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Population differentiation of inconnu, *Stenodus leucichthys*, in the Mackenzie River system

by

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Abstract

In northwestern Canada, populations of inconnu (*Stenodus leucichthys)* are anadromous in the lower Mackenzie River region, migrating extensively between coastal feeding areas and upstream spawning sites, whereas populations in the Great Slave Lake area are year-round residents of freshwater.

To test predictions that relate life history characteristics of fish populations to habitat use and migratory behavior, I compared representative populations of freshwater and anadromous inconnu. Life history patterns did not fully conform to model predictions; in particular, growth and fecundity were higher in the freshwater vs. anadromous inconnu. Although some of the differences between populations can be related to differences in migratory distance, departures from expected patterns also appear to be associated with "atypical" characteristics of the freshwater feeding habitats, e.g., relatively high productivity and a longer growing season.

Phenotypic differences I observed between populations can result from differences in genotype, differences in the environment, or some combination of the two. I incubated eggs of freshwater and anadromous inconnu and reared the offspring in a common environment to determine the importance of these two influences on several biologically relevant traits. Rates of egg development and salinity tolerance differed among life history types, whereas juvenile growth rates did not, suggesting that the former two are under a significant degree of genetic control but the latter may be more strongly influenced in the wild by environmental differences.

Molecular genetic relationships (mitochondrial DNA) indicate that inconnu in the Mackenzie River basin originated from the Yukon River system. Geographic isolation and past glacial history appear to have been dominant forces shaping the current patterns of genetic variation of inconnu, with life history pattern having had little

influence. Freshwater populations likely arose through multiple, independent divergences from andromous populations following post-glacial dispersal and establishment in different areas.

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1. General Introduction

The diversity of organisms and the wide range of variation that can exist within species continues to fascinate biologists. With the rapid loss of habitat, increased exploitation of harvested species, and consequent loss of biodiversity that is now occurring, understanding of variation at the community and the species levels becomes increasingly important (Primack 1993). If we wish to maintain adaptive phenotypic and genetic variability within species, then we need to have a clear idea of the processes that contribute to this variability.

In this thesis I focus on the processes that lead to differentiation at the intraspecific level. I view these as occurring at two different scales: (a) large-scale processes (e.g., changes in geography, climate, anthropogenic influences) that can lead to initial colonization or separation of individuals from a single panmictic population, and (b) smaller- scale processes that can lead to the subsequent development and maintenance of phenotypic and genetic differences between separated populations (e.g., mutation, genetic drift, natural selection, local environmental differences, phenotypic plasticity).

Fish, particularly salmonids, provide an interesting and useful system to look at such questions. Fish, in general, show a high degree of diversity in both behavior and morphology, relative to other vertebrate taxa, such as birds and mammals (Moyle and Cech 1988), and salmonids as a group show considerable intraspecific variability, with the occurrence of multiple life-history forms or ecotypes being a common phenomenon. Intra-population variation in salmonids can range from obvious morphological differences, such as dwarf and normal forms of lake whitefish, *Coregonus clupeaformis*, and least cisco, *Coregonus sardinella*, that co-exist within lakes (Bernatchez and Dodson 1990; J. D. Reist, pers. comm.), to more subtle differences in habitat use, migratory behavior, and life history traits. Some examples here include the existence of benthic and pelagic forms of lake cisco, *Coregonus artedii*, and the occurrence of both anadromous and landlocked/freshwater forms of arctic charr, *Salvelinus alpinus*, brown trout, *Salmo trutta*, and Atlantic salmon, *Salmo salar* (e.g., Behnke 1972, 1980; McCart 1980; Jonsson 1985). This variability is, in part, believed to have resulted from a genome duplication event that occurred in the common ancestor of modern salmonids and may explain the unparalleled success of this group in utilizing both marine and freshwater environments, as well as their ability to survive in small demes where inbreeding might normally be a problem (Allendorf and Thorgaard 1984).

Most work on intraspecific variation of salmonids has focused on Pacific salmon (*Oncorhynchus* spp.), which are an important resource along the west coast of Canada and the United States (Randall *et al.* 1987; Foote *et al.* 1989; and others). Other species, such as Atlantic salmon, the charrs (*Salvelinus* spp.), and lake whitefish (see above references), have also been studied to a lesser extent. I have chosen to direct my research towards the study of a northern piscivorous whitefish (Coregoninae), the inconnu, *Stenodus leucichthys* (Güldenstadt). This species shows many parallels with its southern counterparts, but also some key differences. Inconnu live in an environment with colder temperatures and hence a shorter growing season. They migrate only into estuarine and coastal areas rather than undertake the true oceanic migrations characteristic of Pacific and Atlantic salmon species, and they exhibit iteroparous instead of semelparous spawning behavior.

My initial research (Howland 1997) compared various aspects of habitat use and migratory patterns of different inconnu populations in the Mackenzie River system to investigate the proposed existence of two different migratory types (McPhail and Lindsey 1970; Scott and Crossman 1973). Inconnu in the lower Mackenzie River north of Norman Wells were thought to feed and overwinter in coastal areas but spawn in freshwater, resulting in lengthy spawning migrations, whereas those in the Great Slave Lake area were assumed to be year-round residents of freshwater, undergoing much shorter spawning migrations within lake/tributary systems (Fig. 2-1). Evidence from gill netting, radio-tracking, and a synthesis of historical data showed that feeding/overwintering habitats and the timing and distance of spawning migrations for inconnu from these two areas differ substantially, while an analysis of otolith microchemistry confirmed the existence of both anadromous and freshwater types, providing direct support for this hypothesis (Howland 1997).

With the knowledge that inconnu in the Mackenzie River system differ both in migratory type and habitat use, I combined the theories and techniques of different disciplines, including life history theory, physiology and population/molecular genetics, to examine how relationships among large- and small-scale processes can lead to differentiation at the intra-specific level and how such differences are manifested in populations.

Chapter 2 documents the demographic characteristics of inconnu from the Mackenzie River system and examines the variation and potential trade-offs in fitness-related traits, such as size at age, age at maturity, size at maturity, mortality, and fecundity, that have evolved in inconnu populations with differing migratory behavior and habitat use. Life history differences between inconnu populations are examined in relation to what has typically been found in other species with anadromous and freshwater life history types in an attempt to better understand the processes that can generate this variation.

In chapters 3 and 4, a common garden experimental approach is used to determine the relative contributions of genetic versus environmental factors to variation in the life histories of inconnu. Quantitatively inherited traits, such as egg development rate, juvenile growth, and salinity tolerance, are compared in wild inconnu of freshwater and anadromous origin that were hatched and reared in a common environment.

In Chapter 5, an analysis of genetic relationships among existing inconnu populations is used to examine factors that may have been important in the initial dispersal, colonization, and establishment of populations of inconnu in the western Arctic and thus gain insights about how freshwater and anadromous forms initially evolved in this region.

This thesis extends our understanding of inconnu and will provide necessary biological information for its management in the Mackenzie River system. Inconnu are an important resource to residents of the north and the results of this research will aid in the development of sustainable management strategies for this species. The inconnu is biologically unique, as it is the only piscivore to have evolved within a family of invertebrate feeders and is exceptionally fast-growing and highly migratory relative to other northern whitefish, making it an interesting species in terms of its life history. Existing life history studies on migratory fish have mainly compared truly anadromous forms (migrate between the ocean and freshwater) to landlocked forms. The inconnu is not truly either; those in the lower Mackenzie migrate only to coastal waters whereas Great Slave Lake inconnu have no physical barriers preventing their migration to the coast (and do migrate between lake and river). Thus, this research also provides a test of the robustness of existing life history theory, which has dealt mainly with true anadromy versus freshwater residency. Furthermore, life history analyses of fish rarely go beyond looking at phenotypic differences. By combining field studies with the common garden experimental approach and the biochemical techniques of population genetics, this study provides an original contribution to our understanding of how anadromous fish populations evolve.

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2. Is there a universal relationship between migratory behavior and other aspects of life history? A comparison of anadromous and potamodromous populations of a northern coregonid, the inconnu (*Stenodus leucicthys*), in the Mackenzie River system

Introduction

A large proportion of the commercially important fish species in north-temperate latitudes are anadromous, i.e., migrate between freshwater spawning areas and marine feeding areas. Many of the same species also have freshwater populations that are resident (non-migratory) (see references below) or potamodromous (migratory) (Tallman et al. 2002). In fish species having both anadromous and freshwater forms, there are often significant differences in life history between the two forms, suggesting that migratory behavior has a strong influence on other life history characteristics. In particular, observed differences in life history traits are theorized to be necessary to offset the costs of long-distance migration (Roff 1988, 1991; Stearns 1992). Such variation in life history has been observed for the charrs, *Salvelinus* spp. (Hutchings and Morris 1985; Tallman et al. 1996), Atlantic salmon and trouts, *Salmo* spp. (Jonsson 1985), Pacific salmon, *Oncorynchus* spp. (Randall et al. 1987), and sticklebacks, *Gasterosteus* spp. (Snyder and Dingle 1989).

By migrating seaward, anadromous fish in colder regions are able to gain access to highly productive feeding areas such as oceans and estuaries (Gross et al. 1988); as a result, anadromous fish often have higher growth rates, and hence a larger size at a given age, than freshwater forms. A large body size is necessary for long distance migrants because larger fish are better able to tolerate the energetic demands of migration (Glebe and Leggett 1981). Body size can increase not only by growing faster, but by growing for a longer period of time; anadromous fish achieve this by delaying maturity, which results in larger body size at the time of the first spawning migration. Because fecundity usually increases with body size (Bagenal 1978; Roff 1984, 1992; Stearns 1992), mature anadromous fish can be expected to have higher age-specific fecundities than freshwater forms; anadromous forms of fish may also show higher size-specific fecundities relative to their freshwater counterparts (e.g., Wootton 1973; Tallman 2002). Higher size-specific fecundities presumably offset a longer juvenile period and increased adult mortality resulting from long migrations. Freshwater forms should not require as large a body size because their migratory costs are typically reduced (potamodromous) or eliminated (resident). Because freshwater forms generally feed in areas of lower productivity and have slower rates of growth, the relatively small size gains accrued through delayed maturity may not offset the fitness costs of a longer generation time and extended exposure to predation, thus maturing early, but at a smaller size, may be the best available strategy for increasing lifetime reproductive success (Stearns 1992).

Both freshwater (potamodromous) and anadromous forms are known to exist for inconnu, (*Stenodus leucichthys*) an arctic whitefish (coregonid) occurring in the Mackenzie River system of northwestern Canada. Based on otolith microchemistry analyses, inconnu in the lower Mackenzie River and tributaries, such as the Arctic Red, are anadromous whereas those in Great Slave Lake and its southern tributaries represent a potamodromous form (Howland et al. 2001; Fig. 2-1). Although there are no barriers to fish movement, inconnu tagged in the lower Mackenzie River and its delta have never been recaptured or detected upstream of Fort Simpson (Stein et al. 1973; Jessop and Lilley 1975; Howland et al. 2000). Likewise, those tagged in the Great Slave Lake area have never been recaptured in the Mackenzie River or delta (Day and Low 1993). Tag recaptures and detections (Stein et al. 1973; Jessop and Lilley 1975; Howland et al. 2000) indicate that anadromous inconnu migrate from 600 to 1500 km between feeding/overwintering areas (the Mackenzie estuary and along the coast of the Beaufort Sea) and spawning areas (including the upper Arctic Red and Peel Rivers). In contrast, potamodromous inconnu (thought to feed and overwinter primarily in the shallow waters off the south shore of Great Slave Lake) have been found to undergo much shorter spawning migrations of between 40 and 300 km into rivers such as the Slave and Buffalo, which drain into the south end of Great Slave Lake (Fuller 1955; Howland et al. 2000).

Although inconnu in the Mackenzie River are known to exhibit variation in migratory behavior, little is known about their fundamental demographic and life history traits. To fill this knowledge gap and test the predicted relationships between fitness-related traits and migratory behavior, I quantified and compared life history characteristics of two representative populations in the Mackenzie River system, one anadromous (Arctic Red River), and one potamodromous (Slave River). This comparison should also provide information about local (small-scale) processes that may promote differentiation among populations.

Study area

Inconnu are distributed from the lower reaches of tributaries draining into Great Slave Lake, including the Slave River, north to the lower Mackenzie River and associated tributaries, the Mackenzie Delta, and the Beaufort Sea (from Shingle Point to Tuktoyaktuk (see Fig. 2-1) (Scott and Crossman 1973).The Mackenzie River runs 1,650 km from its head at the west end of Great Slave Lake to the Beaufort Sea coast, receiving outflow from numerous tributaries, the largest of which are the Arctic Red, Peel, and Liard rivers (Fig. 2-1). Before entering the Beaufort Sea, the river forms the Mackenzie Delta, a 12,170 km^2 network of lakes and channels (Brunskill 1986). The Mackenzie Delta serves as a highly productive summer feeding area, juvenile rearing area and migratory corridor for anadromous coregonids (Reist and Bond 1988). Once the Mackenzie River enters the Beaufort Sea, its freshwater plume extends northeast along the Tuktoyaktuk Peninsula, west to Shingle Point, and encircles the numerous islands in the outer delta, creating a narrow zone of freshened water (Reist and Bond 1988).

Arctic Red River is one of several large tributaries that originate in the Western Cordillera. The river's upper reaches, some 600 km upstream of the Beaufort Sea, contain ideal spawning habitat; the water is clear and swift, and flows over coarse gravel substrate with numerous rapids (KLH, personal observation). As the river nears the Mackenzie it begins to widen, flows more slowly, and becomes turbid and silty.

Great Slave Lake, the fifth largest lake in North America, has three major basins. The large west basin, into which the Slave River flows, is turbid, relatively shallow (mean depth 41 m) and productive (Rawson 1950) and is utilized as a summer feeding area and overwintering area by inconnu. Like the Arctic Red River, the lower Slave River is large and turbid. Impassable rapids are located at Fort Smith, approximately 300 km upstream of where the Slave River enters Great Slave Lake; inconnu are believed to spawn at the base of these rapids (Fuller 1955; McLeod et al. 1985).

Methods

Field

Life history data were collected from inconnu captured by gillnets in the Arctic Red and Slave Rivers in 1992-1996 and 1993-1995, respectively (see Howland et al. 2000). Fish were either fully sampled fresh in the field, partially sampled (measured, weighed) and then frozen, or frozen directly and sampled later in the laboratory. I measured fork length (±1mm), total body mass (± 1g), and gonad mass (± 1g) of individual fish. Aging structures (scales, pectoral fin rays, and otoliths) were removed for later analysis, sex was determined from inspection of gonads and stage of maturity was qualitatively addressed (Howland 1997); ovaries of all mature females were preserved in 5% formalin for fecundity estimates. In cases where only frozen length and body mass data were available, the following relationships between fresh and frozen measurements were used to convert all samples to fresh length and body mass: Fresh fork length (mm) = 11.5 + 1.01 [Frozen fork length (mm)], r^2=0.98, $p<0.0001$; Fresh body mass (g) = 46.9 + 1.04 [Frozen body mass (g)], r^2=0.99, $p<0.0001$).

Laboratory

Aging and back-calculation - Inconnu were aged from otoliths using a modified version of the "break and burn" method (Chilton and Beamish 1982; Howland et al. 2004). The majority of fish captured in this study were spawners migrating to or from spawning sites; thus, few fish were under 6 years of age. To obtain information on younger ages, I back-calculated size at age from otoliths using the biological intercept method (Campana 1990). I digitally imaged a sub-sample of otoliths (n=5 samples per 100 mm length interval for each population), measuring the radius along the longest growth axis, from the nucleus to the distal edge, using WinRing v. 2.0 (Reynolds and Compton). I found a significant linear correlation between fork length and the otolith radius for both populations (Arctic Red River: $r^2 = 0.74$, $p < 0.0001$, Slave River: $r^2 = 0.73$, $p < 0.0001$). Digital images were then captured from a random sample of approximately 80 otoliths of each sex within each population. A sample comprising the best 50-60 otoliths for each sex-location combination were selected for back-calculation based on the following criteria: 1) clear banding pattern for at least first 4-7 years of growth; 2) center and first annulus clear; 3) relatively straight longitudinal axis which intersects the annuli at an approximate right angle. Distances between annual growth increments were measured from images. Measurements were taken at the outer edge of each clearly visible annulus perpendicular to the longitudinal growth axis. With these data I back-calculated body size from growth increments on otoliths as follows: $La = Lc + (Oa-Oc)(Lc-Lo)(Oc-Oo)^{-1}$, where La = back-calculated length at annulus a, Lc = length of fish at capture, Oa = size of otolith at annulus a, Oc = size of otolith at time of capture, Lo = length of fish at hatch, Oo = size of otolith at hatch (Campana 1990). The length of fish and size of otoliths at hatch were set at 12 mm and 0.15 mm, respectively, based on measurement of laboratory hatched inconnu (Howland et al. 2002).

Fecundity and Egg size - Preserved inconnu ovaries were prepared for estimation of fecundity and egg size following the methods of Healey and Nicol (1975). Because inconnu have extremely high fecundities (Nikol'skii 1954; Alt 1977), I estimated fecundity from sub-samples. For each female, three sub-samples (200 eggs each) and remaining eggs were air-dried to a constant mass and weighed (mg). Fecundity was then estimated as: (total dry ovary mass/average dry sub-sample mass)*(number of eggs in sub-sample) (Healey and Nicol 1975).

I compared egg size between populations using estimated dry mass and fresh egg diameter. Mean individual dry egg mass was determined from the sub-samples of 200 eggs. Egg diameter was determined by aligning a random sample of 10 eggs along a ruler and dividing the total diameter by 10 (Morin et al. 1982). An average egg diameter for each female was determined from 2-4 sets of these measurements. Diameters of fresh eggs were measured for a random sub-sample of females in each population. The diameters of eggs in this sub-sample, along with all remaining samples, were then measured in the lab following preservation and a regression equation, developed from 35 samples was used to convert these data to fresh diameters: Fresh Diameter (mm) = 0.16 + 0.85 [Preserved Diameter (mm)], $r^2=0.90$, $p<0.0001$.

Statistical Analyses

Preliminary plots of size and age indicated differences between sexes, therefore, analyses were done separately for males and females. For all analyses I initially separated data by year within each population and then pooled the data across years if no significant differences were found. The assumption of normality was assessed using normal probability plots and the Kolmogorov-Smirnov test, while homogeneity of sample variances was assessed using residual plots and Levene's Median Test (Brown and Forsythe 1974). In cases where these assumptions were not met, data were either log-transformed or non-parametric tests were used. The goodness of fit and coefficients of determination (r^2) for all regressions were assessed using the method of least squares. Comparisons of regression functions among years and between populations were made using appropriate dummy variable regression models (Neter et al.1990). Differences were considered significant at a probability level of 0.05.

Length, Age, Age at Maturity - Since all but one comparison of length and age among years (length of anadromous males 1992 versus 1994, $p>0.05$) were non-significant (Kruskall-Wallis tests, Kolmogorov-Smirnov two-sample tests), data were pooled and comparisons of means between populations were made using the Mann-Whitney U Test, while the Kolmogorov-Smirnov two-sample test was used to compare length- and age-frequency distributions between populations.

In my study, fish were collected within the rivers en route to or from spawning areas; thus, samples consisted mainly of mature spawning fish. I was therefore unable to determine age-at-maturity using traditional methods (e.g., Morin et al. 1982; Luksenburg and Pedersen 2002; Meyer et al. 2003). Instead, I compared size and age at first maturity, as well as the mean and modal size and age of mature spawners, in each population.

Size at Age - The relationship between length (mm) and age (years) for each collection year within populations was examined using a quadratic model with untransformed data. Comparisons among collection years within each population were made using the following dummy variable regression models:

$$Yi = \beta_0 + \beta_1 xi_1 + \beta_2 xi_1^2 + \beta_3 Xi_2 + \beta_4 Xi_3 + \beta_5 Xi_4 + \beta_6 xi_1 Xi_2 + \beta_7 xi_1 Xi_3 + \beta_8 xi_1 Xi_4 + \beta_9 xi_1^2 Xi_2 + \\ + \beta_{10} xi_1^2 Xi_3 + \beta_{11} xi_1^2 Xi_4 + \varepsilon_i \text{ (Anadromous)}$$

$$Yi = \beta_0 + \beta_1 xi_1 + \beta_2 xi_1^2 + \beta_3 Xi_2 + \beta_4 Xi_3 + \beta_6 xi_1 Xi_2 + \beta_7 xi_1^2 Xi_2 + \beta_8 xi_1 Xi_3 + \beta_9 xi_1^2 Xi_3 + \varepsilon_i \\ \text{(Potamodromous)}$$

Where Yi is the expected length of fish i, X_{i1} is the age of fish i, xi_1 = the deviation of Xi from the arithmetic mean of Xi, $Xi_2 = 1$ if 2nd year of data or 0 otherwise, $Xi_3 = 1$ if 3rd year of data or 0 otherwise, $Xi_4 = 1$ if 4th year of data or 0 otherwise, β_0 is the intercept of the regression, β_1 is the linear effect of age on Yi for the 1st year of data, β_2 is the quadratic effect of age on Yi for the 1st year of data, $\beta_3, \beta_4, \beta_5$ indicate, respectively, the differences in the height of the regression functions for the 2nd, 3rd and 4th years of data relative to the regression for the 1st year of data at any given level of age, $\beta_6, \beta_7, \beta_8$ indicate, respectively, interaction effects between the linear portion of the regression functions for the 2nd, 3rd and 4th years of data and the linear portion of the regression function for the 1st year of data at any given level of age, $\beta_9, \beta_{10}, \beta_{11}$ indicate, respectively, interaction effects between the quadratic portion of the regression functions for the 2nd, 3rd and 4th years of data and the quadratic portion of the regression function for the 1st year of data at any given level of age, ε_i = unknown error in Yi. In all quadratic regressions I minimized the effects of multicollinearity by expressing the independent variable as a deviation from the mean (Neter et al.1990).

All comparisons of length-at-age among collection years were non-significant; therefore, data were pooled across years and combined with back-calculated length-at-age data within each population for further analyses. The combined data were fit using quadratic models with $\log_{10}$ (age + 1) and a y-intercept of 12 mm (size of inconnu at age 0). The quadratic functions were compared between populations using the following dummy variable regression model:

$$Yi = \beta_0 + \beta_1 \log_{10}(Xi_1+1) + \beta_2 \log_{10}(xi_1+1)^2 + \beta_3 \log_{10}(Xi_1+1)Xi_2 + \beta_4 \log_{10}(xi_1+1)^2 Xi_2 + \varepsilon_i$$

Where Yi is the expected length of fish i, X_{i1} is the age of fish i, xi_1 = the deviation of Xi from the arithmetic mean of Xi. $Xi_2 = 1$ if anadromous or 0 otherwise, β_0 = 12 mm, β_1 is the linear effect of age on Yi for the anadromous population, β_2 is the quadratic effect of age on Yi for the anadromous population, β_3 indicates the interaction effect between the linear portion of the regression functions of the anadromous and potamodromous populations at any given level of age, β_4 indicates the interaction effect between the quadratic portion of the regression functions of the anadromous and potamodromous populations at any given level of age, ε_i = unknown error in Yi. The same model was also used to compare sexes within populations, where $Xi_2 = 1$ if males or 0 otherwise.

Mean fork length (back calculated and original data combined) at each age was compared between populations using two-tailed t-tests. Growth at each age was calculated for individual fish using back calculated fork length as follows: $G_i=(L_i - L_{i-1})$, where G_i is growth (mm) at age i and L_i is fork length (mm) at age i. Mean growth at each age was then compared between populations using two-tailed t-tests.

Condition (mass-length relationships) - Since initial plots of body mass on length showed a high degree of overlap for different collection years within each population, I pooled data across years for further analyses. Data were separated by stage of maturity (as determined from examination of the gonads) for inconnu from the anadromous population since immature, mature and spent/resting individuals were expected to differ with respect to their body mass-length relationships. Only mature (pre-spawning) individuals were used in comparisons between anadromous and potamodromous populations, since this was the only stage of maturity for which I had data from both populations. The following dummy variable regression model was used to compare regression functions between populations for $\log_{10}$ mass (g) and $\log_{10}$ fork length (mm):

$$Yi = \beta_0 + \beta_1 Xi_1 + \beta_2 Xi_2 + \beta_3 Xi_1 Xi_2 + \varepsilon_i$$

Where Yi is the expected $\log_{10}$ mass of fish i, X_{i1} is the fork length of fish i, $Xi_2 = 1$ if anadromous or 0 otherwise, β_0 is the intercept of the regression, β_1 is the effect of fork length on Yi for the anadromous population β_2 indicates the difference in the height of the regression function for the potamodromous population relative to the regression for the anadromous population at any given level of fork length, β_3 indicates the interaction effect between the regression function for potamodromous population and the regression function for anadromous population at any given level of fork length, ε_i = unknown error in Yi.

Mortality - Since previous comparisons showed no significant effect of collection year on age frequency, catch curves (natural log of age-class frequency against age) were plotted using pooled data for each population and a least squares regression was fitted to the descending limb of each curve. This regression included the catches with the greatest abundance plus one year, to the oldest age class with n>1. Instantaneous mortality rate (Z), annual survival rate (S) and annual mortality rate (A) were calculated as follows: Z = positive slope of the regression, $S = e^{-Z}$, A = 1-S (Ricker 1975).

Fecundity - The relationships between fecundity and age, somatic mass, and fork length for each year of data within populations was initially examined using simple linear models with log transformed fecundity data. The regression functions were compared among collection years for each independent variable using dummy variable regression models.

All comparisons of age-, somatic mass- and fork length-specific fecundity among years by population were non-significant; therefore, data were pooled across years for further analyses. The following dummy variable regression model was then used to compare regression functions between populations for $\log_{10}$fecundity with each independent variable:

$$Yi = \beta_0 + \beta_1 Xi_1 + \beta_2 Xi_2 + \beta_3 Xi_1\, Xi_2 + \varepsilon_i$$

Where Yi is the expected $\log_{10}$fecundity of fish i, X_{i1} is the fork length, age, or somatic mass of fish i, $Xi_2 = 1$ if anadromous or 0 otherwise, β_0 is the intercept of the regression, β_1 is the effect of fork length, age or somatic mass on Yi for the anadromous population β_2 indicates the difference in the height of the regression function for the potamodromous population relative to the regression for the anadromous population at any given level of fork length, β_3 indicates the interaction effect between the regression function for potamodromous population and the regression function for anadromous population at any given level of fork length, ε_i = unknown error in Yi.

A stepwise (forward and backward) procedure was performed with $\log_{10}$fecundity and age, somatic mass and fork length for each population. When all independent variables were considered simultaneously for a given population, $\log_{10}$fecundity was significantly associated with fork length (Arctic Red River, $p<0.0001$; Slave River, $p<0.0001$) but not age (Arctic Red River, $p=0.259$; Slave River $p=0.687$) or somatic mass (Arctic Red River, $p=0.057$; Slave River, $p=0.318$).

Egg Size - Mean sizes (fresh diameter (mm) and (µg)) of eggs from females classified as running ripe and within 2 weeks of the expected time of spawning (Arctic Red River, September 21-26; Slave River, October 2-14) were compared using t-tests.

Results

Age structure and age at maturity - All potamodromous inconnu captured in this study were mature; however, 7.9 % of anadromous inconnu were classified as immature. For consistency, only mature individuals were used in comparisons of length and age between population comparisons reported below.

The ages of inconnu from the Arctic Red (anadromous) and Slave (potamodromous) rivers differed markedly (Fig. 2-2). Inconnu from the anadromous population were older (mean ± 1 SD: females, 16.6 ± 3.3 years; males, 13.4 ± 3.0 years), and had a wider age distribution than those from the potamodromous population (females, 8.5 ± 1.7 years; males, 6.9 ± 1.0 years; Mann-Whitney U Tests, $p<0.001$; Kolmogorov-Smirnov Two Sample Tests, $p<0.0001$). Maximum ages of captured inconnu were 31 (females) and 30 (males) for the anadromous population and 24 (females) and 12 (males) for the potamodromous population.

The youngest mature inconnu in the anadromous population were aged 10 (females) and 6 (males), whereas those from the potamodromous population first matured at ages 7 (females) and 5 (males) (Fig.2-2). The modal age of mature fish from the anadromous population (16 for females, 14 for males) was twice that of the potamodromous population (8 for females, 7 for males) (Fig. 2-2).

Length structure and length at maturity - Despite the substantial differences in age, lengths of mature anadromous and potamodromous inconnu were generally more similar (females: 839 ± 67 mm and 828 ± 50 mm; males: 716 ± 48 mm and 724 ± 39 mm; Mann-Whitney U Test: females $p=0.08$, males $p=0.002$; Kolmogorov-Smirnov Two Sample Test, females $p=0.16$, males $p=0.04$) (Fig. 2-3). Anadromous inconnu reached maximum lengths (mm) of 1122 (females) and 879 (males). Similarly fish from the potamodromous population reached maximum lengths (mm) of 1100 (females) and 885 (males).

Minimum length at maturity (anadromous, 577 mm; potamodromous, 565 mm) was similar for male inconnu from both populations but was substantially greater in anadromous (685 mm) as compared to potamodromous females (545 mm) (Fig. 2-3). Modal lengths at maturity were similar in both populations (males 700-799 mm, females 800-899 mm) (Fig. 2-3).

