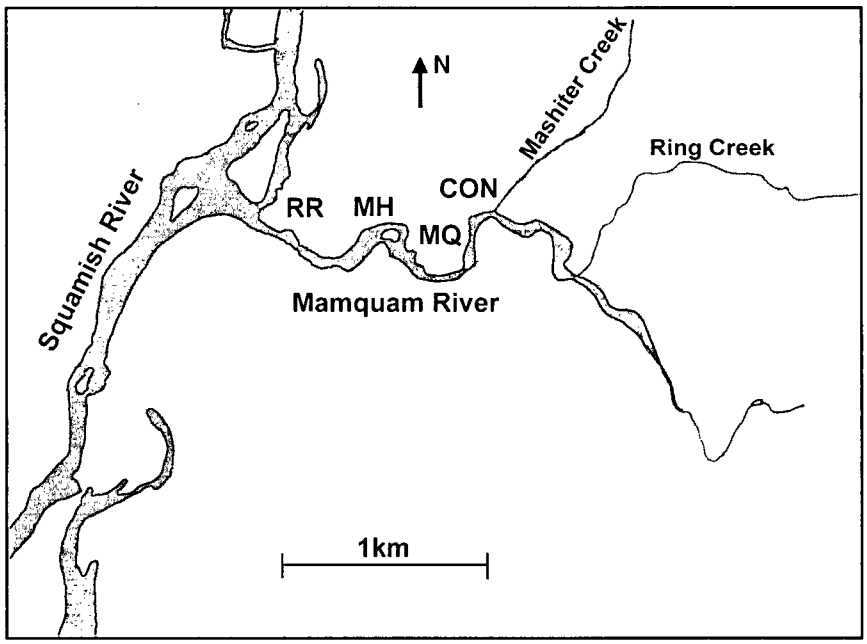


Figure 4. Locations of the four sampling sites on the Mamquam River. RR refers to Railroad and CON to Confluence, both off-channel sites. MH to Mashiter and MQ to Mamquam, both side-channel sites.

Table 6. Details of the live-specimen collection. 'Passes' refers to seine net sweeps.

Sampling Location	Habitat Type	Date	Total Collected	Area Sampled	Effort	Water Temperature
Railroad	Main	June 12	250	40m2	10-12 passes over 1.5 hrs	9°C main
Mashiter	Channel	June 18	280	30m	4-5 passes over 1 hr	8°C channel 7°C main
Mamquam	Channel	June 13	84	3 redds	2hrs	7°C channel 8.5°C main
Mamquam	Channel	June 24	145	10m	2 passes over 15 min	7°C channel
Confluence	Main	July 7	120	40m	15 passes over 2 hrs	

Main-channel sites:

The collection site deemed 'Railroad' is main-channel habitat located within 200m of the confluence of the Mamquam and Squamish Rivers. It is also 200m downstream from a local railway bridge. Displaced debris, gravel and sandbars provide evidence of great fluctuations in river course and flow in the past at this site. The actual sampling location was a slough just of the main channel with considerable reduced flow. Depth in the are ranges from 15cm to 1m. Instream cover includes decaying logs, boulders, reeds and overhanging branches. The riparian vegetation is red alder (*Alnus rubra*) and sitka spruce (*Picea sitchensis*). During the sampling both sticklebacks (*Culea spp.*) and chum fry (*O. keta*) were caught in addition to coho.

The second main-channel collection site, named 'Confluence', is located upstream of both the Mashiter and Mamquam channels near the confluence of Mashiter Creek with the Mamquam River. The sampling area is obviously subject to high flow periods and the bank is severely undercut as a result. Coho fry tended to aggregate in these undercuts. Riparian vegetation is predominately willow (*Salix sp.*), which provides some in-stream shade. The substrate is gravel with sandbars and washed-down debris is common. Relative to the other collection sites, fish were difficult to find here with the most intensive sampling effort still yielding less fish than the other sites. Other species collected during the sampling included sculpins (*Cottus spp.*) and chum fry.

Off-channel sites:

Mashiter Channel is approximately 400m in length. After its initial construction, the DFO has continued to refine the design, adding a pool in the summer of 1997. It meanders through dense alder and western red cedar (*Thuja plicata*) meeting the main channel upstream from the Railroad site. The width of the channel is approximately 5 to 6m and depth ranges from 15 to

45cm. Although the channel is groundwater fed, dense algae mats present during summer probably result from nutrient surface run-off from the neighbouring Squamish Golf Course. The substrate is mixed with some gravel, but largely consisted of sand and mud.

Mamquam Channel joins the main channel just upstream from the blind end of Mashiter Channel, adjacent to the golf course. It is approximately 500m in length and varies in width from 5 to 6m and depth from 15 to 40cm. Riparian vegetation is alder and cedar, which largely shade the surface area of the water. The channel is groundwater fed and typically 2-3 degrees cooler than Mashiter Channel in the summer. Unlike Mashiter Channel, algae mats do not form in summer suggesting it has lower levels of primary production. The substrate is largely gravel with boulders and submerged branches providing instream cover. At the blind end of the channel is a deep pool and there are two additional pools between it and the mouth. Mamquam Channel is younger than Mashiter and the DFO continued to construct additional channels linked to Mamquam in the summer of 1997.

2.1.3 Fin-clip collection

In addition to the live specimens described above, fin clips were taken from fish collected during electroshocking passes and from counting fences in channels on the Mamquam, Cheakamus, and Vedder Rivers. All these clips were collected during the autumn of 1997 and winter of 1998 (Table 7). Forty samples were collected from each river and for each fish clipped length and mass was recorded. Mamquam samples came from the Mamquam Channel (described above) and were collected from the counting fence operated for Dr. Guillermo Giannicos' (Institute for Resources and Environment, UBC) concurrent study. Cheakamus samples came from the Upper Paradise Channel located off Paradise Valley Road near the North Vancouver Outdoor School north of Squamish. They were collected in September by electroshocking and in December and January from a counting fence. Vedder River samples were collected during September while

electroshocking in the Hopedale Channel near Chilliwack. No main-channel collections were made during the fall due to high level flows and unsafe sampling conditions. Juvenile coho are unlikely to be present in these habitats during those conditions.

Table 7. Details of the fin clip collection.

Location	Date	Total Collected	Method
Hopedale Channel Vedder River	September 24, 1997	40	Electroshocked
Upper Paradise Cheakamus River	September 24, 1997	20	Electroshocked
Upper Paradise Cheakamus River	December 15, 1997	10	Counting Fence
Mamquam Channel Mamquam River	December 15, 1997	20	Counting Fence
Upper Paradise Channel Cheakamus River	January 9, 1998	10	Counting Fence
Mamquam Channel Mamquam River	January 9, 1998	20	Counting Fence

2.2 Common Garden Experiment

2.2.1 Rearing Environment

From mid-June to early July 1988, juvenile coho were collected, as described in Section 2.1.2, from 2 off-channel and 2 main-channel sites on the Mamquam River near Squamish, B.C. The fish were transferred to 100L tanks at the DFO West Vancouver Laboratory (Figure 5). Fish from each sampling site were divided into 2 tanks in order to test for tank effects on growth rate. The only exception was the Confluence population from which there were not enough fish collected to divide the population and still maintain consistent density among tanks. Each tank

held approximately 100 fish. The fish were hand-fed to excess on Moore-Clark Size 0 starter feed several times daily until they reached a size of approximately 6cm fork length. After that they were fed twice daily to excess on Moore-Clark Size 1 until the cessation of the experiment. The aquarium operates on a timed photoperiod corresponding to daylight hours and surface – feeding coho only feed during these hours. All tanks had continuously flowing freshwater from combined groundwater and surface-water (Cypress Creek) sources. Temperatures within tanks fluctuated depending on the water source temperature, reflecting seasonal trends. All tanks were monitored 2 to 3 times weekly to ensure consistency across tanks and tank inflows were adjusted accordingly if necessary. No effort was made to ensure constant temperature throughout the rearing period since fish do not experience that in the wild. Tanks were oxygenated with aeration stones and cleaned two to three times weekly by siphoning. Mortality in all tanks throughout the growing period was low and no cannibalism was observed. The largest cause of loss was a series of weather-related overflow events in the fall resulting in some escaped fish.

Figure 5. The aquarium environment.

2.2.2 Measurements and Identification

At the beginning of the rearing period each fish was measured for mass and fork length. However, in order to determine the specific rate of growth for fish over the rearing period, every individual had to be uniquely marked with a tag interpretable at the beginning and end of the experiment. Since the fish were caught at the alevin or button-up stages they ranged in size from 29 to 45mm at the beginning of the experiment. Passive induction transponders (P.I.T.) or floy tags were not feasible markers at this stage as the fish were too small. Sequential coded-wire tags, however, provided an alternative. Sequential coded-wire tags are manufactured by the Northwest Marine Technology. They are magnetic stainless steel wires approximately 3mm in length engraved with six data fields in a binary code readable under magnification. Each tag is unique and up to 10, 000 separate sequences are possible. Studies have shown that tags when inserted properly in the cartilinogous snout the tags do not affect growth, behaviour or survival of fish (Jefferts et al. 1963, Jewell and Hager 1972).

Thetag was inserted in the snout of the fish using a coded wire tag injector. To determine if tagging was successful the fish were passed through the quality control device; a two-way sluice with a magnetic detector able to separate tagged from untagged fish. Since duplication can occur in the binary code of the unique sequence, two flanking tags were filed for each tag inserted in a fish. Flanking tags were matched with initial size data for each individual. These tags were read under a microscope at 16-40X amplification and sequences were decoded using a Windows based decoder program provided by Northwest Marine Technologies. After the end of the rearing period every individual was measured again for mass and fork length and kept for later identification. To match data from the beginning and end of the rearing period, the sequential coded wire tags were dissected from the terminated fish. The decoded sequences were matched

with original flanking tags. Specific rate of growth was calculated for every surviving individual from which a tag was successfully dissected and matched.

2.2.3 Data Analysis

Descriptive statistics for mass, fork length, and growth measurements were calculated for each sub-population using JMP version 3.2.2 and SAS software (SAS Institute Inc.). Further statistical tests for population differentiation were also conducted using these packages. Initial testing for normality (Shapiro-Wilks W test) and homogeneity of variances (Levene test) indicated that some populations deviated from expected normal distributions and had non-homogeneous variances for mass, fork length and growth ($p<0.05$). Consequently, these data were normalised by log transformations (ln (100x) for initial and final lengths and ln (1000x) for relative growth rates) in order to meet the assumptions of parametric tests. These data were multiplied by a factor of either 100 or 1000 to ease analysis in the case of small numbers. Further analyses were conducted on transformed data.

To compare means between sub-populations for initial and final fork length and mass an Analysis of Variance (ANOVA) was conducted (Sokal and Rohlf 1981). The linear model was

$$Y_{ijkl} = H_i + S(H)_{ij} + R_k + R \times S(H)_{ijk} + \varepsilon_{ijkl}$$

corresponding to a 2-way nested experimental design (Table 8). Since there were unequal sample sizes among cells and one missing cell, sums of squares (SS) were calculated according to Hockings' (1985) algorithm for 'effective hypothesis tests' which perform more efficiently under these conditions. The algorithm is not affected by reordering of levels or effects. The ANOVA was a mixed model with habitat (H) as fixed effect and site within habitat S (H) and replicate (R) as random effects. Appropriate F-tests were determined by computing the expected mean squares for the model. Accordingly, habitat was tested against site within habitat (F = MS

H/MS S (H)) and both replicate and site within habitat were tested against the interaction effect of replicate by site within habitat. However, given the unequal sample sizes, test denominators were synthesised. The interaction effect of replicate by habitat was confounded within the interaction effect of replicate by site within habitat due to the missing cell.

Table 8. Experimental design for the ANOVA showing sample sizes in each treatment/rep combination.

	Main-Channel		Off-Channel		
	Railroad	**Confluence**	**Mashiter**	**Mamquam**	**Total**
Rep 1	81	98	98	87	364
Rep 2	97	-	98	82	277
Total	178	98	196	169	641

Comparing mean growth between sub-populations was done using the ANOVA model. Specific growth rate (G) is the percentage increase in fork length or mass for each fish over the growing period and was approximated by the following formula (Ricker 1979):

$$G = \frac{(\ln l_2 - \ln l_1)}{(t_2 - t_1)}$$

where l_1 and l_2 represent initial and final mass or length and t_2-t_1 represents the growing period in days. Relative growth rate was also calculated by the formula:

$$\frac{\left((l_2 - l_1)/l_1\right)}{(t_2 - t_1)}$$

Generally, t_2-t_1 = 160 although there were slight tank to tank differences. In addition to ANOVA, coefficients of variation were calculated to compare the distribution of fork lengths

between July and December. The relationship between relative growth rate and final fork length was also examined by correlation analysis using Pearson correlation coefficients (Sokal and Rohlf 1981).

2.3 Microsatellite Analysis

2.3.1 Polymerase Chain Reaction

Four sub-populations within the Mamquam River were assayed for microsatellite variation. These were sampled from locations called Railroad (RR) and Confluence (CON), both main-channel (MC) habitat, and Mashiter (MH) and Mamquam1 (MQ1), both off-channel (OC) habitats. Samples were also collected from fish reared in the common garden experiment described above. All fish were collected in June and July as described above. In addition, a second group from the Mamquam channel was sampled in winter. Samples from populations in nearby rivers were also analyzed to allow comparison with isolated populations.

