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Physiological, endocrine, and genetic bases of anadromy in the brook charr, *Salvelinus fontinalis*, of the Laval River (Québec, Canada)

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Synopsis

Brook charr, *Salvelinus fontinalis*, often display alternate life history styles in coastal areas. In the Laval River, some brook charr remain freshwater residents, while others undergo seasonal migrations between freshwater and saltwater environments. In the present paper, we examined physiological (electrolyte concentrations, gill Na^+, K^+-ATPase activity, and thyroid hormone levels) as well as genetic differences (neutral genetic markers) between anadromous and river-resident fish from the Laval River. We also examined how artificial rearing conditions affected seasonal variations in the osmoregulatory physiology of a domestic strain derived from wild anadromous fish. Sympatric anadromous and resident forms of brook charr of the Laval River exhibited differences in gill Na^+, K^+-ATPase activity, plasma thyroxine (T_4), and triidothyronine (T_3) concentrations. In domestic anadromous charr, rearing conditions during development had no negative impact on osmoregulatory ability or on gill Na^+, K^+-ATPase activity. These results argued for an important hereditary component of gill Na^+, K^+-ATPase activity. However, the spring increase in T_4 was present only in wild fish. Significant differences observed at microsatellite loci further suggested that at least some level of reproductive isolation may have occurred between anadromous and resident charr in the Laval River.

Introduction

Brook charr, *Salvelinus fontinalis*, often display alternate life history styles in coastal rivers of eastern Canada. Many populations remain resident in freshwater environments during their whole life. Others, mostly northern populations, exhibit external signs of smolting like silvering and a decrease in condition factor and move into seawater in the spring, spending one to four months feeding in estuarine or coastal waters before returning to rivers for reproduction (White 1940, Wilder 1952, Castonguay et al. 1982, Doyon et al. 1991). In addition, major seasonal variations in the ability of domestic brook charr to tolerate direct transfer to saltwater have been shown to be related to different levels of gill Na^+, K^+-ATPase activity (Besner & Pelletier 1991, Pelletier & Besner 1992) or to saltwater temperature conditions (Claireaux & Audet 2000). The increase in gill Na^+, K^+-ATPase activity has often been shown to be correlated to smoltification or increases in hypo-osmoregulatory ability (Zaugg & McLain 1972, Bœuf & Harache 1982, McCormick et al. 1985, Olsen et al. 1993). A thyroxine (T_4) surge at the time of smoltification has been observed in many salmonids; though not related to seawater adaptability (Folmar & Dickoff 1981, Bœuf et al. 1989). Relationships with seasonal changes in aggression, homing, or migratory activity (downstream or upstream migration) have also been described (Yamauchi et al. 1985, Tsukamoto et al. 1988, Youngson 1989, Morin et al. 1994, Hutchison & Iwata 1998). Lebel & Leloup (1992) and Leloup & Lebel (1993) showed increases in plasma triiodothyronine (T_3) concentrations and T_3–T_4 ratio

following transfer to saltwater in rainbow trout, suggesting an increased requirement of T_3 for the development of hypo-osmoregulatory mechanisms.

The origin of life history style polymorphism and the relationship between anadromous and resident forms when they coexist within a single river are not yet elucidated in brook charr (Power 1980, Lejeune 1987). In species where only one sex (usually males) exhibits a polymorphism in life history (e.g. Gross 1985), anadromous and resident forms clearly belong to a single gene pool and no divergence can occur in autosomal genes. However, when males and females of both types coexist (as is the case in brook charr), assortative mating of anadromous and resident individuals and/or reduced fitness of hybrids would lead to some level of divergence (Foote et al. 1992). Significant divergence at selectively neutral loci between sympatric anadromous and resident forms within the same river has been reported in *Salmo salar* (Vuorinen & Berg 1989, Birt et al. 1991), *Onchorhynchus nerka* (Foote et al. 1989), and *Salmo trutta* (Skaala & Naevdal 1989), although the latter result was not supported by subsequent studies (Hindar et al. 1991, Cross et al. 1992). In Arctic charr, *Salvelinus alpinus*, controlled breeding experiments have shown that the progeny of anadromous and resident parents could give rise to both forms (Nordeng 1983), suggesting that the heritability of life history type was low. Jones et al. (1997) used allozymes to compare allele frequencies in anadromous and resident brook charr and found no significant divergence. However, the low resolution of the markers they used may have hampered the detection of a low differentiation. Experimental crosses of anadromous and resident parents have revealed that different systems of genetic determination of smolting were involved in chinook (Clarke et al. 1994), sockeye (Foote et al. 1992), or Atlantic salmon (Bailey et al. 1980).

The existence of river-specific adaptations in migratory behavior and physiology has important implications not only for the study of the ontogeny of anadromy but also for the need to protect river-specific stocks of anadromous salmonids (e.g. Taylor 1991, McCormick 1994). A decline in both anadromous brook charr and Atlantic salmon, *Salmo salar*, in many rivers of eastern Canada has increased the need of developing population management tools. In this paper, we compared anadromous and river-resident forms from the Laval River using physiological variables involved in the smolting process. We then determined whether the physiological and endocrine differences observed between anadromous and resident fish could be explained by some level of reproductive isolation using both neutral genetic markers and a transplant experiment under laboratory conditions.

Materials and methods

Wild fish collection

Both anadromous and resident brook charr inhabit the Laval River on the north shore of the St. Lawrence estuary, Québec (48°44′N; 69°05′W). This river has not been stocked with domestic strains, and anadromous brook charr is exploited by sport fishing. Wild anadromous brook charr were captured at three strategic periods of the anadromous life-cycle: i.e. in freshwater, just prior to downstream migration (n = 11, angling); in the estuary (n = 13, monofilament gillnets, stretched meshes of 1.5 cm); and in freshwater again, following upstream migration (n = 20, fish fence located 3.6 km upstream) (Table 1, Figure 1). Adipose fins from all

Table 1. Sampling date, temperature, salinity, and fork length of the fish used in this study.

