ASPECTS OF NATURAL AND ARTIFICIAL HYBRIDIZATION BETWEEN BROWN TROUT AND ATLANTIC SALMON IN NEWFOUNDLAND

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by

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Abstract

Two projects were undertaken to obtain information at the molecular, organismal and ecological levels of organization concerning the causes and dynamics of natural hybridization between brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) in Newfoundland.

(1) Protein electrophoresis and mitochondrial DNA analysis were used to detect the frequency and direction of natural hybridization between these species in nine Newfoundland rivers. In total, 37 hybrids were discovered in a sample of 792 juvenile fish for a regional frequency of 4.7%. Local frequencies ranged from 0 to 18.7% and were significantly heterogeneous. All of the hybrids sampled were produced from matings between female brown trout and male Atlantic salmon.

(2) The relative viability of hybrids produced using anadromous brown trout, Atlantic salmon grilse and sexually mature Atlantic salmon parr from a Newfoundland river was investigated. The sperm of sexually mature salmon parr performed equally well compared to anadromous salmon sperm when fertilizing both salmon and trout eggs. Hatching success was high in all crosses and controls. By first feeding, hybrids produced using brown trout eggs had suffered higher mortality and were smaller in size than the reciprocal hybrid and both parental controls. Hybrids produced using salmon eggs exhibited high viability and by first feeding were greater in size than both parental controls. A comparison of hatching time and length of hatching period suggests a paternal influence in embryo development.

Possible reasons for the breakdown of prereproductive isolating mechanisms between Newfoundland brown trout and Atlantic salmon are considered in light of the contrasting results obtained from the two

investigations. Reproductive characteristics of the populations involved appear to have a major influence on the dynamics of hybridization between these species in Newfoundland. It is proposed that an abundance of sexually mature Atlantic salmon parr in Newfoundland streams is responsible for both the frequency and direction of hybridization observed in this study. Possible reasons for the differences in viability of reciprocal hybrids and the significance of results with respect to the aquaculture industry are also discussed.

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Chapter 1

General Introduction

1.1. Organismal Hybridization

Hybridization, at the organizational level of the organism, has been defined by Mayr (1971) as *the crossing of two individuals belonging to two unlike natural populations that have secondarily come into contact*. This includes both heterospecific and conspecific hybridization; the latter involving individuals who belong to the same species, but whose respective populations have undergone some degree of genetic divergence and are considered to be different subspecies, races or forms. Although the definition refers to populations as *natural*, artificial hybrids can be produced when gametes of different populations, be they natural or domesticated, are brought into contact through human intervention. The term *secondarily* specifies that hybridizing individuals have come into contact as a result of conditions which contradict those maintaining the reproductive integrity of a population. This is brought about by the loss or disturbance of some physical or biological barrier which has existed between two populations or species and has permitted them to diverge genetically over time.

The extent of hybridization may vary depending on the case. In general, natural hybridization in the animal kingdom is rare. When produced, hybrids are usually low in frequency, low in viability, sterile or sufficiently dissimilar to either parental population that backcrossing is even less likely to occur (Mayr 1971). When hybridization is rare, the ecological or genetic isolation between closely related populations is considered to be strong. In contrast, a lack of isolation between

hybridizing populations can result in *introgression*, in which there is a transfer and incorporation of genetic material from one species into the genome of another (Billington et al. 1988; Campton 1987; Dowling et al. 1989). A *hybrid swarm* is produced when two species or populations hybridize on a continuous basis with constant exchange of genetic material between the two parental extremes (Gyllensten et al. 1985a; Mayr 1971). *Hybrid zones* are narrow regions in which genetically distinct populations meet and hybridize, forming a cline between the two parental extremes (Barton and Hewitt 1985).

Mayr (1971) considered hybridization to be of little significance with respect to the evolution of higher animals. However, hybridization and introgression can ultimately lead to a decrease in overall diversity (Nelson and Soule 1987). Therefore, an understanding of the process is essential for biological conservation.

1.2. The Study of Hybridization

Studies of hybridization generally fall into three categories of research covering the genetics, performance and ecology of hybrids. Although the focus of a particular study may fall into one of these, the results are often relevant to all three.

Genetic research concentrates on either the product of hybridization or causes of hybrid sterility. Embryos resulting from a hybrid mating can be parthenogenic, in which the pronucleus of either the sperm (gynogenesis) or the ova (androgenesis) is eliminated during fertilization, or hybrid, in which there is a genetic contribution from both parents (Chevassus 1983). Zygotes can also be diploid, triploid or tetraploid depending on the ploidy of the parental gametes or as a result of polyspermy (Chevassus 1983). The fertility of a hybrid can vary from completely sterile, with

abnormal gonadal (Buss and Wright 1957), gametic (Lincoln 1981) or zygotic (Buss and Miller 1967) development, to completely fertile with the ability to produce viable F2 and F3 generations, backcross with either parental species, or even hybridize with a third species (Buss and Wright 1957). Cytological studies of hybrid sterility have investigated problems that arise during meiosis. Abnormalities during synapsis, meiotic spindle formation or the first anaphase of meiosis, are often the cause of inviable gametes (White 1973).

A second branch of research focuses on the organismal level of organization and is concerned with the relative appearance and performance of hybrids with respect to their parental species. These types of studies measure the viability of hybrids from zygote to adult and assess characteristics such as morphology, growth rate, fertility and disease resistance. Heterosis, a phenomenon in which highly heterozygous individuals, such as hybrids, exhibit superior performance compared to their relatively homozygous parents (Brown 1970; Mayr 1971; Kozlov 1972; Mil'shtein and Popova 1972; Leary et al. 1984), is often the subject of such hybridization experiments. The development of new breeds, sterile hybrid strains or the transfer of beneficial traits from one species to another can be of significant economic importance.

The study of hybridization at the ecological level of organization examines population isolating mechanisms and their apparent breakdown. 'Postmating' reproductive isolation is achieved through hybrid sterility, hybrid inviability, physiological differences between hybridizing individuals or biochemical differences between hybridizing gametes which prevent zygote formation (Mayr 1971; Garbers 1989). This type of isolation is for the most part the result of stochastic processes which have resulted in the genetic divergence and subsequent incompatibility of hybridizing genotypes. It does not prevent hybridization from occurring, but the fact that this incompatibility exists,

and that crossing individuals waste gametes and leave fewer offspring, drives the selection of 'premating' reproductive isolating mechanisms. Premating isolation involves the mechanical, behavioural and spatial barriers which prevent attempted matings between unlike individuals (Mayr 1971). The result of a breakdown in premating isolation is hybridization and the study of natural hybridization is, for the most part, the study of the causes or conditions which lead to this breakdown and the subsequent effects.

1.3. Theories of Hybridization

1.3.1. *In general*

In many cases, closely related species are similar in their reproductive morphology and behaviours. Their respective breeding times and places may overlap as can their physical requirements for successful reproduction. In such cases, premating isolation is susceptible to disturbances and may breakdown. Mayr (1971) lists several conditions or circumstances which may facilitate the breakdown of premating isolation and explain some of the broader trends observed in the animal kingdom.

Hybridization tends to be more common among externally fertilized organisms, such as fish, than it is among internally fertilized species, such as terrestrial vertebrates. During external fertilization, sperm may be carried by water currents to the ova of another species spawning nearby. Also, the ova from one female can be fertilized by several males at once as the ability of a female to restrict access to her ova is much more limited than it is for internally fertilized females.

The strength, or weakness of the mating bond between individuals may

influence the potential for hybridization. In many species, offspring require a considerable amount of care on behalf of one or both parents if reproduction is to be successful. Consequently, it is important that individuals be discriminating when selecting a mate in order to avoid mistakes which may later prove costly. In other species, mating bonds are weak with parental involvement ending shortly after copulation. Individuals may not spend as much time selecting a suitable mate and accidental hybridization may be more common.

If there is considerable disparity in the abundance of different species, rare species may have trouble finding suitable mates. As reproductive drives build, rare individuals may become receptive to inappropriate stimuli and mate with the wrong species rather than not mate at all.

If species are spatially, or geographically isolated, there will be no selective reenforcement of other premating isolating mechanisms. Circumstances which bring such populations together are often the cause of hybridization. Degradation or limitation of available habitat may force different species to use the same area for reproduction. Introduction of a species into an area where it is not normally found, often results in its hybridization with related, native species.

1.3.2. *In fish*

Much of what has been summarized by Mayr (1971) concerning the causes of hybridization in the animal kingdom, was first identified by Hubbs (1955), who, with his colleagues, spent fifteen years documenting cases of natural hybridization between fish species. In general, Hubbs (1955) found hybridization to be much less frequent within the marine environment than among freshwater fish species. This broad trend was attributed to the relative instability of lakes and rivers with respect to the sea. Also, during times of change, dispersal routes to suitable habitat are

not as limiting in the marine environment as they are in freshwater. Hubbs (1955) also predicted that the incidence of hybridization should increase with latitude, such that the highest frequencies will occur in the holarctic freshwater regions, which have been strongly effected by glacial advances during the Pleistocene, and decrease toward the equatorial regions.

In addition to the above, Hubbs (1955) identified four ecological circumstances associated with an increased tendency of fish species to hybridize. (1) Fish species reproductively isolated by environmental cues, may breed together where a disturbance, either natural or human induced, has rendered the environment intermediate. (2) Spawning habitat is limited such that distinct species of breeding fish are forced into close proximity. (3) There is considerable disparity in the abundance of related species. (4) A species has been introduced into an area where it is not native. These conditions are frequently cited as possible reasons for the breakdown of reproductive isolation when natural fish hybrids are discovered. However, it is usually difficult to single out a predominant cause in a particular case. This is partly due to the fact that naturally occurring hybrids are rare and difficult to find. In many cases, the only information available is the occurrence of hybrids and sometimes the frequency.

1.4. Biochemical Approach to the Study of Natural Hybridization

Since the introduction of biochemical techniques to population genetics, many suspected hybrids have been confirmed and many new cases of hybridization discovered. Contrary to the contention of Hubbs (1955) that fish hybrids are almost universally intermediate in morphology to their parental species, Nyman (1970) found that in many cases, morphological characters are similar to either parent and to a much

lower degree, intermediate. Leary et al. (1983) observed that the hybrid product of brook trout (*Salvelinus fontinalis*) and bull trout (*Salvelinus confluentus*) had consistently high meristic counts with respect to the parental controls and attributed this to their relatively slow embryonic development. Also, many closely related species may overlap in their morphometric and meristic characters making the positive identification of a hybrid difficult (Nyman 1970). In protein systems, enzymes are expressed by single codominant genes and hybrids usually express a complete summation of both parental genotypes (Nyman 1970). Thus the application of protein electrophoresis has provided a more sensitive means by which hybrids can be identified.

Recently developed molecular techniques, which can be used to characterize the mitochondrial DNA (mtDNA) of a given species, have provided a means of obtaining additional information about natural hybridization (Campton 1987). MtDNA is a circular, double-stranded, extra-nuclear genome that does not recombine. It is generally considered to be maternally inherited (Gyllensten et al. 1985b; Hutchison et al. 1974). Gyllensten et al. (1991) have shown that paternal inheritance of mtDNA can occur in higher vertebrates, but that less than one in 1000 molecules are so derived. Also, repeated backcrossing of female hybrids with the paternal species is required (23 to 26 generations in mice) before enough paternal mtDNA is present in an individual to be detected using highly sensitive techniques such as the polymerase chain reaction (PCR) (Gyllensten et al. 1991). Characterization of a given species' mtDNA can be achieved by observing the patterns generated on a gel when the molecule is cut with a particular restriction enzyme (restriction fragment length polymorphisms or RFLPs) (Gyllensten and Wilson 1987), or by direct nucleotide sequencing of a mtDNA gene (Kocher et al. 1989; McVeigh et al. 1991). Therefore, by typing the mtDNA of a given hybrid, the maternal species can be determined.

Several studies have already exploited these properties to identify cases of introgression and understand more about the causes and dynamics of natural hybridization. Avise and Saunders (1984) used RFLPs to determine the maternal species of naturally occurring hybrids between species of sunfish (genus *Lepomis*). They discovered a tendency for hybridization to occur between parental species differing greatly in abundance and a tendency for the rare species to contribute the female parent. The apparent association between relative species abundance and direction of hybridization was attributed to the reproductive behaviour of these species. Within this genus, males construct and protect nests and are subsequently selected by females. In the absence of conspecific males, rare females may choose to mate with congeneric males instead (Avise and Saunders 1984). Herkel et al. (1990) found that naturally occurring hybrids produced by northern pike (*Esox lucius*) and chain pickerel (*Esox niger*) always had chain pickerel as a maternal parent. However, previous experiments in the lab had shown that hybrids produced using northern pike females do not progress beyond the zygotic stage whereas the reciprocal cross is perfectly viable (Buss and Miller 1967) . Gyllensten et al. (1985a) combined allozyme and mtDNA data to show that the males and females of introduced and native cutthroat trout subspecies (*Oncorhynchus clarki lewisi* and *O. clarki bouvieri*) contributed equally to hybrid swarms. Billington et al. (1988) discovered evidence of introgression between two *Stizostedion* species in the great lakes. In this case, two fish possessing walleye (*Stizostedion vitreum*) nuclear genotypes were found to have sauger (*Stizostedion canadense*) mtDNA genotypes.

1.5. Hybridization in the Subfamily Salmoninae

Fish representing the subfamily Salmoninae, are commonly referred to

as salmonids or the salmons, trouts and charrs. Several examples of natural hybridization can be found within this subfamily. In northern Labrador, arctic charr (*Salvelinus alpinus*) have been reported to hybridize with both brook trout (*S. fontinalis*) (Hammar et al. 1991) and lake trout (*Salvelinus namaychush*) (Hammar et al. 1989). Brook trout is also known to hybridize naturally with brown trout (*Salmo trutta*) to produce the sterile 'tiger trout' (Brown 1966). Campton and Utter (1985) identified natural hybrids between steelhead trout (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarki*) in two Puget Sound streams. Natural hybridization has also been discovered between chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) in Northern California (Bartley et al. 1990). Numerous examples of natural hybridization between brown trout (*S. trutta*) and Atlantic salmon (*Salmo salar*) have been reported in both Europe (Payne et al. 1972; Solomon and Child 1978; Crozier 1984; Garcia de Leanize and Verspoor 1989; Hurrell and Price 1991; Jansson et al. 1991) and North America (Beland et al. 1981; Verspoor 1988). The ability of salmonids to produce viable interspecies and intergeneric hybrids under artificial conditions has also been well documented (Chevassus 1979).

Salmonids are of substantial economic value, supporting both a commercial and recreational fishery. Their importance has also grown recently with respect to the worldwide aquaculture industry. Consequently, there has been a strong desire on the part of government agencies and sport fishermen to enhance existing populations as well as try to increase species diversity in popular fishing rivers. Hatchery reared fish have often been introduced into rivers or lakes where they do not naturally occur. Such introductions can put native fish populations at risk through the potential of hybridization and introgression. A disturbing example of this was the introduction of coastal rainbow trout (*O. mykiss*) into several inland lakes throughout the western United

States. Hybridization and subsequent introgression of introduced fish with native cutthroat trout (*O. clarki*) has resulted in the permanent loss of several unique, locally adapted gene pools (Benke 1972).

Examples such as the above have resulted in a much more cautious approach to the management of salmonids, with a greater emphasis on the protection of a population's genetic integrity (Billingsley 1981; Stahl 1987). However, a rapidly growing salmonid aquaculture industry has raised concern over the potential effect of accidental introductions. Damage to sea pens can result in the release of several thousand fish at a time. The impact of these 'domesticated' strains on wild populations is the subject of considerable scientific debate (Hansen et al. 1991).