Genomic DNA was extracted from blood and fin clip samples using one of two techniques depending on the tissue. For blood, 3-6µl of blood extracted from the fish tail was lysed in a 100mmol NaOH solution. Immediately after blood was extracted, the sample was placed in a 0.5ml eppondorf tube with 200µl of 100mM NaOH, vortexed and then cooled on ice. Within 3 hours, the sample was incubated at 100^{o}C for 10 minutes and then frozen at -20^{o}C for later use. Before using the extraction products in PCR the tube was centrifuged at 14000 g for 3 minutes. Dr. Bob Devlin (DFO West Vancouver Lab, pers comm.) provided this protocol. For fin clips, DNA was extracted using a chelix resin protocol (John Nelson, SeaStar Biotech, pers comm). A small piece of fin clip (~2 X 5mm) was placed in a 0.2mL eppendorf tube with 200ul of extraction buffer containing 5% chelix resin, 0.1% Tween 20 and 0.1mg/mL protinease K. Each

tube was incubated at 94°C for 15 minutes in an MJ thermocycler. Samples were frozen at -20°C for later use and centrifuged at 14000g for 3 minutes before using in PCR.

From genomic DNA, microsatellite alleles at 5 loci were amplified by the polyermerase chain reaction (PCR) (Sukai et al.1985, Ehrlich 1989). OTS 105 and 106 were isolated at the DFO Pacific Ecology Lab (John Nelson, SeaStar Biotech, pers comm) and Bt73, 6a2 and 3b10 were isolated at the University of Victoria (Christian Smith, University of Victoria pers. Comm; Smith et al. 1998). Primer sequences, repeat sequences, and annealing temperatures are described in Table 9. PCR reactions contained 0.24pmol of each primer, 80umol of each nucleotide, 1 unit of Gibco TAQ, 100nmol of genomic DNA in buffer for a volume of 25ul. The total amount of DNA averaged 2ul from blood samples and 5ul from fin samples. The PCR buffer consisted of 20mM Tris-HCl pH 8.8, 10 mM KCl, 10mM $(NH_4)_2SO_4$, 2mM $MgSO_4$, 0.1% Triton X-100, and 0.1mg/mL bovine serum albumin. PCR reactions were run for 35 temperature cycles on an MJ thermocycler using both 0.5mL and 0.2mL eppendorf tubes. Temperature cycles were preceeded by a 3-minute denaturing step at 94°C. After the denaturing step, the solutions were cooled to 80°C, then TAQ was added to hot start the PCR. The first 5 temperature cycles followed a touchdown pattern so that the initial cycle annealing was 5°C above optimal, the second 4°C above and so on till the optimal annealing temperature was reached on the 5th cycle. After all cycles were completed the reactions were held at 72°C for 5 minutes to complete the annealing process. Products were stored at -20°C until electrophoresis.

Table 9. Primer sequences and annealing temperatures for the five loci examined.

Locus	Direction	Primer sequences (5'-3')	Annealing temperature
Ots 105	F	GAGGATCTATCAACATTATC	50°C
	R	GCAGCACCAGCTTCCC	
Ots 106	F	GGTTTTCAAAGGGTTCTCC	48°C
	R	GGTATTTATGTTTTTTTATTGGT	
Bt73	F	CCTGGAGATCCTCCAGCAGGA	45°C
	R	CTATTCTGCTTGTAACTAGACCTA	
6a2	F	AGGATGGCAGAGCACCACT	58°C
	R	CACCCATAATCACATATTCAGA	
3b10	F	GGAGTGCTGGACAGATTGG	55°C
	R	CAGCTTTTTACAAATCCTCCTG	

2.3.2 Polyacrylimide Gel Electrophoresis

In order to visualise alleles, the PCR products were size fractionated on a polyacrylimide gel electrophoresis (PAGE) system. The electrophoresis unit incorporated two upright polyacrylimide gels placed between upper and lower buffer chambers. The upper chamber contains an anode and the lower a cathode. Since DNA carries a net negative charge, it migrates through the gel matrix towards the positive cathode when an electric field is applied. Alleles differentiate according to size with smaller alleles migrating faster through the matrix. The concentration of polyacrylimide depended on the locus being examined (Table 10). Each gel contained 2xTAE buffer, 50ul TEMED, 10% APS and 7-10% 19:1 Bis-Acrylimide for a total volume of 50mL. In order to form the gel, the liquid polyacrylimide was poured between 2 glass plates. Before polymerisation was complete a 30-teeth comb was placed between the plates at the top edge in order to form sample wells.

After the gels solidified, two glass plates were clamped onto each electrophoresis unit using bull clips and 1x TAE was added to the upper and lower buffer chambers. Loading dye (50mM EDTA pH 8.0, 30% glycerol, and 25% bromphenol blue) was added to 10-12uL of PCR product

and then each sample was loaded into a separate well. In addition, three 20bp ladders, one 1Kb ladder, and a standard fish were loaded in each gel in order to estimate allele sizes. Gels were run at 70V for 15-18 hrs depending on the locus (Table 10). After the electrophoresis run, gels were stained with Ethidium Bromide (0.5 mg/ml EtBr), rinsed in water, and then visualised over a UV light table. Gels were photographed using either a Polaroid camera or a digital imaging system, both fitted with yellow lens filters. Finished products for a sub-sample of products for each locus are displayed in Figure 6-Figure 10.

Table 10. Polyacrylimide gel electrophoresis conditions and product size for each locus examined.

Locus	% Polyacrylimide	Running time (hrs)	Voltage (V)	Product Range (base pairs)
Ots 105	10	18	70	120-140
Ots 106	7	18	70	220-240
Bt73	8	18	70	120-150
6a2	10	18	70	90-200
3b10	10	15	70	100-170

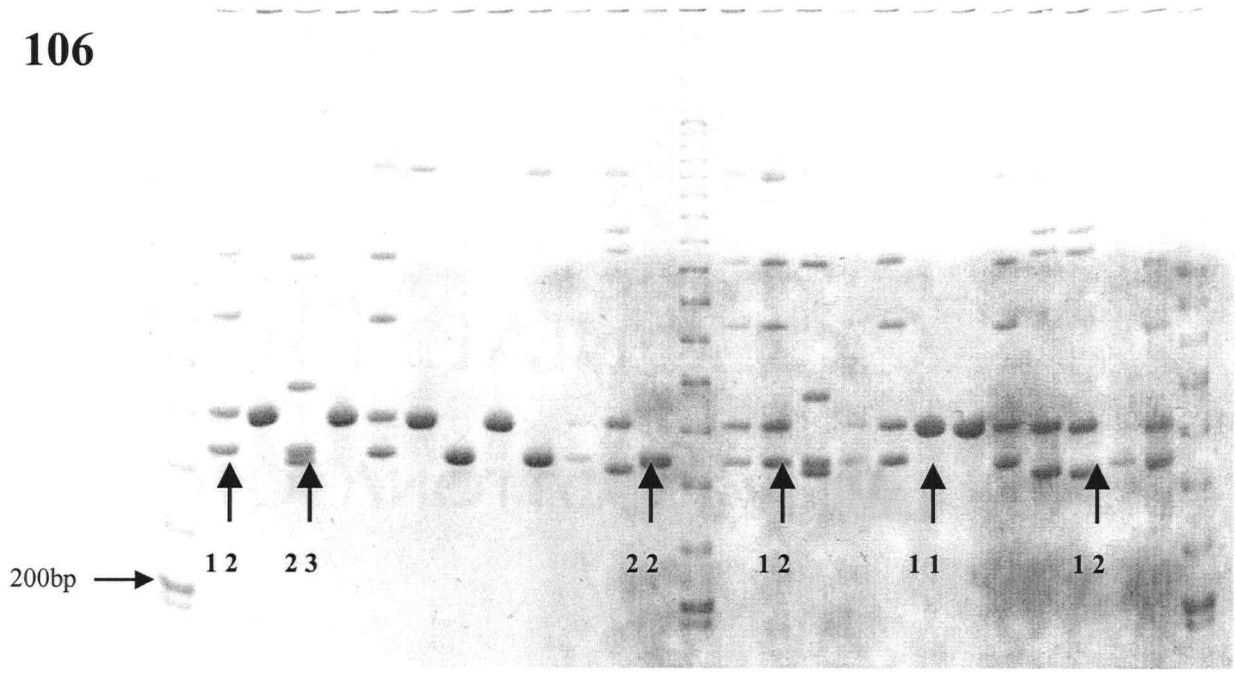


Figure 6. Sample of PCR products from locus OTS 106. For this locus, there are three alleles in the 240-260 base pair range. The numbers show various homozygote and heterozygote combinations of the three.

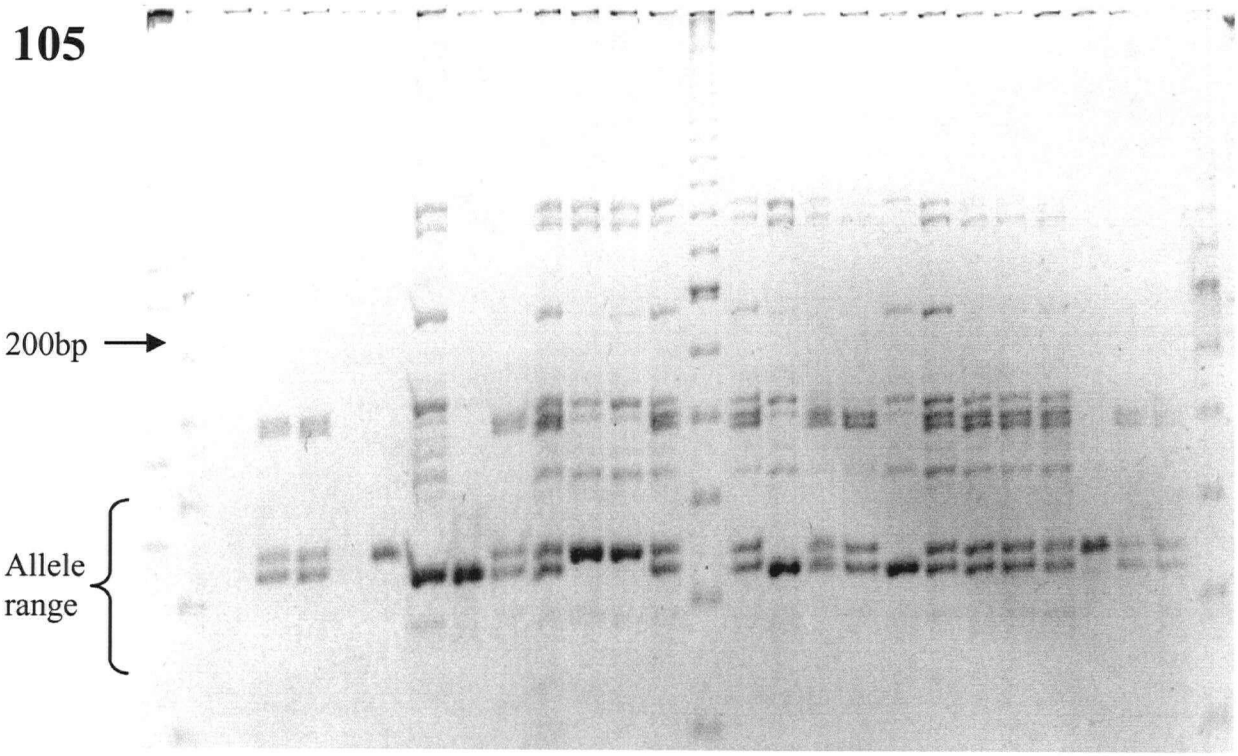


Figure 7. Sample of PCR products from locus OTS 105.

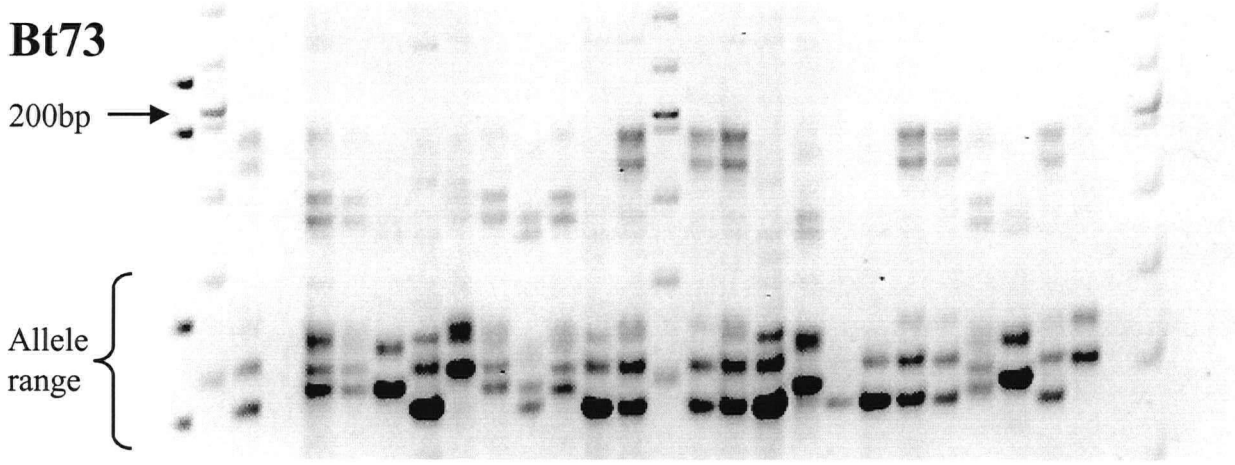


Figure 8. Sample of PCR products from locus Bt73.