	Wild freshwater resident		Wild anadromous			Domestic anadromous		
	Spring (FW)	Spring (FW)	Spring downstream migration (FW)	Summer estuary (SW)	Summer upstream migration (FW)	Spring (FW)	Summer (SW)	Summer (FW)
Sampling date	03 June	23–28 July	5–20 May	20–29 May	28 July–04 August	17–18 May	09 June	02 August
Temperature (°C)	4.0–7.0[1]	18.0–20.0[1]	5.0–7.0	4.0–7.0[1]	20.7–23.2[1]	7.5	7.5	15.2
Salinity (‰)	0[1]	0[1]	0–2[1]	19–27[2]	0[1]	0	19–21	0
Length (cm)	24.13 ± 3.0	19.34 ± 3.6	33.8 ± 5.7	33.9 ± 4.6	43.9 ± 4.8	33.0 ± 2.6	35.9 ± 4.1	34.28 ± 3.7

FW = fresh water, SW = salt water.
[1]Data measured near to site of capture.
[2]Data range including tide effect and fresh water supply in Baie Laval.

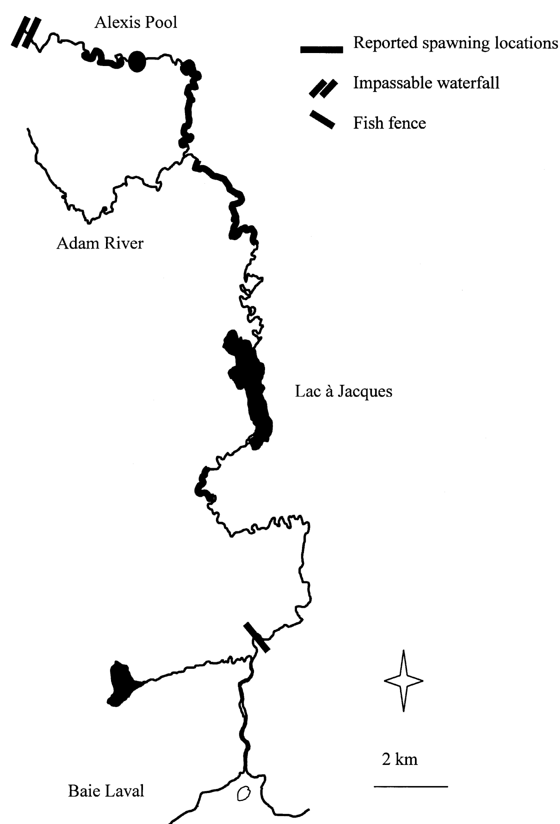

Figure 1. Map of Laval River. Reported spawning grounds for anadromous brook charr are indicated as bold lines. Spawning of anadromous fish is also likely to occur in the Adam River.

anadromous fish were collected for genetic analysis. Resident brook charr were identified on the basis of visual observations (pigmentation, size, and secondary sexual characteristics) and were caught by angling in the Laval River at Alexis Pool or in a tributary of the Laval River (Adam Stream, readily accessible to anadromous fish) at the time of down- and upstream migration of anadromous fish (Figure 1). Time periods were chosen as to increase the probability of observing physiological differences between the two forms. Adipose fins from 39 resident fish from the river's main stream and 10 from Adam River were collected for microsatellite analysis of resident fish. Fish presented no external signs of injuries related to capture. Following capture, fish were allowed to rest in retention cages [cylindrical Vexar cages ($1 \times 0.4 \times 0.6$ m) through which river water was freely flowing] near the capture site for 24–48 h before tissue samples were taken.

Domestic strain

From 1993 to 1996, 22 anadromous breeders from the Laval River were captured and brought to the ISMER aquaculture station in Pointe-au-Père, Québec (48°31′N; 68°29′W), where they were raised under natural temperature, photoperiod, and salinity conditions (Savaria 1998). In the present study, domestic fish were fed once a day in the morning and were given a daily ration of 1% of their wet weight. Adipose fins of 68 fish from the domestic strain (F1) were collected for genetic analysis. Laboratory-reared fish (age 2+) were sampled in fresh water during spring (at the same time as the wild fish were migrating downstream). Following the spring sampling, domestic fish were gradually transferred to salt water (2‰ per day over 10 days) and sampled 7 days after 20‰ salinity was reached. They were gradually reacclimated to fresh water at the end of July (during upstream migration of wild fish in the river) and another 10 fish were sampled.

Sampling procedure and analytical techniques

All sampling was performed between 10:00 and 13:00 h. Fish were placed in a bucket containing anaesthetic (0.02% MS-222 [3-aminobenzoic acid ethyl ester]) for 1–2 min. Body weight and fork length were measured; blood was drawn by caudal puncture using ammonium-heparinized syringes and centrifuged at 7000 *g* for 3 min. Plasma aliquots were prepared, frozen on dry ice, and stored at −80°C for further analyses. For each fish, plasma osmolality, chloride, sodium, cortisol, glucose, thyroxine (T_4), and triidothyronine (T_3) concentrations were measured. The second left gill arch was dissected from each fish and stored at −80°C for later gill Na^+, K^+-ATPase activity analysis.