Size at Age and Growth – The relationship between fork length and age differed substantially between anadromous and potamodromous populations as indicated by the polynomial regressions fit to combined back-calculated and original length-at-age data (dummy variable regression: males, $p<0.0001$; females $p<0.0001$; Fig. 2-4). The higher linear coefficients of the regression equations for the potamodromous population (males, $\beta_1 = 793$; females, $\beta_1 = 855$) suggest that at younger ages they grow more rapidly than the anadromous population (males, $\beta_1=622$; females, $\beta_1=678$). In contrast, the higher negative quadratic coefficients (males, $\beta_2 = -0.77$; females, $\beta_2 = -1.5$) suggest that later in life, growth of potamodromous inconnu slows down more rapidly than that of the anadromous type (males, $\beta_2 = -0.17$; females, $\beta_2 = -0.22$). In the age groups where sufficient sample sizes were available for direct comparisons (ages 10 or less), mean length (Fig. 2-5) and growth (Fig. 2-6) at a given age were generally greater in the potamodromous population.

Condition (mass-length) - Within the anadromous population, mature females had a greater body mass per unit length than spent females, while juveniles were intermediate. There was little difference between spent and mature males. Among mature individuals, potamodromous inconnu had a greater body mass per unit length than anadromous inconnu for both sexes (dummy variable regression: females, $p<0.0001$, males $p<0.0001$; Fig. 2-7).

Mortality - Potamodromous inconnu (especially the females) had exceptionally high mortality rates relative to those from the anadromous population (Fig. 2-8).

Fecundity - Fecundity was correlated with fork length (anadromous $r^2=0.76$, $p<0.001$; potamodromous $r^2=0.47$, $p<0.001$, Fig. 2-9a), age (anadromous $r^2=0.41$, $p<0.001$; potamodromous $r^2=0.13$, $p<0.001$, Fig. 2-9b) and somatic mass (anadromous $r^2=0.72$, $p<0.001$; potamodromous $r^2=0.41$, $p<0.001$; Fig. 2-9c). Mass-specific fecundity did not differ ($p=0.26$) between populations, but length- and age-specific fecundities were higher ($p=0.02$) in the potamodromous population. However, when all three variables were considered simultaneously within a given population, fork length was the only significant predictor of fecundity. The relationship between fecundity and fork length for each population was: anadromous, $\log_{10}\text{fecundity} = 1.807 \times 10^{-3}$ length (mm) + 3.463; freshwater, $\log_{10}\text{fecundity} = 1.482 \times 10^{-3}$ length (mm) + 3.842. The predicted fecundities at a common length (835 mm) for each population were: anadromous = 93,723 eggs/female; potamodromous = 120,080 eggs/female.

Egg Size - Egg diameter and mass both increased linearly from early summer to fall just prior to spawning (egg diameter: anadromous r^2=0.91, p<0.001; potamodromous r^2=0.72, p<0.001; egg mass: anadromous r^2=0.89, p<0.001; potamodromous r^2=0.70, p<0.001) ranging from 1.35-2.51 mm and 0.72-4.34 mg (anadromous population) and 1.6-2.46 mm and 1.37-4.27 mg (potamodromous population). Egg size was highly variable among individuals at any given time, suggesting that maturation rate varies among females within each population (Fig. 2-10). There was no difference in mean egg size of running ripe females from anadromous (dry egg mass, 3.74 ± 0.35 mg; fresh egg diameter, 2.38 ± 0.05 mm) and potamodromous populations (dry egg mass: 3.67 ± 0.35 mg, t-test: p=0.548; fresh egg diameter, 2.32 ± 0.09 mm, t-test: p=0.052).

Discussion

The comparison of life history traits between inconnu from the anadromous Arctic Red and potamodromous Slave River populations showed patterns that only partially conform to those typically observed in other species with andromous and freshwater life history types. Previous studies(e.g., Hutchings and Morris 1985; Jonsson 1985; Randall et al. 1987; Snyder and Dingle 1989; Tallman et al. 1996) have supported the prediction that anadromous species or populations grow faster and mature later to reach larger body sizes than their freshwater counterparts (Roff 1991). Larger individuals should, in theory, be better able to endure the energetic demands of long distance migrations (Glebe and Leggett 1981; Roff 1988). In colder regions migrating seaward is considered beneficial by providing access to highly productive feeding areas, resulting in increased growth rate and body size of anadromous relative to freshwater forms (Gross 1988).

In my study, age at maturity was substantially higher in the anadromous population of inconnu, similar to predictions and previous studies; however, this delay in maturity did not appear to confer any size or growth advantage over the potamodromous population. Size at maturity and the average size of mature individuals were similar for both populations of inconnu, while growth was greater in the potamodromous population. I suggest that delayed maturity in the anadromous form of inconnu is simply a necessity for reaching a critical size for migration or sexual development. Given that both forms of inconnu in this study mature at approximately the same average size, it is reasonable to conclude that some physiological minimum size is required to sexually mature, migrate and successfully reproduce. Because of this, growth rate and age at maturity are negatively correlated, with faster growing individuals within a species generally maturing earlier than slower growing fish (e.g., Thorpe 1987; Hutchings 1993; Gross 1996; McCarthy et al. 2003). Since potamodromous inconnu from the Slave River grow at a faster rate they would be expected to reach this physiological size limit and be capable of maturing at an earlier age than anadromous inconnu from Arctic Red River which is also consistent with our results on age at maturity.

I offer two hypotheses why the potamodromous population exhibited a faster rate of growth, and the associated larger size at age and earlier age at maturity than the anadromous population, despite the presumed benefit of migration, i.e., the higher productivity of cold water marine environments relative to freshwater habitats. The first reason for differences in growth between populations may be independent of migratory behaviour but rather, simply related to compensatory growth under different rates of exploitation. Numerous studies have shown that growth tends to increase, while age at maturity tends to decrease, over time with exploitation (e.g., Healey1975; Rijnsdorp 1993; Rochet 1998). The two populations in my study are subject to different forms of exploitation, although the intensity of each fishery has not been quantified. Catch curves, however, suggest that total mortality is higher in the potamodromous population than in the anadromous population. To determine whether differences in total mortality reflect differences in fishing mortality or life history (i.e., natural mortality), I compared the age and length distributions as well as size-at-age of inconnu from the present study to pre- and post-exploitation data from inconnu captured in the Buffalo River, a Great Slave Lake population that was subjected to high levels of exploitation in the late 1970s which resulted in marked recruitment failure and declines in relative abundance (Day and Low 1993; Appendix 1). The results of this comparison suggest that although exploitation may be having a moderate effect on life history traits of the potamodromous population of inconnu from the Slave River, it is not the main factor driving the observed life history differences between potamodromous and anadromous populations in this region (Appendix 1). Furthermore, previous studies have shown that catch-per-unit-effort during the period of my study was greater in the potamodromous population (Howland et al. 2000). This result suggests that abundance was higher in the potamodromous population and that exploitation rates would not likely have caused increased growth or decreased age-at-maturity relative to the anadromous population.

A second explanation for differences in growth between populations is related to the habitats occupied by the two populations. The non-sympatric potamodromous population of inconnu in this study inhabits a relatively productive freshwater system located at lower latitude than that of the anadromous population. This contrasts with the more typical studies of sympatric freshwater-resident and anadromous populations. Growth generally slows while age at maturity increases with latitude due to lower temperatures and a shorter growing season (Roff 1992; Stearns 1992; Hutchings 1994). The potamodromous population of inconnu is located ca. 1000 km further south and, based on the open water period, has a longer growing season (ca. 1.5 mo) than the anadromous population (Howland 2002). This climatic difference may have a particularly strong influence on the juvenile growth stage, which is highly temperature and food dependent (Diana 1995). Freshwater invertebrates, the main dietary item of juvenile inconnu (Fuller 1955; Alt 1965) are generally more abundant in warmer waters and during the open water period (Cole 1994). Indeed, the observed size-at-age relationships in my study indicate that it was only during the juvenile phase that the potamodromous population exhibited greater rates of growth than the anadromous population. Following maturation, growth rates are slightly higher in the anadromous population, perhaps reflecting differences in reproductive investment between the life history forms. That such climatic differences would translate into differences in growth is supported by the results of previous lab experiments (Howland 2002) which did not reveal any selection for increased growth to compensate for the shorter growing season in the higher latitude anadromous population (eg., Conover and Schultz 1995; Brown et al. 1998).

Although the two forms of inconnu had a similar size at maturity, the potomodromus form had a higher size-specific fecundity. This may be because the potamodromous form invests less energy into migration, due to a shorter distance, and can therefore divert more somatic reserves towards reproduction. Fecundity can vary directly with available energetic reserves; for example, supplemental feeding increases fecundity in salmonids such as lake trout (Martin and Olver 1980) while starvation typically results in decreased fecundity (Behnke 1992; Bromage et al. 1992).

Differences in fecundity between forms may also be related to the constraints of body shape. Inconnu from the potamodromous population generally had a larger body mass per unit length, which suggests a larger girth, relative to the anadromous form (Fig.

9c and 12a). Since a strong link between fecundity and body size has been well demonstrated for a variety of fish species (e.g., Reznik 1983; Snyder and Dingle 1989; Poizat et al. 2002), females from the potamodromous population likely can carry a greater number of eggs per unit body length relative to anadromous inconnu. The lack of difference in somatic-mass-specific fecundities between populations supports this hypothesis. There does not appear to be any trade off between egg size and fecundity as egg sizes at time of spawning were similar between populations.

Despite differences in size-specific fecundity, the two life history strategies may be similar in terms of lifetime reproductive success. The strong link between longevity and age at maturity has been well demonstrated (e.g., Beverton 1963; Murphy 1968; Hutchings 1994), with early maturity generally expected to result in a shorter lifespan, especially in cases where there is a large reproductive investment (e.g., Williams 1966; Stearns 1976; Hutchings 1994). I see good evidence for this in the present study. The potamodromous population has a much shorter lifespan, and a higher mortality, than the anadromous population, so although they produce a greater number of eggs per spawning event, they likely spawn fewer times per lifetime than the anadromous form. Based on age at maturity, age structure and mortality rates, most individuals in the potamodromous population may spawn only once or possibly twice in a lifetime, depending on the length of time between spawning events. Evidence suggests that some may spawn in consecutive years (Howland et al. 2000) while others spawn in alternate years (Fuller 1955; Day and Low 1993). In contrast, anadromous inconnu appear to spread reproductive effort out over a much longer time frame and may spawn 3-5 times in their lifetime depending on time interval between spawning events.

In conclusion, the inconnu populations examined here may not have fully conformed to expectations of existing theory because they differ in key respects from previously studied systems. In particular, both potamodromous and anadromous inconnu migrate to spawning areas, albeit for different distances, whereas most examples in the literature involve a non-migratory resident population that exists sympatrically with an anadromous population (e.g., Jonsson 1985; Snyder and Dingle 1988; Klemetsen et al. 2003). The potamodromous inconnu population examined in this study also inhabits a relatively productive feeding area (the West basin of Great Slave Lake; Brunskill 1986), unlike resident forms of previously studied species, which often occur in small, relatively unproductive lakes or rivers. The productive food supply combined with a longer growing season due to their more southerly location may allow these potamodromous inconnu to grow at higher rates than the freshwater populations in other species. Thus, although some differences in life history characteristics between anadromous and freshwater populations can be linked to differences in migratory behavior, characteristics of the feeding areas (and, to a minor extent exploitation) can also have an influence that accentuates or otherwise alters those differences.

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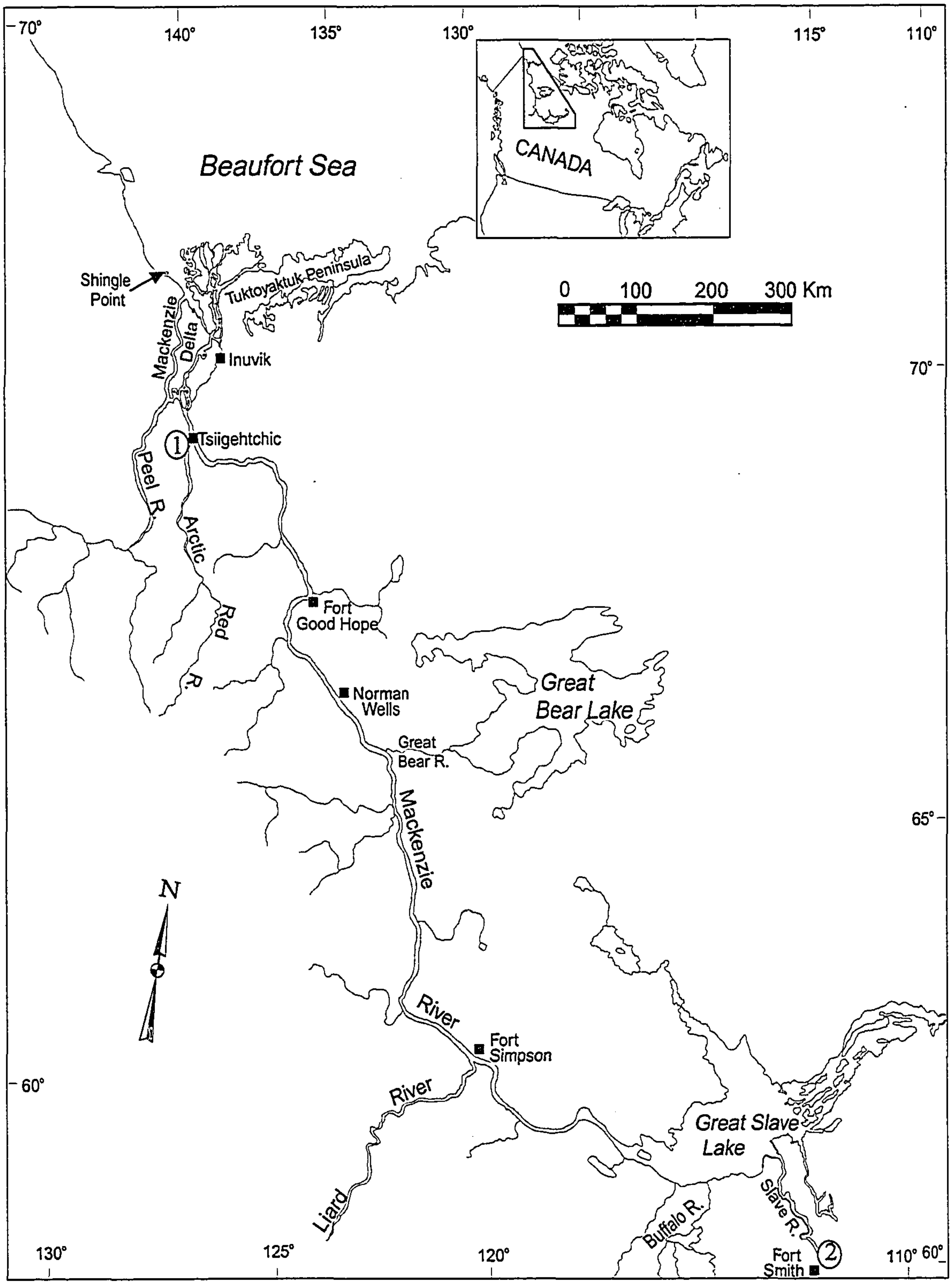

Figure 2-1. Study area showing the Arctic Red and Slave Rivers

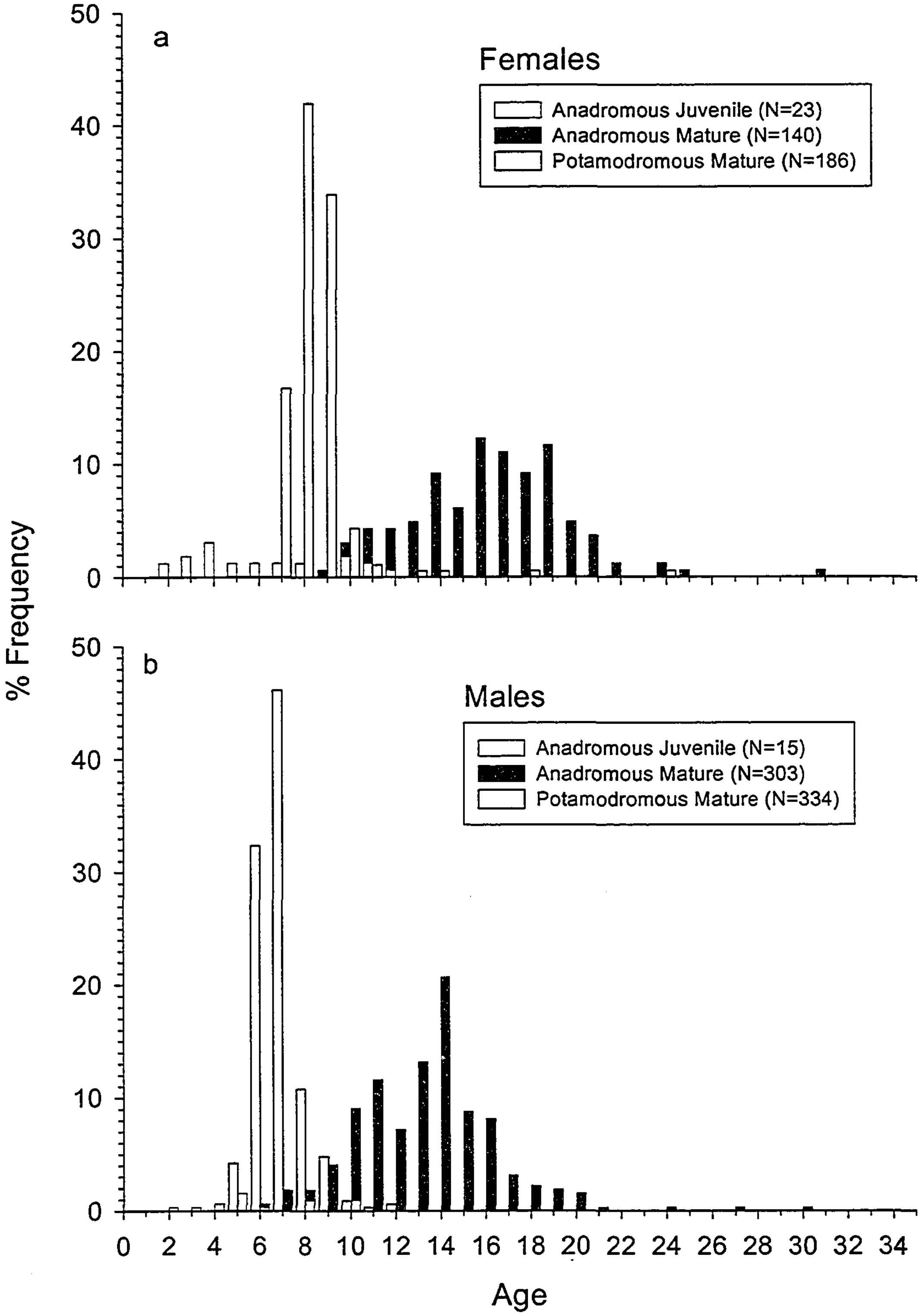

Figure 2-2. Age frequency distributions by life history type [anadromous mature (■) and juvenile(▨), potamodromous (□)] for a) male and b) female inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994.

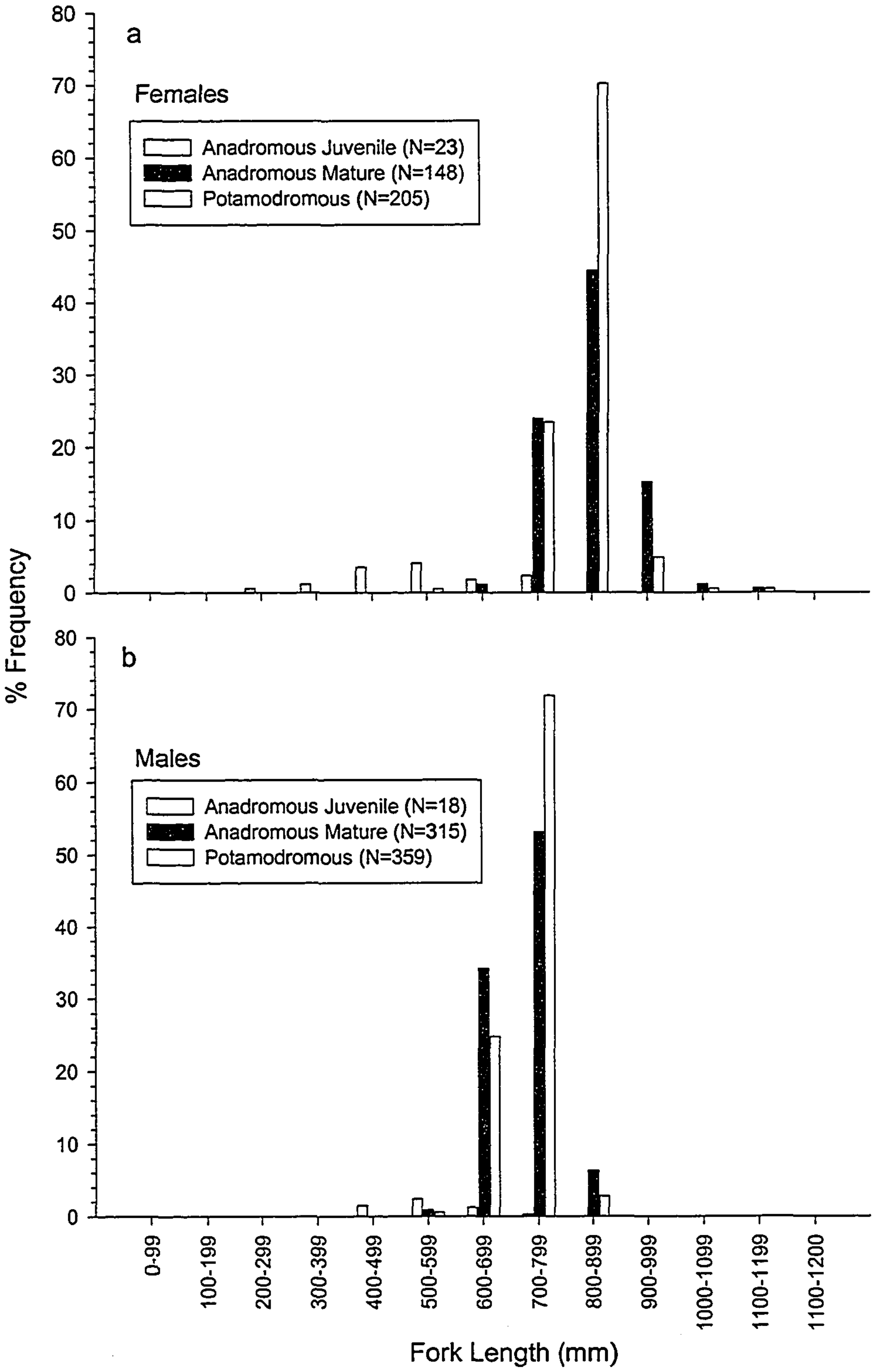

Figure 2-3. Length frequency distributions by life history type [anadromous mature (■) and juvenile(▨), potamodromous (□)] of a) female and b) male inconnu captured from the Arctic Red and Slave Rivers between 1992 and 1994.

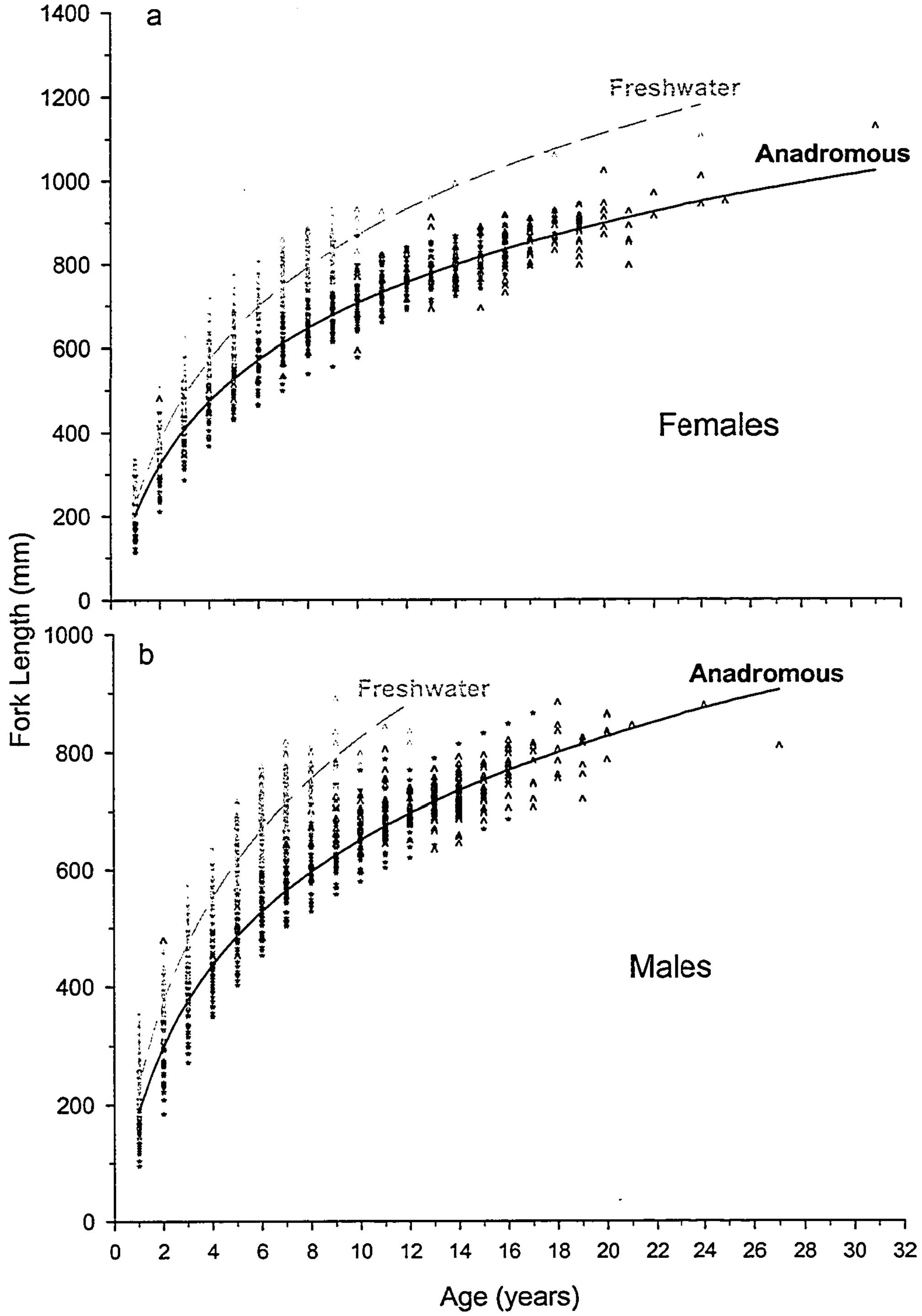

Figure 2-4. Regressions of fork length on age for a) female and b) male anadromous and potamodromous inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994 based on original (^) and back-calculated (*) length at age data.

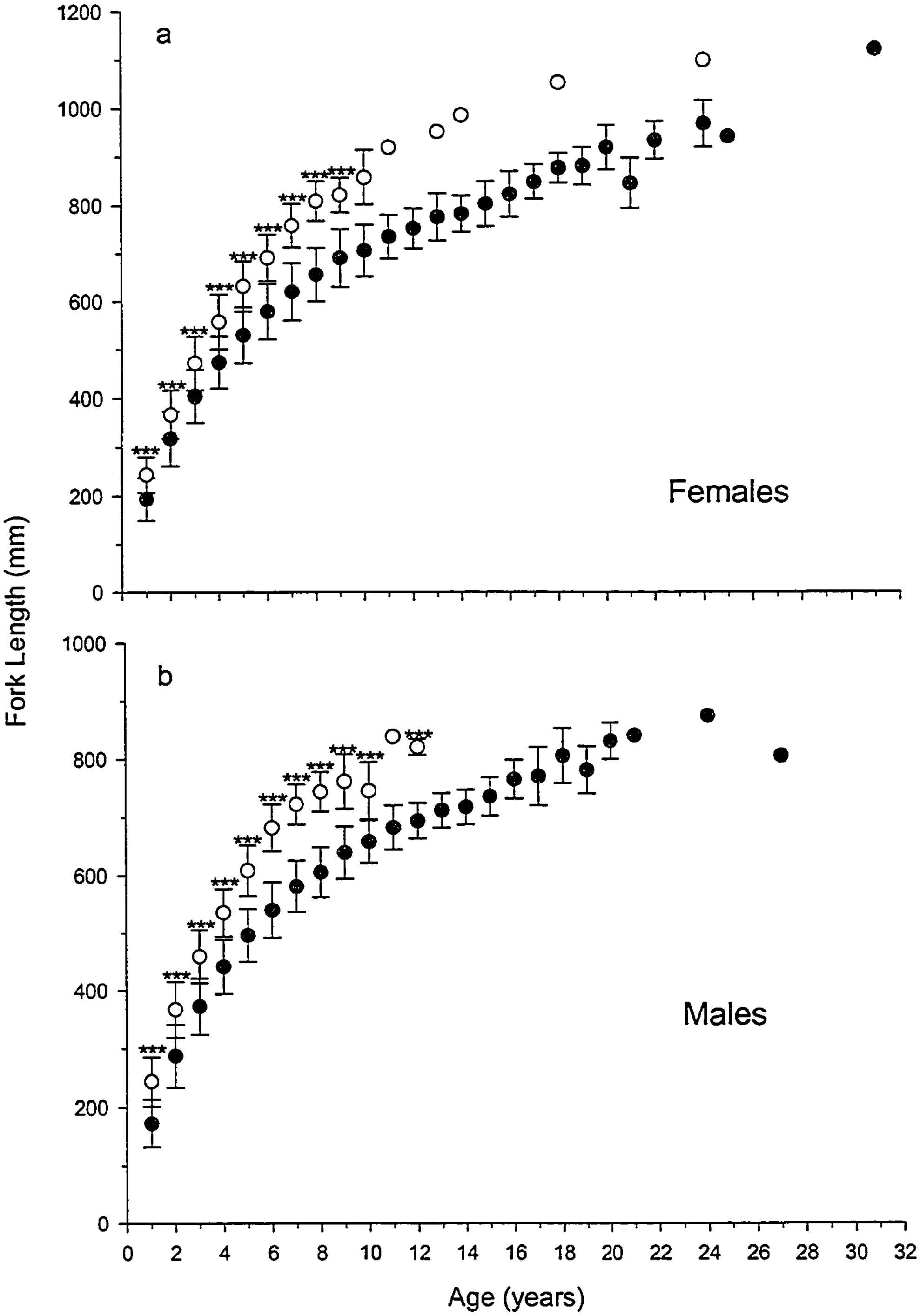

Figure 2-5. Mean ± SD fork length at age by life history type [anadromous (●) and potamodromous (O)] for a) female and b) male inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994. Values are based on original and back-calculated length at age data; *** *p*<0.001; ** *p*<0.01; * *p*<0.05, significant difference between life history types (two tailed t-test).