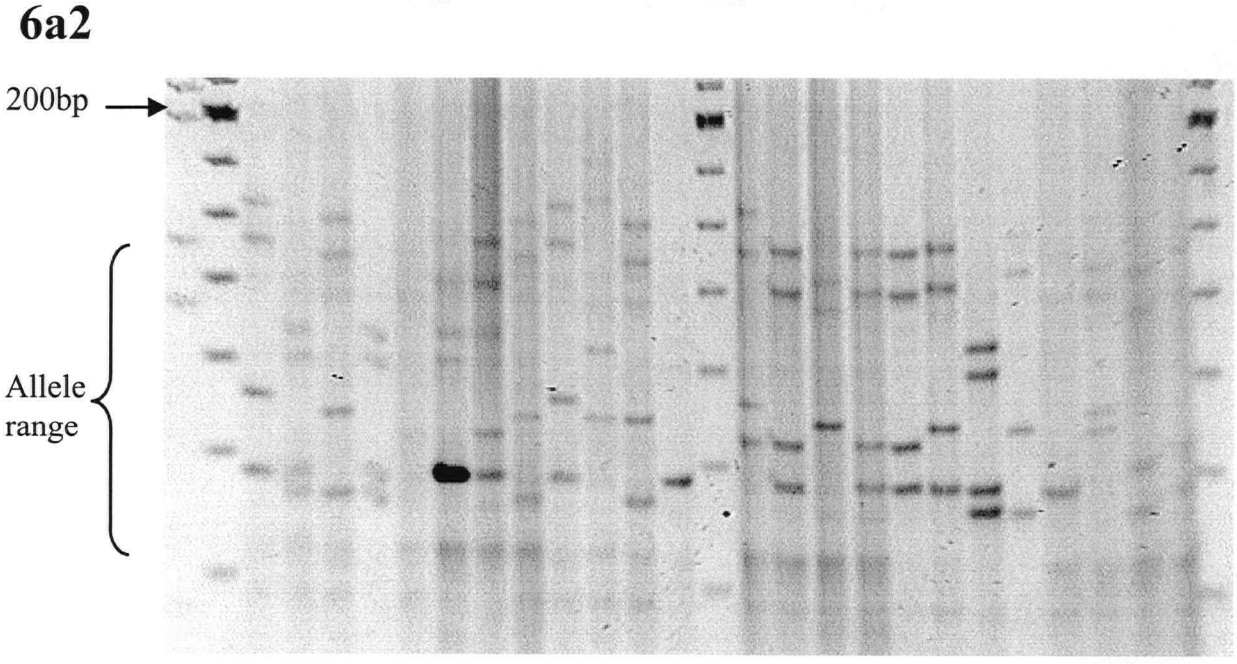


Figure 9. Sample of PCR products from locus 6a2.

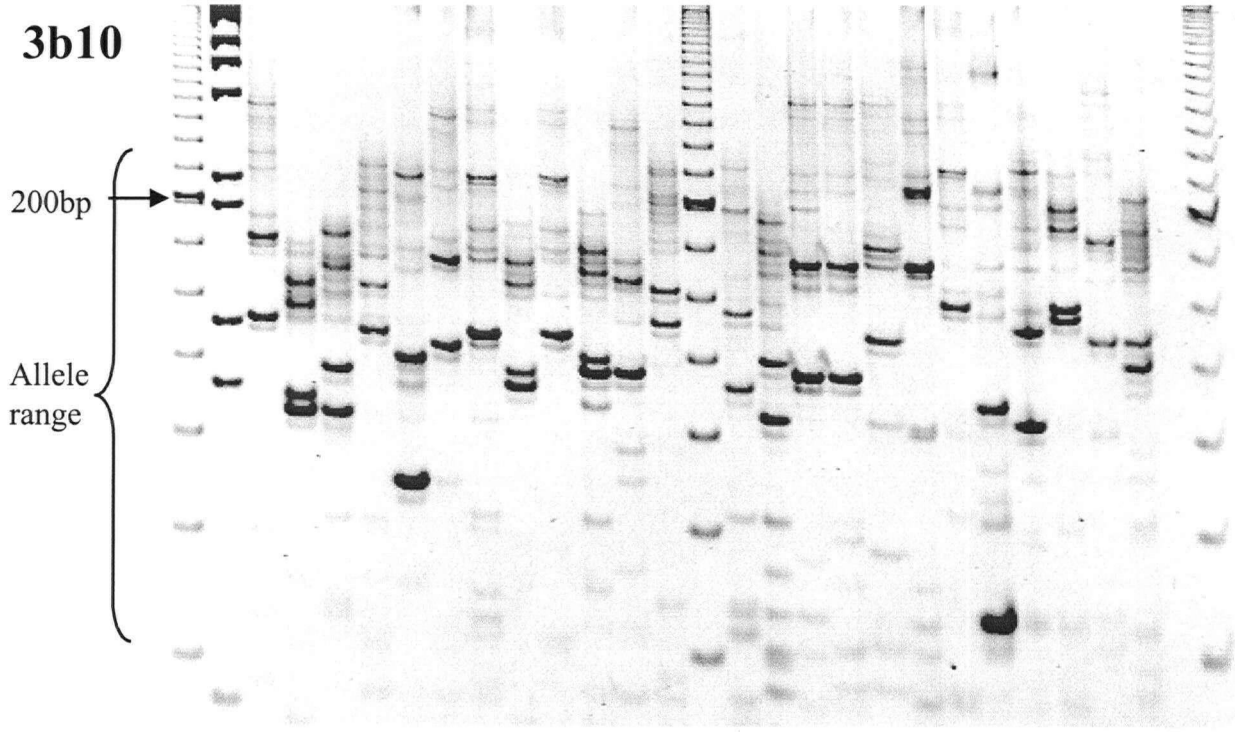


Figure 10. Sample of PCR products from locus 3b10.

2.3.3 Scoring and Analysis

Scoring of gels was completed both manually and automatically depending on the level of polymorphism observed for the locus. For example, OTS 106 and 105 had 3 and 2 alleles respectively, thus making them relatively easy to score manually. On the other hand, Bt73, 6a2 and in particular 3b10 showed higher degrees of polymorphism. These loci were scored initially using BioImage Whole Band software. BioImage estimated allele size using a molecular grid created by the 20bp ladders run on each gel. Since the software identifies alleles by intensity, it can identify more than two 'alleles' per lane. Extra bands result from heteroduplexes and other PCR by-products created during the amplification process. Heteroduplexes result when DNA strands from 2 different alleles hybridise. Therefore once BioImage had identified potential alleles it was necessary to manually select products for scoring. PCR products scored as alleles

were identified by their intensity and also by experience. The bands of true alleles were more intensely stained and since each locus had a finite number of observed alleles, heteroduplex patterns were studied across gels and used to verify choices.

Although BioImage estimated allele size, the qualitative nature of an allele was analyzed. That is, whether or not a products appearing between lanes and gels are the same allele. For this reason, alleles at each locus were named 1,2,3 and so on depending on the number identified. Size information was used to verify scoring consistency. For the three most polymorphic loci, 6a2, Bt73, and 3b10 alleles were identified using a binning procedure. By creating an allele frequency histogram and factoring in repeat sizes, bin sizes were identified. For example, 31 peaks were observed for locus 3b10. Samples falling with a 2bp range on either side of the peak were classified into a group (bin) corresponding to the allele for that peak. In total 31 bins were identified for 3b10, 9 for 6a2 and 4 for Bt73. Scoring choices were also compared to previous studies conducted by John Nelson and Christian Smith using the same loci (SeaStar Biotech, University of Victoria, pers. comm.). Alleles identified in this study corresponded to their observations thus providing further verification of scoring choices.

Table 11. Number of alleles or bins observed at each locus.

Locus	Number of alleles observed
Ots 105	2
Ots 106	3
Bt73	4
6a2	8
3b10	31

Genetic diversity among and within populations was described using allele frequency data for the 5 microsatellite loci examined. Similar methodologies to Small et al. (1998a,b), who

compared microsatellite diversity from widespread coho populations within BC, were used. Allele frequencies and observed and expected heterozygosities were calculated using GENEPOP version 1.2 (Raymond and Rousseau 1995b). Further statistical analyses and parameter estimation was also conducted using GENEPOP. To test the null hypothesis that each population was in Hardy-Weinburg equilibrium, H-W tests were performed for each locus in each population. For loci OTS 105, OTS 106 and Bt73 that had 2, 3 and 4 alleles respectively the exact H-W tests were performed according to Louis and Dempster (1987). For loci 3b10 and 6a2 having 31 and 8 alleles an unbiased estimation of H-W probability was calculated using the Markov chain method described in Guo and Thompson (1992). All output P-values were corrected for simultaneous tests (Lessios 1992, Small 1998a). GENEPOP was used to test for linkage disequilibrium among loci using Fisher's exact test (Raymond and Rousseau 1995). This test was also used for pairwise and global tests for population differentiation in allele frequencies. F-statistics were calculated following Weir and Cockerham (1994), as this method is expected to give better estimates for small values of G_{st} (Chakraborty and Leimar 1987).

The program PHYLIP (Felenstein 1993) was used to estimate genetic distances among populations and to construct an unrooted neighbour-joining dendogram of their relationships. The allele frequency matrix for 4 loci was re-sampled 100 times using the sub-routine SEQBOOT. Locus 6a2 was omitted from the data set due to missing observations for one population. Using the resulting bootstrapped data, GENDIST was used to estimate Cavalli-Sforza and Edwards' (1967) chord distances among populations and then NEIGHBOR was run to construct unrooted neighbour joining trees for each matrix. CONSENSE created a consensus tree from the bootstrapped data and DRAWGRAM was used to graph the result. Cavalli-Sforza and Edwards' (1967) chords were used to construct the tree since they make no explicit assumptions about drift and mutation.

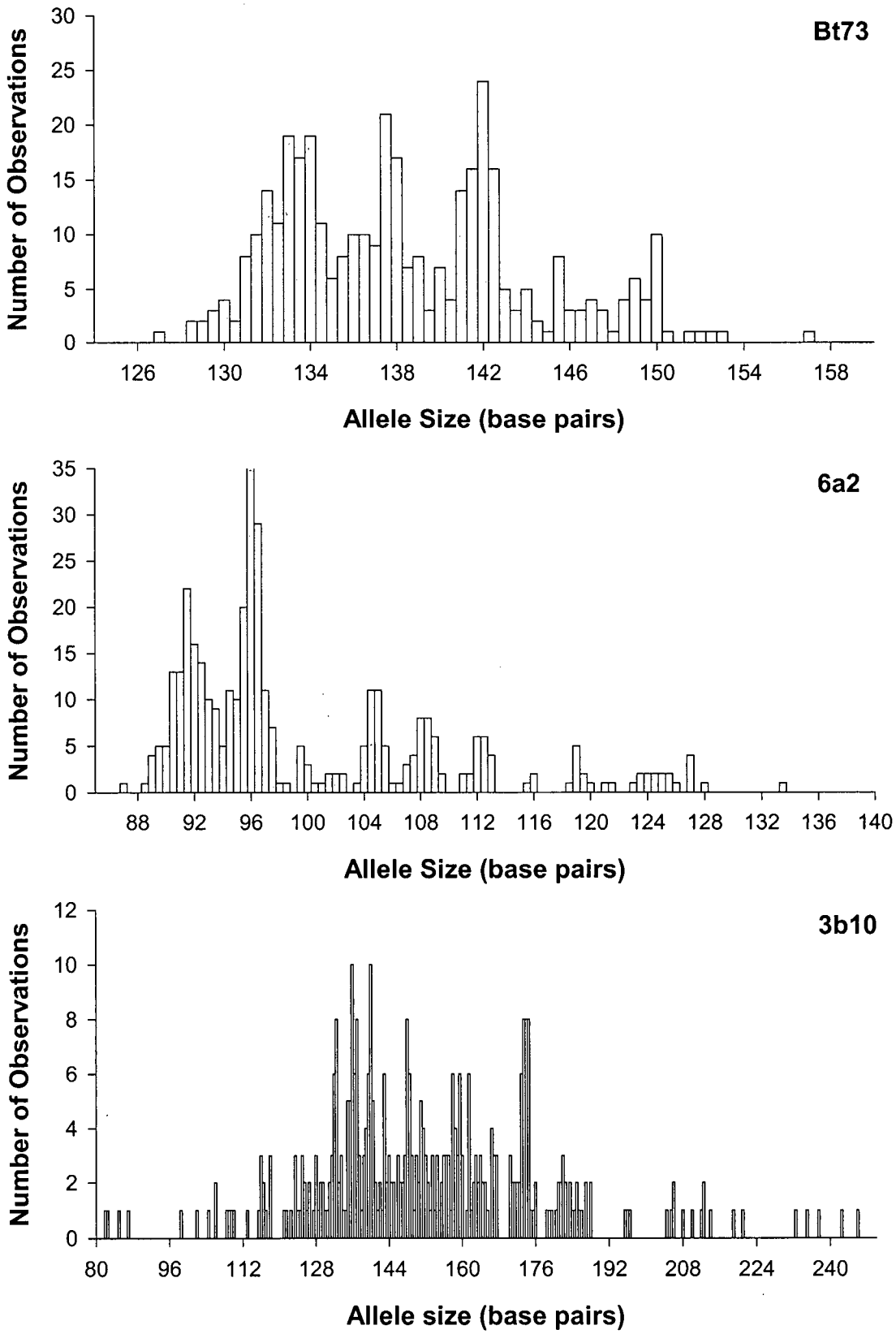


Figure 11. The allele-frequency distributions for loci Bt73, 6a2 and 3b10.

2.3.4 Heritability estimates

To demonstrate local adaptation for a particular trait one must show that the trait has a genetic basis (i.e. it is heritable) and that variation among individuals for the trait is associated with variation in fitness. Since the point of this study is to explore the genetic basis of phenotypic differences in juvenile coho observed among different habitats (i.e. local adaptation), it is necessary to establish the magnitude of heritability for the traits. Heritability (h^2) is the amount of phenotypic variation in the individual due to additive genetic variation (as opposed to variation due to the environment or non-additive genetic effects such as epistasis or dominance). The response (R) to selection (s) on a particular trait is directly related the heritability of the trait ($R = h^2S$). In order to estimate heritability, geneticists typically quantify the covariance for a particular trait among relatives in a known pedigree after rearing them in a common environment (Falconer and MacKay 1996). For organisms, such as coho, that are not easily reared in captivity this method has obvious drawbacks and lab-derived heritabilities are available for few strains. Also, organisms in the wild are subject to higher levels of environmental variation such that lab-derived heritability may be overestimated or underestimated (Houle 1992). To further complicate the situation, specific changes in the environment may have different effects on different genotypes through genotype-by-environment (G X E) interaction (Lewontin 1989). Moreover, phenotypic plasticity may be a genetic trait itself that is selectively advantageous in highly variable environments.