Plasma osmolality was measured with a 3MO micro-osmometer (Advanced Instruments, Needham Heightsand) plasma chloride concentration with a chloride analyzer 925 (Corning Diagnostic Corp., Medfield). Plasma sodium concentration was determined with an atomic absorption spectrophotometer (Perkin Elmer model 460, Wellesley). Plasma cortisol concentration was measured using a radioimmunoassay method (ImmuChem coated tube Corticol ^{125}I). Plasma glucose concentration was measured using a hexokinase enzymatic method (Sigma procedure no. 16 UV). Plasma T_4 and T_3 concentrations were measured by an enzymatic immunoassay method (Medicorp procedure T3-EIA and T4-EIA, respectively). Diagnostic kits (cortisol, T_3, and T_4) were chosen to minimize

potential matrix effects and parallelism was tested before using them in our study. Large brook charr were sampled and their plasma separated into aliquots for determination of intra- and interassay coefficients of variation and to prepare serial dilutions of fish plasma for specificity checking. Serial dilutions of fish plasma were assayed and the slope of the displacement curve compared with the slope of the standard curve. Gill Na^+, K^+-ATPase activity was determined by the method described by Zaugg (1982) and Heinonen & Lahti (1981) as modified by Pelletier (1987).

For each fish, total DNA was extracted from ethanol-preserved adipose fins using standard phenol-chloroform protocol. Genotypic data were obtained for six microsatellite loci (SFO-12, SFO-18, SFO-23, SFO-8 developed especially for brook charr (Angers et al. 1995), MST85 originally developed for brown trout, *Salmo trutta* (Presa & Guyomard 1996), and SSA-197 developed for Atlantic salmon, *Salmo salar* (O'Reilly et al. 1996) as described in Hébert et al. (2000).

Statistical analysis

Physiological data were routinely expressed as mean ± S.E. (n), where n represents the number of fish. The normality and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. Some data sets had to be transformed prior to statistical analysis to homogenize variance: plasma cortisol, T_3, and T_4 concentrations, and gill Na^+, K^+-ATPase activity were transformed as $\log(x + 1)$. The data were analyzed by two-way ANOVA ($\alpha = 0.05$), form (anadromous or freshwater resident) and period of sampling (in fresh water during spring or summer) were used as factors when seasonal physiological changes between the two forms were compared; origin (wild or domestic) and period of sampling were factors when physiological and endocrine variables were compared between wild and domestic anadromous brook charr. A *posteriori* t-tests of comparison of means (Sokal & Rohlf 1981) with $\alpha = 0.05$ were applied following ANOVAs. When the two-way ANOVA indicated significant interactions between origin and period of sampling for a given variable, an *a posteriori* one-way ANOVA (period of sampling) followed by an a posteriori Tukey HSD test for unequal sample size were done. For those parameters for which transformations failed to give homogeneity of variances, we applied the Games and Howell test (Sokal & Rohlf 1981). A covariate effect with fish size (weight and length) was also tested for all variables.

Genetic data analysis

Microsatellite polymorphism was quantified by the number of alleles and observed and expected heterozygosity. The f-values (Weir & Cockerham 1984) were used to estimate deviations from Hardy–Weinberg proportions of homozygotes and heterozygotes as measured by Wright's F_{IS}. F_{ST} was estimated by θ (Weir & Cockerham 1984) to quantify genetic divergence among groups of fish. The permutation procedure implemented in GENETIX 4.0 (Belkhir et al. 1998) was used to test whether f and θ were significantly different from zero (2000 permutations). The chord distance of Cavalli-Sforza & Edward's (1967) (D_{CE}) was used to build a 2000 iterations bootstrapped neighbor-joining tree and provide a visual representation of genetic divergence patterns among groups of fish.

Results

We observed no significant relationship between covariates (length or weight) and any of the variables, which ensures that our results were not biased by size differences among groups.

Anadromous versus freshwater-resident wild fish

Plasma osmolality and chloride and sodium concentrations were higher in wild anadromous (downstream and upstream migrating fish) than in river-resident fish ($p < 0.001$; Figure 2). No significant effect of sampling period or interaction between these two factors were detected. Plasma cortisol and glucose concentrations were generally elevated but did not differ between resident and anadromous fish and exhibited no seasonal variation pattern (cortisol: 24.88 ± 2.05 (36) μg $100\,ml^{-1}$, glucose: 196.53 ± 11.81 (47) mg $100\,ml^{-1}$).

Interactions between form and sampling period were found for gill Na^+, K^+-ATPase activity ($p < 0.01$). Seasonal differences in gill Na^+, K^+-ATPase activity were present only in anadromous fish for which activity was higher during downstream migration than during upstream migration ($p < 0.01$; Figure 3). Gill Na^+, K^+-ATPase activity of anadromous charr sampled during downstream migration was also higher than in

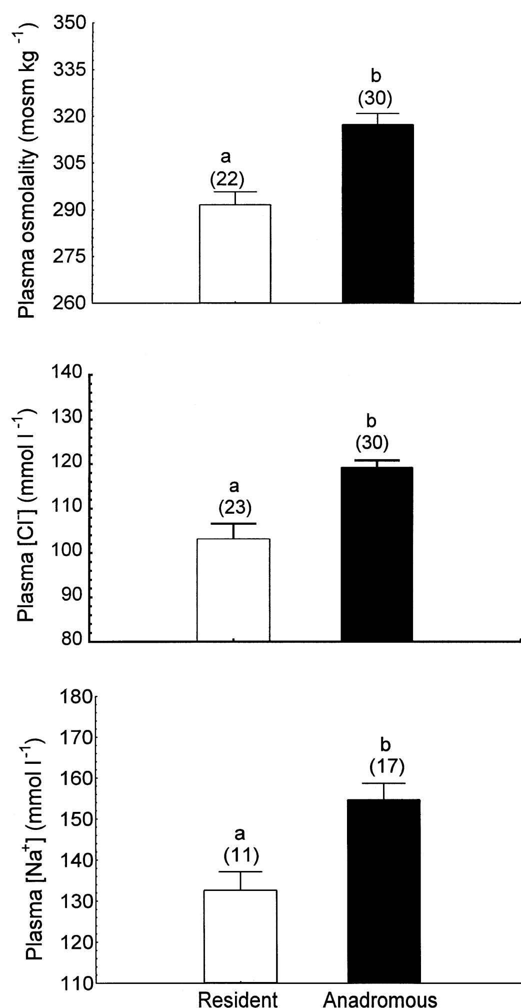

Figure 2. Plasma osmolality and chloride and sodium concentrations in freshwater resident (open bar) and anadromous (solid bar) brook charr of the Laval River. Two sampling periods were pooled. Means with different letters indicate a significant difference between the two forms. Sample size is given in parenthesis.

resident fish sampled during the same period. However, no significant difference was observed between anadromous and resident fish caught during summer.