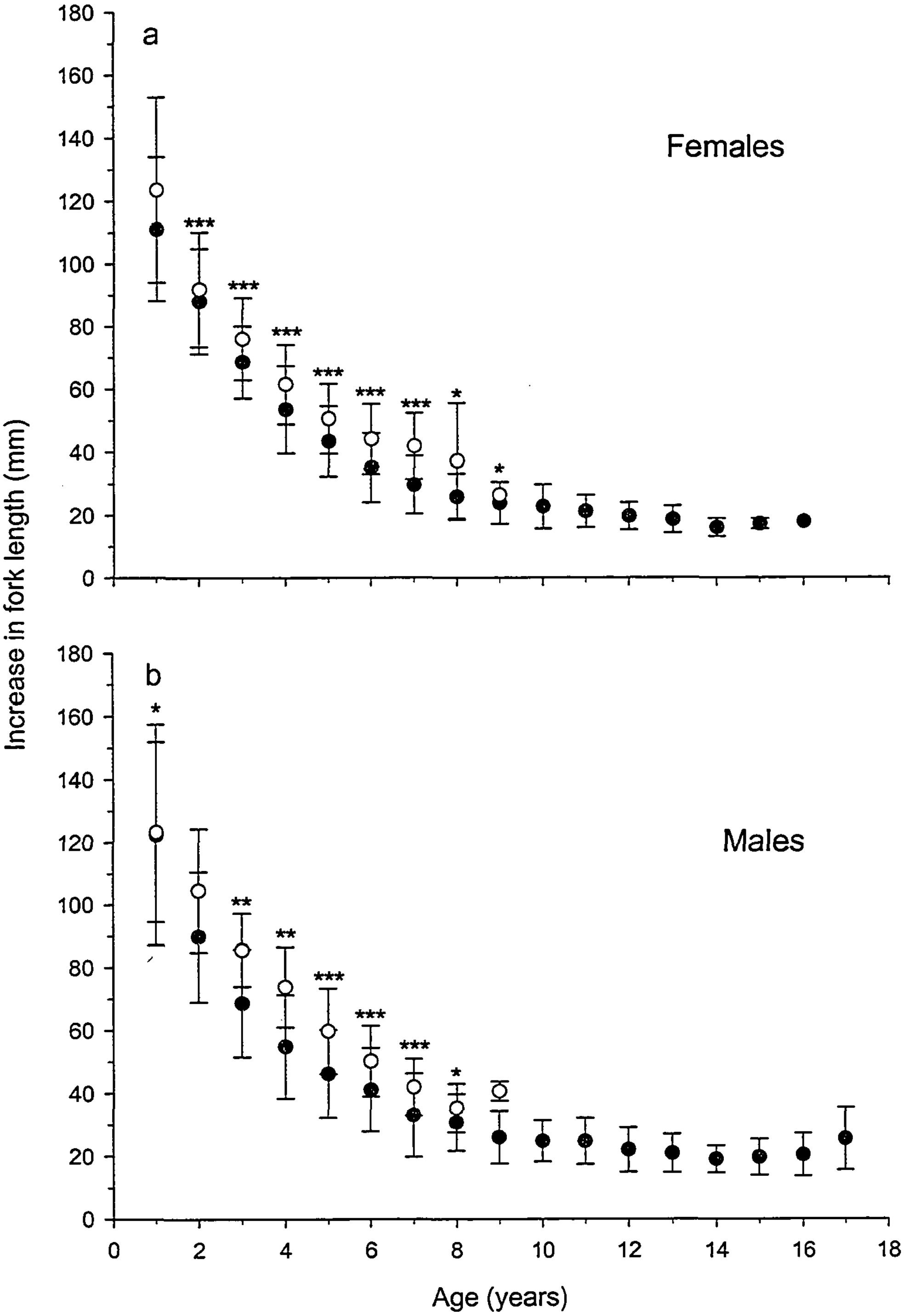

Figure 2-6. Mean ± SD growth at age by life history type [anadromous (●) and potamodromous (O)] for a) female and b) male inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994. Values are based on original and back-calculated length at age data; *** $p<0.001$; ** $p<0.01$; * $p<0.05$, significant difference between life history types (two tailed t-test).

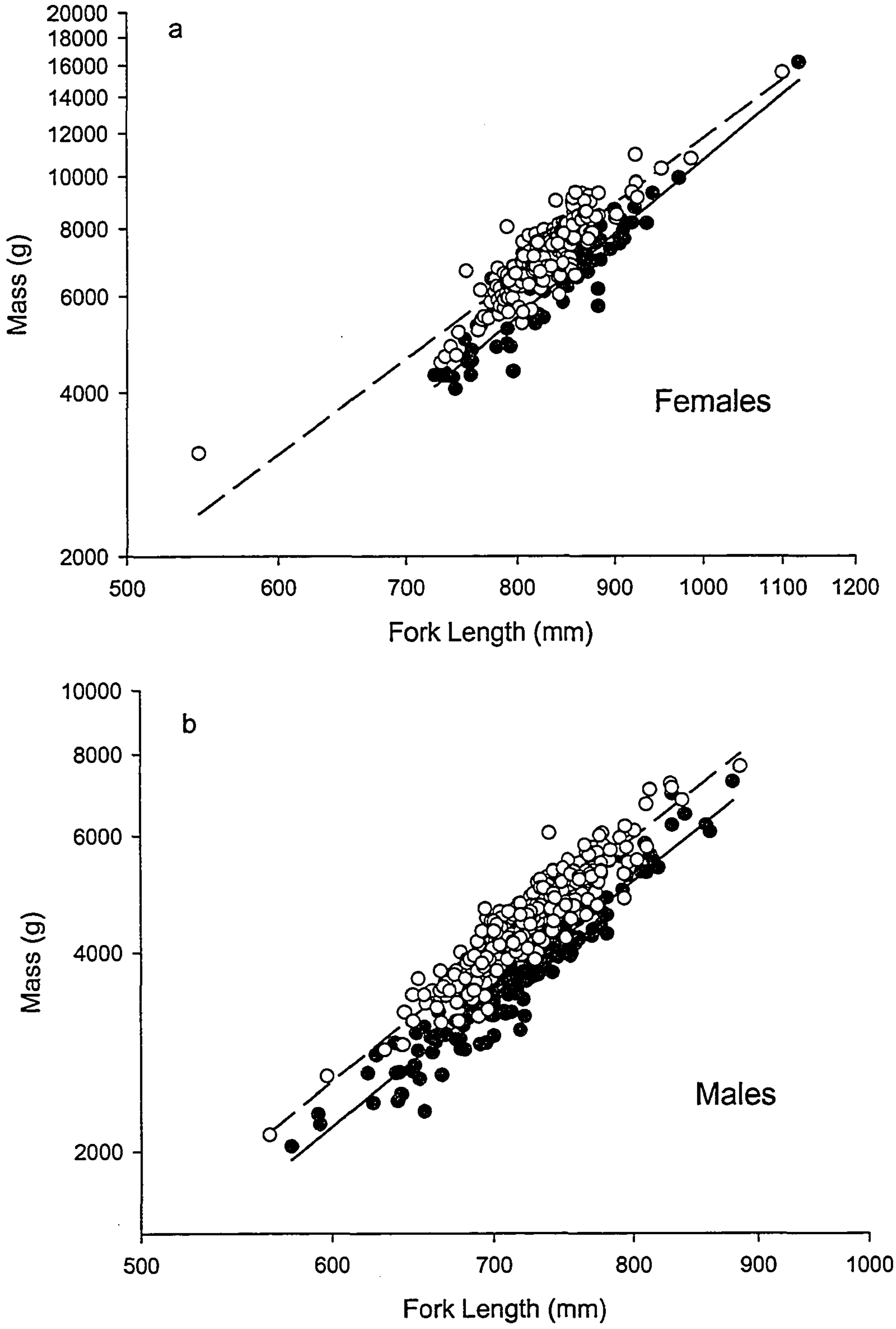

Figure 2-7. Regressions of $\log_{10}$ mass on $\log_{10}$ fork length by life history type [anadromous (●), potamodromous(O)] for mature a) female and b) male inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994.

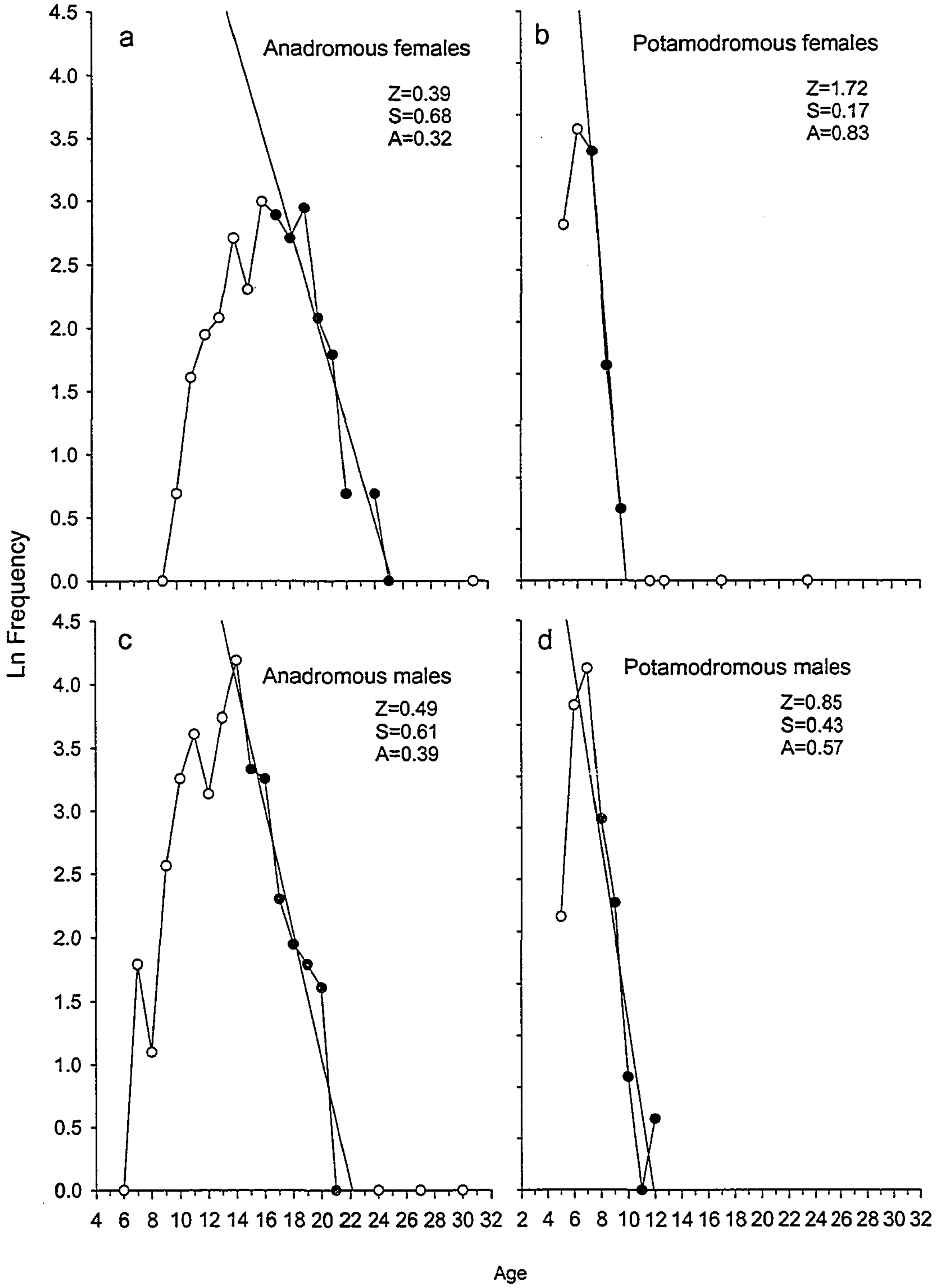

Figure 2-8. Catch curves by sex and life history type for inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994: a) anadromous females; b) potamodromous females; c) anadromous males; d) potamodromous males. Regressions include the age class with the greatest frequency plus one year, to the oldest age class with n>1, Z = positive slope of the regression, $S = e^{-Z}$, A = 1-S.

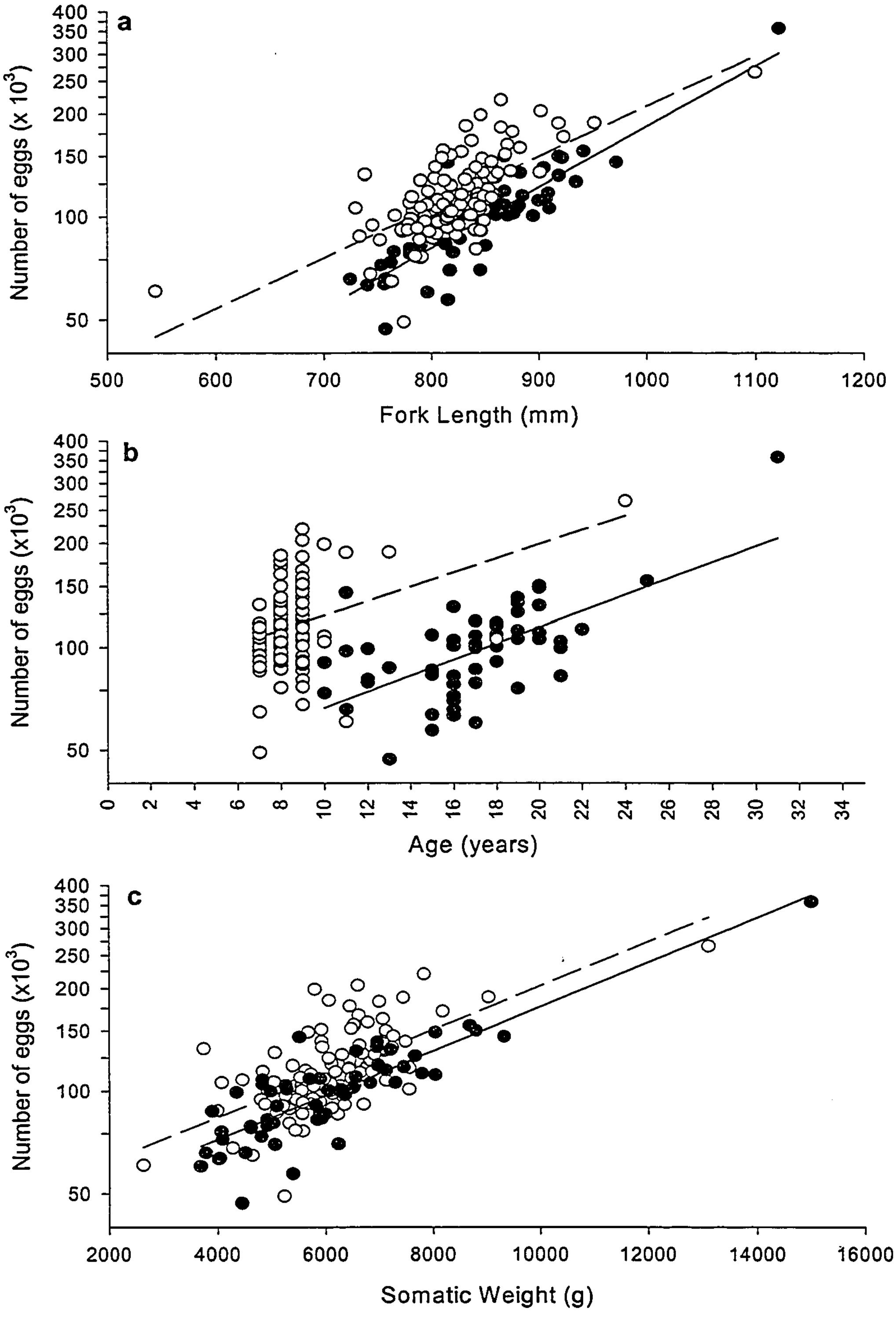

Figure 2-9. Regressions of fecundity ($\log_{10}$ transformed number of eggs) on a) fork length, b) age and c) somatic weight by life history type [anadromous (●) and freshwater(○)]for inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994.

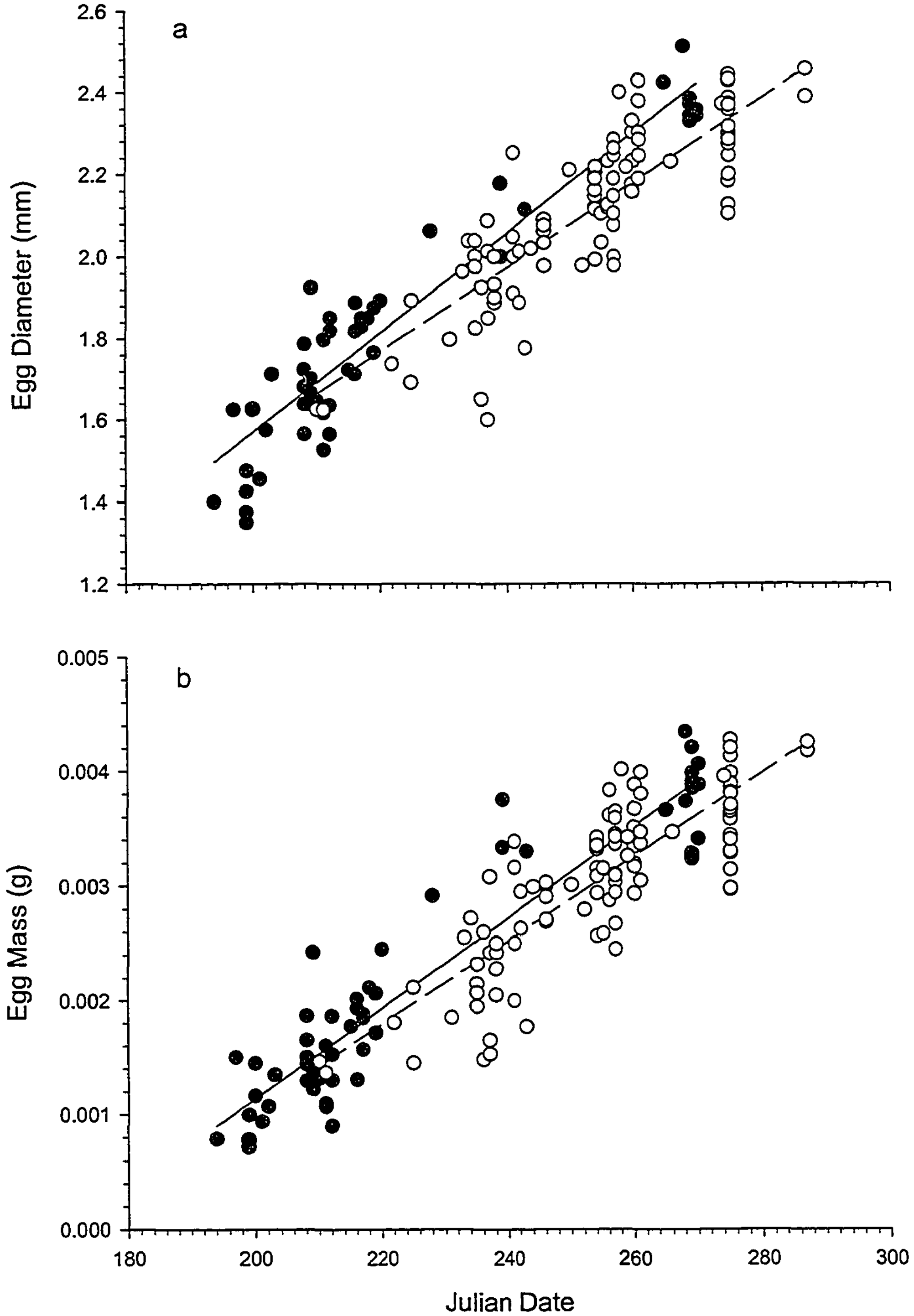

Figure 2-10. Regressions of a) egg diameter and b) egg mass on julian date by life history type [anadromous (●) and freshwater(O)] for inconnu captured in the Arctic Red and Slave Rivers between 1992 and 1994.

Appendix 1: Influence of harvest on the life histories of the study populations

Introduction

Differences in exploitation rates among life history types can be a confounding factor in this comparative study, since exploitation could cause a shift in life history trajectories independent of natural processes (Ricker 1975; Haddon 2001). Because I am examining the potential role that differences in migratory behavior and the environments occupied by the two focal populations play in the life history patterns I observed, I need to assess the impact of harvesting on my findings. The two populations in this study are subjected to different types of fisheries. Anadromous inconnu from the Arctic Red River are harvested by subsistence fisheries both during immature and resting phases of their life cycle and during spawning migrations (Howland et al. 2000). Potamodromous inconnu from the Slave River together with inconnu from other tributaries are harvested as by-catch by the commercial lake whitefish fishery on Great Slave Lake primarily during the mature phase of their life cycle (Day and Low 1993). This fishery has been operating since 1945 (Day and Low 1993). Subsistence fishing for inconnu in the Slave River during upstream spawning migrations is now minimal due to the advent of the ski-doo and resultant decline in the use of this species for dog food (G. Low, DFO Hay River, Personal Communication).

Because the harvests of each of these fisheries are not directly known, I examined their effects on the observed life histories by comparing the age and length frequency distributions of inconnu captured from the Arctic Red and Slave Rivers between 1992 and 1996 (my study) to distributions of inconnu captured in the Buffalo River, a population of inconnu in Great Slave Lake known to have been subjected to particularly high levels of exploitation (Day and Low 1993). The Buffalo River population experienced high levels of targeted commercial fishing due to excessive harvest in the late 1970s, such that a closure was placed on the fishery in 1983. Successively more restrictive closures (encompassing a larger geographic area) were put in place in 1993 and 1995 to afford further protection to this population (G. Low, DFO Hay River, unpublished). This comparison addressed the following questions: 1) Does the Slave River inconnu population show size and age patterns that suggest high levels of exploitation (i.e., that are similar to Buffalo River post-exploitation) and 2) Are observed life history differences between potamodramous and anadromous inconnu maintained regardless of exploitation level.

Methods

Histograms of historical age and length data, as well as size at age were compiled from inconnu captured at the mouth of the Buffalo River during 1947-48 when the population was largely unexploited or lightly exploited (hereafter referred to as unexploited) and 1983-84, when the population had been heavily exploited in the late 1970s (after Day and Low 1993).

Since sexes were not determined for the Buffalo River inconnu collected in the 1940s, I combined data from males and females for inconnu collected in later years as well as for those collected in the Arctic Red and Slave Rivers, for comparison. I note, however, that for the years when information was available, the ratios of males to females (mean ± standard deviation) were similar among locations (Arctic Red River 1992-1996, 1.93 ± 0.7; Slave River 1993-1995, 1.77 ± 0.57; Buffalo River 1976-1992, 1.82 ± 0.97).

Results and discussion

Length frequency – Both the Slave and Arctic Red River populations had length frequency distributions that were similar to the Buffalo River during its unexploited phase, with a modal size range of 700-799 mm. In contrast, the length structure of the Buffalo River population following heavy exploitation was shifted towards smaller fish, with a modal size range of 500-599 mm (Fig. 2-11). These results indicate that levels of exploitation in the Slave and Arctic Red rivers are lower than in the present-day Buffalo River population, whose shifted and slightly truncated size distribution indicates growth over fishing.

Age frequency – The age structure of the inconnu from the Arctic Red River population differed substantially from those of the Slave River as well as the Buffalo River (both in the unexploited and the heavily exploited phases of the fishery). Both the modal and the maximum ages were greatest in the Arctic Red River population (modal age: Arctic Red River = 14 y, Slave River = 7 y Buffalo River unexploited = 9 y; Buffalo River heavily exploited = 5 y; maximum age: Arctic Red River = 35 y, Slave River = 24 y

Buffalo River unexploited = 24 y; Buffalo River heavily exploited = 10 y; Fig. 2-12). These results suggest light to moderate levels of exploitation may be occurring in the Slave River population.

Size at age – As with age structure, the size at age of anadromous inconnu from the Arctic Red River was clearly lower than potamodromous inconnu in the Slave and Buffalo Rivers (both in the unexploited and the heavily exploited phases of the fishery) (Fig. 2-13). The size at age of Slave River inconnu was slightly greater than for the unexploited Buffalo River sample, suggesting that light to moderate levels of exploitation may be stimulating growth of fish in this population. Size at age of the heavily exploited Buffalo River sample was not greater than the unexploited sample despite changes the change in age structure that occurred with increased fishing pressure (Fig. 2-12). This again suggests growth over fishing in the latter Buffalo River population.

The above results show that the age structure and size at age of the Slave River population differ somewhat from the unexploited Buffalo River sample collected in 1947-1948. These differences are most likely the result of moderate levels of exploitation in the Slave River population relative to the previously unexploited state of the Buffalo River population. Thus, exploitation may partly explain the younger age structure and greater size at age of potamodromous inconnu from the Slave River relative to anadromous inconnu from the Arctic Red River. The above results also show, however, that with respect to age structure and size at age, differences between the anadromous population (Arctic Red River) and all potamodromous populations (unexploited and exploited) greatly exceeded any exploitation-related differences between potamodromous populations.

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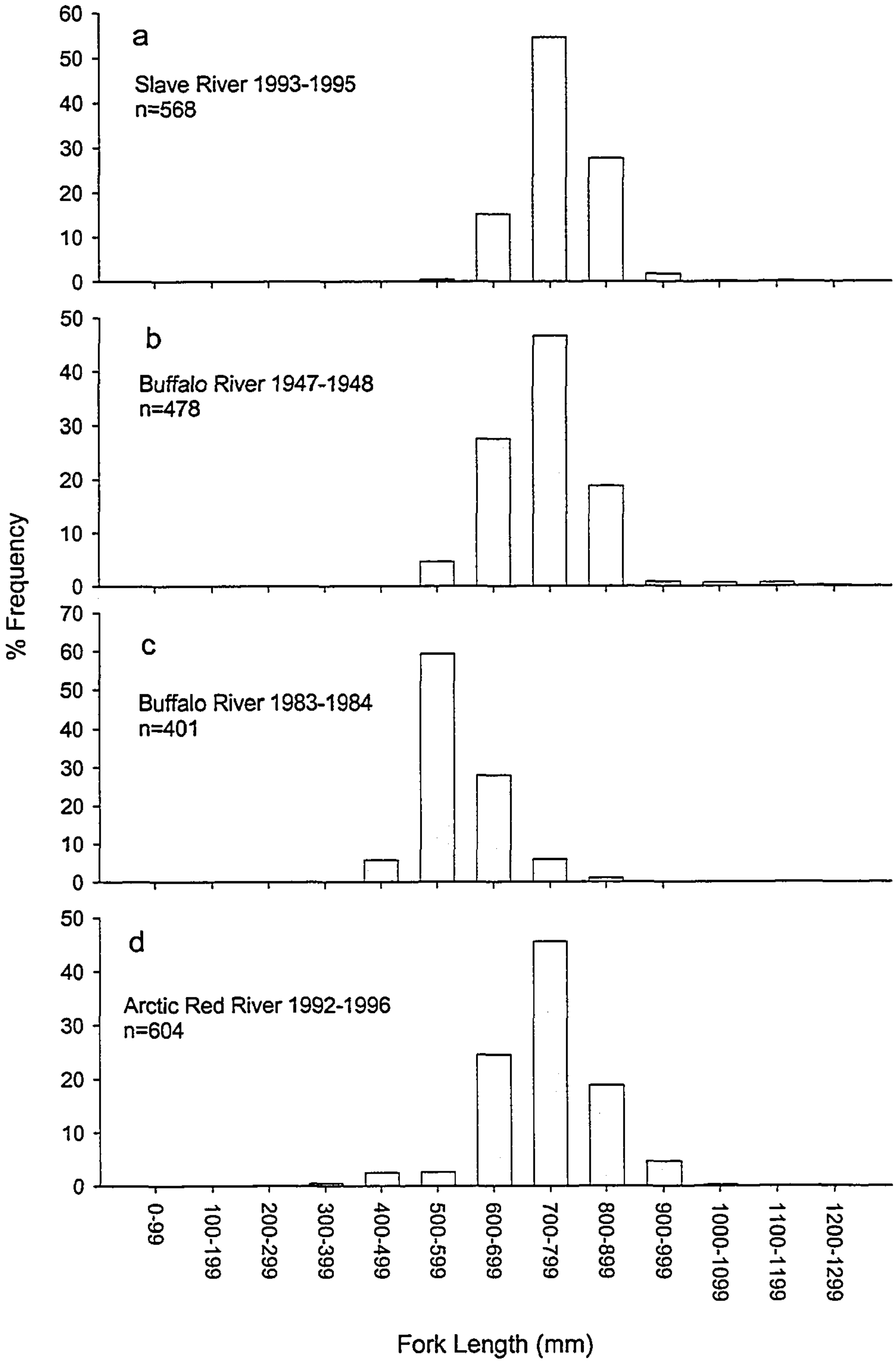

Figure 2-11. Length frequency distributions of inconnu sampled from a) Slave River 1993-1995, b) Buffalo River unexploited 1947-1948 (after Day and Low 1993), c) Buffalo River heavily exploited 1983-1984 (after Day and Low 1993) and d) Arctic Red River 1992-1996.