1.6. Atlantic Salmon and Brown Trout

1.6.1. *In general*

Brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) are the two predominant species of the genus *Salmo*. They are found naturally throughout the North Atlantic drainage with Atlantic salmon occurring in both Europe and North America (Scott and Crossman 1973) and brown trout being historically restricted to the eastern Atlantic (MacCrimmon and Marshall 1968). Both species have been introduced to regions outside their natural range where self-sustaining populations have been established (Crossman 1984; McDowall 1984). Naturalized populations of brown trout can now be found throughout North America (MacCrimmon and Marshall 1968).

Brown trout and Atlantic salmon have a similar life-history and reproductive biology. Both spawn in the fall, with females burying their fertilized ova in the gravelly substrate of a freshwater stream. Embryos

develop during the winter, hatching by early spring as 'alevins' and emerging from the gravel as 'fry' once their egg-sacs have been absorbed. A variable number of years are spent in the fluvial environment as juveniles or 'parr' before migrating downstream into either a marine environment (in the case of anadromous fish) or a lake (in the case of lacustrian or land-locked populations). Some brown trout and Atlantic salmon complete their life-cycle in the fluvial environment. After spending one or more winters at sea or in a lake, mature adult fish ascend a river (usually their natal stream) to reproduce. Unlike the Pacific salmons, which characteristically die after spawning, both brown trout and Atlantic salmon are iteroparous, and may spawn again in later years. Both species also display an alternative life-history pattern in which males can mature sexually within the river as parr and successfully fertilize larger, adult females (Jones and King 1952; Dalley et al. 1983; L'Abee-Lund et al. 1989).

Atlantic salmon exhibit relatively little variability when compared to brown trout. Throughout its range, Atlantic salmon is morphologically uniform, whereas brown trout is polytypic and often classified into distinct morphological and ecological races (Benke 1972, Ferguson and Mason 1981). In a genetic analysis of Atlantic salmon across its entire range, Stahl (1987) found nine out of 38 sampled loci to be polymorphic and a genetic distance between European and North American population of 0.04. In comparison, Steven and McAndrew (1990) surveyed brown trout populations in Scotland alone and found that 13 out of 34 loci were polymorphic with a genetic distance between some populations of 0.05.

Gyllensten and Wilson (1987) analysed restriction fragment length polymorphisms (RFLPs) of the mitochondrial DNA (mtDNA) to determine a genetic divergence of approximately 6.0% between brown trout and Atlantic salmon. Similarly, McVeigh et al. (1991) observed a 5.2%

nucleotide sequence divergence of the mitochondrial cytochrome b gene between these two species. The latter study also discovered a bias toward transition base substitutions over transversions and no amino acid substitutions within the cytochrome b gene. This is considered characteristic of species that have diverged recently from a common ancestor (McVeigh et al. 1991). In contrast to its uniform morphology, the karyotype of Atlantic salmon is variable with a diploid chromosome number ranging between 54 and 58 and an arm number of 72 or 74 (Hartley 1987). Brown trout have a stable diploid chromosome number of 80 with 100 arms (Chevassus 1979). The Atlantic salmon karyotype has probably been derived from a brown trout like karyotype through centric fusions and pericentric inversions (Benke 1972; Hartley 1987).

The ability of brown trout and Atlantic salmon to produce viable hybrids under artificial conditions was first established in the early breeding experiments of Ashworth and Ashworth (1853, from Dangel et al. 1973). Since that time, several researchers have investigated the potential of this cross for both stock enhancement and aquaculture purposes (Chevassus 1979). The possibility of natural hybridization has always been postulated. However, overlap of morphological characteristics and a tendency for hybrids to resemble either one species or the other made positive identification in the field difficult (Nyman 1970). The existence of naturally occurring brown trout x Atlantic salmon hybrids was first confirmed by Payne et al. (1972), using biochemical makers.

1.6.2. *In Newfoundland*

Atlantic salmon are native to and distributed throughout the island of Newfoundland (Porter et al. 1974; Scott and Crossman 1973). There are two morphologically distinct forms of salmon found in Newfoundland; a resident, freshwater form, commonly referred to as the 'ouananiche', and

an anadromous form. There is evidence of genetic isolation between these types where they coexist (Verspoor and Cole 1989; Birt et al. 1991). Atlantic salmon support both a commercial and recreational fishery in Newfoundland. There is also a small, but growing salmonid aquaculture industry on the island.

Several different strains of brown trout have been introduced to the island of Newfoundland. Between 1886 and 1888 the Loch Leven trout was imported from Scotland by the Newfoundland Game Fish Protection Society and used to stock lakes in and around the St. John's area (Andrews 1965). These fish were also introduced to the Petty Harbour ponds, ponds draining into the Topsail river and South Dildo Pond, Trinity Bay (*The Evening Herald, St. John's, Nfld. Feb. 23, 1892*). German brown trout were brought to Newfoundland in 1892 and planted in Whiteway's pond and Robin's pond near Torbay and into Hodgewater pond, near Whitburn (Frost 1938). It is also reported that brown trout from England were brought to the island sometime in 1905 or 1906 and put into Clement's pond and Miller's pond (formerly Lee's pond) near Portugal Cove (Frost 1938). Figure 1.1 indicates the river systems where brown trout are believed to have been introduced.

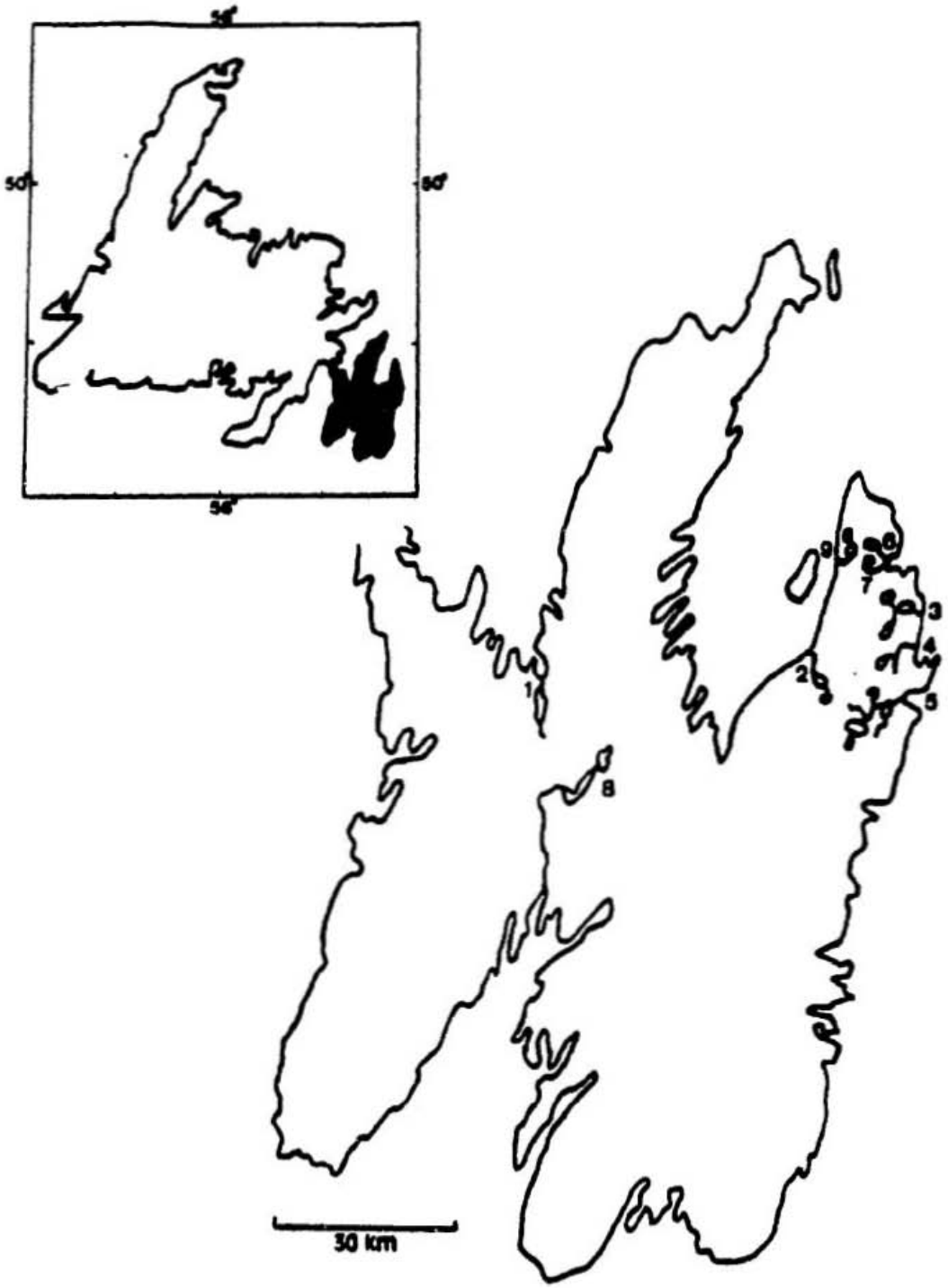
Brown trout in Newfoundland are still commonly referred to as German, Loch Leven, Scottish or English, even though there is no morphological means of telling one type from another (Frost 1938, 1940). Populations of Scottish brown trout can still be found in the Topsail system; isolated by impassible waterfalls. The lakes in Torbay and Portugal Cove, in which the German and English brown trout were introduced respectively, are also isolated by waterfalls and still contain representatives of these strains (Figure 1.1).

Since the original introductions, there has been no management of brown trout on the island of Newfoundland. Adaptation to an

Figure 1.1.

River systems in which brown trout are known to have been introduced. A) Scottish brown trout*; 1. Dildo pond, 2. Topsail ponds, 3. Rennie's Mill river, 4. Waterford river, 5. Petty Harbour ponds. B) German brown trout; 6. Whiteway's pond, 7. Robin's pond, 8. Hodgewater pond. C) English brown trout; 9. Main river. (Frost 1938, *The Evening Herald, St. John's, Nfld. Feb. 23, 1892*)

**Other introductions of Scottish brown trout may have been made in the St. John's area, but are not listed here due to ambiguities in the historical reports*


56°
50°
60°
46°
1
2
3
5
8
30 km

anadromous life-cycle has allowed this species to colonize several rivers both on and off the Avalon Peninsula. Populations of brown trout may be established as far north as Middle Brook, Bonavista Bay and as far west as the Red Harbour River on the Burin Peninsula (Porter et al. 1974) (Figure 1.2). Historically, brown trout have been of little economic importance and are often considered a pest in Newfoundland. However, with recent declines in Atlantic salmon stocks, anadromous brown trout are becoming a popular alternative for the angler.

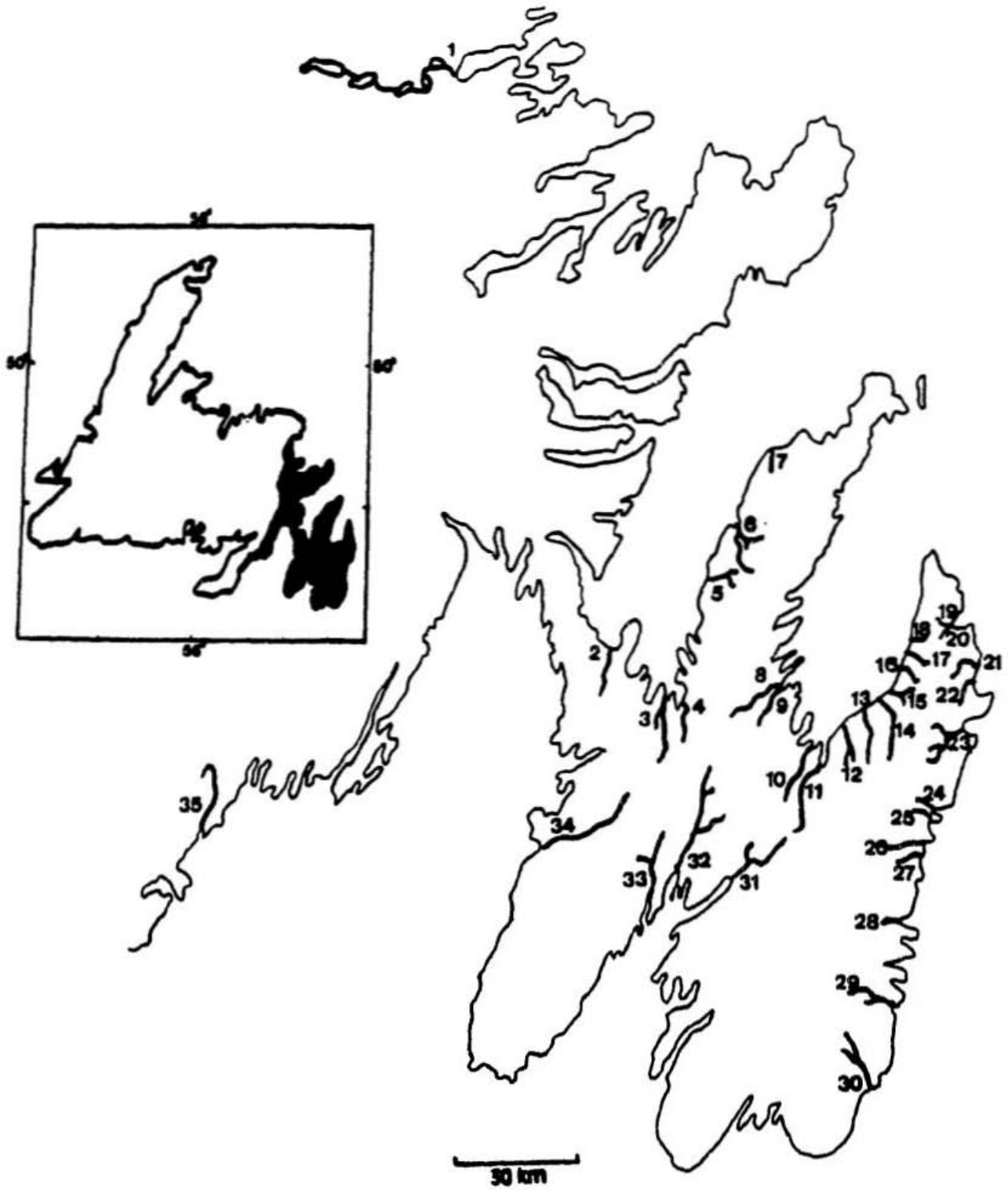
Widespread natural hybridization between brown trout and Atlantic salmon in Newfoundland was first reported by Verspoor (1988). No formal investigation of artificially crossed brown trout and Atlantic salmon from Newfoundland has ever been undertaken.

1.7. Objectives of the present study

The objective of the present study was to gain a deeper understanding of the causes and dynamics of natural hybridization between brown trout and Atlantic salmon on the island of Newfoundland. Two projects were undertaken to obtain information at the molecular, organismal and ecological levels of organisation. (1) In the field, several sympatric populations of brown trout and Atlantic salmon were sampled and analysed using protein electrophoresis in order to determine the frequency of hybridization at both a local and regional scale. The relative maternal contribution of each species to hybrid matings was then established through mtDNA analysis. (2) In the laboratory, artificial hybridization experiments compared the relative viability of the reciprocal hybrids with respect to their parental controls. This information was used to interpret results obtained from the field survey concerning the direction and frequency of hybrid matings in the wild. In addition, the performance of hybrids was assessed with respect to their potential in the aquaculture industry.

Figure 1.2.