Both form and sampling period had a significant effect on plasma T_4 concentration while no interaction between these factors was found ($p > 0.05$). Plasma T_4 concentration was higher in fish (resident and anadromous) caught in spring than during summer (Table 2). It was even higher in residents than in anadromous fish independent of the sampling period. A significant interaction between form and sampling period was detected

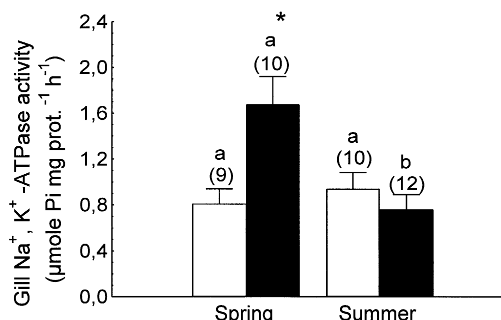

Figure 3. Gill Na^+, K^+-ATPase activity in freshwater resident (open bar) and anadromous (solid bar) brook charr caught during spring and summer. Means with different letters indicate a significant difference between the two periods of sampling for each form. An asterisk indicates significant differences between the two forms for the same sampling period. N is given in parenthesis.

Table 2. Plasma thyroxine concentrations in wild freshwater resident and anadromous fish sampled during spring and summer. Number of fish is given in parenthesis.

	Plasma thyroxine concentration (ng ml^{-1})
[1]Wild freshwater residents	4.49 ± 0.43 (17)[a]
[1]Wild anadromous	2.34 ± 0.17 (29)[b]
[2]Spring	4.12 ± 0.32 (17)*
[2]Summer	2.55 ± 0.30 (29)

[1]The two sampling periods were pooled. Means with different letters indicate a significant difference between the two forms.
[2]The two forms were pooled. Asterisk indicates a significant difference between the two sampling periods.

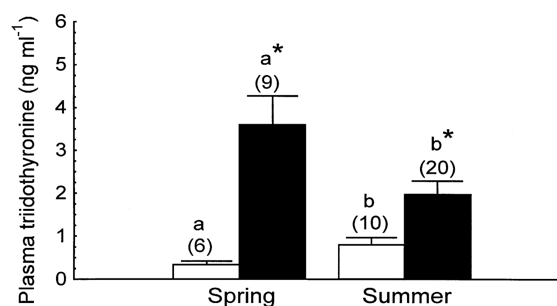

Figure 4. Plasma triidothyronine (T_3) in freshwater resident (open bar) and anadromous (solid bar) brook charr caught during spring and summer. For more details see Figure 3.

for plasma T_3 concentration. Plasma T_3 concentration was higher in anadromous charr during downstream migration than during upstream migration ($p < 0.05$; Figure 4). On the contrary, it was lower in resident charr

caught during spring than in those caught in summer ($p < 0.05$). Plasma T_3 concentration was also generally higher in anadromous fish than in resident fish ($p < 0.05$).

These physiological differences in osmoregulatory status, gill Na^+, K^+-ATPase activity, and hormonal levels were associated with significant divergences at microsatellite markers. Analysis of genetic data strongly supported the hypothesis that anadromous and resident fish were non-randomly mating, and consequently, partially reproductively isolated. Permutation tests were non-significant for all comparisons among anadromous samples, thus suggesting that anadromous fish collected at different time periods belonged to a single gene pool (Table 3). In contrast, resident fish sampled in Alexis Pond and Adam River were strongly genetically differentiated (F_{ST}= 0.101, $p < 0.0005$). All pairwise anadromous-resident comparisons but two were also significant. The Adam River fish were much more differentiated from anadromous fish than were the resident fish collected in Alexis Pond. Since no divergence was detected among anadromous fish, samples were pooled to increase statistical power as small sample sizes may have limited our ability to detect a differentiation. A clear signal of genetic divergence between anadromous and resident fish collected both in Adam River and Alexis Pond was then observed in both comparisons ($F_{ST} = 0.1645$, $p < 0.0005$ and $F_{ST} = 0.019$, $p < 0.0005$). High genetic diversity was found in the resident samples in terms of observed heterozygosity when compared to anadromous groups (Table 4). This allows us to refute the possibility of a family effect, i.e., that resident fish were collected over relatively more limited geographic areas and were derived from a few genitors only, which would bias the estimates of genetic differentiation (Allendorf & Phelps 1981). The NJ-tree (Figure 5) further illustrates the differentiation pattern revealed by F_{ST} analyses. The only strong bootstrap value in the tree (93%) supported the node separating the two resident groups from the four anadromous groups.

Wild versus domestic anadromous fish

Microsatellite marker analysis failed to reject the null hypothesis of no genetic divergence between wild anadromous and domestic fish (Table 4, $p > 0.05$ for all pairwise comparisons), which indicated that

Table 3. Pairwise significant values following sequential Bonferroni correction (Rice 1989) are indicated in bold. N is the number of fish successfully analyzed.