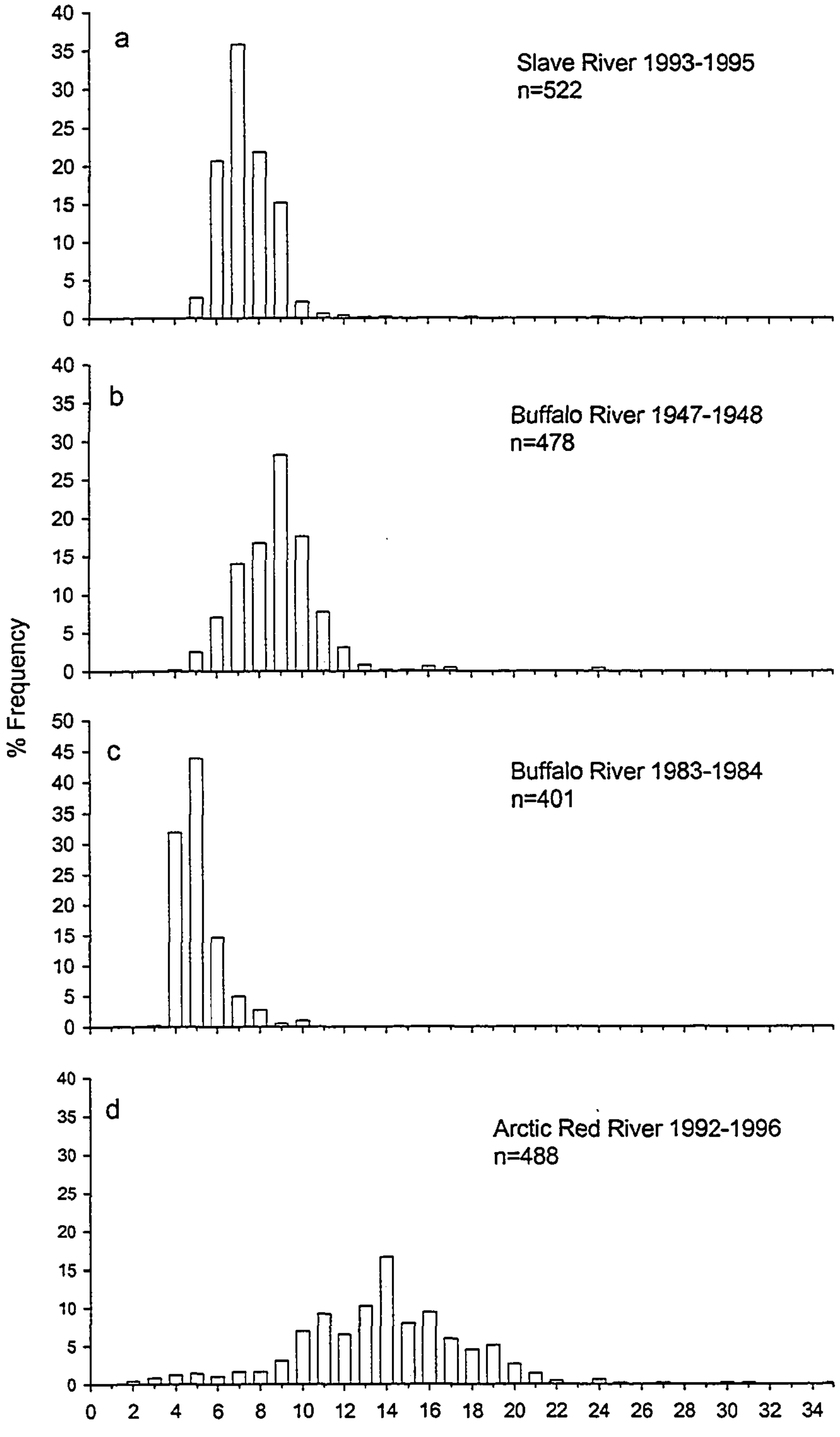

Figure 2-12. Age frequency distributions of inconnu sampled from a) Slave River 1993-1995, b) Buffalo River unexploited 1947-1948 (after Day and Low 1993), c) Buffalo River heavily exploited 1983-1984 (after Day and Low 1993) and d) Arctic Red River 1992-1996.

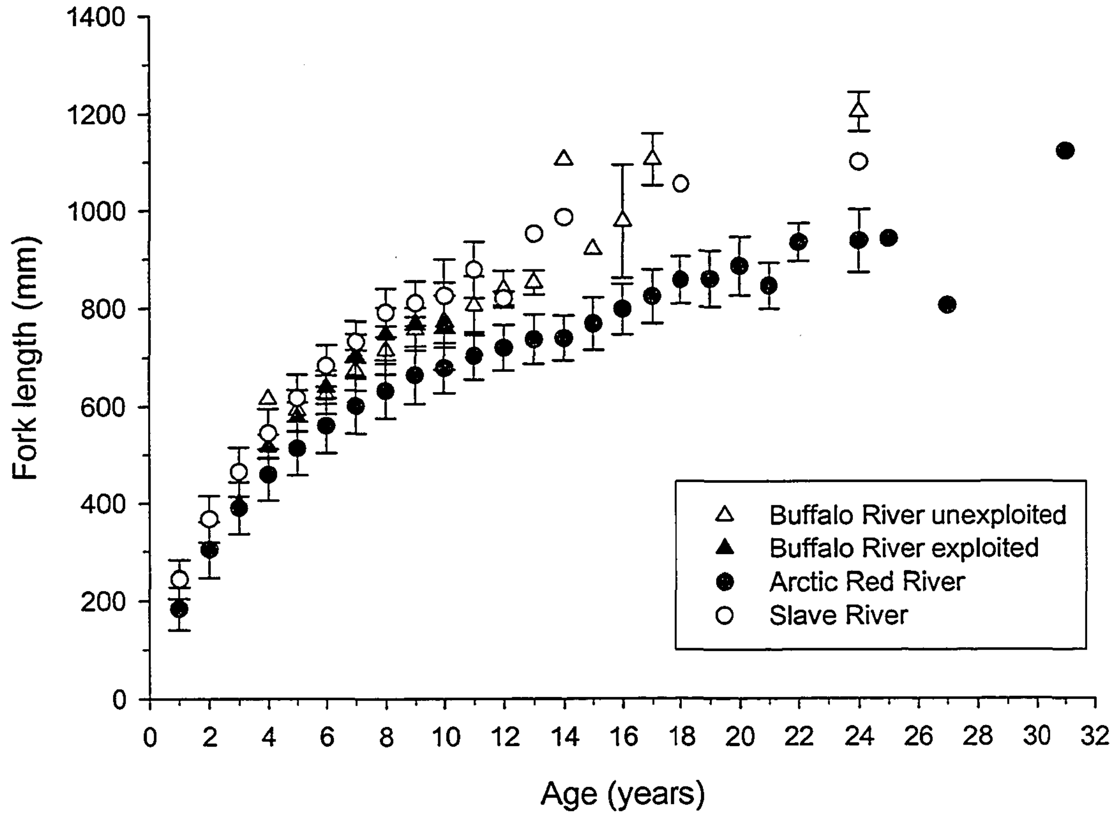

Figure 2-13. Mean ± SD size at age by population [Arctic Red River 1992-1996 (●), Slave River 1993-1995 (O), Buffalo River unexploited 1947-1948 (△) (after Day and Low 1993), Buffalo River heavily exploited 1983-1984 (▲)(after Day and Low 1993). Values for the Slave and Arctic Red rivers are based on original and back-calculated length at age data.

3. The influence of genetic and environmental factors on egg development and juvenile growth in two life history forms of inconnu (*Stenodus leucichthys*)[1]

Introduction

Salmonids, as a group, show considerable intraspecific variability, commonly occurring as multiple life history forms or ecotypes. Such intraspecific variation can range from obvious morphological differences, such as dwarf and normal forms of lake whitefish, *Coregonus clupeaformis* (Fenderson 1964; Bernatchez and Dodson 1990), and arctic charr, *Salvelinus alpinus* (Hindar and Jonsson 1993), which co-exist within lakes, to more subtle differences in habitat use, migratory behavior, and life history traits. Examples here include the existence of benthic and pelagic forms of lake cisco, *Coregonus artedi* (e.g., Lindsey 1981) and, in several species, the occurrence of both anadromous and landlocked/freshwater forms (e.g., arctic charr, brown trout, *Salmo trutta*, and Atlantic salmon, *Salmo salar*) (Behnke 1980; McCart 1980; Jonsson 1985).

Whitefish, in particular, are known for their high phenotypic plasticity (Behnke 1972) and the inconnu, *Stenodus leucichthys*, is no exception. Although variation in migratory behavior, habitat use, and life history traits among inconnu populations has been noted or described (Berg 1948; Nikol'skii 1954; Alt 1988; Howland et al. 2000), mechanisms underlying this variation remain uncertain. Phenotypic differences among populations, and variation among individuals within populations, can be the combined result of variation in genotype, environment, and the interaction between these two factors (Falconer 1989). Whitefish life histories have traditionally been assumed to vary primarily with environment (Scott and Crossman 1971; Behnke 1972). However, few studies on whitefish, and none on inconnu, have looked beyond environmental causes for the phenotypic variation that is so commonly observed.

Based on gill-net surveys, radio telemetry, and otolith microchemistry analyses, I previously identified both freshwater and anadromous types of inconnu in the Mackenzie River system, Northwest Territories, Canada (Howland 1997; Howland et al. 2000). Inconnu of the lower Mackenzie River system north of Norman Wells are mainly anadromous, migrating long distances between the Beaufort Sea and upstream freshwater spawning

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[1] A version of this chapter co-authored by William M. Tonn and Ross F. Tallman has been published in *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* (2002) 57: 253-264.

areas, whereas those in Great Slave Lake and its southern tributaries are year-round residents of freshwater and undergo shorter spawning migrations.

These two forms of inconnu differ not only in migratory behavior, but also in various life history characteristics. Freshwater inconnu mature earlier and grow faster than the anadromous form, especially as juveniles (Chapter 2). Although this contrasts with general predictions based on life history theory for anadromous and freshwater fishes (Snyder and Dingle 1989; Roff 1991), these differences may largely be due to differences in the environments in which the juveniles live. However, the lack of information on the ecology and distribution of juvenile inconnu in the Mackenzie River system (Reist and Bond 1988; Howland et al. 2000), along with the potential counter gradient relationship between latitude and genetic capacity for growth of individuals (e.g., Brown et al. 1998), make it difficult to address this question with naturally occurring juveniles.

In this chapter, I begin to tease apart the contributions of genetic versus environmental factors to such characteristics using a "common garden" experimental approach (Falconer 1989). Eggs of each migratory form were incubated and hatched and the resulting young reared in the same controlled environment to see if egg development time and juvenile growth rates differed despite the common environment.

Methods

Egg collection and fertilization, parental fish

Gametes were collected from inconnu in two areas of the Mackenzie River system: the Arctic Red River is a tributary to the Mackenzie River proper and spawning inconnu have been radio-tracked to its upper reaches (66° 05'W, 132° 20'W), whereas the Slave River is tributary to Great Slave Lake and freshwater inconnu spawn approximately 300 km upstream of the mouth (60° 0'N, 112° 0'W) (Howland et al. 2000). These two populations spawn approximately one month apart, so eggs were collected and fertilized in the field on September 27 and October 21, 1996, from the Arctic Red and Slave rivers, respectively. For both populations, fish caught in gill nets were kept in holding pens until I had accumulated a sufficient number of males and females. The eggs, stripped from four females, were each divided into four groups and fertilized with milt from four males to produce a total of 16 families from each population. Fertilized eggs were airlifted on ice to the University of Alberta. Fork lengths were obtained from all parental fish and a sample of unfertilized eggs from each female was preserved in 5% formalin. To determine diameters of preserved eggs from each female, average egg diameter was obtained by aligning 10 eggs on a ruler. An average egg diameter per female was calculated from three such estimates.

Egg incubation and rearing

Within 12 hours of fertilization, eggs were disinfected with a 1:300 diluted solution of iodophor (Wescodyne ^{R}; Gordon et al. 1987) and placed in Heath trays held at 3°C in a growth chamber. Dechlorinated and aerated water was supplied by a recirculating system. To control fungal growth, infected eggs were removed by hand; as well, the water was treated with UV and antibiotics (Furan), and changed weekly.

Eggs were moved to a rearing facility at 90 days post-fertilization. By this time embryos were well developed and moving within the eggs. Following hatching, fish from both populations were reared under similar conditions, in 20-L plastic containers with flow-through dechlorinated water (pH 7.0-7.2), under a constant photoperiod of 16L:8D. Although I attempted to maintain a constant water temperature, it varied between 4 and 12°C (Fig. 3-1a), due to limitations of the aquarium facilities. Inconnu were fed ad libitum with live brine shrimp nauplii initially, followed by trout starter mash (Moore-Clarke), and later frozen adult brine shrimp. Water temperature, pH, and amount of food offered to fish were recorded on a daily basis.

Measurement of egg development and growth

I followed the development of embryos qualitatively by recording changes such as the first appearance of a notochord and eye pigmentation within each population. For each family, I recorded time of first hatching, completion of hatching, and the total number of individuals hatched at various times in between. I also counted the total number of offspring hatched within each family, allowing me to calculate the cumulative percentage of hatched individuals over time within each family.

To reduce the effects of density on growth and survival (in addition to the ad libitum feeding), families were culled to a maximum of 250 individuals (12.5 fish/L) one week after completion of hatching, and to a maximum of 100 individuals (5 fish/L) at 7 months of age. I monitored density changes in each family by recording mortalities daily. Lengths of individual fish (eye to end of caudal peduncle) were determined by videotaping them in shallow water (< 2 cm), initially with a camera mounted on a microscope and later using a surveillance camera. Taped images were then transferred to a computer and measured using image analysis software (Optimas v5.2), enabling me to follow growth of the larval fish over time with minimal handling stress. I videotaped a sub-sample of 40 fish per family monthly for the first 6 mo, beginning 7-14 days after time of 50% hatch, and bi-monthy thereafter until the termination of the experiment (December 1997), when offspring were approximately one year of age. In families with less than 40 fish, all individuals were taped and measured. To ensure that individual fish were only measured once during any given session, individuals were sampled without replacement. These monthly measurements were used to construct growth curves for individual families and for each population.

Statistical analyses

Although I monitored families from each population separately, I restricted my analyses in this paper to population-level comparisons; thus, family means were used in most analyses. I compared fork length and diameter of eggs from parent fish in each of the two study populations using two-tailed t-Tests. Development and growth rates were calculated on the basis of degree-days (sum of daily temperatures over time) to standardize for slight differences in the temperature regimes to which each population was exposed (due to the 24-d difference in fertilization date).

Development rates of eggs were compared by plotting cumulative percent hatch as a function of degree-days since fertilization. Sigmoid curves were fit to the data and used to estimate time to 50% hatch. A Wilcoxon Rank Sum Test assessed differences between populations in cumulative percent hatch at 408 degree-days, the only common time period for which I had complete data for both populations.

Although I attempted to maintain similar densities among families through periodic culling, mortality rates of families varied resulting in some differences among families over time. To assess whether or not this influenced growth among families, I compared relationships between average densities and average growth rates among families within each of the two study populations during two different time periods, the month following initial culling and the 2-mo period following culling at seven months of age. Growth curves were constructed using the mean length for each family at each time period. Time began at 50% hatch in each population. The assumptions of normality and homogeneity of sample variances were assessed using the Kolmogorov-Smirnov Test (with Lilliefors' correction) and by examining residual plots or using an F-Test, respectively. In cases where these assumptions were not met, data were either transformed or non-parametric statistical tests were used, as outlined below.

Comparisons of growth rates between populations were made using both discrete and instantaneous growth rates. I compared discrete growth rates using Analysis of Covariance (ANCOVA) on square-root transformed data. Instantaneous growth rates for each family were determined for each of the seven time intervals between which fish were videotaped and measured. I then calculated an average instantaneous growth rate for each family and compared rates between populations using a Wilcoxon Rank Sum Test. Differences were considered significant at $p < 0.05$ in all tests.

Results

Parental fish

Parental fish from the Slave River were younger than those from the Arctic Red River, but sizes were similar, and both ages and sizes were well within the range I see in naturally spawning fish from these two rivers (Table 3-1). Sizes of eggs (mean ± 1 SD) produced by the females used in the study also did not differ between populations (Slave River 2.74 ± 0.03 mm, Arctic Red River 2.73 ± 0.08 mm; t=0.39, p=0.71).

Egg development and hatching

Developmental stages of inconnu embryos are similar to those of other teleosts (e.g., Masueti and Hardy 1967). Inconnu eggs are telolecithal and exhibit rapid cleavage at the animal pole, with formation of a blastodisc in the early stages of development.

Development rates of inconnu eggs, determined from the relation between cumulative percent hatch and degree-days (°d) since fertilization, were described for both populations by sigmoid curve equations:

$$\text{Arctic Red River cumulative \% hatch} = \frac{97.35}{1 + e^{-(\text{degree-day} - 43.47)}} \quad (N=120,\ R^2=0.974,\ p<0.0001)$$

$$\text{Slave River cumulative \% hatch} = \frac{99.91}{1 + e^{-(\text{degree-day} - 31.04)}} \quad (N=127,\ R^2=0.996,\ p<0.0001)$$

Development was faster in the Slave River population (Fig. 3-1b and c) and at 408 °d, a higher percentage of larvae had hatched in the Slave River (94.03 ± 8.20%, mean ± 1 SD) than in the Arctic Red River population (4.65 ± 5.90%; Wilcoxon Rank Sum Test, T=360, p<0.001).

Differences in development between the two populations generally increased at each progressive stage (Table 3-2). Hatchlings were first seen in the Slave and Arctic Red River populations after 258 and 324.5 °d and 50% hatch occurred at 376 and 434 °d after fertilization, respectively (Table 3-2; Fig. 3-1c). Hatching occurred over extended periods, 258.2 and 279.2 °d for the Slave and Arctic Red River populations, respectively (approximately 4 weeks at an average temperature of 8.5 °C; Table 3-2; Fig. 3-1a-c). This was primarily due to extensive variation in hatch dates within, rather than among, families.

Growth

Density had a negligible influence on growth rates among families over the course of our study. All regressions of growth rate (mm/day) on density were non-significant (Arctic Red River, period 1: $F_{1,15}$=1.48, p=0.24; period 2: $F_{1,15}$=3.93, p=0.07; Slave River, period 1: $F_{1,14}$=2.83, p=0.12; period 2: $F_{1,14}$=0.19, p=0.67). As a result, density was not considered in subsequent analyses of growth.

Although growth in both populations was slightly exponential, log transformations did not improve the linear fit of the data. Square-root transformations, however, homogenized variances and normalized data. The transformed growth curves for Arctic Red and Slave River inconnu had similar slopes (ANCOVA, $F_{1,243}$=0.062, p=0.303) and intercepts (ANCOVA, $F_{1,242}$=1.252, p=0.264), indicating that size-at-hatch and subsequent rates of growth were similar for both populations (Fig. 3-2). Both populations also displayed similar rates of instantaneous growth (Wilcoxon Rank Sum Test, T=246, p=0.83).

Discussion

Egg development

The observed differences in egg development rate under common conditions suggest that this trait is under considerable genetic control. Although some studies indicate that larval size-at-hatching is correlated with parental size (e.g., Hutchings 1991; Ferguson et al. 1995), the relationship of egg size and development time is not as clear (e.g., Hutchings 1991; Ferguson et al. 1995; Pauly et al. 1998). In our study, neither parents nor eggs differed in size between populations and thus could not have influenced our population-level results.

The different spawning times and rates of egg development from the two populations are most likely linked with seasonal patterns of water temperature and ice conditions in their natal rivers. Inconnu eggs typically incubate over winter on the bottom of rivers. Both populations spawn 1-3 weeks prior to the average time of freeze-up, when surface water temperatures range from 2 to 7 °C (Howland et al. 2000). Following freeze-up, water temperatures in rivers typically drop to just above freezing unless there is significant input from warmer sources such as groundwater or lakes (Prowse and Reedyk 1993). Given the lack of information about the hydrology of my study rivers, I hesitate to estimate natural incubation temperatures and associated hatching times of inconnu in the wild based on my laboratory results. However, the development times I observed in my study (as measured by degree-days; Table 3-2) are remarkably consistent with the length of ice cover on both rivers, assuming an incubation temperature of 2 °C (see Table 3-3), perhaps suggesting that eggs from both population hatch shortly after average time of river break-up.

Regardless of exactly when hatching occurs, I suggest that differences in spawning times and rates of egg development between populations have evolved to match the temperature regimes within each river. Timing of hatch in fish is critical since larvae that hatch too early risk starving before food becomes available (Cushing 1990), whereas those that hatch too late may not have enough time to grow and build up sufficient reserves to survive their first winter (Shuter and Post 1990). Given the short window of opportunity that exists for feeding and growth of young fish in these northern rivers, it is not surprising that development would have evolved to match environmental conditions within specific rivers.

Hatching in both populations occurred over an extended period in our study, which was manifested as variation in development rates within families. Although the pattern in the wild is undocumented, evolving an extended hatch period should be advantageous due to the unpredictability of the rivers in which inconnu reproduce. Over a recent 30-y period, both freeze-up and break-up dates of the Arctic Red and Slave rivers varied substantially (Table 3-3). Although fish may be able to adjust for variability in river freeze-up times by using temperature as a proximate cue for spawning (e.g., Hokanson 1977), they have no way of predicting spring break-up times from one year to the next. Variability in egg development rate would improve the chances that at least some of an individual's offspring within a given year hatch at an optimal time (Den Boer 1968; Lambert and Ware 1984) .

Based on the glacial history of this region (see Lindsey and McPhail 1986), inconnu colonized the Mackenzie River system at most 6 to10 thousand years ago and the freshwater and anadromous forms have likely diverged since that time. In this period of time, adaptive genetic differences in rates of egg development appear to have evolved between populations in response to differing environments. These changes have probably been mediated by differential selective pressures from spawning rivers that differ in the lengths of their open-water growing seasons.

Growth

Density differences among families had no detectable influence on growth rate in our experiment. The ad libitum feeding protocol, limited range in densities due to my culling, and lack of aggression/territoriality amongst individuals likely minimized any potential effects of density.

The similarity in growth rates displayed by juvenile inconnu from both populations when reared under similar conditions suggests that observed size differences in the wild (KL Howland, unpublished data) can be attributed mainly to environmental rather than genetic effects. Based on length of the open-water period, freshwater inconnu in the Great Slave Lake area likely experience a longer growing season than their anadromous counterparts from the lower Mackenzie River region (see Table 3-2). This could have a strong influence on size particularly at the juvenile stage when growth is rapid and highly dependent on temperature and food availability (Diana 1995). This conclusion does not preclude the possibility of a countergradient relationship between growth and latitude, or genetic differences in phenotypic plasticity (reaction norms) for growth, both of which can only be detected if individuals from each population are reared across a range of temperature and food treatments. However, in contrast to examples of countergradient variation in growth (see Conover and Shultz 1995), observed growth in the field is greater among the lower-latitude Slave River inconnu, so there is no empirical suggestion of such variation. Nevertheless, growth may have some degree of heritability (e.g., Bailey and Loudenslager 1986; Nilsson 1994); however, quantitative genetic analyses will be needed be to address this issue.

I suggest that the apparent difference in the degree of evolutionary divergence in growth versus egg development time in these two populations is due, in part, to the predictability of local conditions, and the relative consequences of a mis-match to those conditions. For egg development, the consequences of not hatching at the optimal time in the spring, e.g., too far before or even during ice break-up, may be severe for young fish in these northern rivers. The general time period that is optimal for development and hatching of young (suggested to correspond to the period of ice cover) is predictable over the long-run within a given river and I suggest that average differences between rivers are sufficient to allow for the evolution of population-specific average development rates. However, within populations , development rates remain highly variable, probably because annual variation in ice cover precludes the evolution of a single optimal type. On the other hand, although first-year growth is important for northern fish populations (Shuter and Post 1990), the consequences of differences in growth for populations inhabiting rivers at different latitudes, are not likely to be as severe as those for hatching, so this trait would not be expected to evolve as quickly. Some populations of poikilotherms have evolved faster growth rates at higher altitudes or latitudes to compensate for lower temperatures during the growing season (e.g., Conover and Schultz 1995; Brown et al. 1998). Selection for such differences, however, may not be strong in my study populations, since water temperatures are actually similar between rivers during the growing season (Howland et al. 2000) even though there is a latitudinal gradient in the length of this season.

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Table 3 - 1. Average (± 1 SD) and range of fork length and ages of parental inconnu used in this study and natural spawners (KL Howland, unpublished) from the Slave and Arctic Red rivers.

	Slave River	Fork length (mm) Avg. ± 1 SD (range)	Age (years) Avg. ± 1 SD (range)
♀	Parental fish	861 ± 59 (804-938), N=4	9.5 ± 1.0 (9-11), N=4**
	Natural spawners	807 ± 75 (537-1100), N=220	8.2 ± 1.8 (4-24), N=154
♂	Parental fish	756 ± 21 (734 –775), N=4	6.3 ± 0.5 (6-7), N=4*
	Natural spawners	719 ± 49 (488-906), N=372	6.8 ± 1.2 (4-16), N=230
	Arctic Red River		
♀	Parental fish	877 ± 51 (840-951), N=4	17.25 ± 0.5 (17-18), N=4**
	Natural spawners	840 ± 73 (685-1122), N=264	16.8 ± 3.5 (9-31), N=102
♂	Parental fish	710 ± 34 (665-742), N=4	14 ± 3.7 (9-18), N=4*
	Natural spawners	723 ± 54 (540-879), N=526	13.3 ± 2.9 (6-27), N=250

* $p<0.05$, ** $p<0.001$ for between-population comparisons of parental fish of each sex.

Table 3 - 2. Calendar dates, time from fertilization (in degree-days) and the time difference (in degree-days) to successive stages of embryo development for Arctic Red and Slave River inconnu.

	Arctic Red River		Slave River		Difference
Developmental Stage	Calendar Date	Degree-Days	Calendar Date	Degree-Days	Degree-Days
Fertilization	September 27/96	0	October 21/96	0	0
1st appearance of *notochord*	October 26/96	90	November 18/96	87	3
1st appearance gold eye pigmentation	November 26/96	183	December 11/96	162	21
Appearance of 1st hatchlings	December 30/96	324.5	January 13/97	258	66.5
50% hatch	January 11/97	434	January 30/97	376	58
All families hatched	February 1/97	603.7	February 15/97	516.2	87.5

Table 3 - 3. Average (±1 SD) and range of ice-on/ice-off dates and length of ice covered period for the Slave and Arctic Red rivers, 1965-1995 (data obtained from Water Survey of Canada) and estimated incubation period for inconnu from both rivers (based on data presented in Table 3-2).