Present distribution of brown trout in Newfoundland. 1. Middle brook, 2. Broad lake, 3. Spread Eagle river, 4. Dildo pond, 5. Hearts Delight river, 6. Hearts Content river, 7. Halfway brook, 8. North river, 9. South river, 10. Maloney's river, 11. North Arm river, 12. Quarry brook, 13. Seal Cove river, 14. Manuels river, 15. Topsail river, 16. Broad cove river, 17. Beachy Cove river, 18. Main river, 19. Whiteway's pond, 20. Robin's pond, 21. Rennie's Mill river, 22. Waterford river, 23. Petty Harbour ponds, 24. Bay Bulls river, 25. Lower pond, 26. Mobile river, 27. Tors Cove pond, 28. Cape Broyle river, 29. Renews river, 30. Chance Cove river, 31. Salmonier river, 32. Colinet river, 33. North Harbour river, 34. North East Placentia river, 35. Red Harbour river.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34
35
50 km

Chapter 2

Natural Hybridization Between Atlantic Salmon and Brown Trout in Newfoundland

2.1. Introduction

Naturally occurring hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) was first reported on the Avalon peninsula of Newfoundland by Verspoor (1988). At the time, the observed hybrid frequency of 0.9% was relatively high when compared to values of 0.3 to 0.4% recorded in the United Kingdom (Payne et al. 1972; Solomon and Child 1978; Crozier 1984). This difference was attributed to the fact that brown trout are not indigenous to North America (MacCrimmon and Marshall 1968; Verspoor 1988).

More recently, these species have been found to hybridize at frequencies of 2.3% in Spanish rivers (Garcia de Leaniz and Verspoor 1989), 13% in Swedish rivers (Jansson et al. 1991) and 1.4% in English rivers (Hurrell and Price 1991) where both brown trout and Atlantic salmon are native. Such high frequencies suggest that specific ecological conditions may be responsible for the breakdown of prereproductive isolating mechanisms. In Newfoundland, factors other than the introduction of brown trout may act to encourage natural hybridization between these species.

The study presented in this chapter combines protein electrophoresis and mtDNA analysis to determine both the frequency and the direction of natural hybridization between brown trout and Atlantic salmon in Newfoundland. This information is used in conjunction with information presented in chapter three to identify possible reasons for the breakdown

of prereproductive isolating mechanisms between these species in the wild.

2.2. Materials and Methods

2.2.1. *Sample collection*

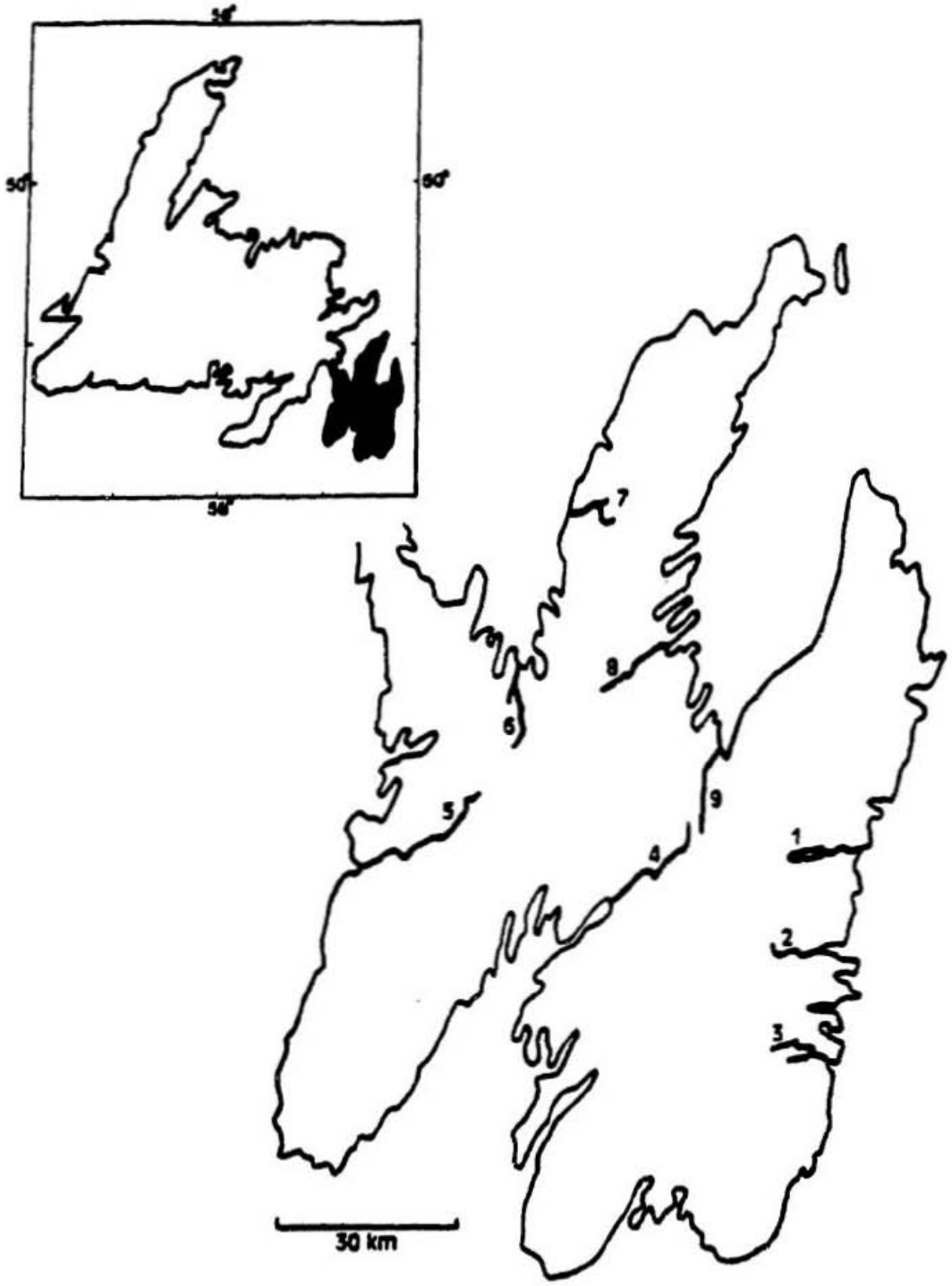
Atlantic salmon and brown trout parr were sampled from nine river systems on the Avalon peninsula of Newfoundland during a period from August 24, 1990 to November 30, 1990 (Figure 2.1). Some of the rivers were sampled at more than one site. The locations of each sample site on all rivers are presented in the appendix. Each of these river systems has been reported to have anadromous populations of both brown trout and Atlantic Salmon (Porter et al. 1974; T. R. Porter and R. J. Gibson, Department of Fisheries and Oceans, St. John's, Nfld., personal communication). No introductions of either brown trout or Atlantic salmon have been made into any of the rivers in this study. All salmon populations are natural and all brown trout populations have colonized the rivers by way of the ocean.

Parr were collected using a 12 volt, back-pack electrofisher. At most sample sites, shocked fish were picked up from the water using a small net attached to the end of the anode then transferred to a bucket of water. This technique was most effective when sampling the river in a downstream direction with the current pushing stunned fish in front of the person sampling. In some cases parr were driven downstream, using the electrofisher, into a beach seine which was stretched across the river and secured on both banks.

Once captured, fish were killed in the field using an overdose of anaesthetic. Four tablets of Alka-seltzer (1300 mg acetylsalic acid, 7664

Figure 2.1.

Location of sampled rivers; 1. Mobile river, 2. Cape Broyle river, 3. Renews river, 4. Salmonier river, 5. North East Placentia river, 6. Spread Eagle river, 7. Hearts Delight river, 8. North river, 9. North Arm river.


30 km

mg heat treated sodium bicarbonate, 4000 mg citric acid; Miles Canada, Etobicoke, Ont.) were added to the water with the fish. Carbon dioxide produced by the tablets first rendered the fish unconscious and killed after approximately ten minutes. Once dead, fish were packed on ice and transported back to St. John's where they were measured, classified as either brown trout or Atlantic salmon, then individually numbered, packaged and stored at -70°C.

2.2.2. *Hybrid identification*

Hybrids were identified using allozyme electrophoresis. Skeletal muscle (approximately 0.2 grams) was homogenized in an equal volume of 0.01 M Tris-HCl (pH 7.5) and subjected to centrifugation at 10,000 x g for one minute. The supernatant of each sample was applied to gels using 0.5 x 0.3 cm wicks made from Whatman (Maidstone, U.K.) number 4 filter paper. To prevent protein degradation, samples were kept on ice at all times during their preparation. Extra supernatant was stored at -70°C.

Samples were subjected to electrophoresis through an 11% horizontal electrostarch gel (Connaught laboratories, Windsor, Ont.) using a discontinuous Tris-citric acid/borate buffer system described by Ridgway et al. (1970). The electrode buffer was 0.06 M LiOH and 0.3 M H_3BO_3 and was recycled after each run by mixing together the portions from the anode and cathode reservoirs. The gel buffer was 0.03 M Tris, 0.005 M citric acid, 0.0006 M LiOH and 0.003 M H_3BO_3. Gels were 20 cm wide, 10 cm long and 0.5 cm in thickness. Gels were run at a constant voltage of 300 V for 2.5 to 3.0 hours and were chilled between two glass cooling plates through which cold water was constantly flowing. After electrophoresis, gels were sliced in half so that two zymograms could be obtained from one gel.

Gels were stained for the enzymes glucose-6-phosphate isomerase (EC

5.3.1.9; *Gpi*-1,2,3) and phosphoglucomutase (EC 5.4.2.2; *Pgm*-1,2) using standard techniques described by Harris and Hopkinson (1976) with some adjustments. For *Pgm*, 25 mg of glucose-1-phosphate (containing 1% glucose-1-6-diphosphate) was dissolved in 7.5 ml of 0.1 M Tris-HCl (pH 7.4) with 0.5 ml of 2% $MgCl_2$, 0.5 ml of 1% nicotinamide adenine dinucleotide phosphate (NADP), 0.5 ml of 1% 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue (MTT) and 0.5 ml of 0.4% phenazine methosulfate (PMS). To this, 5 µl of glucose-6-phosphate dehydrogenase (250 units/ml) was added before mixing in 7.5 ml of 2% agar (at about 55°C). This was poured over the gel and placed in the dark while developing. The stain for *Pgi* was the same except that 10 mg of fructose-6-phosphate was used instead of the glucose-1-phosphate with 1% glucose-1-6-diphosphate. These stains detect a total of five independent genetic loci, four of which are diagnostic for detecting hybrids of brown trout and Atlantic salmon (Vuorinen and Piironen 1984). These enzymes have been used to identify natural hybrids in both Europe (Crozier 1984; Garcia de Leaniz and Verspoor 1989; Hurrell and Price 1991; Jansson et al. 1991) and North America (Beland et al. 1981; Verspoor 1988). Each species is fixed at four of the five loci for proteins with different electrophoretic mobilities. Hybrids express the sum of both parental types (Figures 2.2 and 2.3).

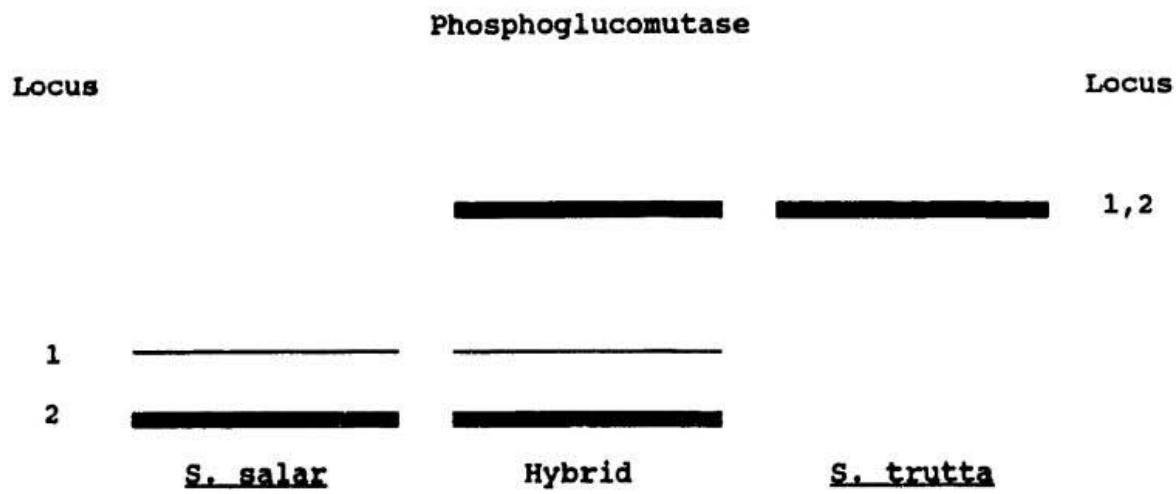
2.2.3. *Identification of hybrid maternal species*

The maternal lineage of hybrid fish was identified by amplifying a segment of the mitochondrial cytochrome b gene using the polymerase chain reaction (PCR) (Saiki et al. 1988), followed by direct nucleotide sequencing. The cytochrome b gene was chosen as it has already been characterised for both Atlantic salmon and brown trout by McVeigh et al. (1991). Diagnostic segments used to identify the mtDNA of each species are shown in Figure 2.4. The preparation of DNA from skeletal muscle,

Figure 2.2.

Zymograms of loci used to identify brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), and their F1 hybrids.
A) Phosphoglucomutase (EC 5.4.2.2; *Pgm*-1,2). B) Glucose-6-phosphate isomerase (EC 5.3.1.9; *Gpi*-1,2,3).