		Wild anadromous			Domestic anadromous	Freshwater residents	
		Downstream	Estuary	Upstream		Alexis	Adam
N		11	13	10	68	39	10
Wild anadromous	Downstream	0	0.05833	0.01369	0.02366	**0.05768**	**0.22818**
	Estuary		0	−0.0613	−0.00511	0.00239	**0.13156**
	Upstream			0	−0.00278	0.02105	**0.16552**
Domestic anadromous					0	**0.01678**	**0.16358**
Freshwater Residents	Alexis					0	**0.10109**
	Adam						0

Table 4. Multilocus estimates of microsatellite variability. N is the number of fish successfully analyzed. A is the mean number of alleles per locus. Hobs. and Hn.b. are observed and expected heterozygosity, the later being corrected for small sample size. f is Weir & Cockerham's (1984) Fis estimate and P(HW) its associated p-value estimated using 2000 randomized data sets.

	Wild anadromous			Domestic anadromous	Freshwater residents	
	Downstream	Estuary	Upstream		Alexis Pond	Adam River
N	11	13	10	68	39	10
A	4.333	6.5	5.1667	10.333	12	5.1667
Hn.b.	0.5435	0.7358	0.6835	0.6855	0.7637	0.6915
Hobs.	0.4271	0.5557	0.6111	0.6115	0.6709	0.6213
f	0.225	0.25325	0.11197	0.10877	0.12284	0.1075
P(HW)	0.004	<0.0005	0.098	0.0005	<0.0005	0.094

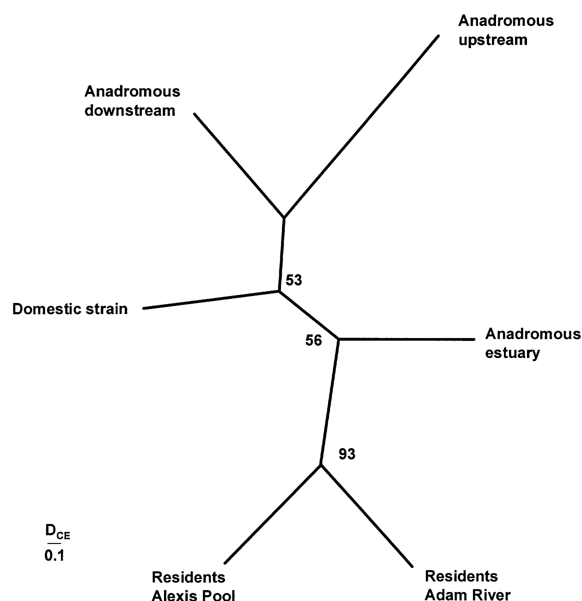

Figure 5. A neighbour-joining tree constructed using the chord distance of Cavalli-Sforza & Edwards (1967). Bootstrap values are based on 2000 replicates.

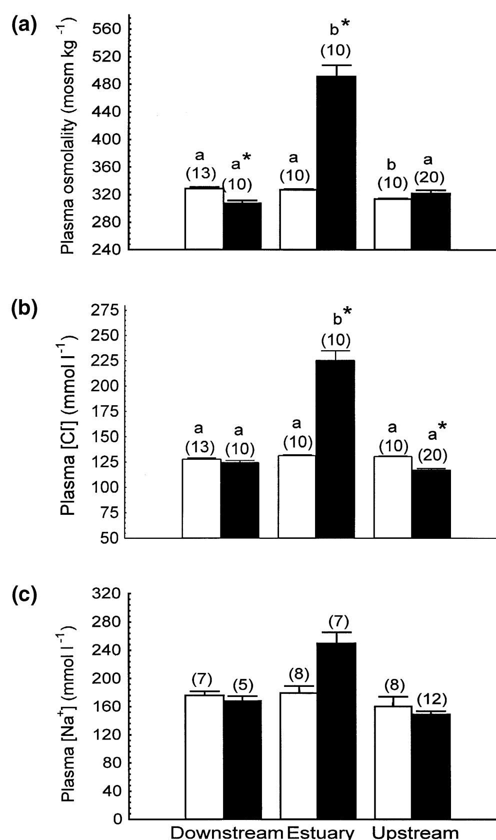

Figure 6. Plasma osmolality (a) and chloride (b) and sodium (c) concentrations in domestic (open bar) and wild (solid bar) anadromous brook charr sampled during three sampling periods. Means with different letters indicate a significant difference between the three periods of sampling in each group. Asterisks indicate significant differences between two groups for a same sampling period. Sample size is given in parenthesis.

the domestic anadromous fish were representative of the wild population. Hence, any physiological differences should be due to environmental differences only.

Significant interactions between sampling period (downstream migration, in estuary, and upstream migration) and origin (wild and domestic) were found for plasma osmolality and chloride concentration. Overall, fish reared under artificial conditions exhibited no sharp differences in osmoregulatory status when compared to wild fish. Prior to the spring downstream migration, plasma osmolality was slightly higher in domestic fish ($p < 0.05$), but this difference was not found at the end of the summer ($p > 0.05$). One notable exception to this statement is the apparent inability of wild fish to keep their osmolality and chloride concentration at a constant level while in saltwater ($p < 0.001$; Figure 6 a,b). In sharp contrast to this result, plasma osmolality in domestic fish remained almost constant throughout the experiment except when transferred back to freshwater at the end of the summer ($p < 0.05$), when a slight decrease was observed.

The plasma cortisol concentration was generally much higher in wild than in domestic fish ($p < 0.001$; Figure 7). Plasma glucose concentration was significantly higher in wild fish caught during downstream and upstream migration than in domestic fish sampled during the same period (Figure 8).

Gill Na^+, K^+-ATPase activity was higher in wild fish caught during downstream migration than during upstream migration ($p < 0.01$; Figure 9). The same tendency was observed in domestic fish, but no significant difference was detected. Gill Na^+, K^+-ATPase activity in both wild and domestic fish sampled in saltwater was significantly higher than in fish sampled in freshwater.