	Arctic Red River		Slave River	
	Average ± 1 SD	Range	Average ±1 SD	Range
Ice-on	Oct. 10 ± 8 d	Sep. 19-Oct. 21	Nov. 5 ± 8 d	Oct. 17-Nov. 22
Ice-off	May 24 ± 8 d	May 7-June 2	May 9 ± 6 d	Apr. 26-May 21
Total period of ice cover	226 ± 13 d	199-251 d	185 ± 10 d	166-203 d
Estimated time to 50% hatch at average water temperature of 2 °C	217 d		188 d	

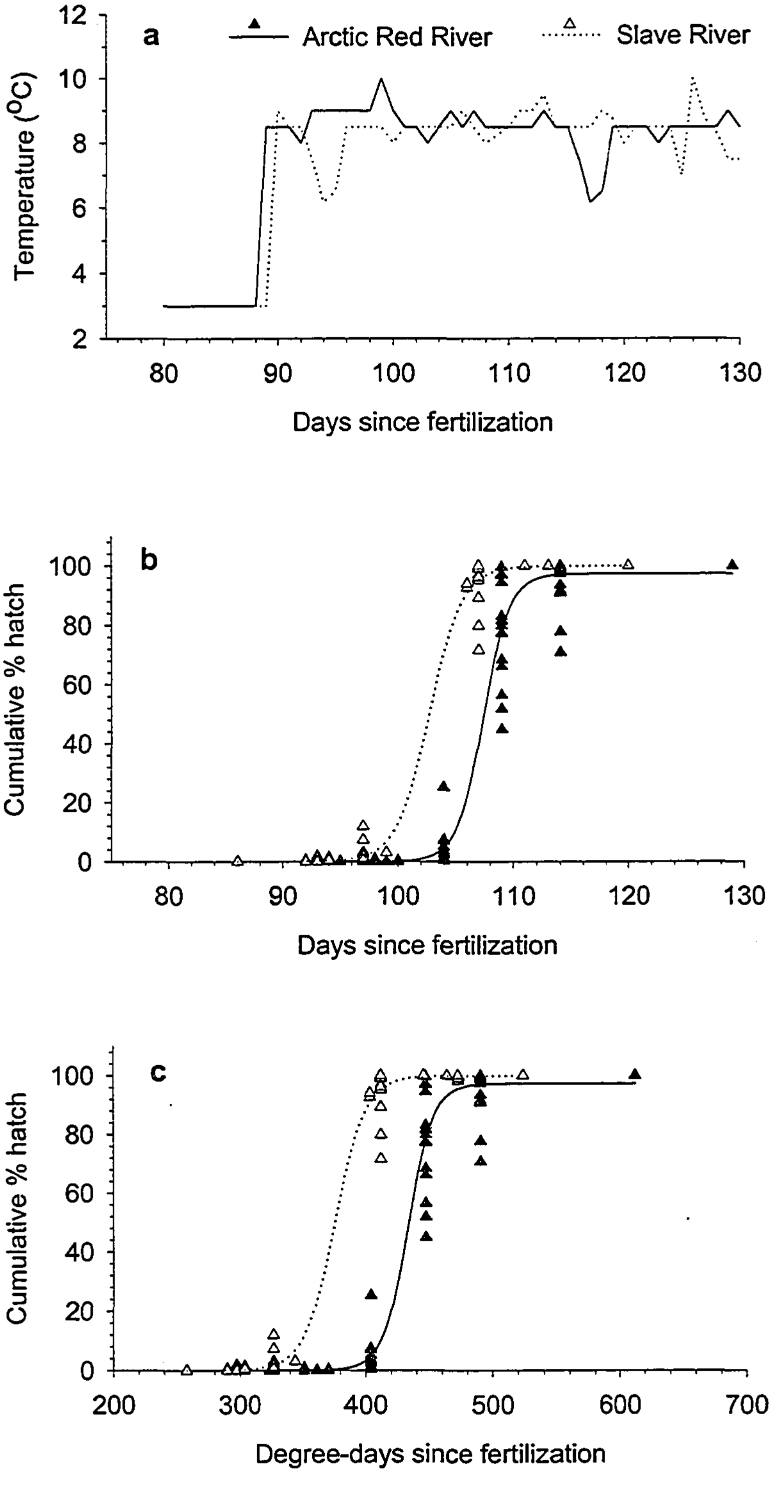

Figure 3-1. a) Daily mean water temperatures over the hatch period, b) cumulative percent hatch versus days after fertilization and, c) cumulative percent hatch versus degree-days for eggs from 16 families of inconnu originating from Arctic Red (solid triangles/line) and Slave rivers (open triangles/dotted line) and raised under common laboratory conditions.

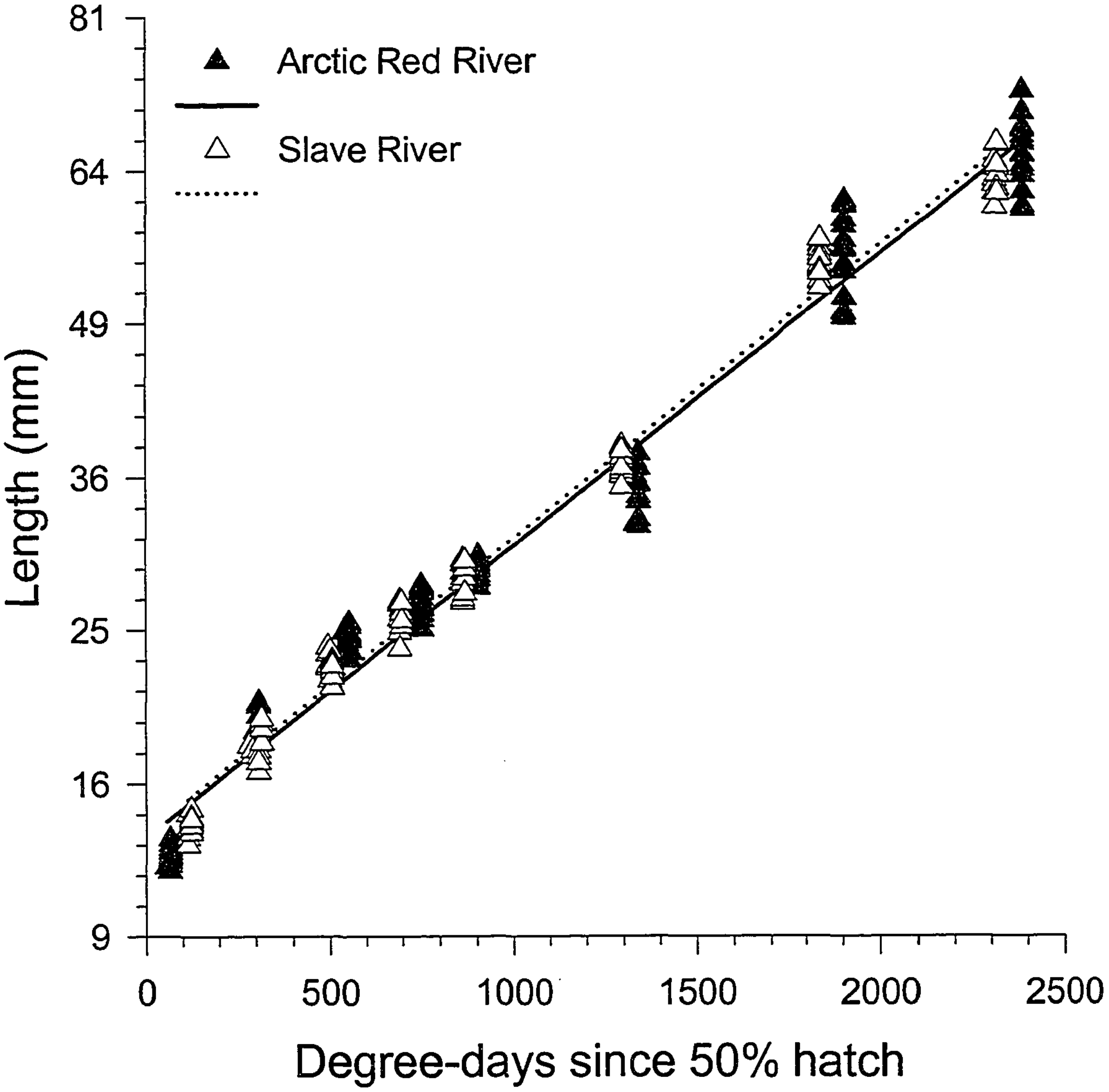

Figure 3-2. Growth curves (square-root transformed length versus degree-days) for juvenile inconnu from the Arctic Red (solid triangles/line) and Slave (open triangles/dotted line) rivers in our common garden experiment. Note that Y-axis is on a non-linear scale.

4. Contrasts in the hypoosmoregulatory abilities of a freshwater and an anadromous population of inconnu[2]

Introduction

Anadromy, the movement between freshwater spawning and marine feeding areas, is a characteristic of many salmonid species (Stearly 1992; Moyle and Cech 1997). Consequently, substantial interest has developed around the osmoregulatory capacities and physiological mechanisms that allow salmonids to move between these distinct environments. Most research on this topic has focused on the Pacific and Atlantic salmon (*Oncorynchus* spp. and *Salmo salar* (L.), e.g., McCormick et al. 1985; Birt et al. 1991; Zaugg and Beckman 1990; Morgan and Iwama 1998), as well as Arctic char (*Salvelinus alpinus* (L.), e.g., Finstad et al. 1989; Nilssen et al. 1997; Eliassen et al. 1998). In contrast, little is known about the salinity tolerance of whitefishes (but see de March 1989; Madsen et al. 1996), many of which also have anadromous populations in the northern parts of their ranges, and even less is known regarding their physiological responses to salinity change.

Inconnu is an arctic whitefish found in large rivers and lakes throughout much of Siberia and the western Arctic of North America (McPhail and Lindsey 1970), where they contribute to both subsistence and commercial fisheries (McCart 1986; Bodaly et al. 1989). Distinct anadromous and freshwater populations have been reported in Siberia (Berg 1948; Nikol'skii 1954) and Alaska (Alt 1977; 1988), and I have recently documented the occurrence of both forms in the Mackenzie River system of northwestern Canada. The freshwater form occurs in Great Slave Lake and its southern tributaries, whereas anadromous inconnu are distributed throughout most of the lower Mackenzie River system north of Norman Wells (Howland et al. 2000); no mixing appears to occur between the two forms. Based on the glacial history of this region (Lindsey and McPhail 1986), inconnu colonized the Mackenzie River system $\leq$ 10, 000 y bp and the freshwater and anadromous forms have likely developed since that time.

Otolith microchemistry reveals that anadromous inconnu in the Mackenzie River system migrate seaward annually after reaching 1-2 years of age (Howland 1997);

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[2] A version of this chapter co-authored by William M. Tonn and Greg Goss has been published in *Journal of Fish Biology* (2001) 59: 916-927.

however, size of first time migrants has, to my knowledge, never been documented. These annual migrations appear to involve feeding in brackish coastal waters of the Beaufort Sea during the summer months, and returning to deeper areas of the Mackenzie Delta or estuary to overwinter (Howland et al. 2000). After reaching sexual maturity (age 7-10, KLH, unpublished data), anadromous inconnu migrate long distances upstream into freshwater tributaries (e.g., Peel and Arctic Red rivers), where they spawn in the fall (Stein et al. 1973; Howland et al. 2000). Inconnu are repeat spawners and are believed to spawn every 3-4 y (Scott and Crossman 1973).

Little is known about the extent of anadromous migrations, e.g., whether inconnu venture into full-strength seawater offshore or to deeper areas along the coast during summer feeding, and whether they undergo seasonal preparatory physiological changes similar to Arctic char and salmon. Survey data suggest that anadromous inconnu from the Mackenzie River system rarely enter coastal waters of salinities > $20^{0}/_{00}$ (Bond 1982; Lawrence et al. 1984; Bond and Erickson 1989; Chiperzak et al. 1991). However, strontium concentrations in otoliths suggest that inconnu occupy waters with salinities similar to those used by anadromous Arctic char (Howland 1997), which are known to undergo seasonal physiological changes and are capable of adapting to full-strength seawater ($32\text{-}35^{0}/_{00}$) during summer (Finstad et al. 1989; Eliassen 1998).

Likewise, the hypoosmoregulatory capabilities of freshwater inconnu have not been studied, and we do not know if the two forms have diverged in their osmoregulatory abilities. Although some studies have found greater seawater adaptability in anadromous forms of salmonids (Staurnes et al. 1992a; Barbour and Garside 1993; Birt and Green 1993; Eliassen et al. 1998), others find no difference (McCormick et al. 1985; Staurnes et al. 1992b; Schmitz 1995; Pirhonen and Forsman 1998). I examined the hypoosmoregulatory abilities of juvenile freshwater and anadromous inconnu from Mackenzie River system that were reared under common laboratory conditions to address the following questions: 1) What salinity levels are inconnu able to tolerate and what can this tell us about their potential distribution in coastal waters? 2) Can inconnu undergo acclimatory physiological changes to occupy saltwater, similar to other salmonids? 3) Given the relatively short time span they have been separated from each other, how do the hypoosmoregulatory capabilities of freshwater and anadromous inconnu differ?

Methods

Experimental animals and rearing conditions

Experimental inconnu originated from two areas of the Mackenzie River system. The Arctic Red River is a tributary to the lower Mackenzie River and contains spawning anadromous inconnu, whereas spawning freshwater inconnu inhabit the Slave River, a southern tributary to Great Slave Lake. Eggs from spawning females of both populations were collected and fertilized in the field in September-October 1996. Fertilization of Arctic Red River eggs occurred approximately 1 mo earlier due to differences in spawning times. Within 12-h of fertilization, eggs were flown to Edmonton, where they were incubated and hatched in the aquatic facility of the University of Alberta during January-February 1997. Juveniles from both populations were reared under similar conditions, at a photoperiod of 16 L:8 D, in multiple 20-L plastic containers with flow-through, dechlorinated water held at an average temperature of 7°C (range 4-12°C due to fluctuations of incoming city water and limitations of the aquatic facility). Inconnu were fed ad libitum with live brine shrimp nauplii, trout starter mash (Moore-Clarke), and later, frozen adult brine shrimp. Salinity tolerance experiments were conducted when fish reached approximately one year of age.

Salinity tolerance experiments

Salinity tolerance of freshwater and anadromous populations were compared in two experiments designed to represent potential scenarios in the wild: 1) an acute transfer from freshwater into various salinities (0 to $25^0/_{00}$), such as might be experienced by fish during coastal storm events, tidal events, or during feeding forays; 2) a step transfer from freshwater to brackish water ($15^0/_{00}$) for 2 wk and then into higher strength seawater ($25^0/_{00}$), which might be experienced during the annual spring migrations of this species from the freshwater deltas and tributaries, into brackish coastal areas and then into higher strength seawater.

For both the acute and step-transfer experiments, dechlorinated water that was aerated, filtered, and recirculated with a pump was used. Fish were transferred to experimental containers (20-L pails for the acute transfer experiments, and 80-L tubs for the step-transfer experiments) and allowed to adjust to their new surroundings in freshwater for 2 d. Concentrated artificial brine (Hagen 50 Fathoms Sea Salt) was then added to the freshwater to obtain the desired salinities. Salinities were measured daily using a hygrometer and held to within ± $1^0/_{00}$. Fish were fed ad libitum throughout the course of the experiments. Excess food and feces were removed daily by siphon and lost water volume was replaced as required.

Acute transfer - This experiment was run from late January to March 1998; the trials of the two populations were staggered by 1 mo (anadromous, January 26-February 3; freshwater, February 27-March 7) to minimize age and size differences resulting from different spawning/hatch times. Experimental fish were 79.4 ± 4.7 mm and 3.2 ± 0.6 g, and 76.3 ± 4.4 mm and 2.7 ± 0.5 g (mean ± SE fork length and wet mass) for inconnu from the Arctic Red and Slave rivers, respectively. Experiments were carried out in a 5°C (± 0.1°C) growth chamber, at a constant photoperiod of 16 L:8 D. Twelve fish were sampled at time 0, prior to the saltwater transfer to serve as a reference control. Then, anadromous and freshwater inconnu were transferred directly to four different salinities: 0 (control), 10, 15, and $25^0/_{00}$ seawater (n=31 fish/salinity/population). Six fish were subsequently sampled from each treatment at 12, 24, 48 and 192 h post-transfer.

Step transfer - This experiment was run from mid-June to late August 1998, again staggering the trials by approximately 1 mo to minimize age and size differences between anadromous and freshwater inconnu. Fish (mean fork length and mass: 86.5 ± 7.1 mm and 3.9 ± 1.0 g, and 87.4 ± 6.1 mm and 4.0 ± 0.8 g, for Arctic Red and Slave river, respectively) were held in 10°C (± 1.0°C) water on a 24 h photoperiod. The fish had been held at this photoperiod for approximately 1.5 mo, following a gradual increase from 16 L:8 D (photoperiodic changes were staggered by approximately one month to account for stage differences between anadromous and freshwater inconnu), simulating natural light conditions they would experience in the north during spring. Inconnu from each population were either acclimated to $15^0/_{00}$ (brackish) for 2 wk and then transferred to $25^0/_{00}$ (n=28 anadromous, n=36 freshwater) or were directly transferred from freshwater to $25^0/_{00}$ (n=22 anadromous, n=30 freshwater). Six fish were sampled prior to the brackish-water acclimation to serve as a reference control. Another 6 fish from each population were sampled from the brackish-water treatment at the end of the acclimation period. Subsequently 6 fish from each treatment were sampled at 12, 24, and 192 h post-transfer.

Sampling and determination of plasma ion concentrations and Na+-K+ ATPase activity

At each sampling individual fish were stunned via cephalic blow and fork length and mass were measured. The tail was then severed and blood collected from the caudal vein using a heparinized hematocrit tube. Blood plasma was separated by centrifugation and stored at -20°C. One gill basket was removed and immersed in a solution of 250 mM sucrose, 10 mM EDTA, 50 mM imidazole (pH 7.3) and frozen at -80°C for subsequent determination of sodium-potassium (Na^+-K^+) ATPase activity.

Changes in blood sodium (Na^+) and chloride (Cl^-) concentration, and Na^+-K^+ ATPase activity were used as indicators of salinity tolerance. Mortality levels and feeding behavior were also incidentally recorded. Plasma [Na^+] was measured using atomic absorption spectrophotometry. Plasma [Cl^-] was determined using a ferric nitrate/mercuric thiocyanate colormetric assay (Zall et al. 1956). Sodium-potassium ATPase activity was measured for the later step-transfer experiment using a slightly modified version of a microassay developed by McCormick (1993); 2.5 μl of homogenate was used and the assay was run at 24°C.

Statistical analyses

All reported values are means (± 1 SE) from sample sizes of n=6 unless otherwise indicated. The General Linear Models (GLM) procedure for factorial data (SPSS 8.0) was used to test for effects of treatment, population, and time on plasma ion and gill Na^+-K^+ ATPase activity levels of fish following transfer to $25^0/_{00}$ seawater. For the acute-transfer experiment, two partial GLM analyses (1 - without 25 ppt treatment, 2 – without 48 h and 192 h treatments) were run to accommodate for missing data in some cells. Comparisons between pre- and post-transfer levels within each treatment were made using one-way analyses of variance (ANOVAs), followed by the Dunnett's test. The assumptions of normality and homogeneity of sample variances were assessed using the Kolmogorov-Smirnov test (with Lilliefor's correction) and F-tests, respectively. In cases where these assumptions were not met, data were either log-transformed or non-parametric statistical tests were used (Kruskal-Wallis ANOVA on ranks). Mortality levels of inconnu from freshwater and anadromous populations were compared with Fisher's Exact Test. Differences were considered significant at a probability level of 0.05.

Results

Acute transfer

Blood plasma ions - Inconnu from both freshwater and anadromous populations responded similarly to a direct transfer from freshwater into salinities ranging from 0 to $25^0/_{00}$ seawater (Figs. 4-1a-d), and no consistent effect of population was seen (partial GLMs, $[Na^+]$ 1 - $P < 0.0001$ 2 – P=0.16, $[Cl^-]$ 1 – P=0.30 2 - P<0.0001. Significant effects of salinity (relative to control group) were observed for both freshwater and anadromous inconnu when transferred to brackish and $25^0/_{00}$ (GLMs with post-hoc Tukey's, P<0.0001 for all pairwise comparisons). Both populations showed an initial increase in plasma $[Na^+]$ and $[Cl^-]$ between 12 and 48 h post-transfer to all salinities > $0^0/_{00}$ (GLMs, $P < 0.0001$). Plasma ion concentrations stabilized or returned to pre-transfer levels by 8 d in 10 and $15^0/_{00}$. At $25^0/_{00}$ plasma ion concentrations increased steadily with time and all fish died between 24 and 48 h post-transfer. Inconnu from both populations appeared to exhibit normal feeding behavior in the freshwater and brackish water treatments, but stopped feeding by 12 h post-transfer to $25^0/_{00}$ seawater.

Step-transfer

Blood plasma ions and mortality – In the second step-transfer experiment, the GLM procedure revealed a significant effect of treatment (acclimated versus non-acclimated, $[Na^+]$ P< 0.0001, $[Cl^-]$ P=0.004), some effect of time ($[Na^+]$ P=0.02, $[Cl^-]$ P=0.05), and no effect of population ($[Na^+]$ P=0.20, $[Cl^-]$ P=0.33) on post-transfer blood plasma ion levels. Individuals from both populations that acclimated to brackish water showed less overall increase in blood plasma ion concentrations (GLMs, $[Na^+]$ P< 0.05, $[Cl^-]$ P<0.0001), had lower mortality (acclimated 5/21 fish died, non-acclimated 14/22 fish died; Fisher's Exact Test, P=0.014) , and exhibited normal feeding behaviour, compared to non-acclimated individuals following transfer to $25^0/_{00}$ seawater.

In the non-acclimated groups, both anadromous and freshwater inconnu showed a steady increase in plasma $[Na^+]$ and $[Cl^-]$ until 24 h post-transfer (Fig. 4-2), as in the earlier acute transfer experiment, but survived longer. Fish did not begin to die until 72 h post-transfer and some individuals were still alive and behaving normally after 8 d. Mortality was similar at 57% (4/7) and 67% (10/15) between 72 hours and 8 d for anadromous and freshwater inconnu, respectively (Fisher's Exact Test, P=0.67). Plasma ion concentrations of survivors stabilized by 8 d, although remaining greater than pre-treatment levels (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*<0.05).

After 2 wk in brackish water, anadromous inconnu maintained plasma [Na^+] and [Cl^-] (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*>0.05), whereas concentrations increased in freshwater inconnu (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*<0.05; Fig. 4-2). Following the subsequent transfer to $25^0/_{00}$ seawater, both groups reached similar plasma [Na^+] and [Cl^-] (GLMs, *P*>0.05), which stabilized by 8 d. Despite reaching similar plasma ion concentrations, the anadromous group suffered no mortality following transfer to $25^0/_{00}$ (0/7 fish died), whereas 35% (5/14) of the fish in the freshwater group died between 72 h and 8 d post-transfer. The small sample size in the anadromous group, however, prevented this difference from being significant (Fisher's Exact Test, *P*=0.12).

Na+-K+ ATPase activity – Although freshwater and anadromous inconnu had similar ATPase activity prior to treatment, activity increased during the 2-wk acclimation to brackish ($15^0/_{00}$) water among the freshwater (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*<0.05), but not anadromous, inconnu (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*>0.05; Fig. 4-3). The difference in ATPase levels between acclimated freshwater and anadromous inconnu carried over following their transfer to $25^0/_{00}$, resulting in a significant population effect at this stage also (GLMs *P*<0.0001). Although their absolute ATPase levels differed at the post-transfer stage, acclimated inconnu from both populations showed a similar pattern of increased Na^+-K^+ATPase activity in the first 12-24 h (GLMs time *P*<0.0001). Levels then remained higher than non-acclimated individuals through to 8 d (GLMs; treatment *P*<0.0001; Fig. 4-3). Non-acclimated survivors also increased Na^+-K^+ATPase activity above pre-treatment levels by day 8 (Kruskal-Wallis ANOVA on ranks with post-hoc Dunnett's test, *P*<0.05), although they remained lower than in acclimated fish (Fig. 4-3).

Discussion

Juvenile inconnu of freshwater and anadromous origin responded similarly to an acute transfer from freshwater into various salinities. At the age of 1 y (ca. 3 g), both populations could survive a direct transfer to brackish water of 10-$15^0/_{00}$, but not to higher strength ($25^0/_{00}$) seawater, for up to eight days. The steady increase in plasma ions, abnormal feeding behaviour, and rapid death of fish transferred to $25^{0}/_{00}$ indicated that at this age, neither population was capable of osmoregulating at this salinity, and all fish likely died of osmoregulatory collapse.

Differences in adaptability to higher strength seawater, however, were manifested at age 1.5 y following acclimation to brackish water. During the 2-week acclimation, anadromous inconnu maintained plasma ion concentrations near pre-treatment levels, something freshwater inconnu were unable to do. Although fish of both forms reached similar ion concentrations following transfer to $25^{0}/_{00}$, all anadromous fish survived whereas several freshwater individuals showed signs of stress in the form of abnormal swimming behavior or increased pigmentation and died following transfer. Thus, under more realistic conditions of brackish-water acclimation, anadromous inconnu withstood higher strength salt water better than their freshwater counterparts. Because both forms were equally capable of regulating ions following transfer to higher strength seawater, mortality in the freshwater form may have been due to their inability to control water loss (Handeland et al. 1998; Hoar 1976).

Although freshwater inconnu had diminished hypoosmoregulatory capabilities following acclimation, they increased gill Na^{+}-K^{+} ATPase activity in response to both the acclimation conditions and to the subsequent transfer to 25 $^{0}/_{00}$. Anadromous inconnu showed only the latter increase, and therefore, had lower levels of post-transfer activity compared to the freshwater group. The higher levels of ATPase activity in the freshwater form were likely a response to a greater transport demand due to higher blood ion levels. Perhaps freshwater inconnu are not as well equipped to excrete ions through other mechanisms (e.g., gill structure, Evans 1984; gut or kidney function, Hoar 1976) and must compensate by increasing gill $Na^{+}K^{+}$-ATPase activity at a lower salinity. I will be addressing this hypothesis, in the future, by comparing the gill morphology of freshwater and anadromous inconnu during salinity changes.

In addition to life history or population differences, a variety of other factors, including fish size, photoperiod, and pre-acclimation may influence the ability of fish to tolerate and/or adapt to sea water. For example, older, larger inconnu used in our later step transfer experiments (1.5 y, ca. 4 g) were better able to tolerate a direct transfer to 25 $^{0}/_{00}$ seawater than the smaller, younger fish from our first experiment, resulting in stabilized plasma ion concentrations and lower mortality among non-acclimated individuals from the later experiment. It is likely that the larger size of fish used in the later experiment, compared to those used earlier, contributed to their improved survival

(Conte and Wagner 1965; McCormick and Naiman 1984; McCormick and Saunders 1987; Hoar 1988).

Seasonal changes in photoperiod, particularly increasing day length associated with spring, are known to increase salinity tolerance by inducing preparatory physiological changes in species, such as Arctic char, that spend their summers at sea (Finstad et al. 1989; Arneson et al. 1992), and in Pacific and Atlantic salmon that undergo parr-smolt transformation in spring (Wagner 1974; McCormick et al. 1987; Berge et al. 1995). Such changes, e.g., elevated cortisol levels, increased chloride cell density, and accompanying gill activity, lead to improved hypoosmoregulatory capabilities (Langdon and Thorpe 1985; McCormick and Saunders 1987; McCormick 1995). It is possible that the longer photoperiod used in our later step transfer experiment also contributed to improved salinity tolerance. The 24-h light to which these fish were gradually adjusted is comparable to what wild inconnu would experience across their range during spring and summer, when they move into coastal areas to feed. My experimental design, however, did not allow me to separate the relative influences of size and photoperiod on the development of salinity tolerance in inconnu.

Nevertheless, my step transfer experiment clearly demonstrated that pre-acclimation to brackish water greatly improves the hypoosmoregulatory abilities of both freshwater and anadromous inconnu. Increases in plasma ion concentrations and mortality were both lower in acclimated than non-acclimated individuals. This improved hypoosmoregulatory ability was associated, in acclimated fish, with initially elevated Na^+-K^+ ATPase activity , which increased steadily over time following transfer to 25 $^0/_{00}$ seawater. Increased enzyme activity was eventually seen in surviving non-acclimated fish, but not until 8 d post-transfer. This time lag probably explains the poor salinity tolerance of non-acclimated individuals, since an increase in gill Na^+-K^+ ATPase activity is considered essential for euryhaline fish to maintain normal electrolyte and water balance in seawater (McCormick et al. 1987).

There is some controversy surrounding the types and sequence of cues that may induce increases in Na^+-K^+ ATPase activity. One-time ocean migrants, such as Pacific and Atlantic salmon species, pre-adapt to a salt water existence while still in freshwater (e.g., Wagner 1974; McCormick et al. 1987; Berge et al. 1995) but McCormick (1994) and McCormick and Saunders (1987) suggested that fish that migrate annually between saltwater and freshwater (e.g., Arctic char and whitefish species) rely primarily on salinity for the induction of appropriate physiological changes. My results, and those of Schmitz

(1995), support this hypothesis, but Arnesen et al. (1992) and Eliassen et al. (1998) found that anadromous Arctic char underwent seasonal increases in chloride cell numbers and gill Na^+-K^+ ATPase activity even when held in freshwater only; still, exposure to seawater could induce further increases in enzyme activity. Although both of the seawater exposure scenarios that relate to our two experiments are plausible in nature, inconnu are more likely to encounter full strength seawater following exposure to brackish water, rather than going directly into full strength seawater. If so, then salinity change may be sufficiently reliable as a trigger for the induction of hypoosmoregulatory mechanisms.