As a result of these limitations, there are obvious advantages to estimating heritability for quantitative traits in the wild. Ritland (1996) developed a method for estimating heritabilities in natural populations that was applied to the data set in this study. The first step involved inferring relatedness among individuals in the population through molecular marker variation. The individuals were genotyped and then using a maximum likelihood procedure relatedness between

individuals was inferred through the number of shared alleles and probability that these alleles are identical by descent (i.b.d.). In the second step, heritabilities for size-related traits (July fork length, December fork length, Relative Growth Rate) were examined using the inferred estimates of relatedness between individuals. The pairwise comparisons of relatedness were plotted against the pairwise covariance for each of the three traits. Approximately twice the slope of this relationship indicates the heritability of the trait. The following equation shows the relationship between the phenotypic similarity of two individuals (Z_i), their coefficient of relatedness (r_i), and narrow sense heritability (h^2):

$$Z_i = 2r_i h^2 + e_i$$

All estimates were derived according to Ritland (1996) and Lynch and Ritland (In press) using a FORTRAN program written by Kermit Ritland.

Chapter 3 RESULTS

3.1 Common Garden Experiment

Of the 924 fish tagged in July, 801 survived to early December (Table 12). The fish were reared for a period of 136-146 days depending on the tank and over this period most fish nearly doubled in fork length and experienced over a 6-fold or greater increase in mass (Table 13, Table 14). Uneven rearing periods resulted from sampling constraints due to the extensive time required to implant sequential coded wire tags in July and also to extract blood samples in December. Mortality varied from tank to tank and was not consistent between replicate tanks collected from the same site. However, the mortality figures also include escaped fish that probably account for the majority of loss. During September and October there were two high flow events leading to fish escapes throughout the laboratory facility. High rainfall increased the input of debris into Cypress Creek inflow resulting in clogged outflows on some tanks. Afterward, several fish from this experiment were observed swimming in drainways in the laboratory. They were excluded from the experiment. Before these events, mortality was recorded in each tank and ranged from 1 to 7 fish. Of the 801 surviving fish, 641 sequential coded tags were successfully dissected, identified and matched with filed tags and original data. For these fish both initial and final fork

length and mass data were available and further analysis was limited to this subset. Tags from the other 160 surviving fish could not be matched either because no tag was found, or because the found tag could not be unambiguously matched.

Table 12. Total survivors, mortalities, and escapees of fish per tank.

Site	Rep	Number of Fish Tagged in July	Number of Surviving Fish	Mortality and/or Escapees	Number of Tags Recovered and Matched
Railroad	1	125	104	20	81
Railroad	2	125	116	9	97
Confluence	1	133	115	18	98
Mashiter	1	150	122	28	98
Mashiter	2	150	149	1	87
Mamquam	1	125	107	18	87
Mamquam	2	117	88	29	82
Total		924	801	123	641

Throughout the growing period the fish were fed to excess. The most voracious feeding was observed during the summer months. As water temperatures became cooler during the winter feeding rates declined. Reduced feeding was evident by behavioural changes. In general, the fish were more disinterested in food and consequently more food drifted to the bottom of the tank. However, during this time smaller fish still fed. Several forms of competitive and territorial behaviour between fish were observed within tanks. Fish were often seen exhibiting lateral displays of aggression. During the more voracious feeding periods some of the larger fish would nip smaller fish although no outright cannibalism was observed. Generally the fish schooled at the bottom of the tank, but some tanks were more aggressive feeders than others were and appeared to consume more food. Variation was also observed in individual behaviour. There were always fish in tanks that did not school and seemed to sneak access to food. There

was no significant correlation between mean growth rates and number of fish in each tank (Pearson correlation = -0.39, N = 7, P>0.05).

The mean initial and final mass for all fish were 1.30 ± 0.034 g and 8.46±0.156 g, respectively. The mean initial and final fork lengths were 46.1 ± 0.35 mm and 89.1±0.5 mm, respectively. Statistics for each tank are displayed in Table 13 and Table 14. The distributions of initial and final fork lengths are displayed in Figure 12. The expected mean squares of the ANOVA model and synthesised test denominators are displayed in Table 15 and Table 12, respectively. There were no significant differences (P>0.05) between off-channel and main-channel fish for either initial or final mass (Table 17, Figure 13). However, there was some site variation for initial fork length (Figure 14). Fish collected from both the Mamquam channel (OC) and Confluence (MC) sites tended to be smaller than their counterparts from the other two sites. However, this difference was not significant (P>0.05). By December there were no significant differences among sites for either mass or fork length (P>0.05). The distributions for initial and final fork lengths for each site are displayed in Figure 15 and Figure 16. Distributions were skewed left initially and then normalised during the growing period. The replicate by site within habitat interaction for growth was significant (P<0.05) indicating there were tank effects.

Table 13. Mean ($\overline{X}$) initial and final fork length, specific growth rate (% mm increase/day), and their standard deviations (s) and coefficients of variation (CV) displayed by replicate.

				Length$_i$ (mm)			**Length$_f$ (mm)**			**Specific Growth Rate *G***		
Habitat	**Site**	**Rep**	**N**	$\overline{X}$	*s*	*CV*	$\overline{X}$	*s*	*CV*	$\overline{X}$	*s*	*CV*
Main Channel	Railroad	1	81	48.1	4.03	8.8	90.6	6.67	7.4	0.46	0.064	13.8
		2	97	47.2	4.80	10.2	86.4	6.26	7.25	0.44	0.066	13.6
	Confluence	1	98	42.7	4.30	10.1	89.5	7.21	8.1	0.51	0.057	11.3
Off Channel	Mashiter	1	98	46.8	3.77	8.1	91.1	6.74	7.4	0.48	0.062	13.1
		2	98	48.1	4.22	8.8	89.1	5.54	6.2	0.44	0.063	14.3
	Mamquam	1	87	44.5	3.89	8.8	88.6	5.78	6.5	0.50	0.062	12.2
		2	82	45.8	3.96	8.7	88.2	5.69	6.5	0.48	0.060	12.5

Table 14. Mean ($\overline{X}$) initial and final mass, specific growth rate (% g increase/day), and their standard deviations (s) and coefficients of variation (CV) displayed by replicate.

				Mass$_i$ (g)			**Mass$_f$ (g)**			**Specific Growth Rate G**		
Habitat	**Site**	**Rep**	**N**	$\overline{X}$	*s*	*CV*	$\overline{X}$	*s*	*CV*	$\overline{X}$	*s*	*CV*
Main Channel	Railroad	1	81	1.45	.449	30.9	9.01	2.136	23.7	1.34	0.238	17.5
		2	97	1.36	.460	33.9	7.71	1.831	23.8	1.30	0.207	16.0
	Confluence	1	98	1.03	.344	33.5	8.44	2.167	25.7	1.46	0.205	14.1
Off Channel	Mashiter	1	98	1.34	.398	29.5	9.18	2.393	26.1	1.38	0.225	16.3
		2	98	1.50	.460	30.8	8.51	1.842	21.7	1.26	0.240	19.0
	Mamquam	1	87	1.18	.404	34.4	8.29	1.179	20.7	1.46	0.240	16.5
		2	82	1.22	.336	27.6	8.09	1.681	20.1	1.39	0.214	15.3

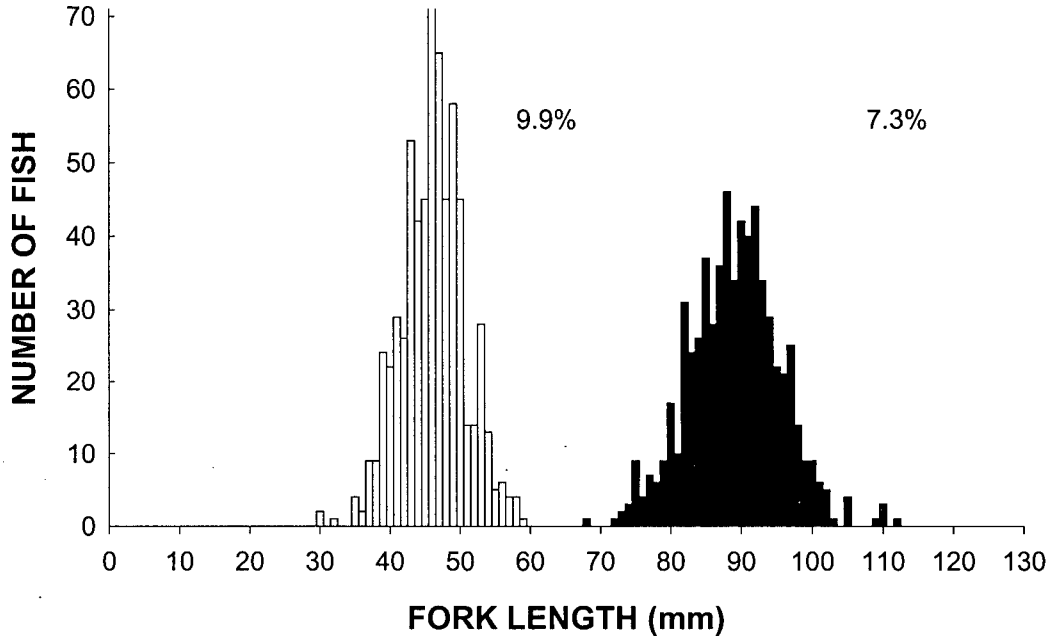


Figure 12. July (open bars) and December (black bars) fork length-frequency distributions and the coefficients of variation for all fish.

Table 15. Observed mean squares for the ANOVA model.

EMS	HABITAT	REP	SITE [HABITAT]	REP*SITE [HABITAT]
HABITAT	180.799	0	90.3994	45.1997
REP	0	181.416	0	45.354
SITE[HABITAT]	0	0	122.427	61.2134
REP*SITE [HABITAT]	0	0	0	60.3092

Table 16. Synthesised denominators used to test effects in the ANOVA model.

Source	MS	DF	Denominator MS Synthesis
HABITAT	1.56504	2.0746	0.7384*SITE[HABITAT]+0.2616*Residual
REP	0.30307	2.4091	0.752*REP*SITE[HABITAT]+0.248*Residual
SITE[HABITAT]	0.37107	1.9826	1.015*REP*SITE[HABITAT]-0.015*Residual
REP*SITE [HABITAT]	0.1086	634	Residual

Table 17 (a-d). ANOVA tables for July and December mass and fork lengths.
a) July mass

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.06312	0.06312	0.0403	0.8588
REP	1	0.53532	0.53532	1.7663	0.2957
SITE[HABITAT]	2	4.16207	2.08103	5.6082	0.1527
REP*SITE[HABITAT]	2	0.73439	0.3672	3.3811	0.0346
ERROR	634	68.855	0.109		
TOTAL	640	79.051			

b) July fork length

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.00541	0.00541	0.0334	0.8714
REP	1	0.05556	0.05556	2.1183	0.2644
SITE[HABITAT]	2	0.43214	0.21607	6.6574	0.1318
REP*SITE[HABITAT]	2	0.0642	0.0321	3.8133	0.0226
ERROR	634	5.337	0.0084		
TOTAL	640	6.46			

c) December mass

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.0008	0.0008	0.0040	0.9548
REP	1	0.18819	0.18819	1.1440	0.3813
SITE[HABITAT]	2	0.50486	0.25243	1.2445	0.4465
REP*SITE[HABITAT]	2	0.40132	0.20066	3.6587	0.0263
ERROR	634	34.771	0.0548		
TOTAL	640	36.682			

d) December fork length

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.00022	0.00022	0.0110	0.9250
REP	1	0.01486	0.01486	0.8913	0.4321
SITE[HABITAT]	2	0.04959	0.02479	1.1962	0.4561
REP*SITE[HABITAT]	2	0.04099	0.0205	4.0370	0.0181
ERROR	634	3.219	0.00507		
TOTAL	640	3.395			

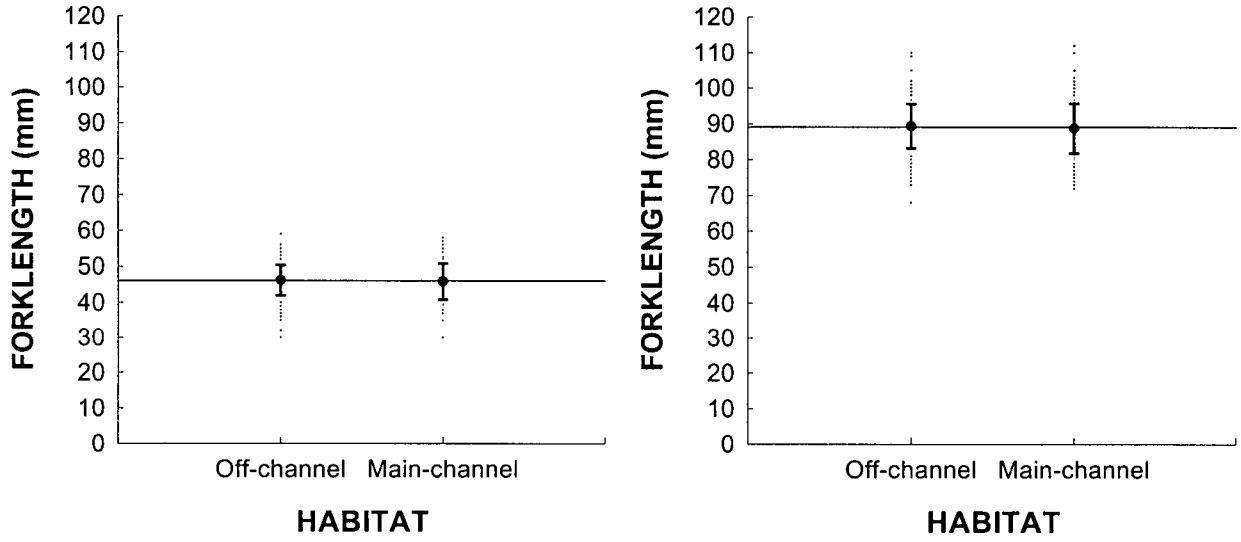


Figure 13. Mean initial (left panel) and final (right panel) lengths for off-channel and main-channel fish. The plot displays the mean of each site (circle), standard deviation (error bars) and outliers (dots). The horizontal line is the overall mean.