A


Phosphoglucomutase
Locus
Locus
1,2
1
2
S. salar
Hybrid
S. trutta

B

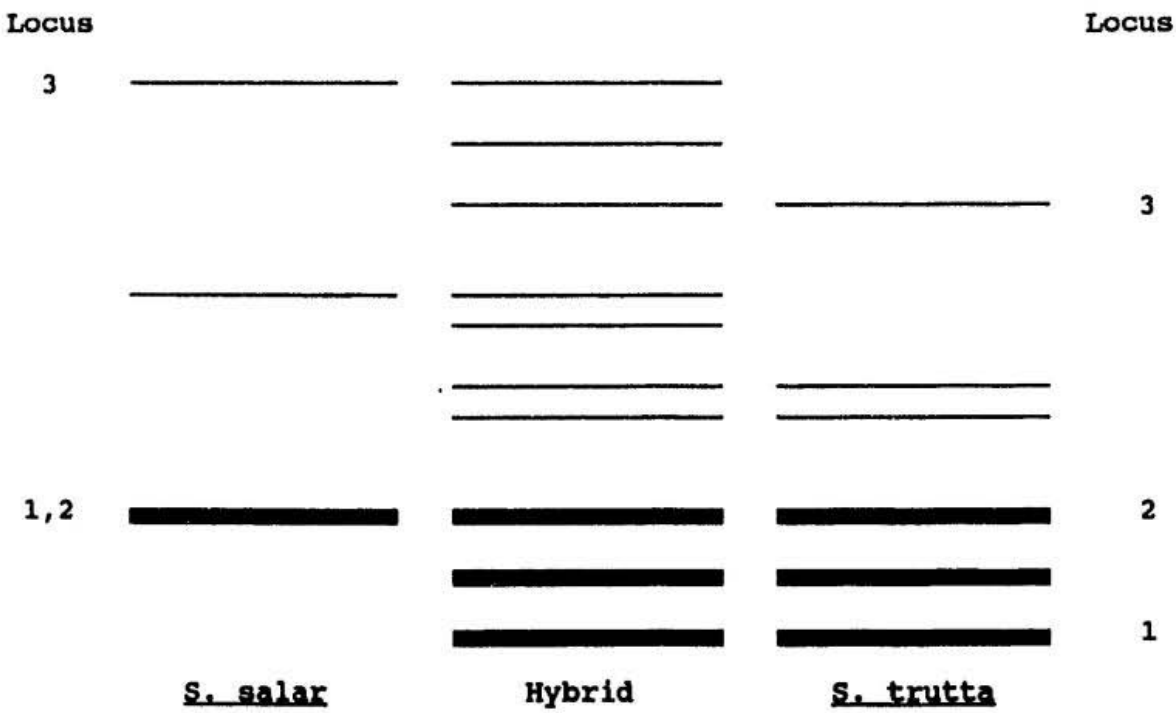
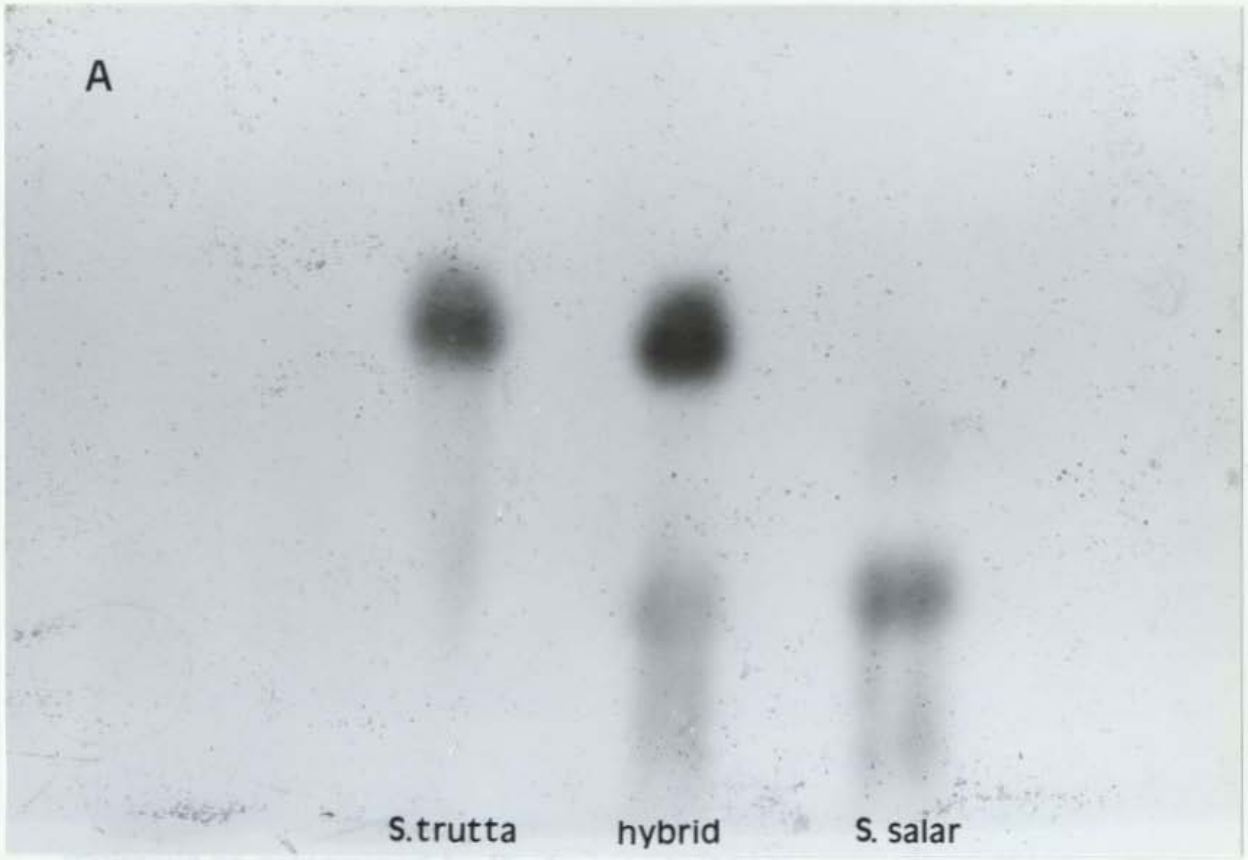

Glucose-6-phosphate isomerase
Locus
Locus
3
3
1,2
2
1
S. salar
Hybrid
S. trutta

Figure 2.3.

Photographs of zymograms used to identify brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), and their F1 hybrids.
A) Phosphoglucomutase (EC 5.4.2.2; *Pgm*-1,2).
B) Glucose-6-phosphate isomerase (EC 5.3.1.9; *Gpi*-1,2,3).


A
S.trutta
hybrid
S. salar

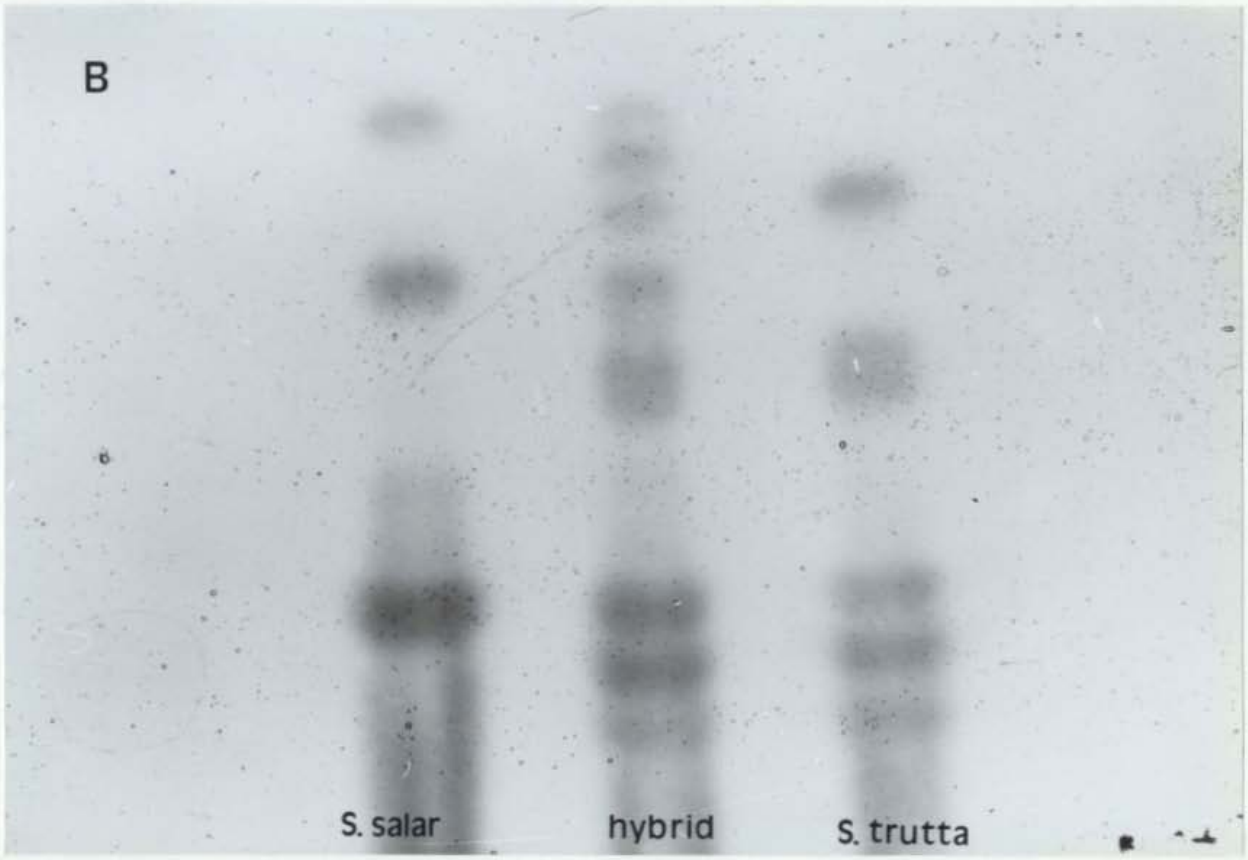

B
S. salar
hybrid
S. trutta

Figure 2.4.

Nucleotide sequences of segments of the cytochrome b genes of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*).
Numbers refer to the relative codon positions for the cytochrome b molecule as determined by Kocher et al. (1989).

* indicates differences used to identify species.


110
100
Salmo trutta: 5'-TTATATAGGTAGGAACCATAGTAGAGTCCTCGGGCGATATG-3'
Salmo salar: 5'-TTATATAGATAGGAACCATAATAAAGTCCTCGGGCGATGTG-3'
90
Salmo trutta: 5'-TATATAAATACAGATAAAGAAGAAAGATGCTCCGTTAGCGT-3'
Salmo salar: 5'-TATATAAATACAGATAAAGAAGAAAGATGCTCCGTTAGCGT-3'
80
70
Salmo trutta: 5'-GAATGTTTCGGATGAGTCAGCCGTAGCTAACATCTCGGCAA-3'
Salmo salar: 5'-GAATCTTACGGATGAGTCAGCCATAGCTAACATCTCGGCAA-3'

amplification and direct sequence analysis of the mitochondrial cytochrome b gene were performed using techniques described in detail by Bartlett and Davidson (1991).

2.2.3.1. DNA preparation

Crude DNA extractions were carried out in 1.5 ml microcentrifuge tubes using 0.2 to 0.5 g of skeletal muscle. Samples were first homogenized in 400 µl of guanidinium extraction buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate pH 7.0, 0.5% Sarkosyl, 0.1 M 2-mercaptoethanol). To this, 15 µl of 2 M sodium acetate (pH 4.1), 400 µl of Tris-saturated phenol and 200 µl of chloroform/isoamyl alcohol (21:1 v/v) were added. The homogenates were vigorously mixed, incubated on ice for 15 minutes, then subjected to centrifugation at 10000 x g for 20 minutes at 4°C. The clear, aqueous phase (about 400 µl) of each sample was transferred to fresh microcentrifuge tubes containing an equal volume of isopropanol, mixed and incubated at -20°C for at least one hour, allowing the DNA to precipitate. Samples were then subjected to centrifugation at 10000 x g for 20 minutes at 4°C. Pellets were washed with cold 70% ethanol, subjected to centrifugation at 10000 x g for 15 minutes and the ethanol discarded. The final DNA pellet was then dried under a vacuum and resuspended in 20 to 50 µl of TE buffer (10 mM Tris-HCl, 1 mM disodium ethylene diamine tetraacetate [EDTA], adjusted to pH 8.0 with NaOH) depending on size of the pellet. Once dissolved, samples were stored at 4°C.

2.2.3.2. Amplification of cytochrome b gene

Amplification of the mitochondrial cytochrome b gene was carried out in two steps using the Cetus DNA Thermal Cycler (Perkin Elmer, Norwalk, CT). First, a symmetric amplification of both the light and heavy DNA strands (double stranded amplification) was carried out. This was

followed by an asymmetric amplification of the heavy DNA strand only (single stranded amplification). In both amplification steps, reactions were run without DNA as a control for possible contamination.

The double stranded amplification reaction mixture consisted of 2.5 µl of 10x amplification buffer (500 mM KCl, 100 mM tris-HCl pH 9.0, 15 mM $MgCl_2$, 0.1% gelatin [w/v], 1% Triton X-100; Promega, Madison, WI), 0.5 µl of 40 mM deoxynucleoside triphosphate (dNTP) stock solution (10 mM dATP, 10 mM dGTP, 10mM dCTP, 10 mM dTTP; Pharmacia, Dorval, Que.), 1.0 µl of 10 µM light strand primer (L-primer: 5'-CCATCCAACATCTCAGCATGATGAAA-3'), 1.0 µl of 10 µM heavy strand primer (H-primer: 5'-CCCCTCAGAATGATATTTGTCCTCA-3'), 20.0 µl of sterile water, 0.2 µl of Taq-DNA polymerase (Promega, Madison, WI), and 1.0 µl of DNA. The reaction mixture was covered with a drop of mineral oil to prevent evaporation then run through a thermal cycle in which the DNA was denatured at 92°C for 45 seconds, the primers were annealed to the single stranded template DNA at 50°C for 45 seconds and the new DNA strand extended along the template at 72°C for 1 minute and 30 seconds. This cycle was repeated 30 times.

The double stranded amplification product was isolated by electrophoresis. Fifteen microliters of each sample was mixed with 5 µl of tracking dye (50% sterile glycerol, 5x gel buffer, 0.05% bromophenol blue) and run through a 2% low melting point, NuSeive agarose gel (FMC BioProducts, Rockland, ME) using a continuous Tris/sodium acetate (TA) buffer system (40 mM Tris, 30 mM sodium acetate, titrated to pH 7.4 with HCl). Gels were 60 ml in volume with 5 µl of ethidium bromide (10 mg/ml) added to make the DNA fluorescent over ultra violet (U.V.) light. They were run at a constant voltage of 65 V for 45 to 60 minutes. The amplified DNA product, seen over U.V. light as a tight band, was cut out of the gel, melted at 70°C in 50 to 100 µl of sterile water (depending on the intensity of the band) and used for the asymmetric amplification of the cytochrome b heavy DNA strand.

The reaction mixture for the single stranded amplification was made with 10 µl of 10x amplification buffer, 2 µl of dNTP stock solution, 4 µl of 0.1 µM light primer, 4 µl of 10 µM heavy primer, 75 µl of sterile water, 0.2 µl of Taq-DNA polymerase and 5 µl of double stranded amplification product. This reaction mixture was not covered by a drop of oil and was run through the same cycle as described above. After amplification, the product was verified by running 5 µl (with 2 µl of tracking dye) through a regular 2% nucleic acid grade agarose gel using the TA buffer system described above. This gel was also 60 ml in volume with 5 µl ethidium bromide so that the amplification product could be seen over U.V. light.

Before sequencing, the single stranded amplification product was desalted to remove any residual nucleotides, primers and buffer salts. This was accomplished by centrifugal dialysis using Centricon-30 filters (Amcon ltd., Oakville. Ont.). The remaining 95 µl of sample was transferred to the top of the filter with 2 ml of distilled water and subjected to centrifugation at 3500 x g for 20 minutes. This was repeated twice, the third time at 6000 x g for 60 minutes. The final desalted product (about 60 µl) was stored at 4°C and used for sequencing.

2.2.3.3. Sequencing of the cytochrome b gene

Sequencing of the amplified cytochrome b gene heavy strand was carried out using the Sanger method (Sanger et al. 1977) and following the manufacturer's directions for the Sequenase, version 2.0, DNA sequencing kit (United States Biochemical Corp., Cleveland, OH). Light primer was annealed to the heavy strand template in a mixture consisting of 7 µl of desalted DNA, 1 µl of 10 µM light primer and 2 µl of 5x Sequenase buffer (200 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 250 mM NaCl). This mixture was heated in the thermal cycler to 65°C for ten minutes then slowly cooled to 30°C over a 30 minute period. To the 10 µl

of annealed DNA, 1 µl of 0.1M dithiothreitol (DTT), 2 µl of labeling mix (0.15 µM dGTP, 0.15 µM dTTP and 0.15 µM dCTP), 0.5 µl of 20 µM S^{35} labeled dATP (5 µCi in a 10 mM Tricine/1 mM DTT buffer; DuPont, Boston, MA), 2 µl dilute Sequenase (DNA polymerase diluted 1:8 in enzyme dilution buffer which was 10 mM Tris-HCl pH 7.5, 5 mM DTT and 0.5 mg/ml bovine serum albumin) and 1 µl Mn buffer (0.15 M Sodium Isocitrate, 0.1 M $MnCl_2$) was added for a final volume of 16.5 µl. After a five minute incubation at room temperature, four aliquots of 3.5 µl each were transferred to four separate termination reaction tubes containing 2.5 µl of either dideoxyGTP (80 µM dGTP, 80 µM dCTP, 80 µM dATP, 80 µM dTTP, 8 µM ddGTP and 50 mM NaCl), ddATP (80 µM of dGTP, dCTP, dATP and dTTP, 8 µM ddATP and 50mM NaCl), ddCTP (80 µM of dGTP, dCTP, dATP and dTTP, 8 µM ddCTP and 50 mM NaCl) or ddTTP (80 µM of dGTP, dCTP, dATP and dTTP, 8 µM ddTTP and 50 mM NaCl). This reaction was incubated at 37°C for five minutes after which 4 µl of stop solution (95% formamide, 20mM EDTA, 0.05% Bromophenol blue, 0.05% xylene cyanol FF) was added to each tube. Immediately prior to loading on sequencing gel samples were heated to 75°C for two minutes then quickly placed on ice.