Conclusions

This study demonstrates that inconnu, like other salmonids, undergo physiological changes in response to salinity by increasing gill Na^+-K^+ ATPase activity, and perhaps chloride cell densities, since the two are known to be correlated (Langdon and Thorpe 1985; Eliasson et al. 1998). Although both anadromous and freshwater inconnu possess the ability to hypoosmoregulate, the former appear better adapted to a euryhaline lifestyle than the latter. Selective pressure likely maintains salinity tolerance in anadromous populations. Anadromous fish that are less able to switch between marine and freshwater environments are probably less successful than those capable of migrating periodically into the more productive marine habitat. Although freshwater inconnu in the Slave River are likely derived from anadromous populations that initially became established in the lower Mackenzie (see Lindsey and McPhail 1986), selective pressure for salinity tolerance in freshwater inconnu is now reduced, resulting in a diminution of this characteristic. Indeed, the varied findings of other comparative studies may be explained, in part, by differences in the length of time various freshwater populations have been separated from their founding anadromous populations. Alternatively, there may be a strong genetic linkage between saltwater adaptability and the behavioral component of migration, which is retained in some freshwater populations, but not others (Schmitz 1995).

Based on their salinity tolerance under experimental conditions, anadromous inconnu may be able to use more coastal habitat and venture farther from shore and the Mackenzie estuary than past surveys indicate. This is consistent with otolith microchemistry work that indicates inconnu occupy saline coastal waters by the age of one year (Howland 1997). Given the large body of literature demonstrating size-dependent salinity tolerance in other salmonid species (cited above), it is reasonable to expect that larger inconnu may tolerate even higher salinities than those examined in the current study.

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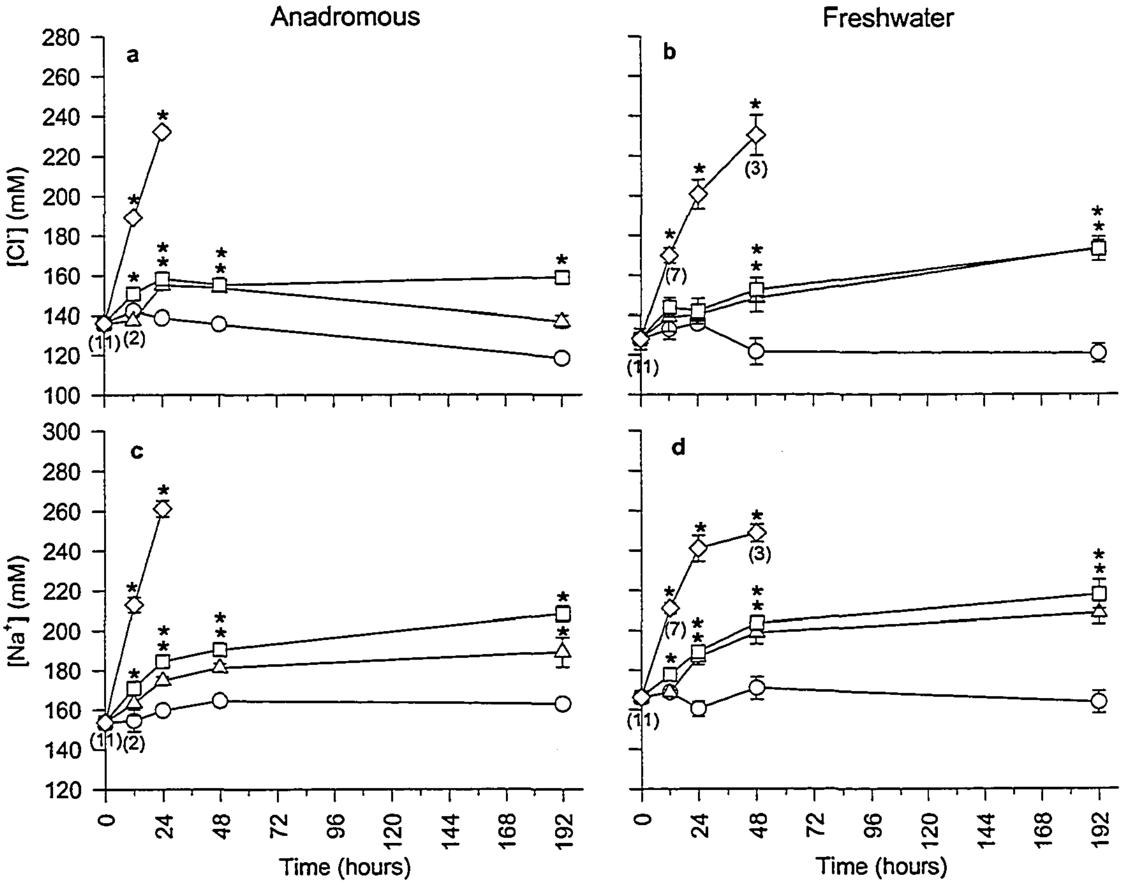

Figure 4-1. Changes in plasma chloride (a and b) and sodium (c and d) concentrations (mean ± 1 SE) of anadromous (a and c), and freshwater (b and d) inconnu transferred directly from freshwater into salinities of 0 (—○—), 10 (—△—), 15 (—□—), and 25 (—◇—)$^0/_{00}$. Asterisks represent a significant difference ($P<0.05$) from the pre-treatment freshwater level (Kruskall-Wallis ANOVA on ranks with post-hoc Dunnett's test). Numbers in parentheses represent sample sizes if other than n=6.

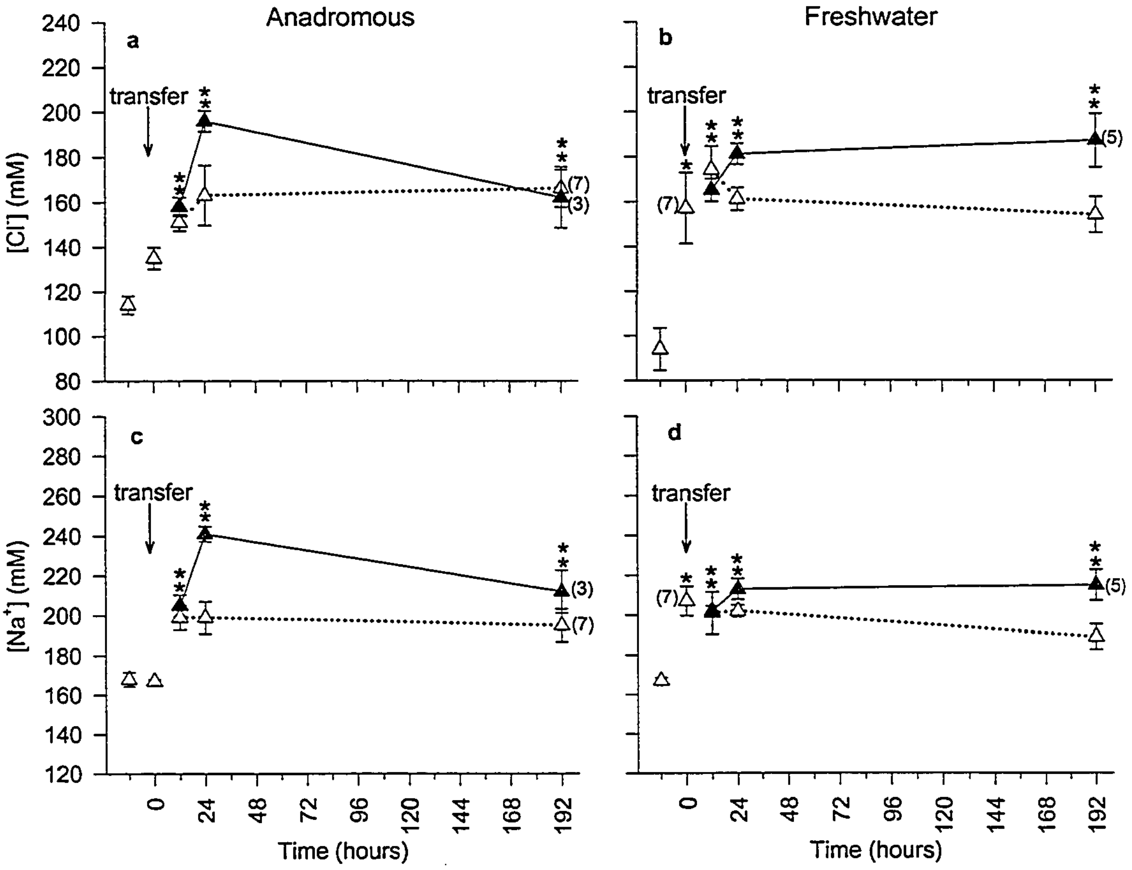

Figure 4-2. Changes in plasma chloride (a and b) and sodium (c and d) concentrations (mean ± 1 SE) of anadromous (a and c), and freshwater (b and d) inconnu following transfer to 25 $^0/_{00}$ seawater, either with (acclimated —△—) or without (non-acclimated —▲—) a 2-wk acclimation period at 15 $^0/_{00}$. Grey triangles represent pre-treatment freshwater levels. Asterisks represent a significant difference (P<0.05) from the pre-treatment freshwater level (Kruskall-Wallis ANOVA on ranks with post-hoc Dunnett's test). Numbers in parentheses represent sample sizes if other than n=6.

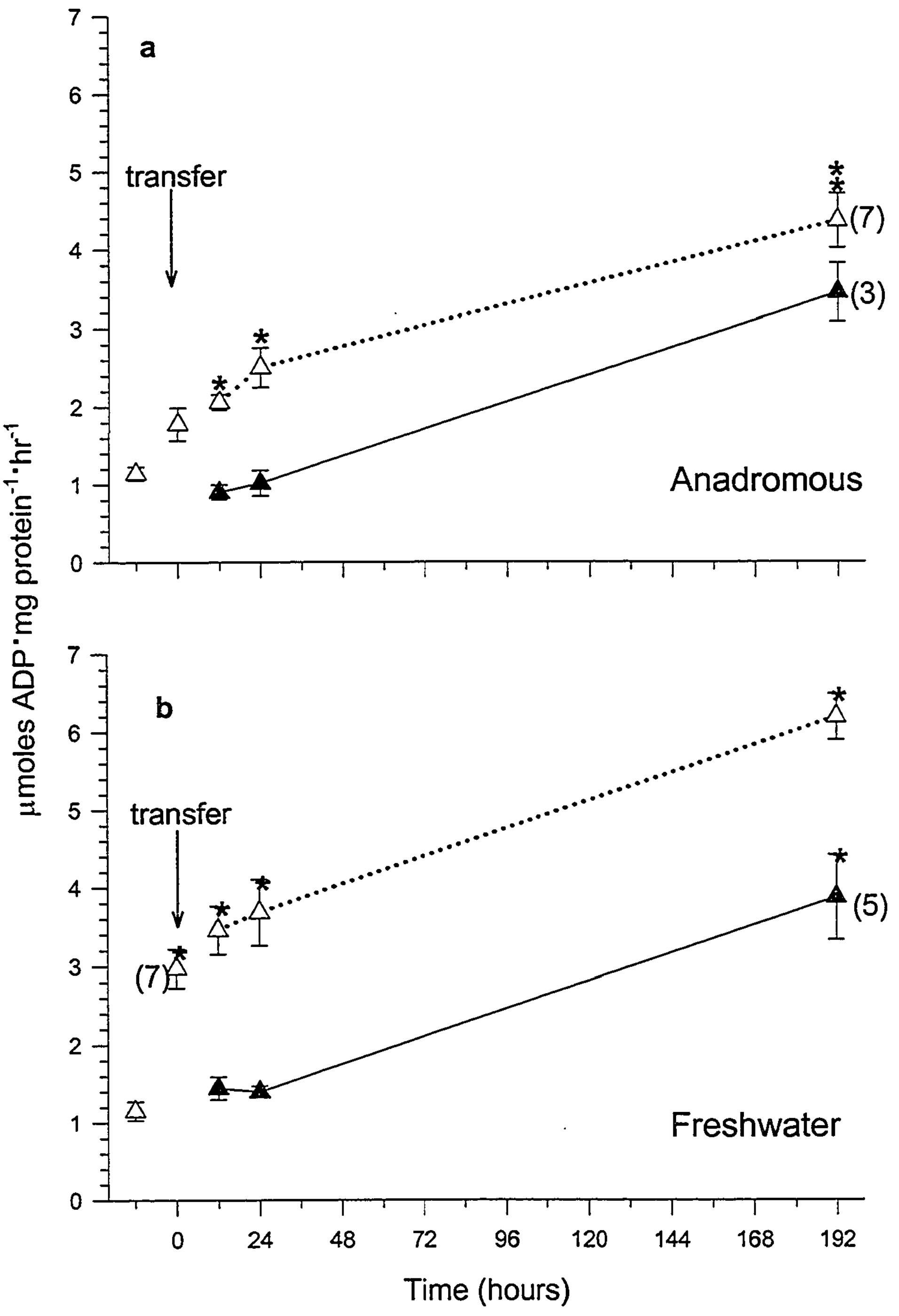

Figure 4-3. Changes in Na^+-K^+ATPase activity of anadromous (a), and freshwater (b) inconnu following transfer to 25 $^0/_{00}$ seawater, either with (acclimated —△—) or without (non-acclimated —▲—) a 2-wk acclimation period at 15 $^0/_{00}$. Grey triangles represent pre-treatment freshwater levels. Asterisks represent a significant difference ($P<0.05$) from the pre-treatment freshwater level (Kruskall-Wallis ANOVA on ranks with post-hoc Dunnett's test). Numbers in parentheses represent sample sizes if other than n=6.

5. Genetic relationships among inconnu, (*Stenodus leucichthys*), populations in the western Canadian Arctic: implications for phylogeography and the origin of life history forms

Introduction

Pleistocene glaciation events played a major role in shaping the current distribution and genetic population structure of northern flora and fauna (Denton and Hughes 1981; Pielou 1991). This was particularly important for freshwater fish. During glacial maxima, many populations were extirpated or forced to move to geographically isolated refugia where ecological or genetic differences could develop; during deglaciations, large quantities of glacial meltwater created many temporary watercourse connections that provided routes for the dispersal, recolonization and mixing of populations (Lindsey and McPhail 1986; Fulton and Andrews 1987; Hewitt 1996). Thus, the present-day fauna in any area may be the result of recolonization from one or several refugia. Evidence to support alternative hypotheses of origin should be preserved in the genotypes of individuals.

The inconnu, *Stenodus leucichthys*, is one of several salmonid species that form part of the present-day northern freshwater fish fauna. Inconnu are commonly found in large northern rivers and associated lakes of northwestern North America and northern Eurasia (McPhail and Lindsey 1970). Within North America, their current distribution spans many parts of the Yukon River system in Alaska and northwestern Canada. They also occur throughout much of the previously glaciated Mackenzie River system, south to Great Slave Lake, the Slave and Liard rivers, and Beaufort Sea coastal drainages from Alaska to the Anderson River in the western Northwest Territories (Scott and Crossman 1973)(Fig. 5-1).

In the Mackenzie River system, Howland et al. (2000) documented both freshwater and anadromous inconnu, raising the question as to the origin of these two life history forms. Were they genetically distinct at the time of their invasion of the upper and lower Mackenzie system, respectively, or are they derived from a single colonization and have diverged subsequently? Fossil remains (Cinq-Mars 1979; Cumbaa et al. 1981) and current distributional patterns (McPhail and Lindsey 1970) suggest that inconnu survived the pleistocence glaciations in the Yukon watershed of Beringia and in Arctic Siberia. Fishes, including inconnu, from theYukon basin may have gained access to the now separated Mackenzie system by one or more routes (Bodaly and Lindsey 1977; Lindsey and McPhail 1986; Fig. 5-1): (1) connections between the unglaciated portion of the Peel River (currently Mackenzie River system) and the Porcupine River (Yukon River system) during the Wisconsinan glaciation or between the Porcupine and Rat rivers (Mackenzie system) through McDougall Pass during post-glacial times, (2) migration along the Arctic coastline from northern Alaskan drainages, and (3) connections between the the Yukon River headwaters and the upper Liard River during the last glacial retreat. Other smaller refugia that may have harboured inconnu during the pleistocene glaciations include the Nahanni (Ford 1976) and Cape Bathurst areas (McPhail and Lindsey 1986). Thus, inconnu could also have gained access to the Mackenzie by: (4) connections between the Nahanni River and the Liard River in post-glacial times and (5) migration along the Arctic coastline from the Anderson River (Fig. 5-1).

Analysis of variation in mitochondrial DNA (mtDNA) has proven to be extremely useful in phylogeographic studies because of its high rate of evolution relative to nuclear DNA, its maternal inheritance, and its lack of recombination (Wilson et al. 1985; Avise 1987; Hillis et al. 1996). Indeed, a number of recent studies have successfully used mtDNA to address questions related to the post-glacial dispersal and historical events responsible for the current distribution and genetic population structure in a variety of North American and European species (e.g., Wilson et al. 1996; Durand et al. 1999; Hansen et al. 1999; Taylor et al. 1999)

In this study I surveyed inconnu populations (mainly North American) for sequence variation in a portion of the mtDNA genome to: **i)** determine factors that may have been important in the initial dispersal, colonization, and establishment of North American populations following the last glaciation, and **ii)** test the hypothesis that freshwater and anadromous forms of inconnu in the Mackenzie River system are of the same origin and have subsequently diverged. Comparisons of molecular genetic information from inconnu populations, both within and outside the Mackenzie River system, provide insights about how this species initially colonized the Mackenzie Basin, and the relative levels of reproductive isolation among current populations.

Methods

Sample collection and preservation

Eighty nine inconnu from one Siberian and 12 North American populations were included in this study (Fig. 5-2, Table 5-1). The North American populations can be grouped into 5 regions which are important from a phylogeographic perspective: Great Slave Lake, Lower Mackenzie River, Wood Bay, Yukon River system, northwest Alaska (Table 5-1). Approximately equal numbers of freshwater and anadromous populations (N=6 and 7, respectively) were included; however, these were not evenly distributed among geographic regions (Table 5-1). Because of the close relationship between *S. leucichthys* and least cisco (*Coregonus sardinella)*, peled (*C. peled)* and broad whitefish (*C. nasus)* (Lockwood et al. 1993; Smith and Todd 1993; Reist et al. 1998), representatives from each of these species were included as outgroups.

A variety of tissues and preservation methods were used. Fresh liver, adipose and pectoral fin clips and scales (with epithelial tissue attached) were preserved in 70% or 95% ethanol. As well, tissues and whole fish were stored at –20 °C for up to two months and then shipped to the Freshwater Institute in Winnipeg where whole fish were processed and tissues were stored at –40 °C (liver) or in a salt-saturated 20% dimethyl sulphoxide, 0.05 M EDTA solution (white muscle). With the occasional exception where there was not enough starting material, or a frozen sample had degraded, all of the above produced mtDNA suitable for PCR amplification.

MtDNA extraction and PCR amplification

Genomic DNA from the Selawik and Kobuk River samples was extracted following the protocol outlined in Miller et al. (1998). For all other samples, genomic DNA was extracted from approximately 25 mg of tissues using Qiagen DNA extraction columns, then air-dried, and resuspended in 50-200 μl of TE buffer.

Two mtDNA segments were amplified with the Polymerase Chain Reaction (PCR) using the following sets of primers, complementary to the flanking conserved tRNA and rRNA genes in these two regions:

Cytochrome B (cytB) (Patton 1993)
5'-GAAAAACCA(CT)CGTTGT(TA)ATTCAACT-3'
5'-GAGCTACTAGGGCAGGCTCA-3'

Control region (Lee et al. 1995)
5'-TTCCACCTCTAACTCCCAAAGCTAG-3'
5'-TATGCTTTAGTTAAGGCTACG-3'

These regions were chosen because they exhibit variability in other salmonids: the cytochrome B region in inconnu (Miller et al. 1998) and sockeye salmon, *Oncorhynchus nerka* (Patton 1993), and the control region in chum, *O. tshawytscha* and chinook salmon, *O. keta* (Cronin et al. 1993).

PCR reactions were carried out in a total volume of 100 µl with 10 µl 10X PCR buffer (Roche), either 1.75 (cytB) or 2.0 (control region) mM $MgCl_2$, 2 µl of dNTP mix (0.2 mM of each of the four deoxynucleotides), 4 µl of each 20 µM primer, 1.5 units of Taq DNA polymerase, and 1.5 µl of whole DNA. Cycling parameters for the cytB segment were: 32 cycles of 95 °C for 45 sec, 53 °C for 30 sec, and 70 °C for 2.5 min, while parameters for the control region were: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec, 52 °C for 1 min, and 72 °C for 2 min, followed by a final 20 min extension at 72 °C.

PCR products were evaluated by electrophoresis and suitable amplification products were purified using the Qiagen Qiaquick Purification Kit. Purified DNA was quantified spectrophotometrically and then diluted to 20 ng/µl with ultra-pure, distilled, autoclaved water. Sequencing reactions were carried out in a 20 µl volume with 30 ng of purified DNA, 0.4 µM of primer, 4 µl of half term (BioCan) and 4 µl of d-rhodamine dye terminator mix (Applied Biosystems). The original PCR primers were used to sequence the entire cytochrome b segment and the tRNA proline end (left domain) of the control region, while two internal primers were developed to sequence the tRNA phenylalanine end or right domain (DLPHE-1 5'-TGTCAAACCCCGAAACCAGG-3'), and the central region (DLPHE-2 5'- TATCGAGGCTTCCTGGTTTCGC-3') of the control region. Termination PCR conditions were: one cycle of 94 °C for 4 min, followed by 25 cycles of 94 °C for 30 sec, T_{anneal} for 15 sec, and 60 °C for 4 min, with the following annealing temperatures: CytB1/B2 56 °C, D-loop Pro 52 °C, DLPHE-1 61 °C, DLPHE-2 62 °C. The amplified, fluorescently labeled, terminated DNA was purified using Edge Gel Filtration Cartridges (BioCan) and analyzed with an ABI 377 automated sequencer.

Sequence alignment

Sequences were aligned using MacVector (v. 7) sequence analysis software and all variable sites were verified from chromatograms. Several fish (*S. leucichthys* and outgroup species) possessed large indels in the right domain of the control region (Fig. 5-3). Within *S. leucichthys*, indels were identified by eye, removed and analyzed separately (see results for details). The remaining sequence was used in intraspecific analyses as outlined below. The right domain (a 390 bp section flanked by the tRNA phenylalanine gene at the 3' end; Fig. 5-3) was omitted for the interspecific phylogenetic analyses due to its characteristic pattern of repeating motifs and large indels, which made it difficult to determine sequence homology across species.

Phylogenetic Analysis

The genetic relationships between *S. leucichthys* haplotypes were analyzed by constructing a parsimony network based on mutational steps between sequences. Estimates of evolutionary divergence or percent sequence divergence between haplotypes were calculated following Tajima and Nei (1984). Genetic relationships among populations were assessed by constructing neighbor-joining dendrograms (Saitou and Nei 1987) using PHYLIP v. 3.6 (Felsenstein 2001). Genetic distances were calculated in ARLEQUIN v. 2.001 (Schneider et al. 2000) using average pairwise sequence divergence between populations and regions with Kimura's (1980) 2-parameter model, corrected for within population/region variation (Nei 1987). Given that our outgroup species were quite distant (25-35% sequence divergence) relative to our ingroup (< 1% sequence divergence), I chose to analyze our ingroup separately and secondarily attach the outgroup taxa (Hendy and Penny 1989; Nixon and Carpenter 1993). Prior to this, however, I constructed a neighbor joining dendrogram that included our outgroup species to determine the root location.

Analysis of Population Structure

The level of genetic variability within populations and within geographic regions was measured using haplotype diversity, which was calculated according to Nei (1987). Homogeneity among populations within regions and life history types (freshwater and anadromous) and among geographic regions (pair-wise comparisons) was tested with GENEPOP v. 3.1d (Raymond and Rousset 1995) using exact tests on haplotype frequencies. Genetic differentiation among populations was tested with ARLEQUIN v.

2.001 (Schneider et al. 2000) using a two-level hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992), with the first level of the hierarchy being either different geographic regions or life history types (freshwater versus anadromous), and the second level, populations within each of those regions or life history types. Two models of geographic regional structure were tested: 1) the regional structure predicted from zoogeographic history of the western Arctic (Table 5-1 and 2) the structure suggested by exact tests of homogeneity among populations (Yukon River, northwest Alaska and Wood Bay regions combined, and Lower Mackenzie River and Great Slave Lake regions combined). In all analyses, populations with N < 6 (Kluane Lake, Ob River) were excluded.

Results

Sequence variation

The cytochrome B segment was approximately 1150 bases long and showed very low variation among individuals. A total of 35 inconnu representing different populations were surveyed for sequence variation at the 5' end of this segment using the cytB1 primer but no variable sites were identified. A smaller subset of 13 fish were surveyed at the 3' end using the cytB2 primer. Only one variable site (a transition, C ↔ T) was found at position 1013. Because of this low level of genetic variability, I excluded the cytochrome B segment from further analyses.

The control region was approximately 1200 bases long (Fig. 5-3) although some length variants were up to 1500 bases long due to large insertions. The more variable regions in both the left and right domains (Fig. 5-4) were sequenced for all 89 inconnu. The 240 bp central conserved region was sequenced for a subset of eight fish that could be differentiated based on variation in the right and left domains. Because no variation was seen among these fish, this region was not sequenced for the remaining samples. A total of nine haplotypes were identified with all of the sequence variation resulting from transitions (A ↔ G being the most common) (Table 5-2). The number of substitutions among individuals ranged between 1 and 4, corresponding to sequence divergences of 0.08% and 0.3%, respectively. Haplotype **a** was the most common, being present in over half of the fish surveyed. Haplotypes **b** and **d** were each present in 13% of the fish and the remaining haplotypes were each present in less than 10% of the fish (Table 5-2).

Eight inconnu contained insertions of varying sizes in the expansion region of the right domain of the control region. The right domain in inconnu appears to be characterized by a series of 1 to 6 repeated motifs (Fig. 5-5). In the majority of fish, I identified four copies of short sequences (~22 bp) that were similar to the light strand promotor sequence (1, 3, 5, Fig. 5-5), interspersed with four longer sequences (~65 bp) containing the putative transcription binding site (2, 4, 6, Fig. 5-5) (Fig. 5-6a). Length variants consisted of indels that were copies of one or more of these repeated sequence blocks (Fig. 5-6b). These copies were nearly always made up of a continuous string of from 1 to 7 repeated sequence blocks, indicating that they most likely resulted from single mutations. An exception was the sample from the Ob River which contained indels that appeared to have resulted from up to four separate mutation events. These mutations may have become fixed within inconnu from the Ob River population or the Siberian region over time; however, I cannot determine this with a sample size of one. Among the North American inconnu, there did not appear to be any consistent pattern of length variation, suggesting that such mutations, although relatively frequent, have occurred randomly and have not become fixed in these populations. The three outgroup species showed a similar pattern of repeated short and long sequences (Fig. 5-6c-e), most of which showed high congruence with those of inconnu (Fig. 5-5). Although only a small number of individuals were sequenced for each outgroup species (1 to 3 fish), length variants were observed within *C.sardinella* (Fig. 5-6d), indicating that this phenomenon also occurs in coregonid species other than inconnu.

Phylogeny

Haplotypes were separated from each other by anywhere from one to four mutational steps (Fig. 5-7). Some patterns emerged from the parsimony network (Fig. 5-7). Haplotypes **d**, **e**, and **f**, which are closely related to each other and collectively most distant from haplotype **a**, were present only in the Alaskan populations and the Wood Bay sample. Haplotypes **g** and **h**, both uncommon but closely related to each other, were unique to the lower Yukon River population. Haplotype **a**, along with **b** and **c** (both only one mutational site different from **a**) were present in all populations, but were the most abundant in lower Mackenzie and Great Slave Lake areas, respectively.

The neighbor joining dendrogram shows inconnu populations in the western Arctic falling into two main assemblages, with the lower Yukon River population as a common ancestor (Fig. 5-8). On average these two groups differed by 0.42 % sequence divergence. Assemblage I comprises populations from the lower Mackenzie and Great Slave Lake regions which consist entirely of fish with either haplotypes **a**, **b** or **c** (except for one fish with the rare haplotype **i**). Within this assemblage, the Great Slave Lake populations appear to have diverged most recently from those in the lower Mackenzie. Assemblage II is composed of populations from the northwest Alaskan drainages, the upper Yukon River and Wood Bay. Nearly three-fifths of the fish in these populations possess haplotypes **d**, **e**, or **f**, which are absent from assemblage I. The remaining fish in assemblage II possess a mixture of the assemblage I haplotypes. The putative root population (least divergent from other coregonines), lower Yukon River, contains primarily the more common haplotypes from both assemblages (**a** – **d**), lacks haplotypes **e** and **f** from assemblage II, and contains two unique haplotypes, **g** and **h**, not found elsewhere. Comparisons of pairwise genetic distances between regions show that, with the exception of the Wood Bay area, genetic distance increases with geographic distance between regions (Table 5-3)

Population Structure

The Yukon River and northwest Alaskan regions showed the highest haplotypic diversity, with a relatively even distribution of the various haplotypes (Table 5-1, Fig. 5-2). In contrast, populations in the Wood Bay, lower Mackenzie and Great Slave Lake areas had much lower diversity with a particular haplotype dominating in each area. In the extreme, haplotype **a** was the only one present in the Great Slave Lake area (Table 5-1).

Haplotype frequency distributions of populations within each geographic region (Table 5-1) were homogeneous ($P>0.05$ for all regions), whereas frequency distributions of populations within each life history type differed (freshwater, $P=0.001$; anadromous, $P=0.0001$). When populations within regions were pooled, pair-wise comparisons among regions revealed that the Yukon River, northwest Alaskan, and Wood Bay regions were homogeneous, with each differing from the lower Mackenzie and Great Slave Lake populations (Table 5-3). The Alaskan and Wood Bay regions formed one homogeneous group and both differed from the lower Mackenzie and Great Slave populations (Table 5-3). Although these broad groupings were identified statistically, it should be noted that differentiation among regions, as suggested by probabilities, generally increased with geographic distance (Table 5-3).