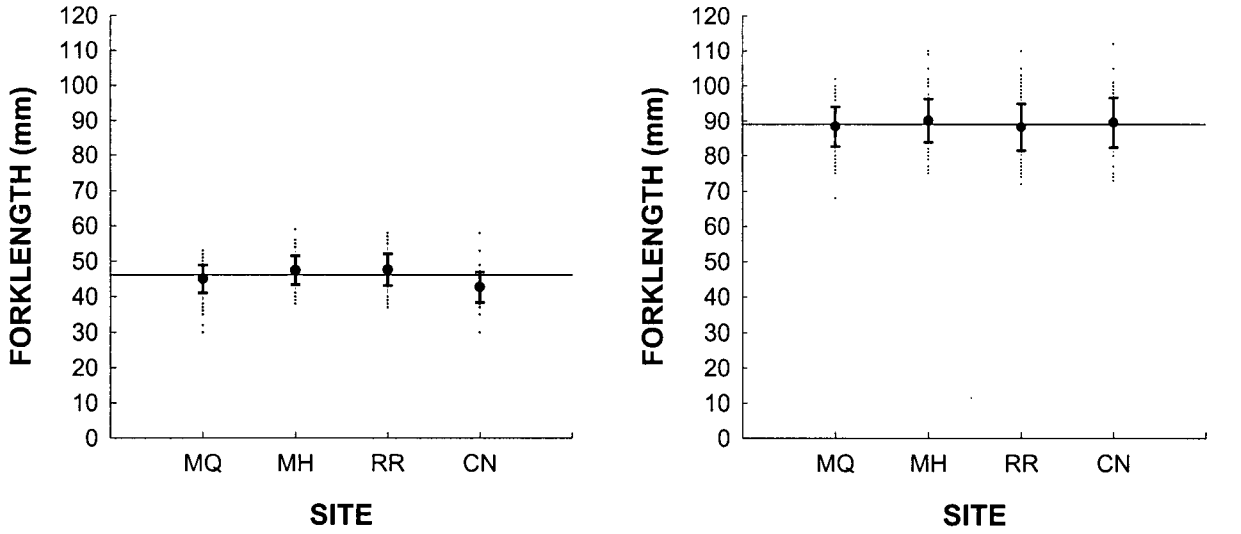


Figure 14. Mean initial (left panel) and final (right panel) lengths for fish collected from different sites within off-channel and main-channel habitats. (MQ and MH refer to Mamquam and Mashiter channels, both off-channel sites. RR and CN refer to Railroad and Confluence, both main-channel sites.)

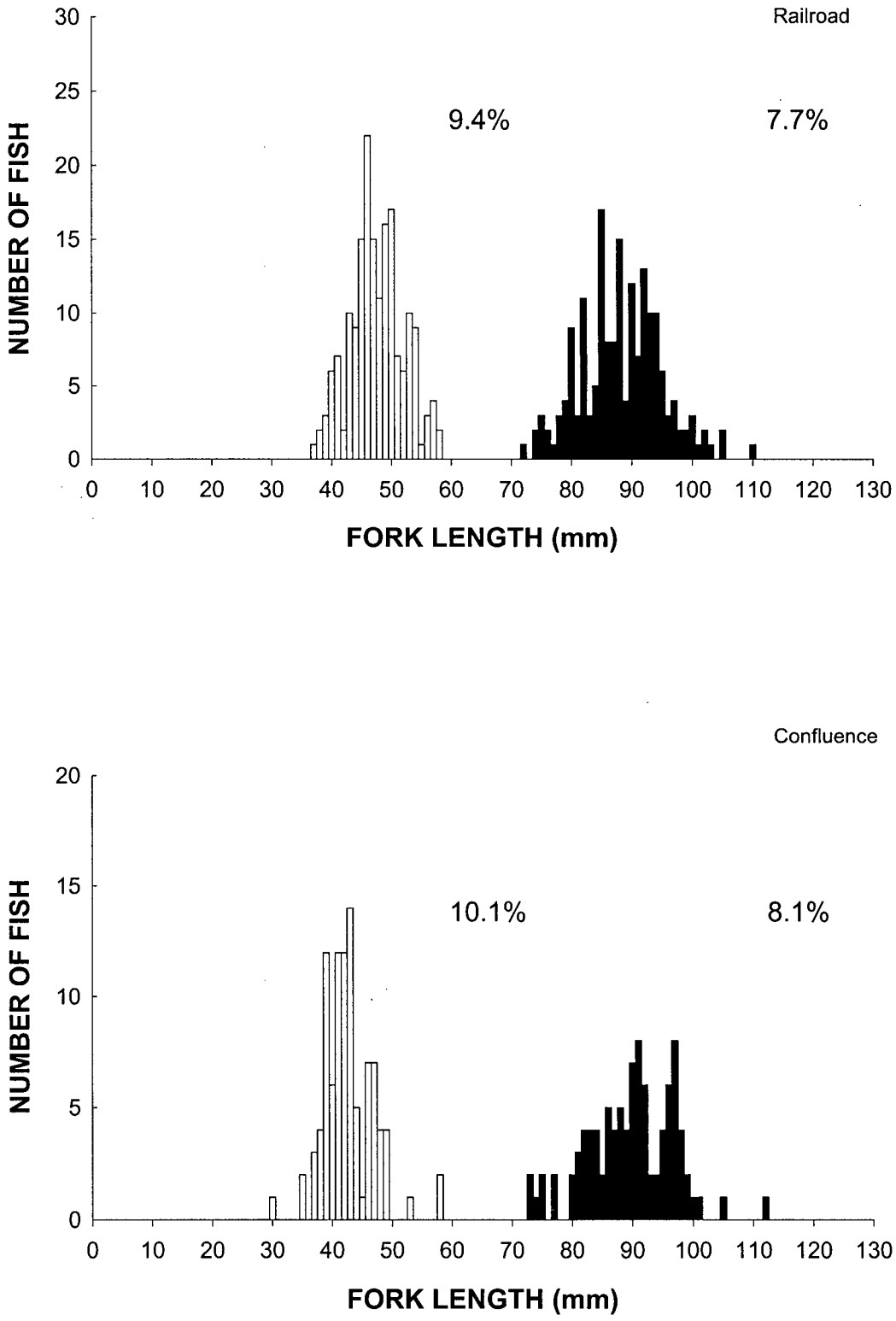
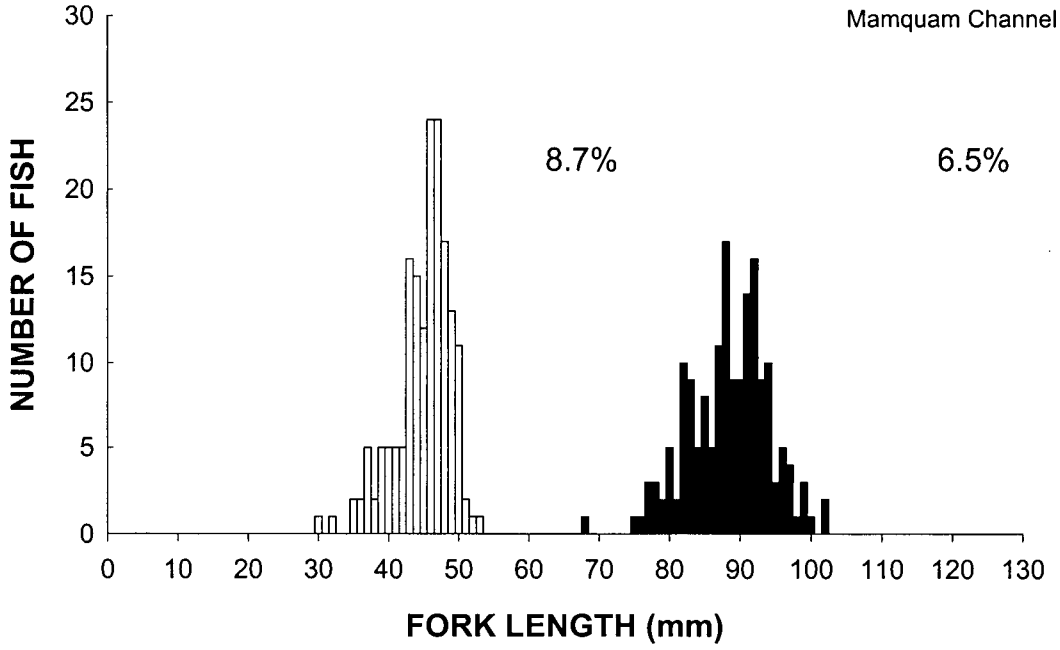


Figure 15. July (open bars) and December (black bars) fork length-frequency distributions and coefficients of variation for main-channel fish.



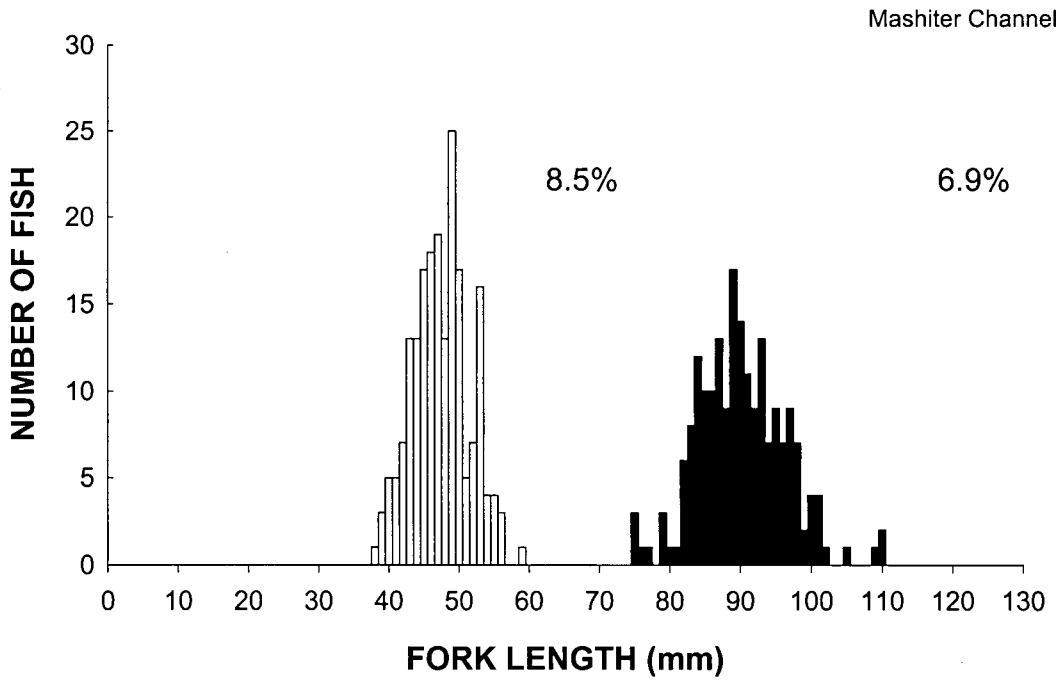


Figure 16. July (open bars) and December (black bars) fork length-frequency distributions and coefficients of variation for off-channel fish.

For all fish, the mean specific growth rate was 0.679 ±0.0101% mm/day. There was no significant difference between off-channel and main-channel fish for specific growth rate (Table 18, Table 19). However, there was a significant difference between sites for fork length (Table 19). Confluence, a main-channel site, and Mamquam, a side-channel site, had significantly higher mean specific growth rates then the other two sites (Figure 17). Given that there was no significant difference between mean and initial fork lengths for all sites, the higher specific growth rates in the Confluence and Mamquam populations indicate that smaller fish in this population grew faster than smaller fish in other populations. There was a negative correlation between initial fork length and specific growth rate (Figure 19). The correlation between final fork length and specific growth rate was positive, but weaker. Correspondingly, there was only a weak correlation between initial length and final length (Figure 18). The fact that mean growth rate for length varied significantly among sites within habitats indicates that a greater growth compensation effect occurred within these groups. Fish from both the Confluence and Mamquam Channel sites were slightly smaller and had greater variation for size in July than the other two sites.

Table 18. ANOVA table results for specific growth rate (mass).

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.01029	0.01029	0.0234	0.8917
REP	1	0.78621	0.78621	19.1571	0.0111
SITE[HABITAT]	2	1.15394	0.57697	15.2590	0.0661
REP*SITE[HABITAT]	2	0.07599	0.038	0.7558	0.4701
ERROR	634	31.872	0.0503		
TOTAL	640	35.142			

Table 19. ANOVA table results for specific growth rate (fork length).

Source	DF	SS	MS	F Ratio	Prob>F
HABITAT	1	0.00083	0.00083	0.0157	0.9113
REP	1	0.07522	0.07522	36.2275	0.0007
SITE[HABITAT]	2	0.1401	0.07005	47.1008	0.0257
REP*SITE[HABITAT]	2	0.00304	0.00152	0.4043	0.6676
ERROR	634	2.385	0.003761		
TOTAL	640	2.780			

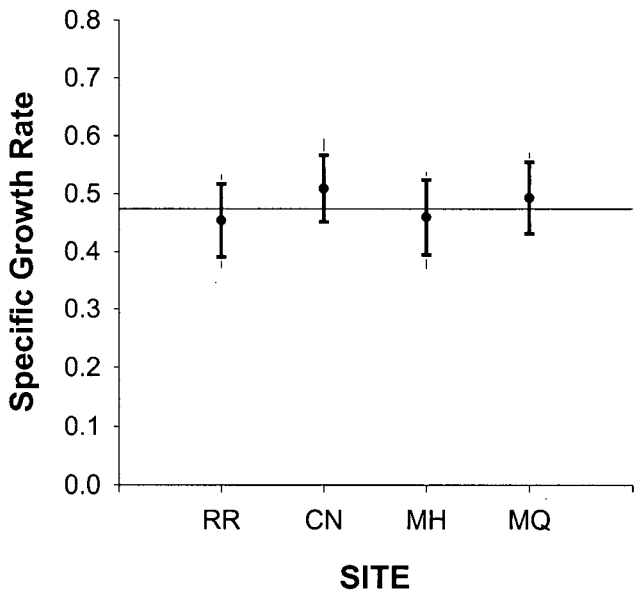


Figure 17. Mean specific growth rate for fork length by site. (RR and CN refer to Railroad and Confluence, both main-channel sites. MQ and MH refer to Mamquam and Mashiter channels, both off-channel sites.)