Sequencing products were separated on a 6% denaturing polyacrylamide gel (7 M urea, 5.7% acrylamide, 0.3% bis-acrylamide, 0.1 M Tris, 0.1 M boric acid, 2 mM EDTA, 0.08% ammonium persulfate, 3.3 mM tetra-methylethylenediamine [TEMED]). Gels were 60 ml in volume and were left to polymerize overnight. Before loading the samples, gels were pre-run until they had warmed to a temperature of 50°C. Samples were separated through the gel at a constant power of 35 watts for 1.5 to 2 hours. After a run, gels were fixed in a one litre solution of 10% methanol and 10% acetic acid for fifteen minutes, dried down on to 3 mm chromatography paper (Whatman, Maidstone, U.K) using a gel drier and exposed to auto-radiography film (X-Omat RP diagnostic film; Kodak,

Rochester, NY) for two to three days before developing. The DNA sequence, determined from the auto-radiograph, identified the maternal species of a hybrid fish (Figure 2.5).

2.2.4. *Statistical Analysis*

Statistical analysis involved a 2 x 9 contingency table analysis to test for regional heterogeneity of local hybrid frequencies. This was determined by calculating a G statistic adjusted for small numbers with William's correction (Sokal and Rohlf 1981). G was considered significant at a level of $P < 0.05$.

2.3. Results

2.3.1. *Hybridization frequency*

Overall, 792 Atlantic salmon and brown trout were sampled, 37 of which were determined to be hybrids for a regional frequency of 4.7% (Table 2.1). Hybrids were found in all river systems except one; the Mobile River. Local frequencies of hybridization at the various sample sites ranged between 0.0 and 18.7% (Table 2.1) and were significantly heterogeneous (2 x 9 contingency table: $G_{adj} = 38.03$, $P < 0.001$, d.f.= 8). This suggests that the conditions responsible for hybridization are variable from one location to another. However, if the sample from the Hearts Delight river, where the hybrid frequency was particularly high, is excluded from the analysis, frequency differences are no longer significant (2 x 8 contingency table: $G_{adj} = 13.49$, $P < 0.10$, d.f.= 8).

All hybrids expressed a complete summation of both parental genotypes at each of the four diagnostic loci. There was no evidence of

Figure 2.5.

Photograph of an auto-radiograph used to determine the DNA sequence for a segment of the cytochrome b gene. Sequences shown are those of a brown trout (*Salmo trutta*), two hybrids, and an Atlantic salmon (*Salmo salar*).

• indicates differences used to identify maternal species.

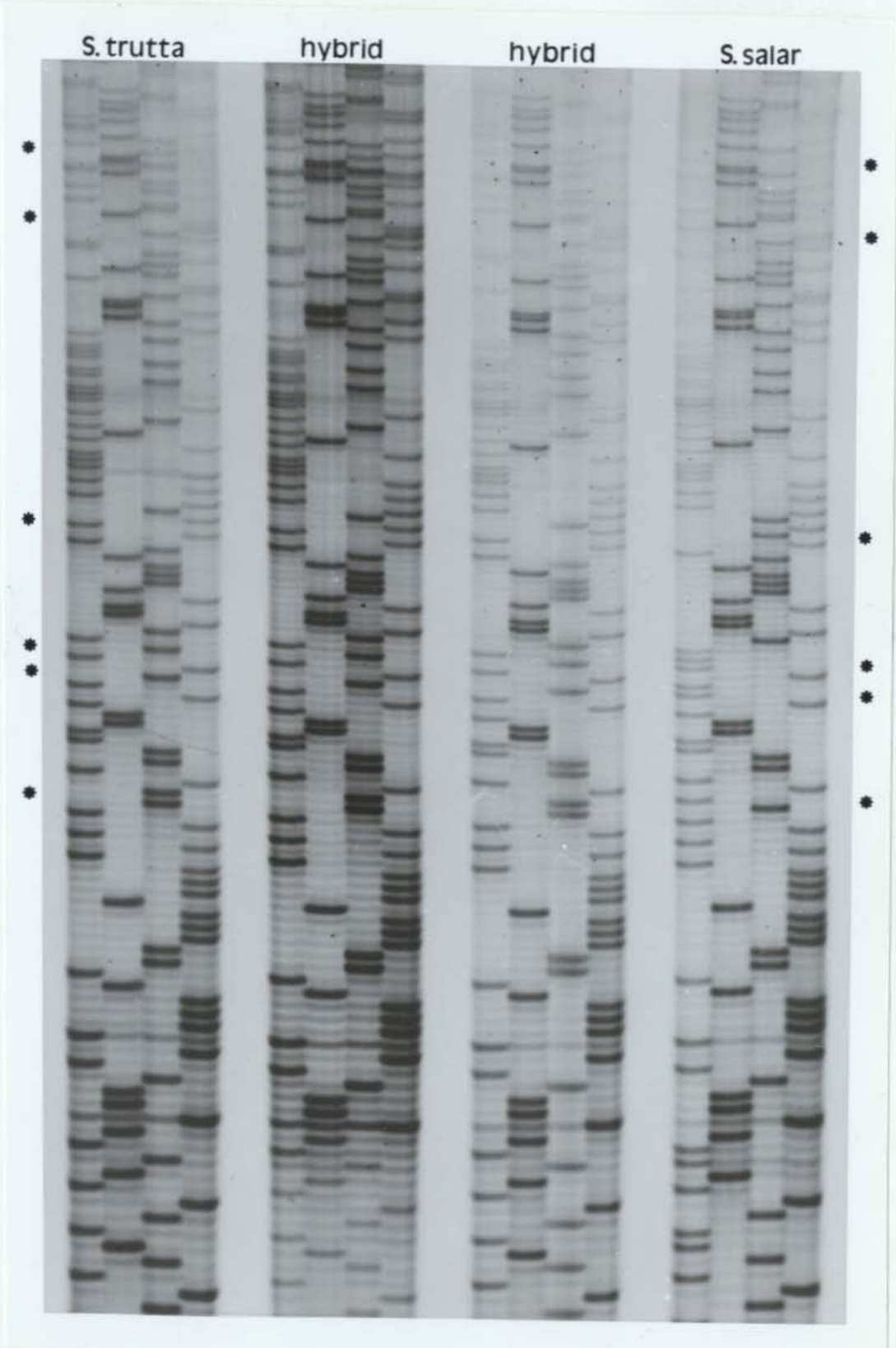

S. trutta
hybrid
hybrid
S. salar

Table 2.1.

Sample sites and the number of Atlantic salmon, brown trout and hybrids collected at each.

Sample site	N	Salmon	Trout	Hybrids No.	Hybrids %
1. Mobile R.					
a.	34	26	8	0	0.0
b.	77	46	31	0	0.0
total	111	72	39	0	0.0
2. Cape Broyle R.	33	15	16	2	6.1
3. Renews R.	78	53	21	4	5.1
4. Salmonier R.	75	15	58	2	2.7
5. N.E. Placentia R.					
a.	34	8	25	1	2.9
b.	43	19	19	5	11.6
c.	32	18	14	0	0.0
total	109	45	58	6	5.5
6. Spread Eagle R.	79	4	74	1	1.3
7. Hearts Delight R.	80	32	33	15	18.7
8. North R.	126	107	13	6	4.8
9. North Arm R.	101	49	51	1	1.0
Overall	792	393	362	37	4.7

introgression. Although Verspoor and Hammer (1991) have suggested that introgression between these species is possible, backcrossing is considered unlikely due to disruptions of meiotic pairing in fertile F1 hybrids (Johnson and Wright 1986).

2.3.2. *Hybrid maternal species*

All of the hybrids had mitochondrial cytochrome b genes characteristic of brown trout. Therefore, all hybrids were the products of matings between female brown trout and male Atlantic salmon.

2.4. Discussion

The overall hybrid frequency of 4.7% observed in this study was much higher than the 0.9% reported by Verspoor (1988) for the same region. The lower estimate in the earlier survey is probably the result of sampling biases. Verspoor (1988) analyzed only those fish identified morphologically as Atlantic salmon and included samples from rivers where no brown trout had ever been reported. Morphologically, hybrids can resemble either species (Jones 1948; Nyman 1970) so surveys must include a random sample of both brown trout and Atlantic salmon in order to obtain an accurate estimation of hybrid frequency. In addition, low hybrid viability may prevent many fish from reaching adult size. The present survey only considered samples of juveniles, whereas Verspoor (1988) included parr, smolts and adult fish. If only samples of parr are considered and those from rivers known not to contain brown trout are excluded, then the hybrid frequency in the survey by Verspoor (1988) becomes 2.2% which is more similar to the results presented here.

Crozier (1984) considered only brown trout and sampled both adult and juvenile fish in determining a hybrid frequency of 0.4% in the Lough

Neagh system of Northern Ireland. The combined surveys of Payne et al. (1972) and Solomon and Child (1978) proposed a 0.3% hybrid frequency for the United Kingdom. However these studies only considered adult Atlantic salmon caught offshore in commercial fishing nets.

It is probably more appropriate to compare results of the present study with those which have sampled only juvenile Atlantic salmon or brown trout. In North America, Beland et al. (1981) sampled juveniles of both species from the Stewiacke River, Nova Scotia and found one hybrid out of 56 fish for a frequency of 1.8%. Hurrell and Price (1991) sampled both Atlantic salmon and brown trout parr when estimating a hybrid frequency of 1.4% for rivers in south west England. Garcia de Leaniz and Verspoor (1989) found that 2.3% of Atlantic salmon parr sampled in Spanish rivers were hybrids. Jansson et al. (1991) discovered hybrid frequencies of 13% in samples of brown trout and Atlantic salmon parr from the River Gronan, Sweden, with local frequencies as high as 23%. However, the last two cases may have had higher than normal hybridization frequencies due to former introductions of Atlantic salmon in the rivers which were sampled.

It is difficult to say whether frequencies of hybridization are actually higher in North America, where brown trout are introduced, than they are in Europe, where both species are naturally sympatric. Hybridization between these species is possible in North America only because of the introduction of brown trout. What is not obvious is how prereproductive isolating mechanisms between these species have been compromised such that extensive hybridization is occurring in both Europe and North America. MtDNA analysis provides additional information and permits a closer examination of possible explanations.

Hybridization between brown trout and Atlantic salmon in Newfoundland appears to be unidirectional, with Atlantic salmon males

always fertilizing brown trout females. This bias in direction could be the result of differences in the chemotactic responses of salmon and trout sperm to the eggs of a different species. The eggs of Echinodermata species (starfish, sea cucumbers and brittle stars) are known to secrete chemical attractants which guide the sperm to the egg and are thought to be species-specific (Miller 1985; Garbers 1989). Trout eggs may be able to elicit a strong chemotactic response in salmon sperm, whereas the factors secreted by salmon eggs may not have as strong an effect on trout sperm. The existence of sperm chemotaxis in teleost fish has yet to be clearly demonstrated (Hart 1990), however indirect evidence of chemotaxis in the Rosy barb (*Barbus conchonius*) has been discovered (Amanze and Iyengar 1990). Whether or not sperm chemotaxis represents a reproductive barrier between closely related fish species is also unknown. The observation of natural hybrids in Europe which have Atlantic salmon mothers (Youngson et al. 1992; H. Jansson, Laxforskning sinstitutet, Älvkarleby, Sweden, personal communication) considerably weakens this hypothesis with respect to Atlantic salmon x brown trout hybridization.

A simpler explanation for this observation would be that there is a difference in viability between the reciprocal crosses such that only hybrids with brown trout mothers are surviving long enough to be sampled. Such was the case for natural hybridization between northern pike (*Esox lucius*) and chain pickerel (*Esox niger*) in which the maternal parent of all hybrid fish was a chain pickerel (Herke et al. 1990). Previous experiments in the laboratory had shown that hybrids produced using chain pickerel females are viable, whereas the reciprocal cross is completely inviable (Buss and Miller 1967).

To address this question with respect to the results presented above, breeding experiments were undertaken to compare the relative viability of reciprocal brown trout x Atlantic salmon hybrids. This study is the subject of chapter 3.

Chapter 3

Artificial Hybridization Between Newfoundland Brown Trout and Atlantic Salmon

3.1. Introduction

Artificial hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) has been of both scientific and commercial interest for over 100 years. Several studies have investigated the morphological appearance of hybrids (Day 1882; Jones 1948), their fertility (Alm 1955; Piggins 1970) and their biochemical genetics (Nyman 1970; Nygren et al. 1972; Vuorinen and Piironen 1984; Johnson and Wright 1986).

Breeding experiments comparing the relative viability of reciprocal hybrids and their parental controls have had variable results. Some find that hybrids produced using brown trout eggs and Atlantic salmon milt perform best (Refstie and Gjedrem 1975), whereas others have had greater success with hybrids produced using eggs from Atlantic salmon or Atlantic salmon grilse (Hofer 1909 [from Refstie and Gjerem 1975]; Alm 1955; Piggins 1970). It is likely that a good deal depends on the strains of fish used as well as the quality of eggs and milt at the time of fertilization. In a review of the literature, Chevassus (1979) concluded that hybrids with brown trout mothers are greater in viability, but that both crosses perform as well as or better than the parental controls.

The following study investigated the relative viability of hybrids produced using anadromous brown trout, Atlantic salmon grilse and sexually mature Atlantic salmon parr from Newfoundland. Hatchability, survival and growth are considered during the fresh water period until

first feeding. Also, some qualitative observations on the performance of hybrids after this stage are presented.

2.2. Materials and Methods

2.2.1. *Notation*

The following notation is used in order to simplify identification of the various families and individual fish. Each individual is identified by its species (T = brown trout, S = Atlantic salmon and SP = sexually mature salmon parr) and a number (except for the sexually mature salmon parr whose milt was pooled). All crosses list the female parental species first. For example, T1 x T4 represents the family produced by the female brown trout 1 and the male brown trout 4. T3 x SP represents a hybrid cross produced using brown trout eggs and the pooled milt from six sexually mature salmon parr. When crosses are referred to in more general terms, numbers are not used.

2.2.2. *Breeding stock*

Breeding stock was obtained from the North East Placentia River located on the Avalon Peninsula of Newfoundland (47° 16' N, 53° 50' W). Anadromous brown trout were captured at a counting fence in late July and transported live to the Ocean Sciences Centre, Memorial University, St. John's. At the time, no secondary sexual characteristics were evident on the fish making identification of males and females difficult. Fourteen fish were collected, three of which turned out to be male. There was one mortality during transport.

Atlantic salmon could not be collected from the counting fence at this

time without suffering high mortality. It was suspected that warm water temperatures in July made the trip too difficult for the fish. Consequently, ripe anadromous Atlantic salmon and sexually mature male Atlantic salmon parr were captured in early November when the water was much colder. They were seined from a nursery stream further up river from the counting fence and transported back to St. John's without any problems. By this time, the brown trout were also ripe and ready to spawn. Figure 3.1 indicates the locations on the river where the brown trout and Atlantic salmon were collected.