Significant interactions between the two sources of variation were found for both plasma T_4 and T_3 concentrations. Domestic fish did not show any temporal variation in either plasma T_4 or T_3 concentrations (Figure 10). In contrast, wild charr exhibited a surge in the T_4 concentration during downstream migration. Furthermore, plasma T_4 concentration in

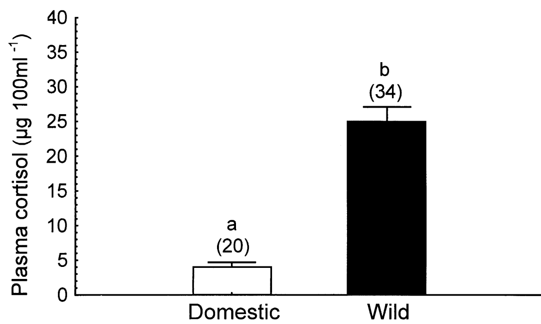

Figure 7. Plasma cortisol concentration in domestic (open bar) and wild (solid bar) brook charr. For more details see Figure 2.

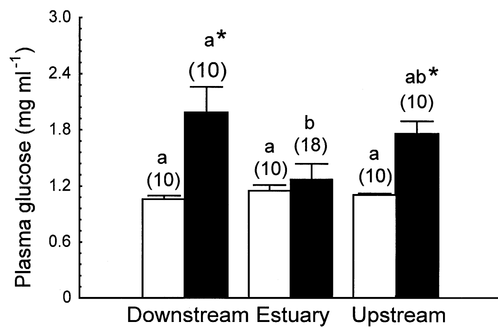

Figure 8. Plasma glucose concentration in domestic (open bar) and wild (solid bar) anadromous brook charr sampled for the three sampling periods. For more details see Figure 6.

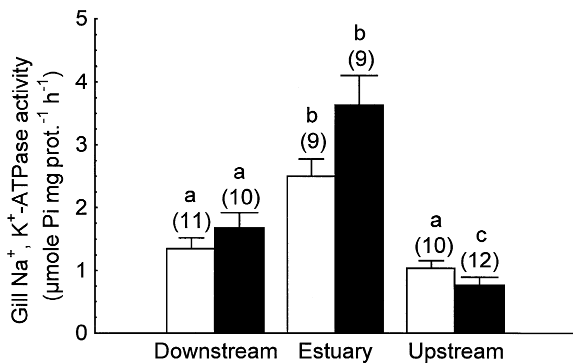

Figure 9. Gill Na^+, K^+-ATPase activity in domestic (open bar) and wild (solid bar) anadromous brook charr for the three sampling periods. For more details see Figure 6.

wild fish caught during the downstream migration was higher than in domestic fish sampled at the same time of the year ($p < 0.05$). The *a posteriori* analysis failed to show significant differences in plasma T_3 concentrations among sampling periods in wild and

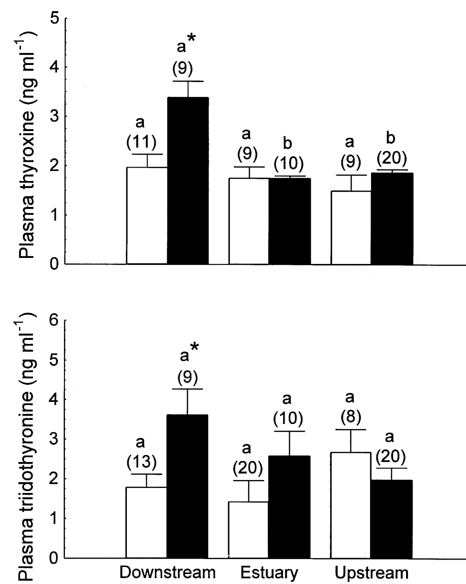

Figure 10. Plasma thyroxine (T_4) and plasma triidothyronine (T_3) in domestic (open bar) and wild (solid bar) anadromous brook charr for the three sampling periods. For more details see Figure 6.

domestic fish. As was the case for T_4, plasma T_3 concentration in wild fish caught during downstream migration was higher than in domestic fish sampled at the same time ($p < 0.05$).

Discussion

Anadromous versus resident brook charr

Our results showed that sympatric anadromous and resident forms of brook charr in the Laval River system represent physiologically and genetically distinct populations. The most important physiological differences were related to seasonal gill Na^+, K^+-ATPase activity and thyroid hormone patterns.

Higher gill Na^+, K^+-ATPase activity during spring was observed in anadromous fish but not in freshwater residents. The higher enzyme activity during downstream migration in anadromous fish could be related to preparation for the seawater environment and during spring could be used to differentiate freshwater resident and anadromous brook charr of the Laval River. Indeed, high levels of gill Na^+, K^+-ATPase activity in freshwater salmonids are usually considered a predictive indicator of the ability for rapid and successful seawater acclimation (Zaugg & McLain 1972, Bœuf & Harache 1982) and as a characteristic of the parr-smolt

transformation in many anadromous salmonids species (Zaugg & McLain 1972, Folmar & Dickoff 1981, Bœuf 1994).