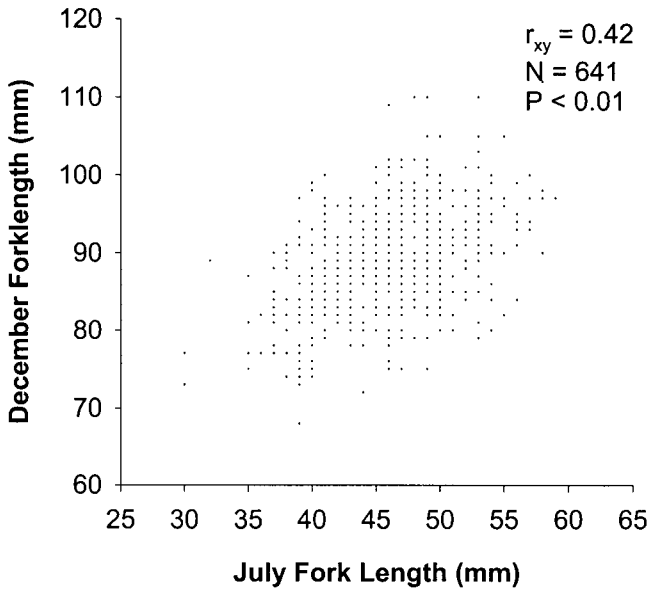


Figure 18. Scatterplot between initial and final fork lengths for all fish. Pearson correlation and associated significance level are presented.

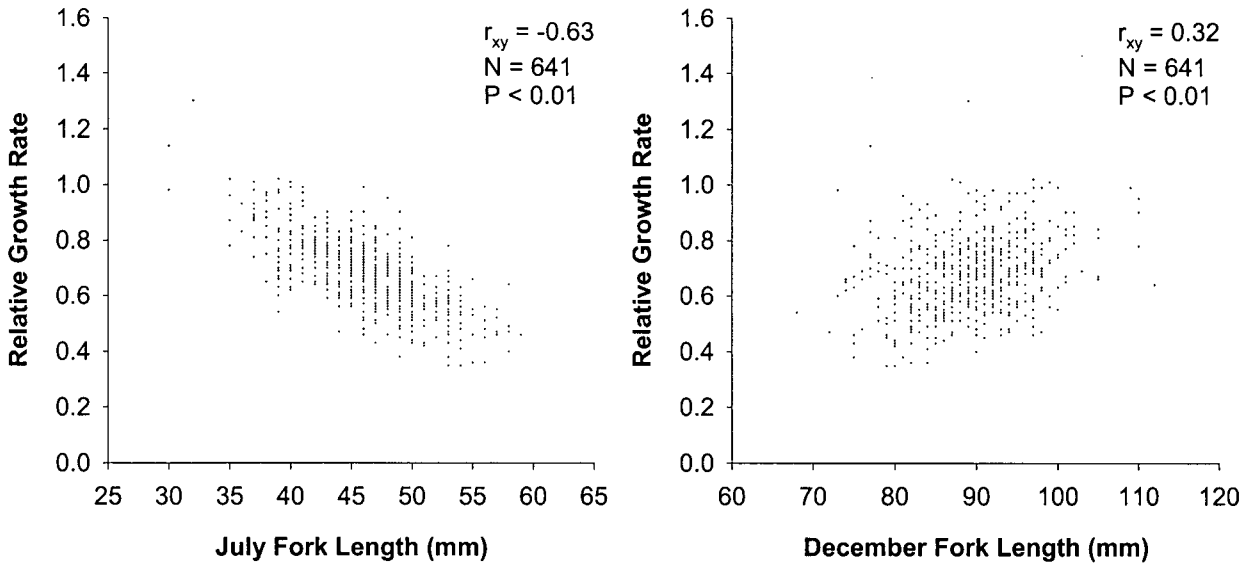


Figure 19. Scatterplots between July fork length and specific growth rate (left panel) and December fork length and specific growth rate (right panel). Pearson correlations and their associated significance levels are presented.

3.2 Microsatellite Analysis

For loci Ots 106, Ots 105 and Bt73, which had 3, 2 and 4 alleles respectively, each allele was observed in every population. For the more polymorphic loci, 6a2 and 3b10, with 8 and 31 alleles respectively, not every allele was present in every population. Frequencies and observed and expected heterozygosities are listed in Table 22 and Table 23. Observed heterozygosities were moderate to high ranging from 0.57-0.87 (Table 20). All populations were in Hardy-Weinburg equilibrium for loci Ots 106, Ots 105 and 6a2. However, Hardy-Weinburg equilibrium was rejected for the Mamquam Channel sub-population in the first sample at locus Bt72 ($P<0.05/7$ – the significance level of 0.05 was corrected for 7 simultaneous tests). Very likely this was due to the sampling methodology for the group which involved sampling redds. Hardy-Weinburg equilibrium was also rejected for 4 populations (Mashiter, both Mamquam Channel samples, and Vedder) for locus 3b10 ($P<0.05/7$) due to an excess of homozygotes. Previous studies and pedigree analysis (Smith and Nelson, pers. comm.) plus lack of Hardy-Weinburg equilibrium for the 4 populations in this study, indicate the presence of a null allele at locus 3b10. A null allele has no microsatellite repeat for the locus examined and thus no PCR product is observed. The null allele invalidated exact probability tests for population differentiation at this locus. However, corrected allele frequencies in the presence of a null allele were calculated using GENEPOP. The program uses a maximum likelihood procedure to estimate corrected allele frequencies according to the EM algorithm when null alleles are present (Dempster et al. 1977). Corrected frequencies were used to estimate Cavalli-Sforza chord distances in the PHYLIP program (Felenstein 1993). Tests for linkage disequilibrium indicated the 5 loci were independent.

Table 20. Mean heterozygosities of the five loci examined.

LOCUS	HETEROZYGOSITY
Ots 106	0.57
Ots 105	0.61
Bt73	0.74
6a2	0.80
3b10	0.87

The single-locus and multi-locus F_{st} values indicated significant differences in allele frequencies among populations (Figure 20). In pairwise tests using Fisher's exact test, Cheakamus and Vedder populations were significantly different ($P<0.05/21$) from all Mamquam sub-populations at least one locus. They were also significantly differently from each other at least one locus ($P<0.05/21$). Within the Mamquam River, the two samples collected from the Mamquam channel in July and December showed no significant differences in allele frequencies. Similarly, two other sub-populations, Mashiter and Railroad, were not significantly different from each other or the Mamquam channel samples. However, the sub-population, Confluence, collected upstream from the others, showed significantly different allele frequencies from every other Mamquam population at least one locus. The dendogram constructed from bootstrapped C-S chord distances (Table 21) reflects the hierarchical nature of the sampling with the exception of the unique Confluence sub-population, which is set apart from other Mamquam groups. Samples were collected from within the Mamquam to compare within-river variation, and also the Cheakamus and Vedder Rivers to compare variation among rivers and regions.

Table 21. Cavalli-Sforza chord distances calculated from the original, not bootstrapped, data set. RR, CON, MH, MQ refer to the Railroad, Confluence, Mashiter, and Mamquam channel sites, respectively. They are all located on the Mamquam River. Mam refers to the second sample collected from the Mamquam channel in December. CHK and VED refer to the Cheakamus and Vedder Rivers.

Population	RR	CON	MH	MQ	Mam	CHK
CON	0.0316					
MH	0.0276	0.0267				
MQ1	0.0376	0.0431	0.0240			
Mam	0.0378	0.0457	0.0248	0.0309		
CHK	0.0422	0.0409	0.0371	0.0488	0.0499	
VED	0.0484	0.0391	0.0282	0.0549	0.0530	0.0346

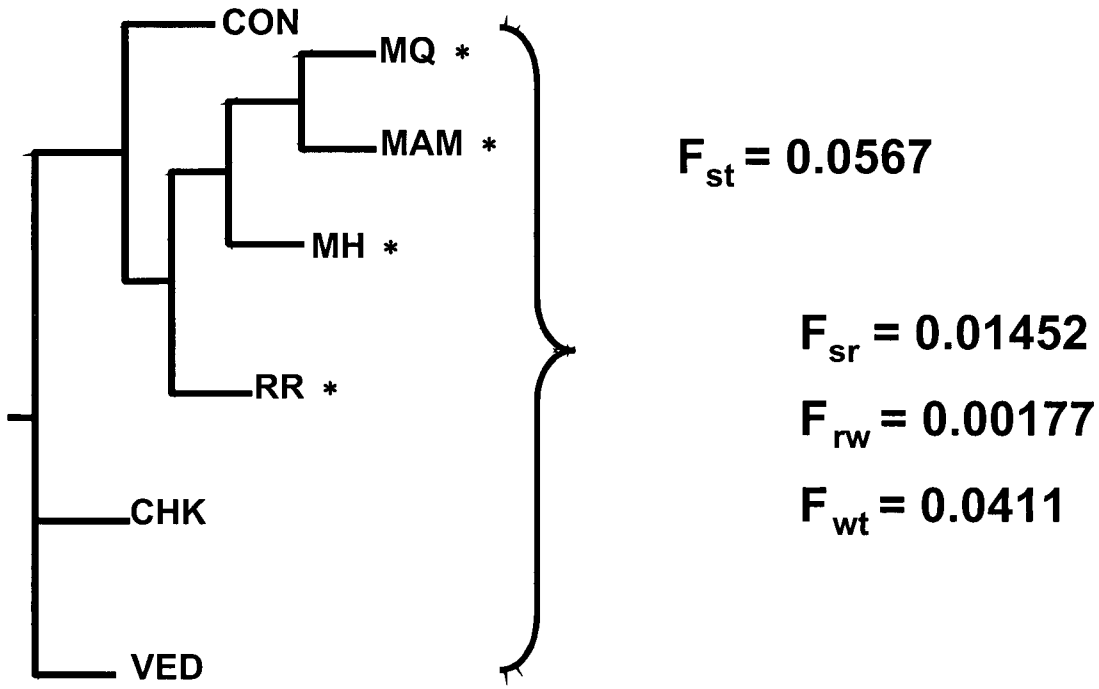


Figure 20. Phenogram displaying the genetic relationships among the populations sampled. It was constructed from Cavalli-Sforza chord distances calculated from 500 bootstrapped samples of the initial allele frequency matrix. The branch lengths do not represent distances. Sub-populations with an asterisk beside them are not significantly different ($P>0.05/21$); all others are (Fisher's exact test $p<0.05/21$). F_{st} values are calculated according to Weir and Cockeram (1994). F_{st} compares all the significantly different sub-populations. F_{sr} is the component due to variation between Mamquam sub-populations. F_{rw} is the component due to between-river variation (i.e. Mamquam, Cheakamus and Vedder). F_{wt} is the regional component (i.e. Squamish v. Lower Fraser). RR, CON, MH, MQ refer to the Railroad, Confluence, Mashiter, and Mamquam channel sites, respectively. They are all located on the Mamquam River. MAM refers to the second sample collected from the Mamquam channel in December. CHK and VED refer to the Cheakamus and Vedder Rivers.

Table 22. Allele frequencies and Observed (H_o) and Expected (H_e) Heterozygosities for Ots106, Ots105, Bt73 and 6a2. Populations out of H-W equilibrium are indicated with an asterisk beside H_o. RR, CON, MH, MQ refer to the Railroad, Confluence, Mashiter, and Mamquam channel sites, respectively. They are all located on the Mamquam River. MAM refers to the second sample collected from the Mamquam channel in December. CHK and VED refer to the Cheakamus and Vedder Rivers.

		Mamquam River						
		Main Channel		Off Channel				
Locus		RR	CON	MH	MQ	Mam	CHK	VED
Ots106	Allele 1	0.462	0.196	0.382	0.451	0.474	0.125	0.118
	2	0.423	0.625	0.382	0.353	0.368	0.625	0.353
	3	0.115	0.179	0.235	0.196	0.158	0.25	0.529
	N	104	56	102	102	38	40	34
	H_o	0.62	0.61	0.80	0.78	0.42	0.35	0.47
	H_e	0.60	0.55	0.66	0.64	0.63	0.54	0.60
Ots105	1	0.539	0.833	0.5	0.413	0.521	0.396	0.453
	2	0.461	0.167	0.5	0.587	0.479	0.604	0.547
	N	102	54	104	104	48	48	64
	H_o	0.61	0.85	0.65	0.48	0.63	0.54	0.53
	H_e	0.50	0.97	0.50	0.49	0.51	0.49	0.50
Bt73	1	0.514	0.346	0.443	0.604	0.514	0.2	0.233
	2	0.286	0.212	0.318	0.177	0.114	0.2	0.2
	3	0.171	0.365	0.216	0.208	0.371	0.5	0.4
	4	0.029	0.077	0.023	0.01	0	0.1	0.167
	N	35	52	96	88	35	10	30
	H_o	0.71	0.77	0.70	0.52*	0.79	0.8	0.93
	H_e	0.65	0.71	0.66	0.57	0.61	0.73	0.74
6a2	Allele 1	0.103	0.341	0.291	0.259	0.241	0	0.071
	2	0.397	0.114	0.417	0.426	0.31	0	0.554
	3	0.034	0	0.063	0	0.017	0	0.054
	4	0.172	0.205	0.042	0.056	0.138	0	0.125
	5	0.121	0.091	0.083	0.185	0.172	0	0.036
	6	0.121	0.182	0.083	0.019	0.086	0	0.018
	8	0.034	0	0	0.019	0.017	0	0.089
	9	0.017	0.068	0.021	0.037	0.017	0	0.054
	N	58	44	49	54	58	0	56
	H_o	0.79	0.82	0.88	0.78	0.79		0.72
	H_e	0.78	0.80	0.71	0.73	0.80		0.67

Table 23. Observed and corrected (bold) allele frequencies plus observed (H_o) and expected (H_e) heterozygosities for 3b10. Allele frequencies are corrected for the presence of a null allele in this locus. Populations out of H-W equilibrium are indicated with an asterisk beside H_o. RR, CON, MH, MQ refer to the Railroad, Confluence, Mashiter, and Mamquam channel sites, respectively. They are all located on the Mamquam River. MAM refers to the second sample collected from the Mamquam channel in December. CHK and VED refer to the Cheakamus and Vedder Rivers.