3.2.3. *Spawning and experimental design*

Fish were artificially spawned on November 15, 1990. Eggs and milt were not pooled except for the milt of six sexually mature male Atlantic salmon parr. Instead, the eggs of each female were divided equally into five families consisting of two controls, two hybrid crosses and a single family using the pooled milt from the salmon parr. No family was duplicated and each was incubated separately so that individuals whose gametes were abnormally low in viability could be identified and eliminated from the results. The size of each family was estimated by counting the number of eggs in one family for each female and the numbers are rounded to the nearest ten. Table 3.1 illustrates the experimental design as well as the size and position of each family in the incubator. Three anadromous females and three anadromous males of each species were used along with the pooled milt of the sexually mature salmon parr. Overall, 30 families were produced.

3.2.4. *Incubation and rearing*

Fertilized eggs were incubated using recirculated water with temperatures ranging from 5.5 to 8.7°C and a mean temperature of

Figure 3.1.

Sites on the North East Placentia river where A) adult anadromous brown trout and B) adult anadromous Atlantic salmon and sexually mature male Atlantic salmon juveniles were collected.

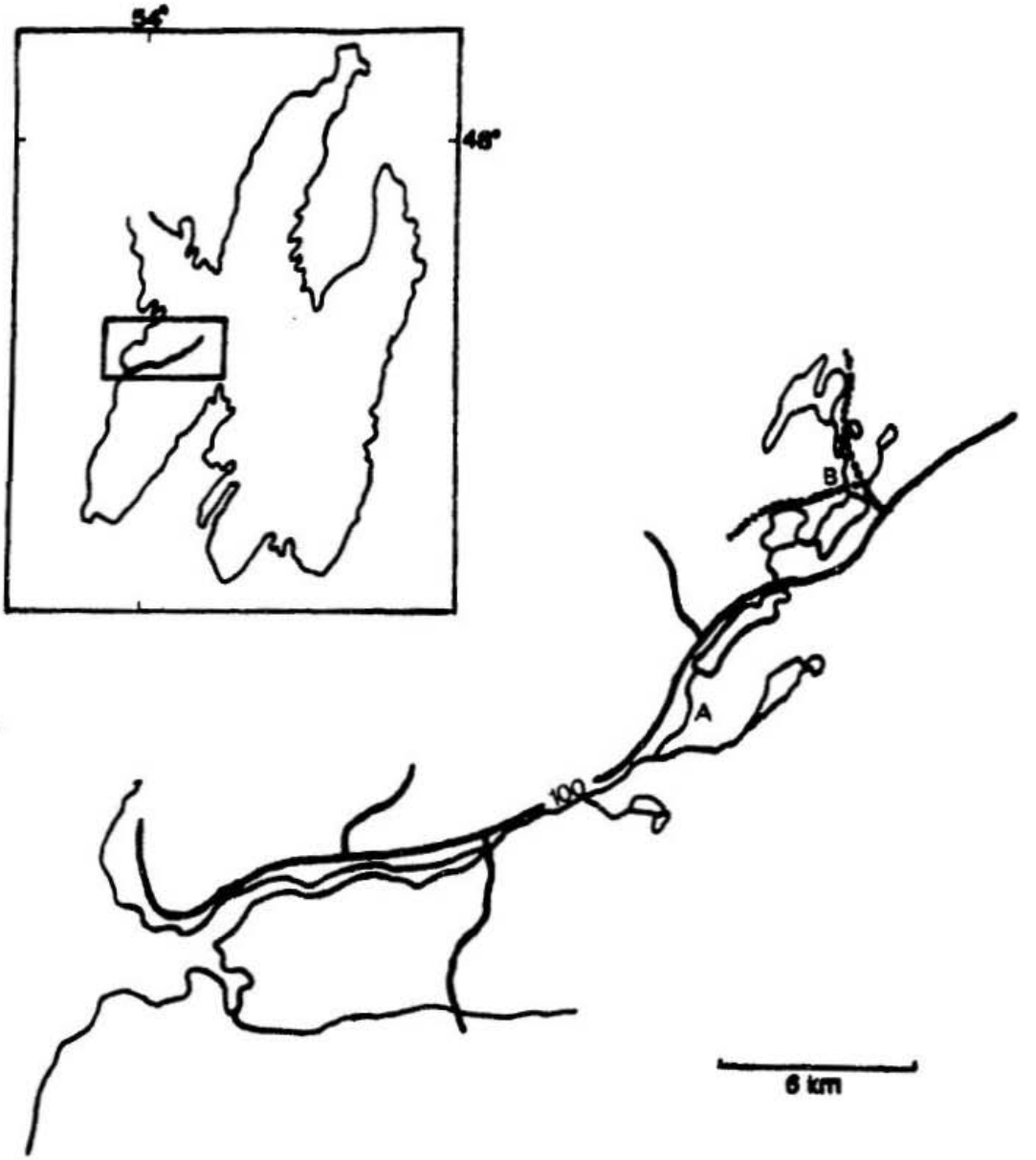

54°
48°
B
A
100
6 km

Table 3.1.

Parents of families, number of eggs per family and relative position of each family in incubator. Incubator consisted of eight egg-trays stacked vertically on top of one another with each tray divided into four cells.

Tray	Cells			
	A	B	C	D
1	S1 x S4 360	S1 x S5 360	S2 x S4 600	S2 x S6 600
2	S3 x S6 370	S3 x S5 370	S2 x SP 600	S1 x SP 360
3	S1 x T4 360	S1 x T5 360	S2 x T4 600	S2 x T6 600
4	S3 x T4 370	S3 x T2 370		S3 x SP 370
5	T1 x S4 370	T1 x S5 370	T2 x S4 500	T2 x S6 500
6	T3 x S6 310	T3 x S5 310	T1 x SP 370	T2 x SP 500
7	T1 x T4 370	T1 x T5 370	T2 x T4 500	T2 x T6 500
8	T3 x T4 310	T3 x T5 310		T3 x SP 310

7.5°C. Dead eggs were removed twice a week and counted. After hatching, dead fish were counted and removed every second day. Alevins were left in the incubators until their egg-sacs were almost completely absorbed, then moved to rearing tanks where they started receiving food.

Rearing tanks were 0.5 m^2 with a water depth of 15 cm. Water was recirculated and maintained at a temperature of approximately 10°C. Due to limited space, families were combined into four groups consisting of brown trout, Atlantic salmon and the two reciprocal crosses. The families produced using parr were eliminated altogether. Equal densities could not be maintained so quantitative measurements were no longer taken. However, qualitative observations concerning feeding and survival are presented.

Automatic feeders were used to deliver an excess amount of food to the tanks eight times per day. At first, fry were given Atlantic salmon starter feed (Corey Feed Mills, Fredricton, N.B.) and periodically were given finely grated frozen beef liver to help encourage active feeding. As fish grew, the size of feed was increased accordingly. Uneaten food and any dead fish were removed from the tanks daily.

3.2.5. *Length measurement*

Total length measurements of fry were obtained just after transfer to the rearing tanks. To avoid any injury to the fish, a diapositive photograph was taken of a sample from each group in a tray of shallow water with a ruler below. Measurements were taken from the photograph using a stereo microscope.

3.2.6. *Statistical analysis*

Comparisons of the hatchability and survival for reciprocal hybrids and

control groups were made using a Chi-squared contingency table analysis with a Yates correction. Due to the estimation of family size, high number of eggs and environmental differences from one tray to the other, a conservative level of significance was chosen at $P < 0.001$. However, the Chi-square values for all comparisons are presented. Differences in mean length were compared using a posted ANOVA corrected using the Bonferroni method and considered significant at a level of $P < 0.001$. All statistical calculations were performed using the computer program GraphPad InStat (Graphpad, SanDiego, CA).

During the alevin stage, 50 fish were removed from several hybrid families (Table 3.2) for additional genetic analysis which is not presented here. These individuals have been excluded from the results and any statistical analysis for this stage.

3.3. Results

The hatching success and death rate of fish during the alevin stage (from hatching until transfer to rearing tanks) for each family are presented in Table 3.2. Eight families were eliminated from the study due to abnormally high mortality. These included all of the families involving the female T2 or the male T6 which showed very low viability in both hybrid and conspecific matings. This was likely the result of poor egg and milt quality at the time of spawning. Two other families, S1 x S5 and S2 x S4, also showed unusually high mortality during the alevin stage. It is suspected that their position at the centre of the top egg tray (Table 3.1), where water entered the incubator, may have been responsible. The mortality of these families are presented in Table 3.2, however they have been excluded from any statistical comparisons and from the summary of results presented in subsequent tables.

Table 3.2.

Parents of families (c), number of eggs fertilized in each family (no), mortality from fertilization to hatching (mh) and mortality from hatching to egg-sac absorption (mf) with percentages in brackets.

Tray		Division A	Division B	Division C	Division D
1	c	S1 x S4	S1 x S5	S2 x S4	S2 x S6
	no	360	360	600	600
	mh	6 (1.7)	8 (2.2)	18 (3.0)	10 (1.7)
	mf	9 (2.5)	112 (31.8)	438 (75.3)	61 (10.3)
2	c	S3 x S6	S3 x S5	S2 x SP	S1 x SP
	no	370	370	600	360
	mh	9 (2.4)	10 (4.7)	22 (3.7)	5 (1.4)
	mf	6 (0.3)	0 (0.0)	4 (0.7)	2 (0.6)
3	c	S1 x T4*	S1 x T5*	S2 x T4*	S2 x T6
	no	360	360	600	600
	mh	29 (8.1)	17 (4.7)	28 (4.6)	447 (74.5)
	mf	7 (2.5)	9 (2.6)	4 (0.8)	0 (0.0)
4	c	S3 x T4*	S3 x T5*		S3 x SP
	no	370	370		370
	mh	5 (1.4)	70 (18.9)		11 (3.0)
	mf	4 (1.3)	4 (1.6)		0 (0.0)
5	c	T1 x S4*	T1 x S5*	T2 x S4	T2 x S6
	no	370	370	500	500
	mh	46 (12.4)	26 (7.0)	388 (77.6)	380 (76.0)
	mf	67 (20.7)	128 (37.2)	7 (6.3)	6 (5.0)
6	c	T3 x S6*	T3 x S5*	T1 x SP	T2 x SP
	no	310	310	370	500
	mh	33 (10.6)	17 (5.5)	13 (3.5)	381 (76.2)
	mf	67 (29.5)	53 (21.8)	103 (28.8)	5 (4.2)
7	c	T1 x T4	T1 x T5	T2 x T4	T2 x T6
	no	370	370	500	500
	mh	8 (2.2)	32 (8.6)	300 (60.0)	497 (99.4)
	mf	4 (1.1)	19 (5.6)	2 (1.0)	1 (33.3)
8	c	T3 x T4	T3 x T5		T3 x SP
	no	310	310		310
	mh	17 (5.5)	48 (15.5)		49 (15.8)
	mf	1 (0.3)	9 (3.4)		87 (33.3)

* 50 alevins were removed from these families for further genetic analysis and are not included in the summary table or any statistical analysis for this stage.

The survival for each type of cross and control from fertilization until hatching is summarized in Tables 3.3. Hatching success was high in all crosses and controls. The sperm of sexually mature Atlantic salmon parr performed equally well compared to that of the anadromous salmon sperm when fertilizing both salmon and trout eggs. Salmon eggs fertilized by either anadromous salmon or parr milt did significantly better than all hybrid crosses and the brown trout control. Chi-square values for comparisons between groups are presented in Table 3.4

During the alevin stage (Table 3.5), the viability of the S x T hybrid was equivalent to both the T x T parental control and the S x SP control. The S x S parental control had significantly lower survival than the S x T cross and the S x SP cross. This may be due to the environmental conditions in the top tray of the incubator where the majority of S x S alevin mortality occurred (Table 3.2). The T x T control was equal to the S x S control, but lower in viability than the S x SP cross (Table 3.6). The T x S and T x SP hybrid alevins were significantly lower in viability than the reciprocal cross and all control groups (Table 3.5 and Table 3.6). The majority of these T x S hybrids died from what appeared to be a failure to absorb their egg-sacs. Egg-sacs were not deformed and conditions such as blue-sac disease or gas-bubble disease (Roberts and Shepherd 1986) were not apparent. There was no significant difference between the survival of hybrids produced using salmon milt or parr milt. Chi-square values for comparisons made between groups during this stage are presented in Table 3.6.

The time of hatching and period over which hatching occurred is presented in Table 3.7. In general, eggs fertilized using brown trout sperm hatched approximately 40 degree-days (five to six days) earlier than eggs fertilized with Atlantic salmon milt. Also, the former group started hatching and finished over a shorter period than the latter group. This suggests a paternal factor in embryo development.

Table 3.3.

Mean percentage survival (m) from fertilization to hatching including the number of families (n) and the range of percentages (r).

Sperm		Eggs	
		Brown trout	Atlantic salmon
Brown trout	n	4	5
	m	92.1	92.5
	r	84.5 - 97.8	81.1 - 98.6
Atlantic salmon	n	4	4
	m	91.1	97.9
	r	87.6 - 94.5	97.3 - 98.3
Salmon parr	n	2	3
	m	90.4	97.3
	r	84.2 - 96.5	96.3 - 98.6

Table 3.4

Chi-square and P values (in brackets) for the comparisons of survival from fertilization to hatching including the total frequency of survivors (s) and mortalities (m) for each type of cross.

		S x S	S x SP	S x T	T x SP	T x S
m 105 s 1255	T x T	54.2 (<0.001)	30.6 (<0.001)	0.22 (0.64)	1.00 (0.31)	1.23 (0.27)
m 122 s 1238	T x S	72.7 (<0.001)	43.8 (<0.001)	3.17 (0.07)	0.000747 (0.98)	–
m 62 s 618	T x SP	60.1 (<0.001)	36.0 (<0.001)	2.31 (0.13)	–	–
m 149 s 1912	S x T	52.4 (<0.001)	28.8 (<0.001)	–	–	–
m 38 s 1292	S x SP	1.69 (0.19)	–	–	–	–
m 35 s 1665	S x S	–	–	–	–	–

Table 3.5.

Mean percentage survival (m) from hatching to egg-sac absorption including the number of families (n) and the range of percentages (r).

Sperm		Eggs	
		Brown trout	Atlantic salmon
Brown trout	n	4	5
	m	97.4	98.2
	r	94.4 - 99.7	97.4 - 99.2
Atlantic salmon	n	4	4
	m	72.9	96.7
	r	63.8 - 78.2	89.7 - 100.0
Salmon parr	n	2	3
	m	68.9	99.3
	r	66.7 - 71.2	99.3 - 100.0

Table 3.6

Chi-square and P values (in brackets) for the comparisons of survival from hatching to egg-sac absorption including the total frequency of survivors (s) and mortalities (m) for each type of cross.

		S x S	S x SP	S x T	T x SP	T x S
m 33 s 1222	T x T	6.93 (0.0085)	18.4 (<0.001)	2.67 (0.10)	309.4 (<0.001)	336.9 (<0.001)
m 315 s 723	T x S	341.4 (<0.001)	430.2 (<0.001)	470.7 (<0.001)	1.32 (0.91)	–
m 190 s 428	T x SP	297.5 (<0.001)	412.9 (<0.001)	436.6 (<0.001)	–	–
m 28 s 1634	S x T	21.8 (<0.001)	8.47 (0.0036)	–	–	–
m 6 s 1286	S x SP	43.8 (<0.001)	–	–	–	–
m 76 s 1589	S x S	–	–	–	–	–

Table 3.7.