We found several differences in the thyroid hormone pattern between the two forms. The plasma T_4 concentration was generally higher in residents compared to anadromous fish while the reverse was observed for plasma T_3 concentration. One may suggest that T_4 to T_3 conversion was higher in anadromous compared to resident fish. Indeed, the T_3–T_4 ratio was fifteen times greater in anadromous than in river resident fish during spring and five times greater during summer. T_4 is metabolized through several intracellular pathways, but a significant proportion can be converted to T_3. T_3 has about a ten-fold greater affinity than T_4 for putative nuclear receptor sites (e.g. Cyr & Eales 1996) and this is why T_3 is generally considered the active form of the thyroid hormones (e.g. Eales & MacLatchy 1989, Eales & Brown 1993, Leatherland 1994). The present finding suggests that the surge of T_4 is not inevitably accompanied by an increase in T_3 production despite the apparent surge in available T_4 substrate in river-resident fish. A decrease of T_4 to T_3 transformation may have contributed to the T_4 surge in river-resident fish. According to Bœuf et al. (1989), it is likely that T_4 is converted locally to T_3 within a restricted target tissue and the T_3 so generated then interacts with nuclear receptor sites in that tissue. Therefore, this local conversion may be too low to have a substantial effect on the plasma T_3 pool. Previous studies have also reported higher plasma T_4 concentrations in non-migrant than in migrant hatchery-reared steelhead trout, *Oncorhynchus mykiss*, but no significant differences in plasma T_3 concentrations were observed between these two forms (Ewing et al. 1994). However, the distinction between the two forms of this species is not clearly defined and the authors separated the two forms only on the migration tendency criteria. In Arctic charr, Høgåsen & Prunet (1997) observed higher plasma T_4 concentrations in migrants than in residents. However, in their study the anadromous fish were trapped in the river and resident fish were caught in a lake where the water flow conditions were different, it has been clearly established that thyroid activity fluctuates in response to various environmental stimuli (e.g. Barron 1986). In our study, river-resident and anadromous fish came from the same river system and were sampled during the same periods of the year, suggesting that the thyroid hormone activity differences between the two forms are related to behavioral or endogenous differences relating to life history styles. However, the hypothesis that environmental conditions were associated with differences in hormone levels between the two forms could not be completely rejected. Indeed, resident and anadromous fish were captured at different sites where water characteristics like temperature, flow conditions, and velocity were not identical.

Seasonal patterns of plasma thyroid hormones also differed between the two forms. Plasma T_4 and T_3 concentrations were higher in anadromous charr in spring compared to summer while the reverse was observed for T_3 in freshwater residents. The T_3–T_4 ratio was similar between anadromous fish caught during spring (1.05) and summer (1.07), but it was three-fold lower in residents caught in June (0.07) than in residents sampled in late July (0.20). Thyroid hormones have been shown to stimulate various behavioral changes during smolting, like salinity preference, territorial behavior, phototaxis, rheotaxis, and induction of downstream migration (e.g. Iwata 1995). Thyroid hormones, particularly T_3, are also associated with metabolism and energy balance (Eales & MacLatchy 1989). Because reproduction is energetically demanding, direct and indirect interactions between thyroid hormones and reproduction are anticipated (e.g. Cyr & Eales 1996). A fall in thyroid status during gonadal growth, vitellogenesis, or spermatogenesis may reflect a decrease in metabolic reserves accented by upstream migration in anadromous fish (e.g. Cyr & Eales 1996). The absence of large movements by river residents could contribute to maintaining thyroid hormone status in summer. The relationship between the thyroid and the reproductive system, with higher thyroid activity during growth phase and the start of gonadal development followed by a decrease during gonadal growth, were demonstrated in many studies (e.g. Cyr & Eales 1996). Hence, differences in seasonal plasma T_3 changes between the two forms could then have been related to differences in sexual status of each form at the time of capture. Another study currently in progress in our laboratory could shed some light on this question.

Plasma osmolality and chloride and sodium concentrations were generally higher in anadromous than in freshwater-resident fish. Concentrations observed in anadromous fish were similar to those previously reported for domestic brook charr in fresh water (Besner & Pelletier 1991, Audet & Claireaux 1992). However, plasma electrolyte concentrations in freshwater-resident fish were about 10–15% lower than expected. Stress related to capture or confinement in resident fish could have impaired the osmoregulatory capacity. Plasma cortisol and glucose concentrations in

wild fish (river-residents and anadromous) were very high compared to levels previously reported in non-anadromous domestic brook charr reared in fresh water (Audet & Claireaux 1992, Claireaux & Audet 2000), suggesting an important stress related to angling. However, we observed no difference in plasma cortisol or glucose concentrations between wild resident and anadromous fish, causing us to reject the hypothesis that the stress of capture differs between the two forms. The absence of relationship between covariates (length and weight) and plasma electrolyte concentrations eliminates the possible influence of size in conservation of hyper-osmoregulatory ability. Why a hydromineral imbalance is present only in resident fish remains unexplained.

The pattern of neutral genetic variation suggested that interbreeding between anadromous and resident fish is probably limited in the Laval River. Because it is not precisely known whether anadromous fish spawn in the Adam River (though fully accessible from the river's main channel), the differentiation found among anadromous fish and resident fish sampled at that location can be interpreted as reflecting the very fine scale genetic structuring in brook charr in a coastal system (Jones et al. 1996, 1997, Hébert et al. 2000) and the non-random distribution of anadromous and resident phenotypes in the river. Of greater interest in the present study is the significant differentiation between resident fish sampled in Alexis Pond and anadromous fish as both samples were collected in the river's main channel. A sampling bias, whereby the progeny of a limited number of genitors were sampled in Alexis Pond, could possibly account for that differentiation (Allendorf & Phelps 1981). In this case, families would differ in the phenotypes they produce (anadromous or resident), an indication of a strong hereditary component of the migration form. However, high levels of diversity (number of alleles and heterozygosity) were found in the Alexis Pond sample, thus leading us to reject this hypothesis. A second explanation would be that anadromous fish mainly stemmed from spawning grounds in the lower section of the river and may be genetically differentiated. Since fish were collected during their migration, it was not possible to determine their original spawning ground. But the fact that most suitable spawning locations are located in the upper reaches of the river argues against this explanation. A third, more likely explanation would be genetic isolation of both phenotypes in sympatry. Assortative mating and male mate choice have been shown to be involved in the maintenance of genetic divergence between anadromous (sockeye) and nonanadromous (kokanee) Pacific salmon (Foote et al. 1989). Such a mechanism could be invoked in the brook charr as well, although there is currently little data available on the spawning behavior of brook charr in coastal rivers, especially when anadromous and non-anadromous forms spawn at the same place and at the same time. Additional direct behavioral observations on spawning grounds would be a particularly valuable next research step.