Both models of geographic regional structure based on (1) zoogeographic history

(Table 5-1) and (2) the pair-wise tests of homogeneity, respectively, were supported by the AMOVA; however, they differed with respect to the partitioning of genetic variance (Table 5-4). In model 1 approximately 27% of the genetic variation could be attributed to variation among regions, whereas in model 2 this figure was 38%. In both models only a minimal proportion of the genetic variation (2-6%) was due to variation among populations within regions (Table 5-4). Most variation occurred within populations (56-71%), reflecting the diversity of haploypes, particularly within the Alaskan populations, which made up almost half of the samples analyzed. There was no support for genetic structuring by life history type; most of the genetic variation in this model could be attributed to variation within populations (64%) and among populations within each life history type (24%) (Table 5-4).

Discussion

Intraspecific divergence

The level of sequence divergence (0.08 to 0.4%), as well as total number of mitochondrial DNA haplotypes (N = 9) identified in my study, was low relative to freshwater fish species from unglaciated areas (e.g., Bermingham and Avise 1986) but comparable to levels observed in other salmonid species from recently deglaciated areas (Bernatchez and Wilson 1998; Redenbach and Taylor 1999; Taylor et al. 1999). Northern freshwater species such as inconnu can be expected to show lower levels of divergence than their southern counterparts, since they occur in areas where there was extensive glaciation and limited refugia. Northern regions experienced multiple glacial retreats and expansions over time, and are among the most recent to have been colonized following the final retreat of the Late Wisconsin glaciation (approximately 10,000 ya, Bodaly and Lindsey 1977). Such repeated disturbances likely extinguished most of the mitochondrial genetic diversity originally present in these species (Avise et al. 1984). Furthermore, in the case of inconnu, the relatively recent colonization over most of the Mackenzie River system would have allowed little time for new haplotypes to be established through mutation.

Genetic structuring among populations

Despite the low levels of genetic divergence, I found clear structuring with respect to frequency of mitochondrial DNA haplotypes and partitioning of genetic variance among inconnu populations of the western Arctic. Based on my findings, geography and past glacial history appear to have been dominant forces involved in shaping current patterns of genetic variation. AMOVA as well as exact tests of homogeneity suggested structuring by the following geographic regions: Wood Bay, northwest Alaska, Yukon River, Lower Mackenzie, and Great Slave Lake, with the first 3 and latter 2 regions each being more similar to each other than to other regions. With the exception of Wood Bay, the level of genetic differentiation (genetic distance and heterogeneity) between regions increased with geographic distance. In contrast, populations within regions showed high similarity to each other, despite substantial within-population variability, suggesting that degree of geographic isolation has strongly influenced genetic structuring of inconnu.

Post glacial dispersal patterns

Freshwater fish now occurring in the western Arctic of North America originated from three main refugia: 1) Beringia, made up of the Yukon River and other rivers originating from exposed areas of the Bering Sea and Siberia; 2) Cascadia, to the southwest of the glaciation, bordered by the Pacific coast and the continental divide; 3) Mississippi River basin to the southeast of the glaciation (Lindsey and McPhail 1986) and two smaller refugia in the Nahanni River basin (Ford 1976) and Cape Bathurst areas (Lindsey and McPhail 1986) (Fig. 5-1). Although inconnu occur in the Anderson River drainage of the Cape Bathurst area, results from this study do not support the hypothesis that inconnu populations in the western Arctic originate from here (see later explanation). Inconnu have an entirely northern distribution and do not presently occur in areas where the Mississippian and Cascadian refugia were situated; likewise, they are not currently distributed within the Nahanni drainage. They are, however, found throughout the Yukon basin, as well as in Arctic Siberia, suggesting that they survived past glaciations in Beringia (McPhail and Lindsey 1970). Inconnu fossil remains from the Old Crow region of the Yukon Territory, Canada, dating back 60,000 y, also provide evidence that inconnu existed within this area during the Wisconsinan glaciation (Cinq-Mars 1979; Cumbaa et al. 1981).

My genetic data support the hypothesis of a common Beringian origin for extant North American inconnu populations. First, the geographical distribution of haplotypes (in particular the presence of the **a** haplotype across nearly all populations) and the relatively close genetic relationships among inconnu populations suggest a single glacial refuge, most likely in the area of the Yukon River Basin. Second, a relatively higher level of genetic diversity (haplotypic) was observed for the Alaskan populations, particularly in the upper and lower Yukon River. This finding is consistent with the idea that these were source populations that survived past glaciations and thus have had a much longer, stable evolutionary history than populations in the Mackenzie Basin; more, specifically such populations are likely to have historically had a larger genetically effective population size which would have greatly reduced genetic drift and the consequent loss of less common haplotypes. Finally, based on the neighbor-joining dendrogram constructed from pair-wise genetic distances between populations, inconnu formed a monophyletic group, with the lower Yukon population as the most recent common ancestor.

Although the refugial origins of inconnu are clear, the specific patterns of dispersal that have led to their current distribution are more difficult to discern. When considering distribution patterns of freshwater species, particularly those with a Beringian origin, it is not only important to look at melt-water connections that may have developed during periods of glacial retreat, but also to consider drainage patterns that existed during periods of glacial maxima. The most recent glacial maximum took place approximately 18,000 ya and represents the last time that the Bering Strait was exposed creating a land bridge between Asia and North America (Lindsey and McPhail 1986; Pielou 1991). This would have been of particular significance to freshwater fish species, since it provided connections between some rivers that are now isolated from each other, while creating barriers between others. For example, at that time the Kobuk River in northwest Alaska was tributary to the Chuckchee Sea River in Siberia, which flowed north to the Arctic Ocean along with other Siberian rivers, such as the Kolyma and Lena, whereas the Yukon River flowed south draining into the Bering Sea (Lindsey and McPhail 1986). Thus, fish populations in the northwest Alaskan rivers would have had the opportunity to mix with those from Siberia, but would have been isolated from those in the Yukon River.

With the melting of the glaciers and creation of the Bering Strait, these ancient river beds flooded with salt water, isolating freshwater fish populations in the northwest Alaskan rivers from those in Siberia. However, migration between the Yukon and northwest Alaskan rivers would now be possible for brackish-tolerant species such as inconnu, given the relative proximity of their river mouths. Indeed, our genetic data show a close relationship between northwest Alaskan and Yukon River populations. The

Northwest Alaskan populations, however, have a some what lower haplotype diversity, suggesting that they have a more recent origin than expected based on the historical geography of this region. Due to a low sample size, I was unable to compare Siberian and Alaskan populations; however, comparisons of morphological traits reveal similarities on both sides of the Bering Strait (Walters 1955; Lindsey and McPhail 1986).

Within this context we can consider the hypothesized dispersal routes by which inconnu gained access to the now separated Mackenzie watershed (Bodaly and Lindsey 1977; Lindsey and McPhail 1986). The most probable route was via connections between the unglaciated portion of the Peel River (Mackenzie River system) and the Porcupine River (Yukon River system) during the Wisconsinan glaciation or between the Porcupine and Rat rivers through McDougall Pass during post-glacial times. The Peel River was the only portion of the present Mackenzie system to remain unglaciated during the Pleistocene glacial advances (Bodaly and Lindsey 1977) and thus could have harbored inconnu and other species during the last glacial period (Lindsey and McPhail 1986). However, river conditions were presumably harsh and would have prevented the persistence of many fish species at that time (Bodaly and Lindsey 1977). The Peel River population does not show the high diversity of haplotypes found in the Yukon River populations, and is homogeneous with other lower Mackenzie River populations, suggesting that the Peel River was not colonized by inconnu until later, when the rest of the Mackenzie began to open up.

Our genetic data provide strong supporting evidence that inconnu populations throughout the lower Mackenzie River system originated from those in the Yukon River. After Great Slave Lake, inconnu in the lower Mackenzie are most similar in haplotype frequency to those in the Yukon, and based on our neighbor-joining dendrogram, inconnu from the lower Yukon River appear to have been the most recent common ancestor to populations in the lower Mackenzie. In turn, individuals from the lower Mackenzie appear to have colonized the Great Slave Lake area more recently. Inconnu from the latter area are most closely related to lower Mackenzie populations (both with respect to genetic distance and haplotype frequency) and thus the lower Mackenzie is the likely source from which the Great Slave Lake region was colonized. The complete lack of diversity in the Great Slave Lake populations suggests a population bottleneck or a fairly recent invasion and founder effect or with little subsequent immigration. This is supported by geological evidence that shows that, until relatively recently (ca. 6100 ya), the upper Mackenzie River system was blocked to upstream fish migration by impassable waterfalls (Lindsey and McPhail 1986) (Fig. 5-2).

A second possible route of dispersal into the Mackenzie River system was migration along the Arctic coastline from northwest Alaskan drainages. The lack of genetic differentiation between inconnu populations in the Yukon and northwest Alaskan rivers makes it difficult to distinguish which of these routes inconnu used to gain entry to the Mackenzie. Tests of haplotype frequency distribution and genetic distance suggested a close genetic relationship between all the Alaskan populations, and all possessed haplotypes that were also present in Mackenzie populations.

A third possible dispersal route was through connections between the Yukon River headwaters and the upper Liard River created by watershed reversals during the last glacial retreat. I believe that this route of entry was unlikely for several reasons: 1) It would have placed inconnu upstream of the Grand Canyon in the Liard River (an impediment to the migration of most species) (Fig. 5-2), and would have required subsequent extirpation or emigration from this area as none presently occur there; 2) Inconnu in the Great Slave Lake area (population(s) with the closest proximity to Liard River) are more closely related genetically to those in the Lower Mackenzie than to those in the Upper Yukon River system, a pattern which exists for most other species in the Mackenzie River system with known Beringian origins (Pielou 1991); 3) Inconnu in the Great Slave Lake area show extremely low genetic diversity suggesting either a population bottleneck or recent colonization. Colonization via the Liard could only have taken place with the creation of pro-glacial lakes during the glacial retreat (between ca 10,000 and 9,500 ya), considerably earlier than the route from the lower Mackenzie (Lindsey and McPhail 1986).

The Wood Bay population is somewhat unique in that it is most similar genetically to populations in Alaska, yet geographically closer to the Mackenzie River system. Although geographically close, it lacks a direct freshwater connection to the Mackenzie. My neighbor-joining dendrogram suggests that the Wood Bay population is more closely related to Alaskan fish than populations in the Mackenzie Basin (Fig. 5-8). This hypothesis is supported by the presence in the Wood Bay population of the **d** haplotype, which is not present in either the Lower Mackenzie or Great Slave areas and the absence of the **a** haplotype that predominates in the Mackenzie basin. The latter also suggests a lack of subsequent mixing between Wood Bay and the lower Mackenzie. Given that the Wood Bay area was partially open at a time when the Mackenzie Basin was still glaciated (Lindsey and McPhail 1986), colonization of the former most likely occurred via a coastal route of dispersal by fish moving eastward from Alaska. Alternatively inconnu could have remained in this river system from pre-Pleistocene times since the area at the base of Cape Bathhurst, including the eastern margin of the Anderson River Basin, persisted as an unglaciated refugium throughout the Pleistocene glaciations (Lindsey and McPhail 1986).

Origin of distinct life history types

Freshwater and anadromous forms of inconnu occur not only in the recently glaciated Mackenzie River system (Howland et al. 2001), but also in former refugia of Siberia (Berg 1948) and the Yukon River, Alaska (Alt 1977, 1988). Thus, both life-history forms may have been in existence prior to the final glacial retreat some 10, 000 ya. Extant populations may have arisen through a single ancient split into two forms which then independently colonized existing drainages following the most recent glaciation. Alternatively, extant freshwater populations could have arisen through multiple, independent divergences from anadromous populations following post-glacial dispersal and establishment in different waters.

Several aspects of our results support the latter scenario: 1) My homogeneity tests showed that populations of the same life history type, but from different geographic regions were more likely to be different from each other than those of different life history types but of the same region; 2) A low percentage of the genetic variation could be accounted for by life history type, whereas a high proportion of the variation was due to differences among populations within a given type; 3) My neighbor-joining dendrogam did not show the two life history forms to be monophyletic, but rather revealed groupings by geographic region. The general lack of concordance between genetic patterning and life history types suggests that freshwater forms have repeatedly arisen from the anadromous form or vice-versa.

For other species where alternate migratory strategies appear to have repeatedly evolved (Hindar et al. 1991; Taylor and Bentzen 1993; Taylor et al. 1996), freshwater or land-locked populations are generally thought to have arisen from the anadromous form (but see Taylor et al. 1999 for an exception). Anadromous types are likely to be better dispersers because of their tendency to undertake longer migrations and their abilities to withstand a wide range of salinities (McDowell 1988). Freshwater populations may lose such abilities over time due to lack of selective pressure in their new environment. Freshwater populations of inconnu (Howland et al. 2001), and other species (Birt and

Green 1993; Eliassen et al., 1998) can experience a decline in euryhaline abilities over even relatively short periods of time (e.g., less than 6, 000 years for inconnu).

Conclusions

The genetic relationships I observed in this study indicate that inconnu in the Mackenzie River basin originated from the Yukon River system; however, the specific route by which they colonized this region remains unresolved. The degree of geographic isolation and past glacial history appear to have been the dominant forces involved in shaping the current patterns of genetic variation of inconnu, whereas life history pattern (freshwater versus anadromous) has had only a minor influence. Existing freshwater populations likely arose through multiple, independent divergences from anadromous populations in more recent times following post-glacial dispersal and establishment in different waters.

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Table 5-1. Relative haplotype frequencies and haplotype diversity by population and geographic area.

Area	**Populations**	**Life History Type**	**n**	**Relative Frequency of MtDNA Haplotypes (%)**										**Haplotype diversity**
				a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	***h* (±SD)**
Great Slave Lake	Buffalo R.	Freshwater	6	100	-	-	-	-	-	-	-	-	-	0
	Slave R.	Freshwater	6	100	-	-	-	-	-	-	-	-	-	0
	Marion L.	Freshwater	6	100	-	-	-	-	-	-	-	-	-	0
			18	**100**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
Lower Mackenzie River	Arctic Red R.	Anadromous	9	67	22	11	-	-	-	-	-	-	-	0.49 (0.24)
	Peel R.	Anadromous	10	60	30	10	-	-	-	-	-	-	-	0.54 (0.18)
	Campbell L.	Freshwater	10	70	20	-	-	-	-	-	-	10	-	0.46 (0.27)
			29	**64**	**24**	**7**	**-**	**-**	**-**	**-**	**-**	**3**	**-**	**0.51(0.21)**
Wood Bay	Anderson R.	Anadromous	**10**	**-**	**20**	**10**	**70**	**-**	**-**	**-**	**-**	**-**	**-**	**0.46(0.27)**
Yukon River	L. Yukon R.	Anadromous	9	22	22	11	11	-	-	11	22	-	-	0.82 (0.020)
	U. Yukon R.	Freshwater	6	33	-	17	17	17	17	-	-	-	-	0.78 (0.037)
	Kluane L.	Freshwater	2	-	-	50	50	-	-	-	-	-	-	0.50 (0.00)
			17	**24**	**12**	**12**	**18**	**6**	**6**	**12**	**12**	**-**	**-**	**0.85(0.017)**
Northwest Alaska	Kobuk R.	Anadromous	8	25	-	25	38	13	-	-	-	-	-	0.72 (0.051)
	Selawik R.	Anadromous	6	17	33	-	-	50	-	-	-	-	-	0.61 (0.11)
			14	**21**	**14**	**14**	**21**	**29**	**-**	**-**	**-**	**-**	**-**	**0.79(0.025)**
Siberia	Ob R.	Anadromous	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**100**	**-**

Table 5-2. Number of, frequency and sequences of mtDNA haplotypes for inconnu, *Stenodus leucichthys*, and several outgroup species.

Haplotype	n	%	variable nucleotide positions relative to 5′ tRNA proline end of control region																																										
			12	13	14	16	21	22	24	35	38	39	48	54	55	56	57	63	75	114	115	119	128	129	139	162	187	188	243	246	247	248	257	273	303	314	321	375	376	383	401	481	503	504	962
<u>S. leucichthys</u>																																													
a	45	50.6	T	C	T	G	A	C	G	G	C	C	T	A	A	C	C	A	G	T	T	A	G	A	C	A	C	A	C	A	G	G	A	G	C	G	C	A	A	G	C	T	C	A	G
b	13	14.6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
c	8	9.0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
d	13	14.6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	A
e	5	5.6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	A
f	1	1.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	A
g	1	1.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
h	2	2.2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
i	1	1.1	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>Out groups</u>																																													
C. sardinella 1	1		A	A	A	A	G	.	A	A	T	.	C	-	-	A	G	G	A	C	.	.	.	G	.	G	T	G	A	.	.	.	.	.	A	.	T	.	.	T	A	C	A	T	?
C. sardinella 2	1		A	A	A	A	G	.	A	A	T	.	C	-	-	A	G	G	A	C	.	.	.	G	.	G	T	G	A	.	.	.	G	.	.	.	T	.	.	T	A	.	A	T	?
C. sardinella 3	1		A	A	A	A	G	.	A	A	T	.	C	-	-	A	G	G	A	C	C	C	.	G	.	G	T	G	A	.	.	.	.	.	A	.	T	.	.	T	A	C	A	T	?
C. peled	1		A	A	A	A	G	.	.	A	T	.	C	-	-	A	G	G	A	C	.	.	.	G	.	G	T	G	A	.	.	.	.	.	A	.	T	.	.	T	A	C	A	T	?
C. nasus 1	1		A	A	A	A	G	T	.	A	T	T	C	-	-	A	G	G	A	C	.	.	A	G	A	G	T	.	.	G	A	T	.	A	A	A	T	G	G	A	A	C	A	T	?
C. nasus 2	1		A	A	A	A	G	T	.	A	T	T	C	-	-	A	G	G	A	C	.	.	A	G	A	G	T	.	.	G	A	T	.	A	A	.	T	G	G	A	A	C	A	T	?
C. nasus 3	1		A	A	A	A	G	T	.	A	T	T	C	-	-	A	G	G	A	C	.	.	A	G	A	G	T	G	.	G	A	T	.	A	A	A	T	G	G	A	A	C	A	T	?

Table 5-3. Pairwise genetic distances (above diagonal) and exact tests of homogeneity in the distribution of haplotype frequencies (below diagonal) between inconnu from five different geographic areas in northwestern North America. Overall average distances were calculated using Kimura's (1980) two-parameter model, corrected for within region variation (Nei 1987).

	Great Slave Lake	Lower Mackenzie River	Yukon River	Northwest Alaska	Wood Bay
Great Slave Lake	-	0.054	0.448	0.859	1.425
Lower Mackenzie River	0.02344*	-	0.19	0.514	0.933
Yukon River	0.00000**	0.00255**	-	0.029	0.27
Northwest Alaska	0.00000**	0.00038**	0.54081	-	0.094
Wood Bay	0.00000**	0.00000**	0.05758	0.07531	-

* denotes significance at $P<0.05$, **denotes significance at $P<0.005$ using a Bonferroni correction for multiple pairwise comparisons

Table 5-4. Results of two-level hierarchical analysis of molecular variance (AMOVA). Model 1 is the regional structure predicted from zoogeographic history of the western Arctic (see Table 5-1), and model 2 is the structure suggested by exact tests of homogeneity among populations (Yukon, northwest Alaskan and Wood Bay regions combined, and lower Mackenzie and Great Slave Lake populations combined; see Table 5-3). The test determines the probability of finding, by chance, a variance component or phi-statistic more extreme than the observed value.

Source of Variation	Variance	% total variance	ϕ statistic	*P*
By Region (Model 1)				
Among regions	0.154	27.07	ϕ_{CT}=0.271	<0.001*
Among populations within regions	0.007	1.19	ϕ_{SC}=0.016	0.233
Within populations	0.408	71.74	ϕ_{ST}=0.283	<0.001*
By Region (Model 2)				
Among regions	0.27	38.55	ϕ_{CT}=0.385	0.004*
Among populations within regions	0.039	5.58	ϕ_{SC}=0.091	0.029*
Within populations	0.392	55.58	ϕ_{ST}=0.441	<0.001*
By Life Hstory Type				
Among life history types	0.074	12.03	ϕ_{CT}=0.120	0.094
Among populations within life history types	0.148	24.14	ϕ_{SC}=0.274	<0.001*
Within populations	0.392	63.83	ϕ_{ST}=0.362	<0.001*

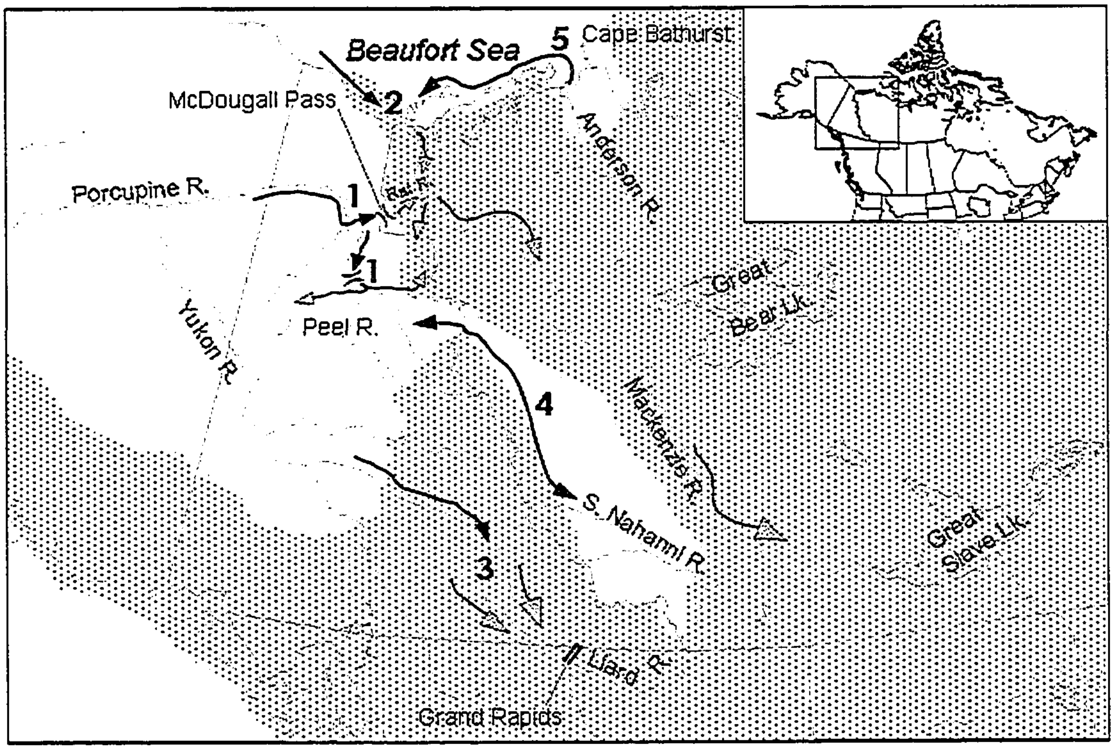


Figure 5-1. Study area showing potential routes by which inconnu from the Yukon drainage may have gained access to the now separated Mackenzie drainage. (1) Connections between the Porcupine River (Yukon system) and the Peel or Rat rivers (Mackenzie system); (2) Migration along the Arctic coastline from Beringia; (3) Connections between Yukon River headwaters and the upper Liard River (Mackenzie system); (4) Connections between proglacial lakes of the south Nahanni River and the headwaters of the Peel River (5) Migration along the Arctic coastline from the Cape Bathurst area. See text for details. Numbers correspond to those listed in the text. Shaded areas represent maximum extent of Wisconsinan glaciation.

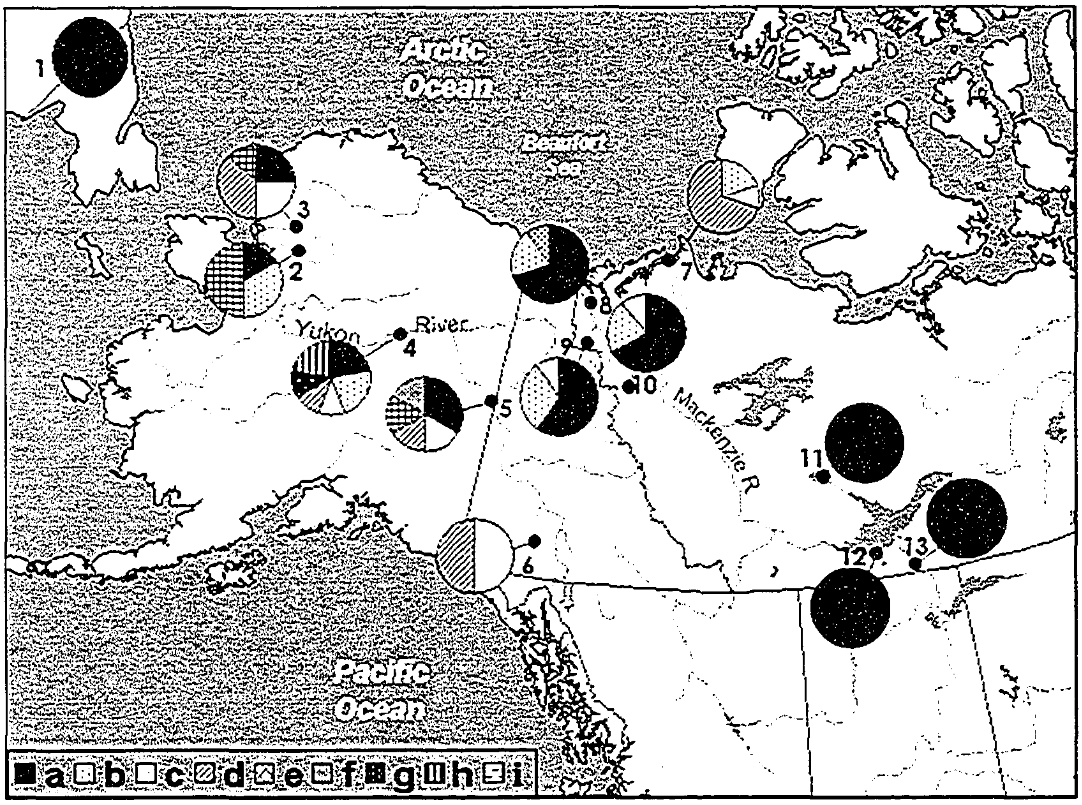


Figure 5-2. Haplotype frequency distribution for the 13 populations analyzed. Patterns correspond to the nine different haplotypes (see Table 5-1). Numbers correspond to different sample collection sites: 1-Ob R., 2-Selawik R., 3-Kobuk R., 4-Lower Yukon R., 5-Upper Yukon R., 6-Kluane L., 7-Wood B., 8-Campbell L., 9- Peel R., 10-Arctic Red R., 11-Marion L., 12-Buffalo R., 13-Slave R.

```
tRNA Proline
                                         *
TTAAACTACC CTCTGGCGCC ACAGTGTACA TGGTGAACCA TATATCCTCA TACAACCTGT GTATATATTA   70
                                                               *
CACCGCTATG TATAATATTG CATATTATGT ACTGACCCAT ATATTATTAT TGCACGTGAG TAGTACATCC  140
                                                  *
TATGTATTAT CAACATAAGT GATTTTAAGC CCTCATACAT CAGCACCATT CCAAGGTTTA CATTAAGCAA  210

GACTCGGATA ATCACCAACG GAACCGTTCT AACCTAGGTA ATTGCTAAAC AACAAACCTC CAGCTAACAC  280

GGGCTCCGTC TTTACCCACC AACTTTCAGC ATCGGTCCCG CTTAATGTAG TAAGAACCGA CCAACGATTT  350
                          **
ATCAGTAGGC ATACTCTTAA TGATAATCAG GGGCAAATAT CGTATTAGGT CGCATCTCGT GAATTATTAC  420

TTGCATCTGG TTCCTATTTC ATGGGCTATC CTTAAGAAAC CACCCCCTGA AAGCCGAATG TAATGCATCT  490

GGTTAATGGT GTCAACCTTA CTGTTCGTTA CCCACCTAGC CGGGCGTTCT CTTATATGCA TAGGGTTCTC  560

CTTTTTTTTT TTTCCTTTCA GCTTGCATAT ACAAGTGCAC ACCGAGAAGT CTAACAAGGT CGAACTAGAT  630

CTTGGTCTCC AGCGGACACA ATAATAATGG CGGAATGATA TTCTATAAAG AATTGCATAA TTGATATCAA  700

GTGCATAAGG TCAGTTTCTT TCTTCATAGA TACCTAAGAT CTCCCCGGCT TTTGCGCGGC TAAACCCCCC  770
                                                   internal primer 2
TACCCCCCTA CGCTGAGCGA TCCTTATTAC TCCTGTCAAA CCCCGAAACC AGGAAGCCTC GATAGCGCTA  840
                                         internal primer 1
TTACCCATCA AACCGTACAT TAATAAACTT TTGTTATACT TAACAAACTT TGGCACCGAC AACCCTATTA  910
                                                        *
TCAAAACTAC CCTTTAATTA AGATATACAT TAATAAACTT TTGTTATACT TGACAAACTT TGGCACCGAC  980

AACCCTATTA TCAAAGCACC CTTTAATTAA GTATGCATTA ATAAACTTTT TGTATACATT AATAAACTTT 1050

TTATTGTACT TACAAACTTT GGCACCGACA ATCCTATTAT CAAAGCCACC CTTTAATTAA AGTATACATT 1020

AATGAACTTT TTGTTATACT TAACAAACTT TGGCACCGAT AACCCTATCA TAAGGACCCC CCCCATTAGA 1090

ATGTACGCTG GCGTAGCCTT AACTAAAGCA TA
                  tRNA Phenylalanine
```

Figure 5-3. Control region sequence for the most common haplotype (**a**) found in inconnu, including sequences for the flanking tRNAs. Variable sites among inconnu are indicated by an asterisk, the central conserved region is underlined, and internal primers used in sequencing reactions are indicated by a dashed line.