Degree-day at which 50% of eggs hatched (h) and period from beginning to 100% hatched (p) (n = number of families).

Sperm		Eggs	
		Brown trout	Atlantic salmon
Brown trout	n	4	5
	h	470	464
	p	15	23
Atlantic salmon	n	4	4
	h	504	511
	p	45	45
Salmon parr	n	2	3
	h	509	511
	p	53	30

Fish were removed from the incubation trays on March 1, 1991; 106 days or 795 degree-days after fertilization. By this time, eggs-sac were almost completely absorbed in all groups and fish were actively swimming to the water surface.

Length measurements for the different groups at the time of transfer are shown in Table 3.8. There was no significant difference in the mean length of trout and salmon. However, the mean length of T x S hybrids was significantly less than the reciprocal cross and both parental groups. In contrast, the mean length of S x T hybrids was significantly greater than both parental controls and the T x S hybrid cross.

After transfer to the rearing tanks the T x S hybrids continued to do poorly. This group of fish also exhibited many morphological abnormalities such as underdeveloped eyes and heads or deformed spines. The S x S fry suffered from a fungal gill infection which eliminated almost the entire group. This was likely brought on by overly crowded conditions (about 1500 fry in one tank) and poor water circulation. Also, the reluctance of the salmon fry to feed at low water temperatures may have resulted in a greater quantity of uneaten food and particulate matter in their tank. Fungus was not a problem for any of the other three groups despite the fact that water was being recirculated. S x T hybrids continued to do very well even at high densities (about 1600 fry in one tank). They did not suffer from fungal infections and started feeding almost immediately. The brown trout parental controls (about 1200 fry in one tank) also continued to do very well and exhibited qualities similar to those of the S x T hybrids.

3.4. Discussion

The results of this study suggest that hybrids produced using the eggs

Table 3.8.

Mean length (l) in mm. of fish immediately after egg-sac absorption including standard deviation (sd), range (r) and number of individuals measured (n).

Sperm		Eggs	
		Brown trout	Atlantic salmon
Brown trout	n	25	25
	l	24	26
	sd	0.64	0.64
	r	22 - 25	25 - 27
Atlantic salmon	n	25	25
	l	20	24
	sd	0.56	0.90
	r	19 - 22	21 - 25

of Newfoundland Atlantic salmon grilse perform as well as either parental control while its reciprocal hybrid, produced using the eggs of anadromous Newfoundland brown trout, is inferior in both its viability and early growth.

Similar results have been obtained by several authors. Hofer (1909, from Refstie and Gjedrem 1975), concluded that hybridization between these species is only possible when Atlantic salmon eggs are used. Piggins (1970), had no success with hybrids produced using anadromous brown trout females. However, hybrids produced using the eggs of Atlantic salmon averaged 50% survival from the ova to yearling stage and showed evidence of hybrid vigor in growth rate during the fresh water period (Piggins 1970). Blanc and Chevassus (1979) found that after the eyed stage, T x S hybrids were relatively low in viability compared to the brown trout controls. Alm (1955) also found that hybrids produced using salmon eggs have a higher hatching rate and survival to first feeding than those produced using brown trout eggs. In contrast to these results, Refstie and Gjedrem (1975) found that both reciprocal hybrids exhibited eyeing and hatching rates that were greater than both parental controls, but that subsequent survival over the next 11 months was higher for the T x S hybrid than for the S x T hybrid. Also, they found that growth during the first 11 months was inferior in both reciprocal hybrids when compared with the parental controls (Refstie and Gjedrem 1975).

A problem with any hybridization study is a potential difference in the natural spawning times of the species and the subsequent difference in gamete quality when fish are artificially spawned. None of the salmon eggs or milt appeared to be of questionable quality. These fish were obtained from the nursery stream very close to, if not during, their natural spawning period and stripped shortly thereafter. Among the brown trout, one female and one male exhibited low gamete quality and were consequently excluded from the results. Also, brown trout controls

had a lower hatching success than the salmon controls. Brown trout had to be captured several months earlier than the salmon and it is unclear how this time in captivity may have affected them. It is also possible that the natural spawning period of the brown trout had passed by the time they were artificially spawned. Heggberget et al. (1989) found that in Norwegian rivers, the peak spawning period of anadromous brown trout was about 15 days earlier than that of salmon. No data are available on the peak spawning time of anadromous brown trout in Newfoundland. Both species have been observed spawning at the same time in the North Harbour and Colinet rivers, Newfoundland (M. O'Connell, Department of Fisheries and Oceans, St. John's, Nfld., personal communication). However, it is certainly possible that in the North East Placentia river, anadromous brown trout spawn earlier than the anadromous salmon. Blanc and Poisson (1983) have stressed the importance of high egg quality to the viability of hybrid salmonids. Consequently, although the quality of brown trout eggs may have been sufficient for producing viable brown trout, they may have been inadequate for the production of hybrids. As Chevassus (1979) has pointed out, intraspecies variability may lead to differences in the results of different authors. Variability in spawning time may account for some of these.

Egg size may also be a factor leading to the observed difference in viability of the reciprocal hybrids. The difference in hatching time and hatching period of eggs fertilized by either brown trout or salmon milt suggests a paternal influence in embryo development. Factors brought into the egg by the sperm may be adapted to a particular egg size. Consequently the smaller brown trout egg may not provide enough space for embryo development. Conversely, the larger salmon egg would be able to accommodate any developmental factors brought in by the brown trout sperm. However, Suzuki and Fukuda (1971) report an opposite correlation in their hybridization experiments using a broad range of salmonid species.

The significance of these results with respect the aquaculture industry and with respect to results obtained in the previous chapter concerning natural hybridization are presented in chapter 4.

Chapter 4

Summary and Overview

4.1. Natural Hybridization

In chapter two, it was discovered that all of the naturally occurring hybrids sampled from the various rivers were the products of matings between female brown trout and male Atlantic salmon. The results of chapter three show that both types of hybrids are viable and suggest that hybrid fish produced using Atlantic salmon females are actually greater in viability than hybrid fish produced using brown trout females. Therefore, if natural hybridization had occurred between brown trout males and Atlantic salmon females, it is likely that the F1 products of these matings would have been detected along with those of the reciprocal cross. Since no such hybrids were observed, it is concluded that natural hybridization between these species in Newfoundland is unidirectional with salmon males always fertilizing brown trout females.

In his extensive review of natural hybridization among fish, Hubbs (1955) identified the degradation of habitat or a shortage of suitable spawning habitat as environmental factors likely to encourage cross fertilization. There is no evidence of habitat degradation in any of the sample sites. However, it is possible that in some streams spawning habitat is limited and both species must share common spawning areas. Similar to an introduction, both conditions bring spatially isolated species together into the same spawning habitat. In this situation, hybridization should occur at random, with hybrid frequencies reflecting the probability of a male from one species encountering a female from the other species. Also, males and females of each species should

contribute equally to hybrid matings. Although the loss of spacial segregation may explain the occurrence of hybridization between these species, it does not explain the observed bias in direction. Additional factors, other than environmental conditions, must be influencing the occurrence and dynamics of hybridization between these species in nature. Something particular to the salmon and trout populations themselves is likely to be responsible.

Hubbs (1955) has also suggested that considerable disparity in species abundance may promote hybridization. There are no data available on the relative abundance of breeding Atlantic salmon and brown trout for the streams sampled in this study. Egglishaw (1970) has shown that the recruitment of Atlantic salmon and brown trout occupying the same stream can be very different. Consequently, juvenile species ratios are not an accurate representation of relative species abundance for adult fish. However, large differences in juvenile species abundance suggest that sample sites in the Spread Eagle and Salmonier rivers are predominantly brown trout streams whereas sample sites in the Renews and North River are predominantly salmon streams (Table 2.1). If hybridization is frequency-dependent, then the direction of hybridization would be expected to reverse when the predominant species changes. This was not observed and thus it is unlikely that disparity in species abundance is a principle cause of hybridization.

In his review of hybridization among vertebrates, Mayr (1970) considered the nature of the mating bond as an important factor influencing the strength of prereproductive isolating mechanisms between species. The reproductive behavior in *Salmo* has been extensively documented (Jones and Ball 1954; Campbell 1977; Webb and Hawkins 1989). After migrating upstream, a female will excavate a form or redd in the gravel substrate into which eggs will be deposited as they are fertilized. Large males, physically defend access to a female from

smaller, less aggressive males. The relative strength or weakness of the mating bond is questionable. Jones and Ball (1954) reported strong aggressive defence of the female by males, especially in brown trout. However, differences in the life-history characteristics of Atlantic salmon and brown trout may explain the unidirectional nature of hybridization between these species in Newfoundland.

A bias in direction would be expected if each species spawned at a different time. Webb and Hawkins (1989) have found that Atlantic salmon females will arrive at a spawning site ahead of males and leave soon after spawning. Males, on the other hand, remain in the stream for a period after spawning, possibly looking for additional mates (Greeley 1932; Webb and Hawkins 1989). Consequently, males of an early spawning species may still be present when females of a later spawning species arrive. In such a case, the later species would be prone to contributing females in hybrid matings and the earlier species, the males. In Norwegian rivers, where salmon and trout are naturally sympatric, temporal difference in spawning time is thought to be the major mechanism of prereproductive isolation; brown trout having a peak spawning time approximately 15 days earlier than salmon (Heggberget et al. 1988). No comparative data are available on the spawning times of anadromous brown trout and Atlantic salmon in Newfoundland, although both species have been observed spawning at the same time on the North Harbour and Colinet rivers (M. O'Connell, Department of Fisheries and Oceans, St. John's, Nfld., personal communication).

In Newfoundland rivers, anadromous Atlantic salmon are generally larger than brown trout. Brown trout may exceed 50 cm in length in many Newfoundland rivers and lakes (Wiseman 1973). However, Lear and Day (1977) found on the North Harbour River, Newfoundland, that 78% of the upstream migrating anadromous brown trout had fork

lengths ranging between 15 and 30 cm while Atlantic salmon were 50 to 60 cm long. O'Connell (1982) reported the mean length of spawning anadromous male brown trout for the North Harbour, North East Placentia and Colinet rivers, Newfoundland, to be 31 cm. Fish this size would weigh under 0.5 kg. Angling statistics for these rivers suggest that Atlantic salmon grilse are normally over 1.5 kg (Porter et al. 1974). This difference in size would allow large anadromous male salmon to outcompete smaller anadromous brown trout males for their mates. Subdominant anadromous male salmon who cannot gain access to conspecific females may be mating with brown trout females instead. However, in Newfoundland rivers, female salmon are generally more abundant than males (O'Connell and Reddin 1983) and competition between anadromous males is likely to be limited.

An alternative life-history strategy employed by male Atlantic salmon and brown trout is to mature sexually within fresh water as parr (Dalley et al. 1983; Jonsson 1985; Gibson and Cunjak 1986; Myers et al. 1986; L'Abee-lund et al. 1989). These individuals compete with one another for superior positions close behind an anadromous female (and attendant anadromous male if there is any present) and are able to sneak in and fertilize some of the eggs as they are shed (Jones and King 1952; Campbell 1977; Myers and Hutchings 1987). Crozier (1984) noted that in Irish rivers where hybrid frequencies were highest, mature parr were also particularly abundant. Garcia de Leaniz and Verspoor (1989) reported exceptionally high early sexual maturation among salmon parr in Spanish rivers where hybrid frequencies averaged 2.3%, but reached as high as 7.7%.

These mature male parr may not be as selective as anadromous males and may indiscriminantly sneak matings with large females of either species. Competition among parr may force subdominant males to seek matings with females of an alternate species instead. Moreover, sexually

mature parr would be present in the stream during the spawning period of both species, whether or not there is temporal segregation of spawning times of the anadromous fish.

There is a considerable amount of interpopulation variation with respect to the proportion of, and age at which salmon and trout mature as parr (Myers et al. 1986; L'Abee-lund et al. 1989). This variability is heritable as well as environmentally influenced such that faster growing fish tend to mature at an earlier age (Thorpe et al. 1983). Atlantic salmon males in Newfoundland tend to mature sexually as parr more often and at an earlier age than brown trout males. In the North Harbour, Colinet and North East Placentia rivers, male brown trout do not begin to mature until they are 3+ years of age, and only at 4+ and older will over 50% of a given year-class be sexually mature (O'Connell 1982). In contrast, Atlantic salmon males of many Newfoundland rivers become sexually mature as early as 1+ years of age (Myers et al. 1986). Gibson and Cunjak (1986) found in the North Arm River and Salmonier River (Table 2.1, Figure 2.1), a higher percentage of Atlantic salmon parr maturing sexually and at an earlier age than brown trout. They attribute this difference to competitive interactions between the two species which have led to slower growth in brown trout and consequently later maturation. O'Connell (1982) has reported brown trout growth to be slower in Newfoundland than in Europe.

The greater tendency for Atlantic salmon to mature sexually as parr at an earlier age than brown trout can explain the bias in direction of hybridization observed in this survey. Since the majority of sexually mature parr in the streams would be salmon, most if not all matings of this type that produce hybrids would be expected to involve Atlantic salmon males and brown trout females. In addition, the selective advantage of small size among 'sneaky' males may favor the younger (and presumably smaller) Atlantic salmon males in these types of

matings. Gross (1985) found that in coho salmon (*Oncorhynchus kisutch*) as the size of a sneaky male decreased, its proximity to a female increased. Therefore, the abundance of sexually mature Atlantic salmon parr in Newfoundland streams appears to be a major factor responsible for both the frequency and direction of hybridization observed in this study.

4.2. Artificial Hybridization

A major purpose of most fish hybridization experiments has been to find desirable crosses for production in the aquaculture industry. The benefits of this strategy include the transfer of beneficial traits from one species to another, heterosis and hybrid sterility; the latter limiting both the economically unfavorable characteristics of sexual maturation as well as the genetic impact of escaped fish on wild fish stocks (Chevassus 1983).

In the experiments described in chapter three, the S x T hybrid exhibited good hatchability and high viability during the alevin stage. This group also started feeding with limited mortality and was less susceptible to fungal infection than the Atlantic salmon control group when kept at high densities. The Atlantic salmon may have done better under more ideal conditions such as lower densities or higher water temperatures. However, the performance of the S x T hybrids under the environmental conditions of this experiment merit further investigation as they appear to exhibit beneficial brown trout characteristics during this stage of their life-cycle. Fish farmers with limited control of water temperatures may find such a cross desirable.

Presently, at 1+ years of age, the S x T hybrids resemble Atlantic salmon. They are less tolerant of high densities than the brown trout and

have consequently suffered from problems such as fin rot and tail biting. However, they also appear to exhibit far less variability in growth rate than the brown trout.

With respect to sterility, Piggins (1970) found the S x T cross to be fertile, producing viable F2 and backcross generations as well as suffering a reduced growth rate during gametogenesis. More often, hybrids mature sexually but produce gametes with reduced viability (Alm 1955; Nygren et al. 1975; Johnson and Wright 1986). It is possible that intraspecies differences are responsible for the variable results. How these hybrids produced using Newfoundland strains of salmon and brown trout perform in sea water and whether or not they mature sexually are the subject of continuing investigations.

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APPENDIX

Figure A.1.

Location of sample sites on A) the Mobile river (sites a and b), B) the Cape Broyle river and C) the Renews river.