Wild versus domestic anadromous brook charr

Genetic differences between resident and anadromous forms suggest that genetic factors could be related to physiological differences and are involved in the induction and regulation of physiological changes related to anadromy in Laval River brook charr. To examine the relative importance of these genetic factors and the environmental conditions on physiological changes related to anadromy, we compared two groups of fish from the anadromous Laval River strain but originating from two different environments (laboratory and nature).

Seasonal changes in gill Na^+, K^+-ATPase activity were similar between anadromous wild and domestic groups relative to wild resident fish, thus suggesting that the propensity of fish to acclimate to seawater is at least partly heritable. Contrary to Shrimpton et al. (1994), who observed that hatchery-reared juvenile coho salmon, *Oncorhynchus kisutch*, showed a lower hypo-osmoregulatory ability compared with wild fish, our results showed that rearing conditions had no direct effects on the hypo-osmoregulatory ability of anadromous charr. In contrast to wild anadromous fish, domestic fish maintained their electrolyte concentrations whether fish were sampled in freshwater or in saltwater. Wild fish caught in the estuary exhibited a surge of plasma osmolality and chloride concentration. In the estuary, fish were captured by net. Net mesh size had been selected to capture fish by their mouths to avoid gill damages as gill histology was to be done on these fish. Bouck et al. (1978) compared the effect of capture on different plasma enzyme activities on domesticated rainbow trout and found that the method of capture had little effect on analyzed variables. In a study on the effect of catch and release, Booth et al. (1995) mentioned that most of the stress-related metabolic effects had disappeared after 12 h. As fish were allowed a 24 h rest before being sampled and no signs of injuries were observed, capture itself was probably not the cause

of the differences in plasma electrolytes. On the other hand, it is possible that the stress of capture combined with the stress of acclimation to the estuarine environment may have impaired the hypo-osmoregulatory ability of wild brook charr. The development of hypo-osmoregulatory activity has been described as being divided into two phases in rainbow trout: an initial crisis phase that lasts around 48 h followed by a longer regulatory phase (Madsen & Naamansen 1989). Domestic fish were sampled 17 days after transfer to salt water. However, we do not know how long after having reached the estuary the wild fish were captured. This could explain the difference in terms of plasma osmolality and chloride between the two groups. Plasma cortisol levels of domestic charr were similar to those reported by Audet & Claireaux (1992) and Claireaux & Audet (2000) and were five-fold lower than in wild charr. With the exception of wild fish caught in the estuary, high plasma cortisol levels were accompanied by a large increase in glycemia whereas the lowest cortisol response measured in domestic fish was associated with less marked changes in plasma glucose. A decrease in hepatic glycogen reserves during winter could have impaired the secondary stress response in wild fish when imposed stress capture over seawater acclimation. In a northern brook charr population, Cunjak & Power (1986) demonstrated a decrease in serum glucose level just after the end of the ice period. Soengas et al. (1992) detected a drop in liver glycogen in rainbow trout along with the increase in gill Na^+, K^+-ATPase activity, corroborating other studies that showed depletion of liver glycogen during smoltification (see references in Soengas et al. 1992). This hypothesis will have to be verified in the future.

In contrast to hypo-osmoregulatory ability, plasma T_4 and T_3 concentrations differed between wild and domestic brook charr sampled during spring. As these fish come from the same population, the differences are not likely to be related to genetic factors. The seasonal changes in photoperiod and temperature were similar for both domestic and wild fish in our study, thus rejecting the hypothesis that photoperiod or temperature could have induced differences between the two groups. McCormick & Björnsson (1994) observed differences in plasma thyroxine concentrations between hatchery smolts, *Salmo salar*, and smolts in the wild that may result from a combination of different rearing environments (artificial versus natural) and the absence of an active migration in captive hatchery smolts. Several environmental stimuli experienced by wild fish during spring are not present in a fish farm environment-stimuli that may have an effect on adaptation to the marine environment. It was previously shown that blood T_4 levels increase in parallel with changes in water quality, rainfall, and water velocity shortly before the beginning of downstream migration (Nishioka et al. 1985, Yamauchi et al. 1985, Youngson et al. 1986). Moreover, diet, quantity of food available, and feeding procedure (i.e. time of day of feeding or administered on fish demand) could have an impact on feeding activity and are likely to change the hormone rhythm pattern (Boujard & Leatherland 1992, Reddy & Leatherland 1994).

Our results showed that, despite very different environmental or rearing conditions and despite different stress levels, the wild and domestic anadromous fish are much more similar to each other than are the wild anadromous and river-resident fish. The genetic divergence observed between anadromous and resident fish further suggests limited interbreeding between forms, therefore implying a genetic basis for the differences in enzyme expression level observed between the two populations. Our results thus support the hypothesis that the physiological differences observed between river resident and anadromous fish are due to population differences in genes controlling the onset of the increase in hypo-osmoregulatory ability rather than the existence of a stable polymorphism between the two life history styles within a single gene pool.

Our study also demonstrates that visual identification is a valuable tool to separate sympatric freshwater resident and anadromous brook charr. However, it is important to verify whether this identification criterium is valid for separating brook charr populations in other river systems. We showed that anadromous brook charr and freshwater resident fish can also be discriminated on a physiological basis. On the other hand, the different seasonal patterns of thyroid hormones between domestic and wild anadromous fish suggest that environmental conditions are an important factor to consider. Our results also clearly demonstrated that hypo-osmoregulatory ability can be retained in captive population of brook charr and could be considered as a potential management tool.

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