	Mamquam River						
	Main Channel		Off Channel				
Bin	RR	CON	MH	MQ	Mam	CHK	VED
1	0.017	0	0	0	0	0.048	0.022
	0.017					**0.031**	**0.022**
2	0	0	0	0	0	0	0
3	0.017	0	0	0	0	0	0
	0.017						
4	0	0	0	0	0.031	0.024	0
					0.031	**0.024**	
5	0	0	0	0.031	0	0.024	0
				0.031		**0.024**	
6	0	0	0	0.016	0	0	0
				0.016			
7	0.067	0	0	0.016	0.031	0.048	0
	0.067			**0.016**	**0.062**	**0.048**	
8	0.017	0.019	0	0	0	0	0
	0.017	**0.019**					
9	0.033	0.037	0.013	0.016	0	0.024	0.043
	0.033	**0.037**	**0.013**	**0.016**		**0.024**	**0.043**
10	0.067	0.019	0.026	0.016	0.031	0.024	0.022
	0.067	**0.019**	**0.026**	**0.016**	**0.031**	**0.024**	**0.022**
11	0	0.056	0.066	0.266	0.031	0.024	0
		0.056	**0.072**	**0.260**	**0.031**	**0.024**	
12	0.067	0.074	0.197	0.172	0.063	0.071	0.196
	0.056	**0.061**	**0.197**	**0.172**	**0.063**	**0.071**	**0.169**
13	0.083	0.111	0.079	0.063	0.125	0.024	0.130
	0.083	**0.100**	**0.079**	**0.071**	**0.125**	**0.024**	**0.138**
14	0.017	0.074	0.026	0.125	0.094	0	0.043
	0.017	**0.061**	**0.026**	**0.120**	**0.094**		**0.050**
15	0.067	0.074	0.066	0.031	0.031	0.095	0.043
	0.067	**0.074**	**0.066**	**0.019**	**0.031**	**0.109**	**0.043**
16	0.05	0.074	0.066	0	0.063	0	0.130
	0.05	**0.074**	**0.059**		**0.063**		**0.130**
17	0.033	0.037	0.053	0.016	0.031	0.119	0.065
	0.033	**0.037**	**0.053**	**0.016**	**0.031**	**0.119**	**0.047**

	Mamquam River						
	Main Channel		Off Channel				
Bin	RR	CON	MH	MQ	Mam	CHK	VED
18	0.083	0.130	0.066	0.063	0.063	0.143	0.065
	0.063	**0.130**	**0.066**	**0.063**	**0.063**	**0.143**	**0.047**
19	0.05	0	0.026	0.031	0.094	0.095	0.065
	0.038		**0.026**	**0.031**	**0.094**	**0.084**	**0.047**
20	0	0.019	0.079	0.016	0.063	0.048	0.065
		0.019	**0.072**	**0.016**	**0.063**	**0.048**	**0.065**
21	0	0	0.026	0	0	0.024	0.022
			0.016			**0.024**	**0.022**
22	0.217	0.093	0.145	0.047	0.063	0.048	0.022
	0.228	**0.093**	**0.140**	**0.047**	**0.063**	**0.048**	**0.022**
23	0	0.019	0.039	0	0.031	0	0
		0.019	**0.039**		**0.031**		
24	0.033	0.093	0	0.016	0	0.024	0
	0.033	**0.093**		**0.016**		**0.024**	
25	0.067	0.019	0	0.016	0	0.024	0.065
		0.019		**0.016**		**0.024**	
26	0	0	0	0	0	0.024	0
						0.024	
27	0	0	0	0	0	0	0
28	0	0.019	0.013	0	0.031	0.024	0
		0.019	**0.013**		**0.031**	**0.024**	
29	0	0	0.013	0.031	0.031	0	0
				0.031	**0.031**		
30	0	0	0	0.000	0.031	0	0
					0.031		
31	0	0	0	0.016	0.031	0	0
					0.031		
32	0	0	0	0	0.031	0.024	0
33	0	0	0	0	0	0	0
Null	**0.047**	**0.074**	**0.035**	**0.030**	**0**	**0.038**	**0.133**
H_o	0.92	0.94	0.91	0.73	0.97	0.95	0.92
H_e	0.87	0.85	0.89	0.77	1.0	0.90	0.78
N	60	54	76	64	32	42	46

3.3 Heritability Estimates

Figure 21 displays the plots of pairwise comparisons between individuals for estimated relatedness (x-axis) and phenotypic similarity for size related traits (y-axis). The top plot is July size (fork length), the middle is December size (fork length) and the bottom plot is relative growth rate. Approximately 10 outliers with estimated relatedness less than -1.2 were removed from the data set because those estimates are artifacts of the method. Positive slopes indicate heritable traits. Many samples were not informative because of incomplete data for quantitative traits. Some of the individuals that were assayed for microsatellite variation were missing or had damaged tags so size and growth data was unavailable. As well, 2 of the 5 loci sampled were not as informative as the other three because they were not as polymorphic. As a result, approximately 1450 pairwise comparisons were used out of more than 4000 possible combinations. Nevertheless, both July size and relative growth rate showed positive relationships while there was no relationship for December size. The regressions for July size and relative growth rate were significant ($P<0.001$).

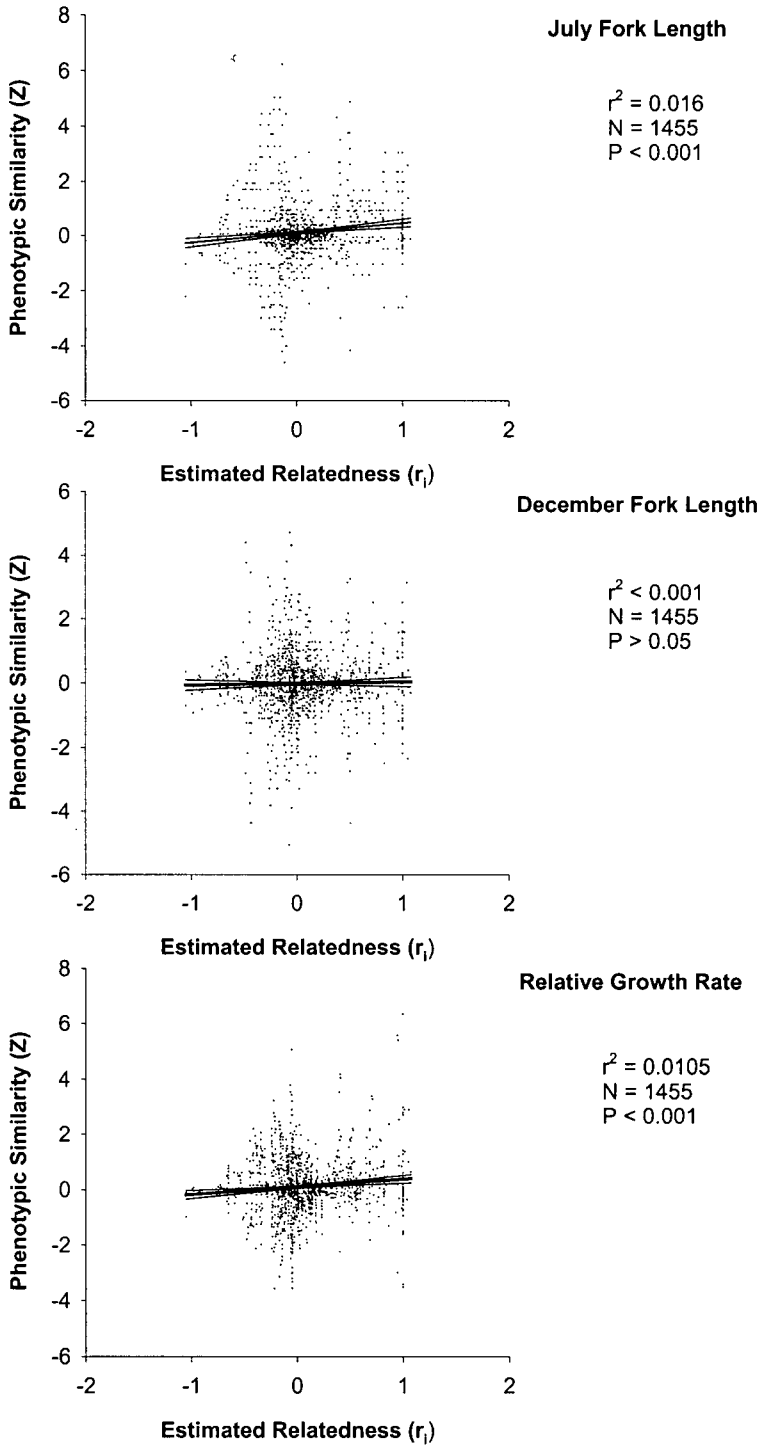


Figure 21. Scatterplots of estimated relatedness and phenotypic similarity for size-related traits. The thick line is the regression line and thin lines are 95% confidence intervals. Coefficients of determination, sample sizes and significance probabilities are indicated.

Chapter 4 DISCUSSION

This study was motivated by field observations indicating that juvenile coho rearing in the Mamquam River showed phenotypic variation in size corresponding to habitat use. Individuals using main-channel habitat for summer nursery grounds were larger than those using artificially created off-channel habitat. Both groups utilise off-channel habitat for overwintering as indicated by increased estimated population sizes in off-channel habitat after September (Sheng et al. 1990). The observation of size differences between the groups does not imply fixed differences in phenotypes since phenotypic plasticity in development can result from environmental variation between rearing habitats. The genetic component of the size variation was studied by rearing individuals from each habitat in a common laboratory environment for six months. Microsatellite DNA variation between groups was also compared. These data were combined in order to examine heritability for size-related traits.

The size differences observed in juvenile coho reared in off-channel and main-channel habitats may result from phenotypic plasticity, developmental constraints, adaptive variation, genetic polymorphism or genetic drift. In this study, the null hypothesis was that environmental variation induces a plastic response associated with environmental differences in rearing habitat.

Three alternative hypotheses were proposed. First, that the initial population is not segregated by size, but that fry show habitat selection or exclusion based on genotype. Secondly, different spawning populations, or the tendency for association between spawning location and genotype, result in phenotypically and genetically distinct populations. And thirdly, that phenotypically and genetically distinct populations result from differences in the timing of spawning. The study showed that although some initial, albeit statistically non-significant, variation in size was observed between fish collected from different sites within off-channel and main-channel habitats, no significant differences were observed between groups after six months rearing in a common environment. For both initial and final sizes, more variation was observed between individuals within groups than among groups. This result indicates that variation observed in juvenile size is either a result of phenotypic plasticity or not associated with fixed genetic differences between groups of fish rearing in main and off-channel habitats in the Mamquam River.

Growth is a complex trait influenced or regulated by many factors including, but not limited to, temperature, oxygen concentration, light, food availability, metabolic rate, and competition (Brett 1979). Within the laboratory, the fish experienced a common environment although one quite different from even the most optimal habitat they would experience *in situ*. The three most significant differences were temperature, food and space. Each of these factors affects growth differently. Although temperature followed seasonal trends, Cypress Creek, the primary water source, is generally warmer than the Mamquam River. The fish experienced temperatures closer to those associated with optimal growth in the tanks (15^{o}C - Brett 1979). Therefore, within the laboratory would be expected to grow faster. However, since the maximum of growth for coho is independent of size with regard to temperature (Brett 1979), all fish would have presumably experienced a proportional increase in growth rate with regards to temperature.

The situation with food and space is somewhat different than temperature because their effect on growth rate is size-dependent. When fed increased rations, smaller fish experience a greater relative increase in growth rate compared to larger fish. Smaller fish are more efficient converters of energy because metabolic rates generally decrease with size, thus food energy is converted to growth. This effect would have been moderated by the amount of intraspecific competition within tanks. Juvenile coho compete for food and territories (Nielson 1995) and fish of high-ranking status can suppress the growth of lower-ranking individuals (Metcalfe and Huntingford 1990). Status is determined by an individual's ability to coerce and dominate others and has been shown to be more directly related to metabolic rate than size in studies of closely related species (Yamamoto et al. 1998). This competition results in growth depensation. Over time faster growing individuals become larger relative to slow growers and the variability for size increases within a population. The results of this study showed the opposite effect, that is, growth compensation. Variation for size was reduced over the growing period probably due to the fact that food was not limiting growth and that the environment was relatively homogeneous.

The conditions within the laboratory resulting in growth compensation over the summer to winter rearing period do not occur *in situ*. Growth depensation generally occurs in populations of underyearling coho (Ricker 1979). Fish rearing in river habitats are subject to high levels of environmental variability and not all habitats provide equal growth opportunity. Both density-independent effects, such as temperature and flow, and density-dependent effects, such as competition, limit growth potential. In addition, fish experience high levels of mortality throughout the juvenile period especially during their first overwintering period (Bradford 1997). Fish trade-off costs incurred from pressures such as defending territories, foraging, avoiding predators, surviving over winter and smoltification. While fast growth has traditionally been viewed as maximising survival, it may not be beneficial in every ecological context. For

example, sustaining maximal growth may leave insufficient energy for predator avoidance; thus slow growth may be favoured in systems with relatively long growing seasons (Conover and Schultz 1997). Conditions favouring one strategy over others may vary from brood to brood and over the life history of a single brood. Thus, through alternative selection mechanisms genetic polymorphism may be maintained within populations. Examples include age and size variation at smoltification and maturation (Thorpe et al. 1983), variation in reproductive tactics (Gross 1985) and variation in juvenile foraging strategies (Nielson 1992).