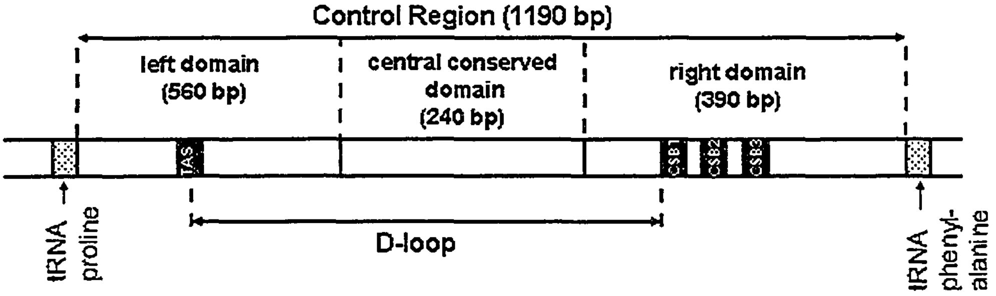


Figure 5-4. Schematic of mitochondrial control region in inconnu, *S. leucichthys,* showing flanking transfer RNAs, the left domain containing the conserved termination association sequence (TAS) and more variable sections where the majority of base substitutions were found, the central conserved domain, and the right domain consisting of conserved sequence blocks (CSB1-3) interspersed with a series of repeated motifs and indels of varying sizes.

Stenodus leucichthys

```
1.    TACATTAATAAACTTTT-GTTA
2a.   ...-....C.......GGCACCGACAACCCTATTATCAAAACTACCCTTTAATTAAGATA
2b.   ...-....C.......GGCACC...................................-..
2c.   ...-....C.......GGCACC..................G.-..............-..
2d.   ...-..G.C.......GGCACC..................G.-..............-..
3a.   .................T.-..
3b.   .G...............T.-..
3c.   .................TA..G
4a.   ...-...-C.......GGCACC.....T............G.C.............AG..
4b.   ...-....C.......GGCACC.....T............G.C.............AG..
4c.   ...-...-C.......GGCACC..................G.-.............AG..
5.    .........G.......T....
6.    ...-....C.......GGCACC..T........C..A.GG..CC...CCC.T.AG.ATGTAC
```

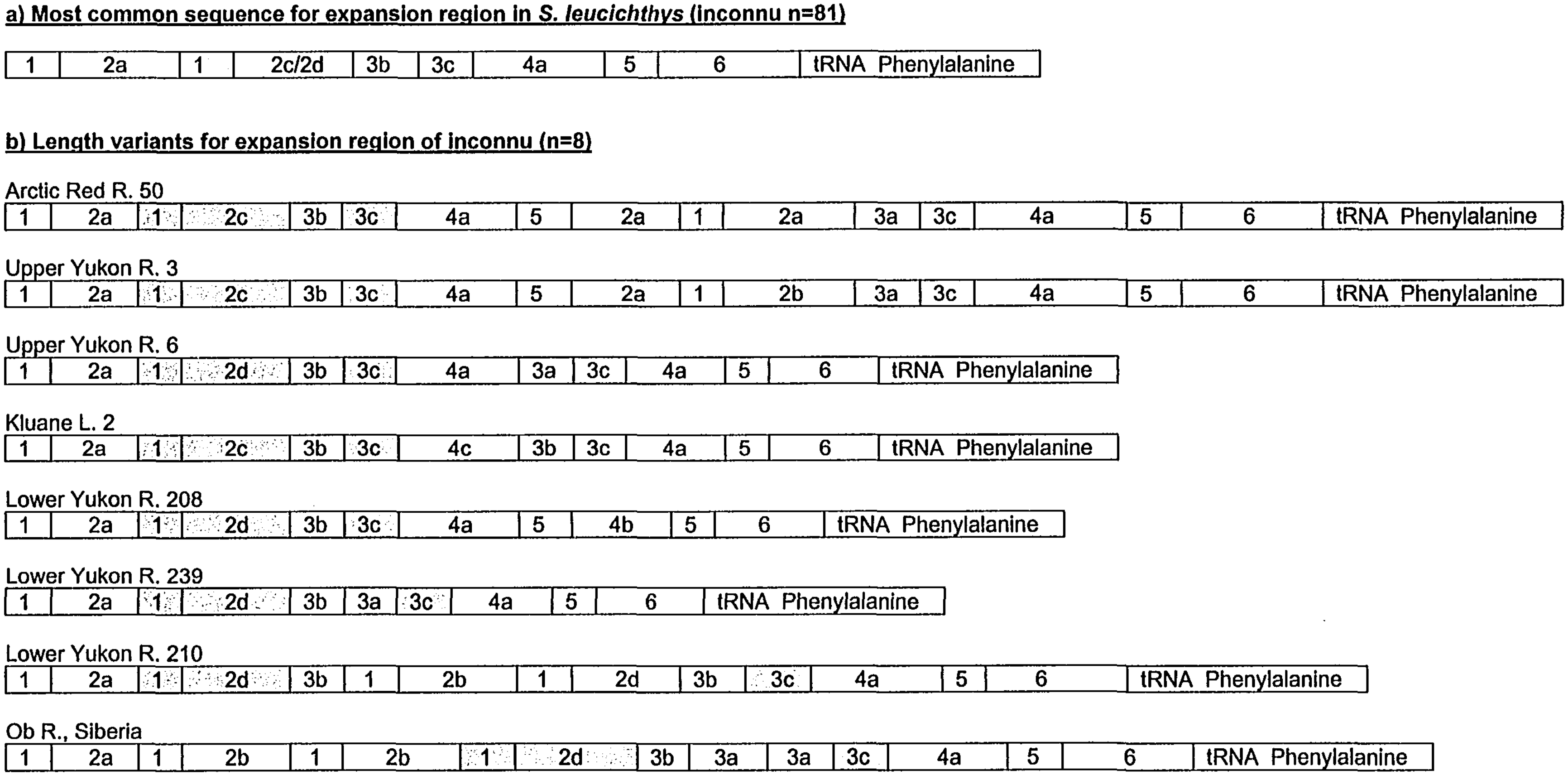
Coregonus sardinella* and *C. Peled

```
1.    .................-TA..
2a.   ...-.C..C.......GGCACC................G.G.C....C........AG..
2b.   ...-.C..C.......GGCACC....................CG...CC-......AG..
2c.   ...-.C..C.......GGCACC.....T..............CG...CC.......AG..
3a.   .C..C.--........TA...
3b.   ....C.--........TA...
3c.   ............AC--.TA..G
3d.   ............A.--.TA..G
4a.   --.-....C.......GGCACC................G.G.C....C........AG..
4b.   --.-............GGCACC..................G.C....C..G.....AG..
4c.   --.-....C.......GGCACC.....T............G.C....C........AG..
4d.   --.-....C.......GGCACC.....T..............CG...CC.......AG..
4e.   --.-....C.......GGCACC.....T..............CG...C-.......AG..
5.    .................T....
6a.   ..-...............GGCACC...........G.C..G.GGG.C..T.C.G.T.A..A-G-AT
6b.   ..-.....C.......GGCACC.........G.C..G.GGG.C..T.C.G.T.A..A-G-AT
6c.   ...-....C.......GGCACC...........C..G.GGG.C..T.C.G.T.A..A-G-AT
```

C. nasus

```
2.    ........C.......GGCACC.....T...........GG.C....CC.......AG..
3a.   ..T.C..--........TA...
3b.   ................--A.--
4.    ...-...-C.......GGCACC.....T...G.......GG.C....C........AG..
5.    ............A....T....
6.    ...-....C.......GGCACC...........C..G..GG.C..T.C.G.T.A..ATATAT
```

Figure 5-5. Sequence of the phenylalanine end of the control region (expansion region) for inconnu and outgroup species. Individual subrepeats for each species are designated numerically. Letters represent different variants of the same sub-repeat. Sequences similar to the light strand promotor-like sequence (TACATTA*Pu*T*Pu*AACTTTT) and the putative transcription factor binding site are underlined.



a) Most common sequence for expansion region in *S. leucichthys* (inconnu n=81)

b) Length variants for expansion region of inconnu (n=8)

Figure 5-6. Expansion region in a) typical inconnu, b) length variants of inconnu, and outgroup species: c) *Coregonus nasus*, d) *C. sardinella,* and e) *C. peled.* Numbers and letters for each species correspond to sequences listed in Figure 5. Dark grey shading indicates repeats found in typical sequence for each species, light grey shading indicates additional repeats found in length variants.

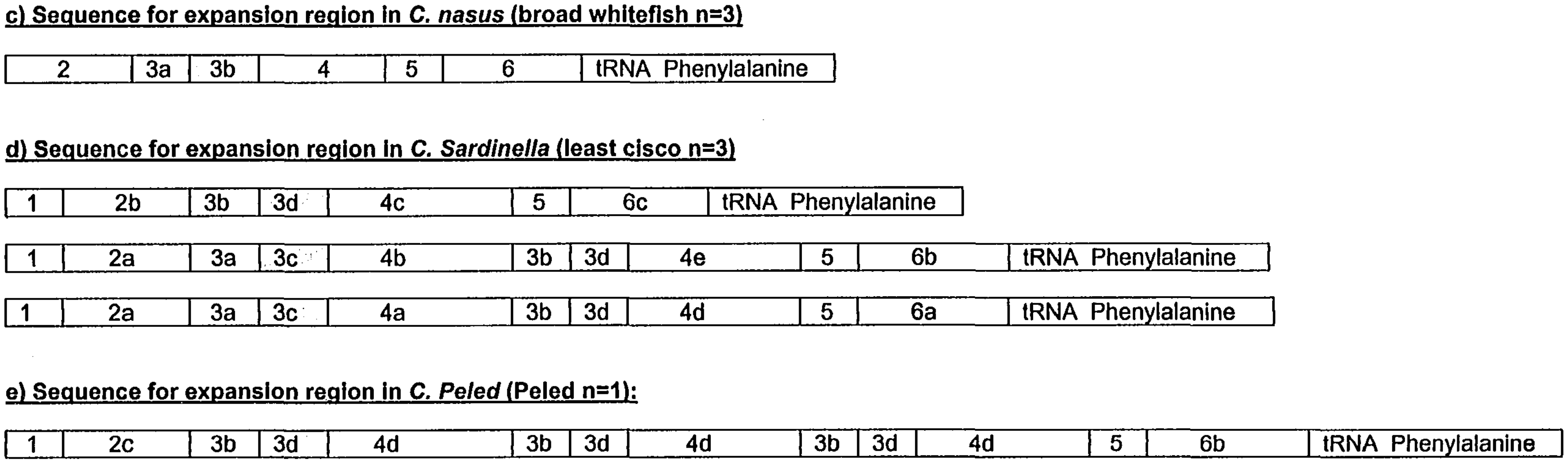


Figure 5-6. Expansion region in a) typical inconnu, b) length variants of inconnu, and outgroup species: c) *Coregonus nasus*, d) *C. sardinella*, and e) *C. peled*. Numbers and letters for each species correspond to sequences listed in Figure 5. Dark grey shading indicates repeats found in typical sequence for each species, light grey shading indicates additional repeats found in length variants.

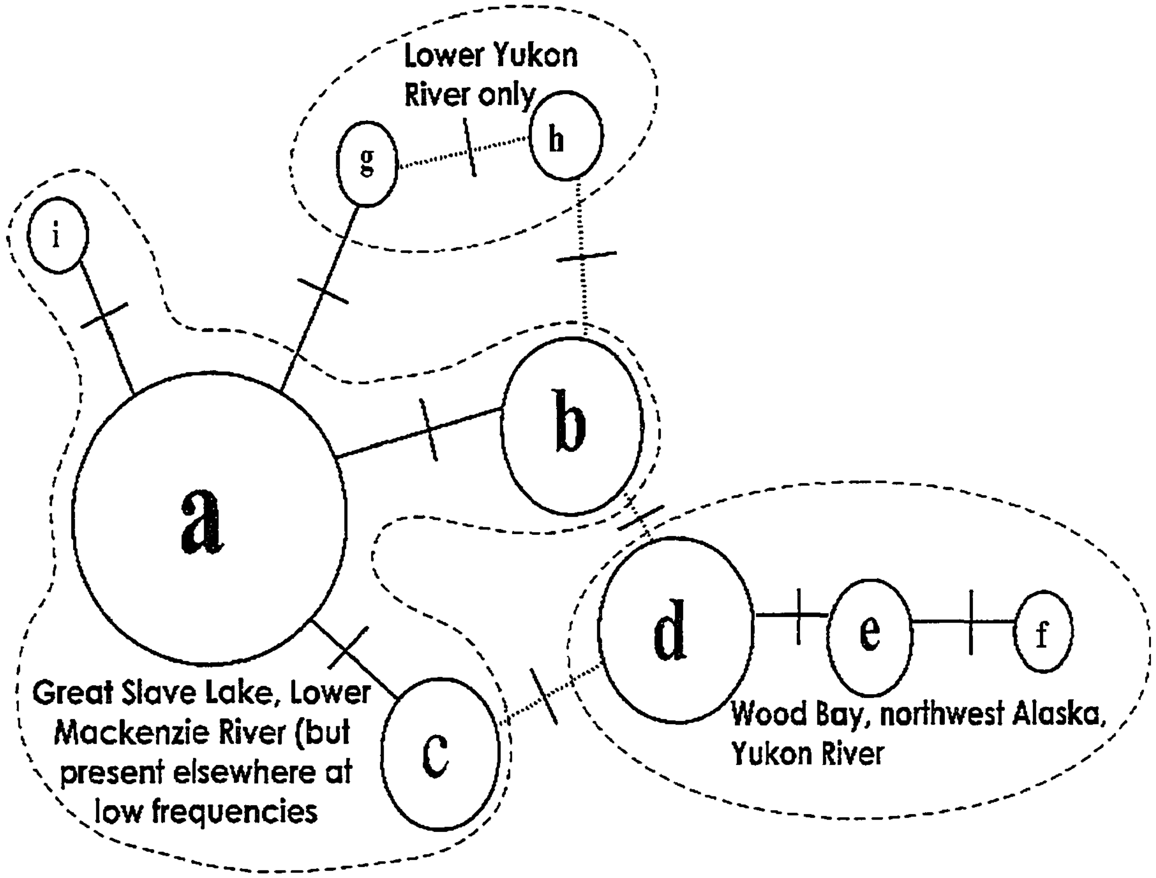


Figure 5-7. Parsimony network showing mutational relationships among haplotypes. Slashes represent number of base substitutions separating two haplotypes. Sizes of circles correspond to the proportion of the total sample represented by each haplotype. Dashed lines enclose groups of haplotypes primarily observed in the geographic locations indicated. Dotted lines represent unresolved relationships between haplotypes whereby a new haplotype could have arisen through a mutation in either of two existing haplotypes.

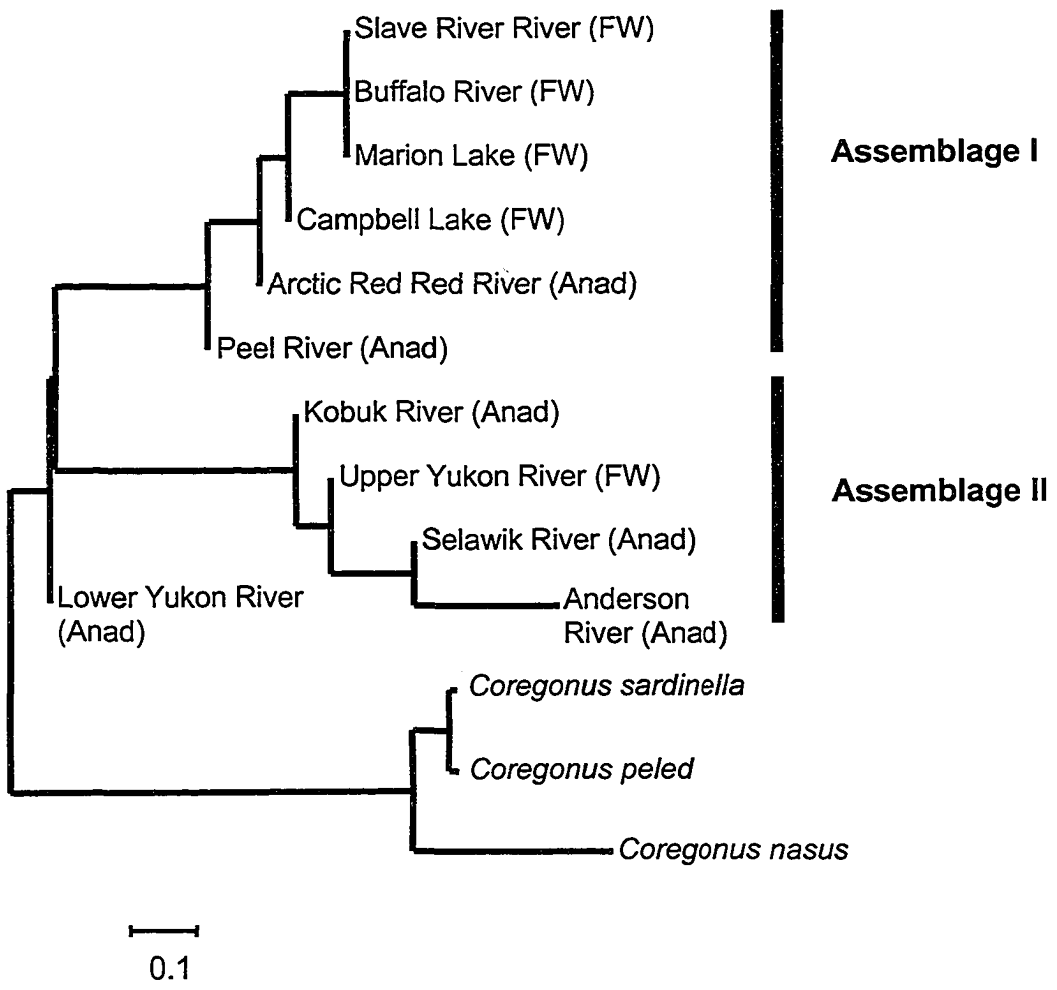


Figure 5-8. Neighbor-joining dendrogram based on average pairwise sequence divergences (Nei 1987) between populations of inconnu. Outgroup branches are not to scale (actual sizes are 40 times greater)

6. Summary and general conclusions

Existing models of life history variation predict strong correlations between migratory behavior and the evolution of life histories in fishes and are supported empirically by studies of freshwater versus anadromous populations of north-temperate species (e.g., Hutchings and Morris 1985; Jonsson 1985; Randall et al. 1987; Snyder and Dingle 1989; Tallman et al. 1996) . These studies have demonstrated that within a species, anadromous populations typically grow faster and mature later than their freshwater counterparts. In theory, a larger body size should be beneficial to long distance migrants and seaward migration, in turn, should confer growth and size benefits through access to highly productive feeding areas (Gross 1988). To examine the robustness of these relations, I initially characterized the life history variation between two representative populations of inconnu, a northern coregonid species known to differ in migratory behavior and habitat use (Chapter 2). Inconnu in the lower Mackenzie River system are anadromous and migrate extensively between coastal feeding areas and upstream spawning sites. In contrast, those in the southern drainages of Great Slave Lake are potamodromous (freshwater), feeding and overwintering in the lake and migrating shorter distances to spawn (Howland et al. 2001). The variation between populations from these two areas was examined in light of what has typically been found in other species with anadromous and freshwater life history types.

My research demonstrated that there were clear differences in the life histories of anadromous and potamodromous inconnu; however, the patterns of variation only partially conformed to model predictions as summarized in Roff (1991). Anadromous inconnu had a later maturity, lower adult mortality, and lived to older ages, as predicted; however, growth rate, particularly during the juvenile phase, was much higher in the potamodramous form. The exceptionally high growth rates of potamodromous inconnu in this system appear to have resulted from access to an unusually productive freshwater food supply, in combination with a long growing season, relative to the marine habitat utilized by anadromous inconnu. Contrary to model predictions, my research also showed that anadromous inconnu had lower size-specific fecundities than the potamodromous form. This may be due to the fact that anadromous inconnu invest more energy into migration, which does not appear to be compensated for through productive feeding, and therefore have fewer reserves available for reproduction. Despite these differences, overall lifetime reproductive success of these two life history strategies may be similar due the greater longevity of the anadromous form.

The inconnu populations examined in this study may not have fully conformed to expectations of existing theory because they differ in key respects from previously studied systems. In particular, both potamodromous and anadromous inconnu migrate to spawning areas, albeit for different distances, whereas most examples in the literature involve a non-migratory resident population that exists sympatrically with an anadromous population (e.g., Jonsson 1985; Snyder and Dingle 1988; Klemetsen et al. 2003). The potamodromous inconnu population examined in this study also inhabits a relatively productive feeding area (Brunskill 1986), unlike resident forms of previously studied species, which often occur in small, relatively unproductive lakes or rivers. The combination of a productive food supply and a long growing season may allow these potamodromous inconnu to grow at high rates relative to the freshwater populations in other species. Thus, although some of the variation in life histories between these populations was linked to differences in migratory behavior, characteristics of the feeding environments appear to have altered this variation.

Although this initial comparison of life histories suggested a strong link between feeding environments and traits such as juvenile growth and age at maturity, the lack of information on the ecology and distribution of juvenile inconnu made it difficult to explore this relationship in naturally occurring fish. The phenotypic differences I observed can result from variation in genotype, the environment or some combination of these two factors (Falconer 1989). Although it has traditionally been assumed that life history traits of whitefish vary primarily with environment (e.g., Lindsey 1981, Shields and Underhill 1993), the relative contributions of environmental and genetic influences to intraspecific heterogeneity are not commonly known. To determine the importance of these two influences in my study populations, I incubated eggs of freshwater and anadromous inconnu and reared the offspring in a common environment to examine traits such as egg development, juvenile growth, and salinity tolerance for persistent differences (Chapters 3 and 4).

In a common environment, rate of egg development differed among life history types, suggesting that this trait is under a substantial degree of genetic control. Both spawning times and egg development rates of inconnu populations appear to be tightly linked with water temperature and ice conditions in their natal rivers, with spawning occurring just prior to freeze up and development being consistent with length of ice cover in each river. Differences in rates of egg development have likely evolved to match different temperature regimes within spawning rivers. I also observed that hatching in both inconnu populations occurred over an extended period of time (ca. 3 weeks). This extended hatch period may have evolved in response to the high year-to-year variability in length of ice cover in these spawning rivers. Although fish may be capable of adjusting to variation in river freeze-up times by using temperature as a proximate cue for spawning (e.g., Hokanson 1977), they have no means for predicting annual spring break up times. An extended hatch period would ensure at least a portion of an individual's offspring hatch at an appropriate time (den Boer 1968; Lambert and Ware 1984)

Growth rates were similar among populations reared in a common environment, suggesting that this trait is more strongly influenced by environmental differences in nature. Thus, observed differences in growth rate of freshwater and anadromous inconnu in the wild are probably a result of phenotypic plasticity to different local environments. However, there is the possibility that growth does have some degree of heritability (e.g., Bailey and Loudenslager 1986; Nilsson 1994). For example, a countergradient relationship between growth and latitude may exist (e.g., Bailey and Loudenslager 1986; Nilsson 1994), or there may be genetic variation in phenotypic plasticity (reaction norms) for growth. More detailed research, in which individuals from each population are reared across a range of temperature and food treatments, would be required to address such questions.

Differences in degree of evolutionary divergence between growth rates and egg development times are likely due to predictability of local conditions and the consequences of mismatch to those conditions. In the case of egg development, the consequences of not hatching at the appropriate time are often lethal for larval fish (Cushing 1990). Since the general time period that is optimal for development and hatching is predictable over the long-run within a given river, there has probably been strong selection pressure for the evolution of population-specific average development rates. For any given year, however, the exact length of the incubation period is unpredictable, thus promoting the evolution of large intra-population variation in developmental rates of inconnu from both spawning rivers. In contrast, although first-year growth is important for northern fish populations (Shuter and Post 1990), the consequences of differences in growth for populations inhabiting rivers at different latitudes are not likely to be as severe as those for hatching, and it may be more greatly constrained by external factors (food supply), so would not be expected to evolve as quickly.

Salinity tolerance, or hypoosmoregulatory capacity, of juvenile freshwater and anadromous inconnu reared in a common environment was compared by exposing them to two experimental treatments: 1) direct transfer from freshwater into 10, 15 or $25^0/oo$ seawater, and 2) transfer to $25^0/oo$ seawater after a 2-wk acclimation period at $15^0/oo$. Saltwater adaptability was assessed by following mortality, changes in plasma [Na^+] and [Cl^-], and in gill Na^+-K^+-ATPase activity up to 8 d post-transfer. Fish from both populations regulated plasma ions following a direct transfer from fresh to brackish water (10-$15^0/oo$), but suffered osmoregulatory collapse with 100% mortality in 48 h when directly transferred to $25^0/oo$ salinity. Acclimation to brackish water improved hypoosmoregulatory capacity in both populations, with acclimated fish showing smaller increases in blood plasma ion concentrations, higher Na^+-K^+ ATPase activity, and lower mortality than non-acclimated fish following transfer to $25^0/oo$ seawater. Anadromous inconnu maintained pre-treatment plasma ion levels during acclimation, whereas these levels increased during acclimation in freshwater inconnu. Juvenile anadromous inconnu are therefore able to adapt physiologically to seawater of at least $25^0/oo$, if brackish-water acclimation is available, but freshwater inconnu have diminished saltwater tolerance, relative to the anadromous form. Thus, although both types can hypoosmoregulate in saline water, the anadromous type has superior capabilities.

Selective pressure likely maintains salinity tolerance in anadromous populations. Anadromous fish that are less able to switch between marine and freshwater environments are probably less successful than those capable of migrating into the more productive marine habitat. Although freshwater inconnu in the Slave River are likely derived from anadromous populations that initially became established in the lower Mackenzie (Chapter 5), selective pressure for salinity tolerance in freshwater inconnu is now reduced, resulting in a gradual loss of this characteristic.

Although my research provided insights into many of the processes leading to and likely maintaining variation among inconnu populations in the Mackenzie (chapters 2-4) there was little evidence in the literature regarding the origin of different life history types in this river system or in other areas where inconnu occur. What processes lead to the initial separation of life history types in the Mackenzie River system? Were they genetically distinct at the time of their invasion of the upper and lower Mackenzie system, respectively, or are they derived from a single colonization and have diverged subsequently? To gain insights about how inconnu initially colonized the Mackenzie River basin and thus how the two life history forms evolved, I examined the genetic relationships of inconnu populations within and outside the Mackenzie River system (Chapter 5). I surveyed 11 northwestern North American, and 1 Siberian population for sequence variation in the control region of the mitochondrial DNA.

Higher genetic diversity in Alaskan populations suggests they have a longer evolutionary history than populations in the Mackenzie Basin and are likely source populations that survived past glaciations in the Beringian refugium. Genetic relationships confirm that all inconnu in the Mackenzie River basin originated from the Yukon River system, although the exact colonization route remains unresolved. Fossil evidence (Cinq-Mars 1979; Cumbaa et al. 1981) and current distribution patterns (McPhail and Lindsey 1976) corroborate these findings.

The degree of geographic isolation and past glacial history have been the dominant forces involved in shaping the current patterns of genetic variation of inconnu, whereas life history pattern (freshwater versus anadromous) appears to have had little influence. The lack of concordance between genetic patterning and life history types suggests that freshwater populations have arisen through multiple, independent divergences from anadromous populations in more recent times following post-glacial dispersal and establishment in different waters; thus, differences may not be reflected in their mitochondrial DNA. Further research using a more rapidly evolving region of the DNA, such as nuclear microsatellites, will be required to test for differences between life history types and spawning stocks within regions. Such techniques may also provide an indication of the level of mixing between life history forms and degree of fidelity to spawning sites exhibited by this species.

My research demonstrates that the processes leading to variation in species are complex and, as shown by the example from this system, can operate primarily at the local level. The life history differences between the potamodromous (freshwater) and anadromous inconnu populations examined in this study appear to have been generated and maintained through a combination of small scale processes, such as environmental variability among habitats, phenotypic plasticity, and genetic variation in adaptive traits. These processes have differentially influenced variation in the life history traits of inconnu depending on the role of specific traits in the life cycle of the species. Although large scale processes, such as the degree of geographic isolation and past glacial history of the region, have had a strong influence on the present distribution patterns of inconnu populations in the western Arctic, different life history forms (anadromous versus potamodromous) appear have diverged locally in more recent times following post-glacial dispersal and establishment in different waters.

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