(* = sample site, scale = 1:50000)

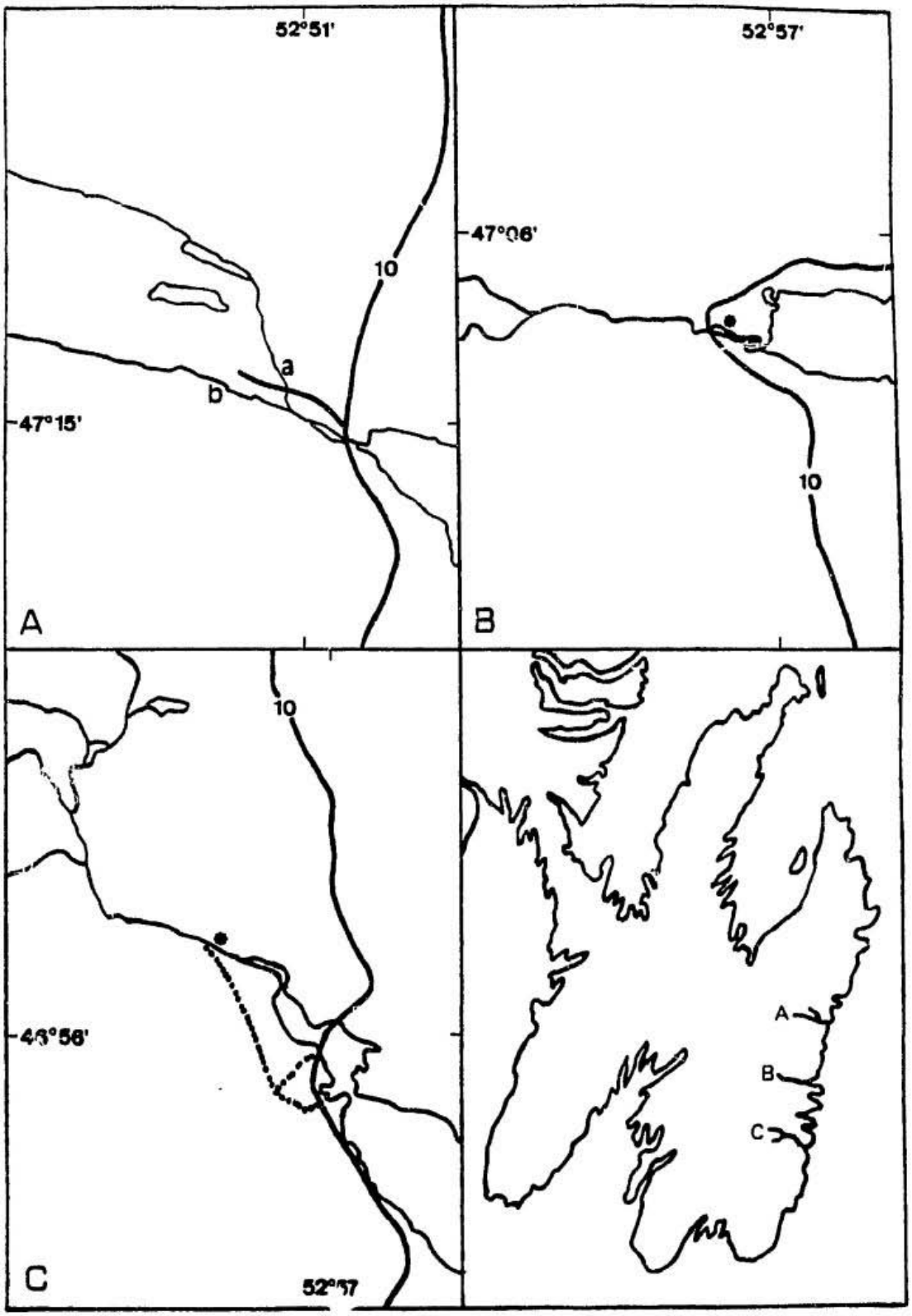

52°51'
47°15'
10
a
b
A
52°57'
47°06'
10
B
10
46°56'
C
52°57
A
B
C

Figure A.2.

Location of sample sites on A) the Salmonier river, B) the Spread Eagle river and C) the Hearts Delight river.

(* = sample site, scale = 1:50000)

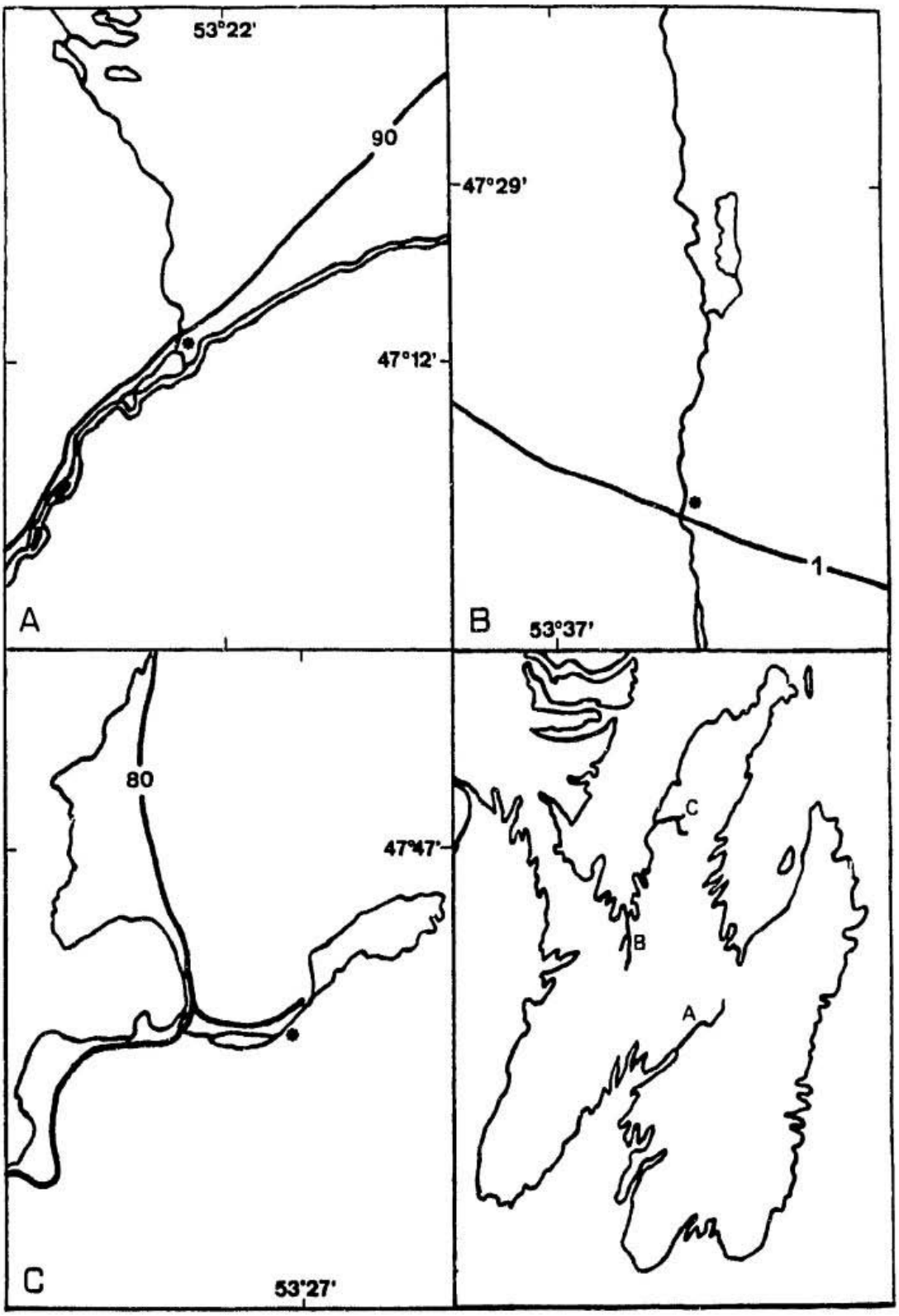

53°22'
90
47°12'
A
47°29'
1
B
53°37'
80
47°47'
C
53°27'
C
B
A

Figure A.3.

Location of sample sites on A) the North River and B) the North Arm river.

(* = sample site, scale = 1:50000)

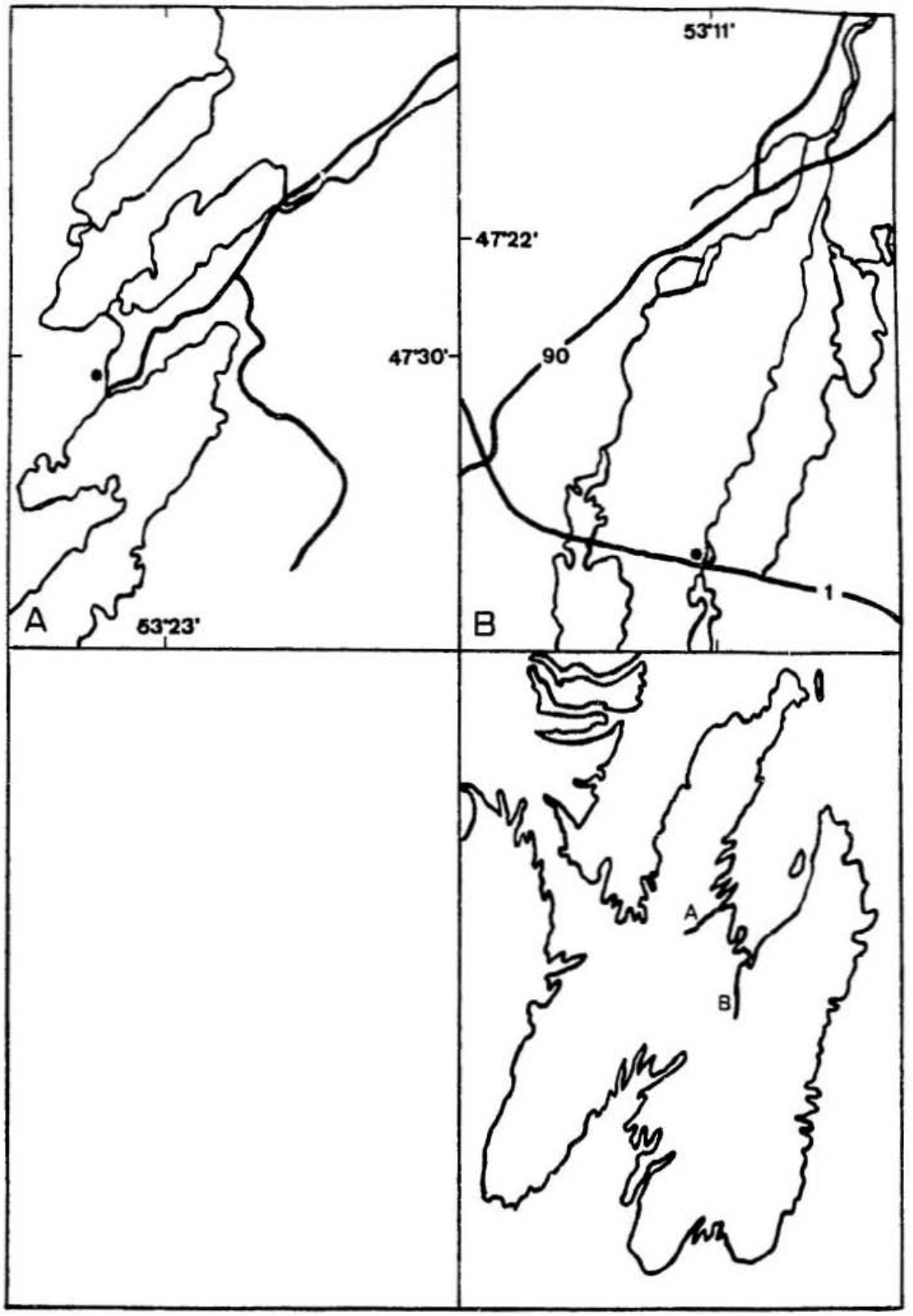
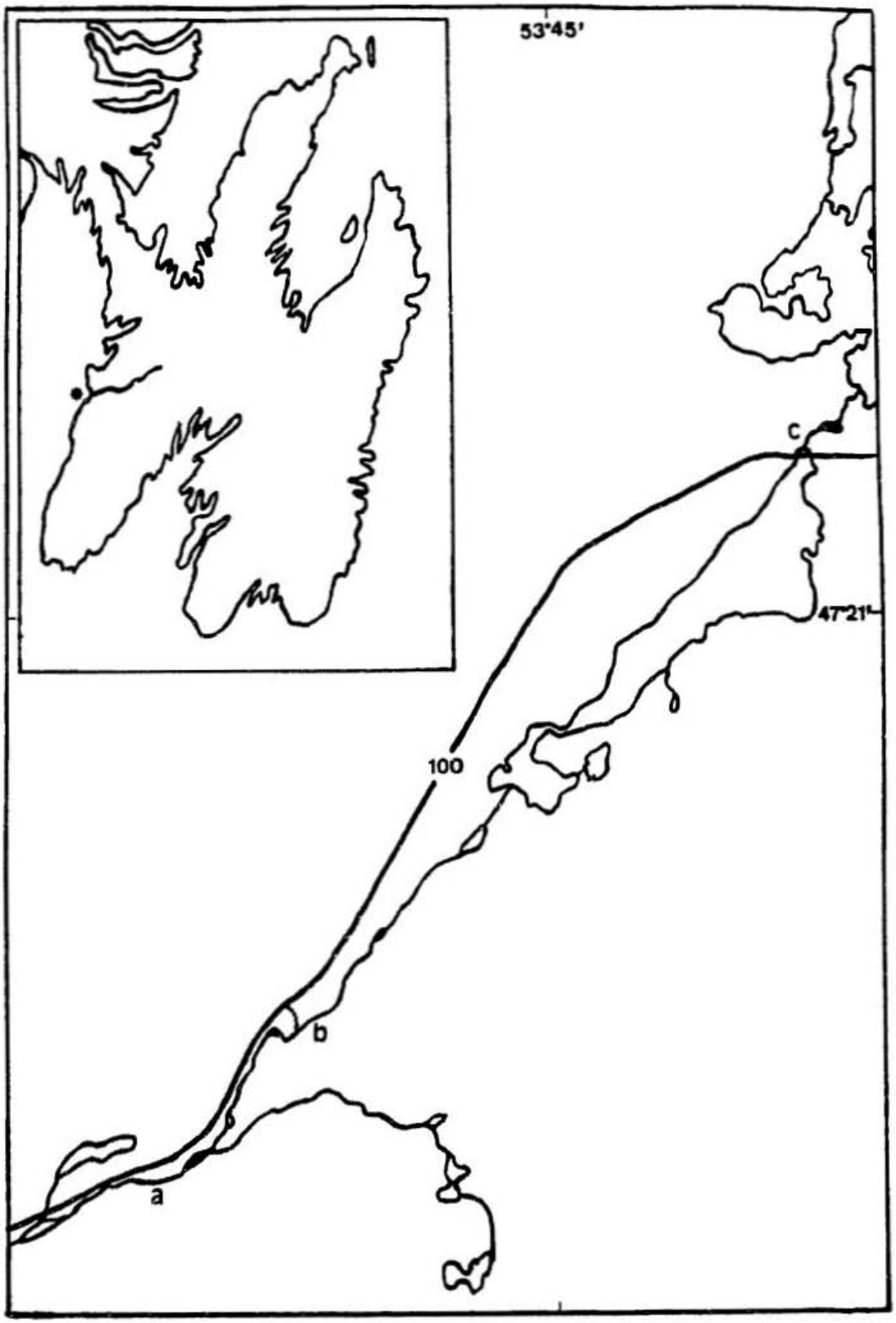

53°11'
47°22'
47°30'
90
1
A
53°23'
B
A
B

Figure A.4.

Location of sample sites on North East Placentia river (sites a, b and c)

(scale = 1:50000)


53°45'
47°21'
100
a
b
c