Although no significant differences in growth were observed between fish from off-channel and main-channel habitats, there were significant differences for growth among sites within habitats. Fish from the Confluence site, a main-channel habitat, and the Mamquam Channel site, an off-channel habitat, had significantly higher growth rates than the other two sites. This difference probably resulted from an increased growth compensation effect in these tanks. Fish from both these sites tended to be smaller at the beginning of the experiment as they were collected last. Therefore, they were probably at a slightly different developmental stage for length reflecting their later emergence times. This possibility is supported by the initial length-frequency distributions for each site. Although mean fork lengths were not significantly different between sites, the distributions of Confluence and Mamquam fish tended to be skewed left at the beginning of the experiment.

More interesting was the variation for growth observed between individuals from within habitats. Under favourable growing conditions, juvenile growth in underyearlings generally follows a seasonal trend corresponding to food abundance, temperature, photoperiod and other factors (Ricker 1979). Fish experience fast growth in the summer that levels off during winter conditions. Although no age data were available for the fish, presumably initial length-frequency distributions corresponded to the timing of emergence so that smaller fish were

younger on average. This may or may not be a reasonable assumption for early on in the rearing period, but clearly it is not reasonable at the end of the summer rearing period as indicated by the plot of final versus initial size. By December, many of the smaller and presumably younger fish of July had caught up to or exceeded their initially larger counterparts, while others remained small in comparison to the mean. The individual growth trajectories within groups, therefore, were quite variable. The question remains whether or not the individual variation for growth was genetically determined since developmental factors that affect growth variation among individuals may be under genetic control. When traits are heritable, genetic variation can be maintained by natural selection.

As it turned out, the plots of phenotypic similarity and relatedness showed positive correlations between estimated relatedness and phenotypic similarity for July fork length and relative growth rate; thus indicating these traits are heritable to some extent. There was no significant correlation for December fork length. Heritability for size in salmon decreases with age due to initial maternal effects. However, in pedigree studies, growth has generally been shown to be heritable through to maturity. The lack of correlation in December probably resulted from the decreasing influence of maternal effects over the growing period combined with the increasing influence of the homogeneous laboratory environment. By December, there was less variation for size in all fish due to the compensation effect. Interpretation of these results is somewhat limited by the data. It is clear from the plots and low r^2 values that the relationships are weak. However, trends are evident and more extensive sampling would probably strengthen the relationships. The approximately 1400 comparisons used in this study were less than optimal. With more loci and a larger sample size stronger relationships would emerge and parameters, such as variance of relatedness, would be estimable.

Further attempts to estimate heritability in the wild using this technique provide one way to gain a better understanding of the genetic basis of phenotypic differentiation in salmon *in situ* (Ritland 1996). Given environmental complexity and complex patterns of relatedness in the wild, only an incomplete understanding of inheritance is gained through lab studies. One obvious limitation of this study is the fact that fish were reared in only one environment. Under these circumsances, there was no way to test for genotype by environment (G x E) interaction for growth among populations. In previous studies of juvenile salmon, G x E was an important factor in development (Tallman 1986, Conover and Schultz 1997). In complex environments, phenotypic plasticity is often an adaptive trait in itself (Schlichting and Pigliucci 1998). Moreover, some argue that norms of reaction of phenotypes across different environments are the object of selection themselves (McNamara and Houston 1996). Thus, the ability of an organism to deal with environmental change over its lifetime may in fact determine its fitness.

Within the constraints of the study, the results suggested that variation for growth was continuous across the Mamquam sub-populations. However, the pattern of variation revealed from the microsatellite analysis was slightly different. Samples collected from the Mamquam Channel, Mashiter Channel and Railroad sub-populations showed no significant differentiation based on allele frequencies, but the sub-population collected from the Confluence site was significantly different from all other Mamquam sub-populations. Interpreting the biological significance of this result is speculative, since a limited number of samples were collected from juveniles over only one brood year. These sampling constraints are likely to bias results towards observing increased heterogeneity between groups (Waples 1991). However, previous studies of microsatellite variation in coho and other salmonids have revealed stable allele frequencies over brood years and minimal bias for juvenile sampling (Small 1998 a, b). The Confluence site is located upriver from the other sites near the confluence of Mashiter Creek and the Mamquam

River. It may be a segregated spawning population representing upriver fish. Genetically distinct sub-populations in closely located populations of Atlantic salmon (Heggbegert et al. 1986) and chum salmon have also been reported (Tallman 1996). Founder effects associated with channel colonisation may also explain the patterns if downstream spawners similar to the Railroad sub-population colonised the channels.

The genetic differentiation within the Mamquam can be put into perspective by comparing within-river variation with variation found among rivers. When all statistically distinct populations are compared (e.g. Confluence, other Mamquam sub-populations, Cheakamus, and Vedder) the multi-locus F_{st} value was 0.0567. This value is reasonably high given the fact that Small (1998) found F_{st} values of 0.051 between upper and lower Fraser River populations. When the F_{st} value was further broken down using hierarchical analysis, most of the variation can be accounted for by variation between regions (e.g. Lower Fraser versus Squamish). This result is consistent with previous studies indicating that coast populations have diverged from Lower Fraser populations (Small et al. 1998b) and related species (Wood et al. 1994). Between river variation accounted for 0.001 of the total F_{st} and, interestingly, within-river variation accounted for more than 0.01. However, it should be noted that uneven sampling complicates the hierarchical analysis (Chakraborty and Leimer 1987).

In summary, the results of the study are somewhat equivocal. The growth experiment provides evidence to reject the second and third alternative hypothesis; namely that size variation is associated with separate off-channel and main-channel spawning populations segregated either spatially or temporally. However, the microsatellite analysis supports the possibility that the Confluence population is a separate spawning group although perhaps without adaptive differentiation for growth rate during the period examined. That variation for growth is not associated with fixed differences between off-channel and main-channel groups was

demonstrated by the laboratory experiment thus supporting the null hypothesis that the size variation was plastic. However, if Confluence is a distinct spawning population and juveniles spawned by Confluence spawners rear in different habitats the phenotypes may still have ecological significance in terms of traits not assessed such as overwinter survival. For example, main-channel winter migrants to the side-channel possessing a size advantage may displace summer residents (Sheng et al. 1990). Moreover, there may be G x E interaction for growth between populations. The first hypothesis, that individuals show habitat selection/exclusion based on genotype is still a possibility given the extensive variation for growth among individuals and heritability for growth. The level of habitat selection, though, would likely be on a much finer scale than between off-channel and main-channel habitat. For example, rifles, pools, rootwads, and other features of streams provide vastly different quality of habitat in terms of resources, cover and flow and individual growth rates for coho vary among these habitats and are correlated with behaviour differences (Nielson 1995).

Population structure was observed within and between rivers, but most of the molecular genetic variation was due to variation within sub-populations. This result is consistent with previous studies of coho (Small 1998a, b) and other Pacific salmonids (Wood et al. 1994). This pattern most certainly reflects the relatively recent shared evolutionary history of populations (McPhail 1997, Healey and Prince 1995). Regional genetic differences are associated with colonisation patterns of freshwater habitat by populations that persisted in glacial refugia (Small 1998a, b Wood et al. 1994). Although populations within regions can be distinguished by their allele frequencies, few contain unique alleles found in only one populations. As a result, the genetic significance of phenotypic differences observed between populations within a region is of great debate (Ricker 1972, Gauldie 1991, Healey and Prince 1995). High rates of natal site fidelity in salmon provide the opportunity for genetic divergence through one of two mechanisms: genetic

drift or selection. Colonisation of new habitat can also result in founder effects that lead to population differentiation. The biological significance of population structuring resulting from chance effects, such as drift or founder effects, is quite different from population structure caused by selection. It also has distinct implications for rehabilitation efforts.

For management purposes, the emphasis for salmon conservation has been placed on classifying populations and groups of populations into discrete units. The reason for this approach is partly due to the tradition of the 'stock concept' and the idea that populations can be exploited through sustainable yield models when intrinsic population survival and growth parameters are known (Beverton and Holt 1957). Accordingly, while many genetic studies of salmonid species attempt to delimit conservation units (ESUs), an implicit second goal is to develop marker tools for identifying unique populations in mixed stock fishery analysis (Fournier et al. 1984). The central goals of these analyses are either to identify stocks that 'belong' to a particular jurisdiction (province, state or nation) or to determine catch quotas in a mixed stock where numerous ecologically distinct populations merge. Thus, in the marine phase of salmon life history when populations are not spatially segregated, genetic tools provide an opportunity to separate the relative contribution of different populations to the marine mixture (Beacham et al. 1988). The primary tool for conserving populations is harvest restriction. This approach to salmon conservation places the emphasis on diversity and uniqueness among regions as the yardstick of biological diversity and focus of conservation effort. However, such criteria may not address all conservation concerns particularly when the level of genetic organization occurs at finer scales than can be resolved through population genetic techniques or when complex interactions between genotype and environment occur.

An alternative approach in salmon management and conservation is to focus on habitat protection (Healey and Prince 1995). This view places more emphasis on the importance of

conserving both adaptive traits and ecological and micro-evolutionary processes (Table 24). Of course, since habitat protection generally requires greater economic sacrifice than other conservation measures it is harder to implement politically. Also, quantifying adaptive variation in wild populations is difficult. However, the fundamental fact about salmon conservation is that salmon are exploited. In coho, past exploitation rates averaged around 80% and reached up to 96% in

Table 24. Contrasting perspectives for salmon management and conservation.

	MANAGEMENT PERSPECTIVE	
	HABITAT	**POPULATION**
GENETIC EMPHASIS	Adaptive variation (selection on heritable traits)	Molecular variation (drift, migration and mutation)
SPATIAL SCALE	Within-population variation	Among-population variation
TEMPORAL SCALE	Short term	Long term
EMPHASIS	Maintaining ecological processes and function, micro-evolution and adaptation	Maintaining long-term evolutionary patterns of diversity
MANAGEMENT ACTION	Habitat protection, enhancement and rehabilitation	Stock enhancement, harvest restrictions

some populations (Labelle et al. 1997). Sustaining exploitation rates at this level provides ample opportunity for selection and adaptation for body size, run timing and marine distribution, influence the rate at which populations and individuals are exploited (Labelle et al. 1997). Moreover, the juvenile phase is increasingly being viewed as a crucial period affecting recruitment of salmon stocks (Chambers and Trippel 1997). Population recruitment can be limited by trade-offs juveniles make when sustaining high predation pressure and competing for limited habitat (Walters and Juanes 1993). Thus, given the complex interactions between salmon population genetics and demography (Figure 22), the dichotomy between population-focused

and habitat-focused approaches to salmon conservation is not really accurate or useful. More research is needed to understand the population genetic and adaptive consequences of dynamic processes associated with exploitation and habitat destruction.

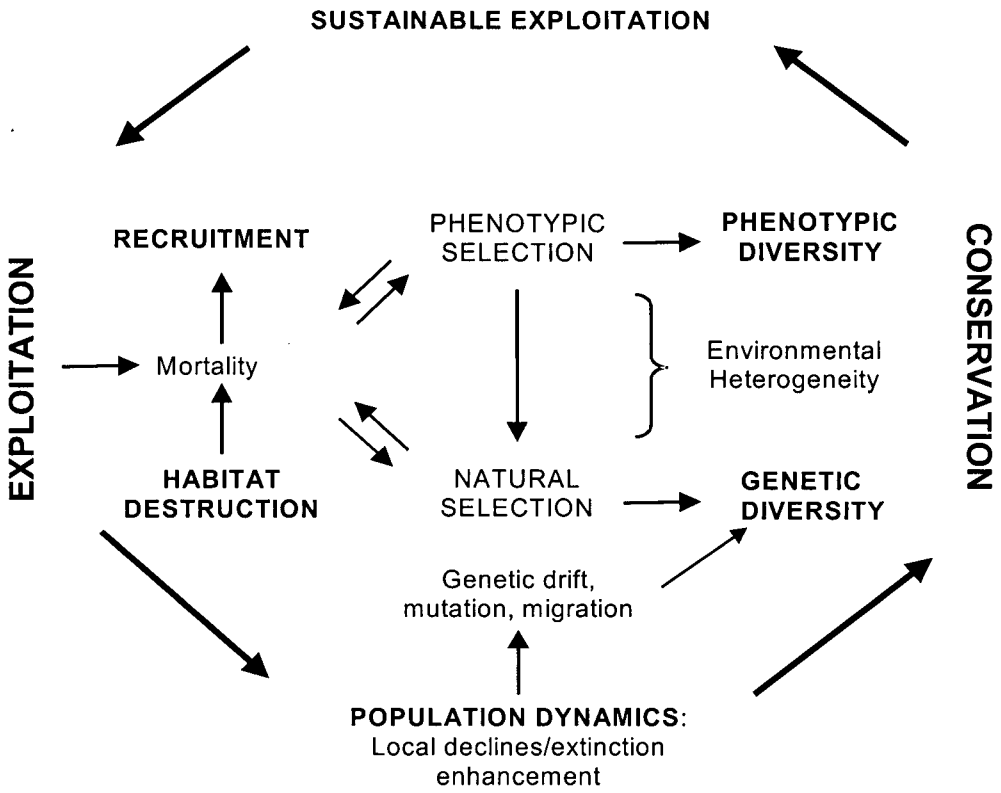


Figure 22. Dynamics affecting salmon population genetics, adaptation and demography.

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