

Reproductive events and associated reduction in the seawater adaptability of brook charr (*Salvelinus fontinalis*): evaluation of gill metabolic adjustments

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Abstract

We tested the hypothesis that along with gill Na⁺K⁺ATPase activity reduction, branchial energetic metabolism of brook charr (*Salvelinus fontinalis*) is altered during sexual maturation. Maturing and sterile fish were transferred from freshwater (FW) to seawater (SW). The activity of gill pyruvate kinase (PK), cytochrome C oxidase (COX), citrate synthase (CS) and lactate dehydrogenase (LDH) was measured at different intervals. Following 1 month in SW, twofold increases in Na⁺K⁺ATPase activity were recorded in all groups, while mean metabolic enzyme activities were sharply reduced (COX: 0.051 ± 0.029 to 0.036 ± 0.018; PK: 1.670 ± 0.565 to 1.210 ± 0.340 LDH: 2.245 ± 0.690 to 1.642 ± 0.381 μmol mg⁻¹ protein min⁻¹). Interestingly, during this period, no mortality occurred. After 5 months, comparison of Na⁺K⁺ATPase:CS and Na⁺K⁺ATPase:LDH ratios of mature and sterile fish held either in FW or SW, indicated that the response of Na⁺K⁺ATPase largely exceeds the response of the metabolic enzyme apparatus in hyperosmotic conditions. Hence, the reduced iono-osmoregulatory capacity and higher mortality observed in SW-maturing fish during the reproductive season appears to be mainly attributable to Na⁺K⁺ATPase activity reduction rather than the alteration of gill metabolic capacity, since no concurrent increase of metabolic enzyme activity with Na⁺K⁺ATPase activity occurred.

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Résumé

Réduction de la capacité d'adaptation à l'eau de mer de l'omble de fontaine (*Salvelinus fontinalis*) en période de reproduction: évaluation des ajustements métaboliques du tissu branchial. Nous avons vérifié l'hypothèse selon laquelle la réduction de l'activité de la Na⁺K⁺ATPase branchiale observée en période de reproduction chez l'omble de fontaine (*Salvelinus fontinalis*), est couplée à des ajustements métaboliques. Des poissons matures et stériles ont été transférés en eau douce (FW) et en eau salée (SW). L'activité de la pyruvate kinase (PK), de la cytochrome C oxydase (COX), de la citrate synthase (CS) et de la lactate deshydrogénase (LDH) a été mesurée à différents intervalles. L'activité de la Na⁺K⁺ATPase après un mois d'exposition à l'eau de mer a doublé chez tous les groupes alors que les activités moyennes de plusieurs enzymes métaboliques ont affiché une réduction marquée (COX: 0.051 ± 0.029 to 0.036 ± 0.018; PK: 1.670 ± 0.565 to 1.210 ± 0.340 LDH: 2.245 ± 0.690 to 1.642 ± 0.381 μmol mg⁻¹ protein min⁻¹) sans qu'aucune mortalité ne soit observée. Après cinq mois en eau salée, la comparaison des ratios Na⁺K⁺ATPase:CS et Na⁺K⁺ATPase:LDH en eau douce (contrôle) et salée, indique que la réponse de la Na⁺K⁺ATPase excède largement la réponse de l'appareil métabolique en condition hyper-osmotique. Ainsi, la réduction des capacités iono-osmorégulatrices et la mortalité plus élevée chez les poissons subissant la maturation sexuelle en eau salée, semblent être attribuables à la réduction de l'activité de la Na⁺K⁺ATPase plutôt qu'à une altération des capacités métaboliques car aucune augmentation ou réduction parallèle de l'activité des enzymes métaboliques avec la Na⁺K⁺ATPase n'a été décelée.

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1. Introduction

Brook charr (*Salvelinus fontinalis*) mariculture has been recently identified as a potential economical activity for the coastal regions of eastern Québec, Canada (Le François et al., 2002). The coastal environment of the St. Lawrence estuary is, however, characterized by the rapid lowering of the water temperature during the fall, subzero temperatures and extensive ice-coverage from December to April, which proscribes year-round sea cage activities. The level of commercial production of brook charr in freshwater (FW) was judged adequate to support the growth of a production strategy based on seasonal on-growing in marine cages. In 2000, a multi-disciplinary research program (Program ÉCO) aimed at the evaluation of the biological, environmental, technical, economic and social aspects of this activity was initiated by the governmental and research authorities.

Successful sea cage farming of brook charr in coastal waters demands the acknowledgment of the physiological constraints of the hypoosmoregulatory abilities of this species (see review by Claireaux and Audet, 2000). In addition to temperature and size requirements prior to seawater (SW) transfer, precocious sexual maturation severely limits the extent of the SW growing season of brook charr (McCormick and Naiman, 1985; Besner and Pelletier, 1991). Numerous studies have highlighted the implication of sexual development in the SW tolerance of various species of anadromous fish. Gonadectomy (Pickering and Dockray, 1972; Aida et al., 1984) and absence of gonadal growth (Le François et al., 1997) improved seawater adaptability (SWA) whereas the administration of gonadal steroids (Lundqvist et al., 1989; Madsen and Korsgaard, 1991; Schmitz and Mayer, 1993) and gonadal development (McCormick and Naiman, 1985; Staurnes et al., 1994; Le François et al., 1997; Persson et al., 1998) impaired SW tolerance in two *Salvelinus* species, brook charr (*S. fontinalis*) and Arctic charr (*S. alpinus*), and in Atlantic salmon (*Salmo salar*) during the typical reproductive season of these species.

Triploidy is currently viewed as a genetic containment measure for mass production of fish in coastal waters. Triploidization is, however, known to cause significant reduction of the SWA in several species of salmonids. Galbreath and Thorgaard (1995) and Withler et al. (1995) observed poor survival in SW trials due to endocrinological differences, delayed smoltification in relation to size and lower stress resistance in sea cages. Furthermore, recent SW growth trials of 0⁺50g diploid (2n) and triploid (3n) brook charr in our facilities revealed significant difference in mortality rates following transfer in SW: 2n : 4–7% and 3n : 19–25% (Le François et al., unpublished data).

In the light of the numerous studies previously cited on the drawbacks imposed by sexual development, the administration of sex hormones or triploidy on SWA in salmonids, we investigated the apparent incompatibility of SW residency and sexual maturation using an alternative method of fish sterilization: ionizing radiation (IR). The occurrence of

changes in Na⁺K⁺ATPase activity (previously published in Le François and Blier, 2000) in relation with adjustments in gill metabolic capacity of brook charr as gonadal growth progresses in hyperosmotic conditions is evaluated.

Gills constitute a major organ for maintaining the ionic and osmotic balance in fish by actively transporting ions (mainly Na⁺, Cl⁻ and K⁺) to preserve the osmolality of intracellular compartments. Na⁺K⁺ATPase, a key enzyme in ion transportation, generates ionic and electrical gradients through ATP hydrolysis to provide the necessary energy (Kirschner, 1993; McCormick, 1995). Gill Na⁺K⁺ATPase activity increases in correlation to salinity tolerance in brook charr (*Salvelinus fontinalis*) (Besner and Pelletier, 1991; Le François and Blier, 2000) and arctic charr (*S. alpinus*) (Finstad et al., 1989; Arnesen et al., 1992; Schmitz, 1995).

Iono-osmoregulation likely requires a significant energy expenditure because of the metabolic cost associated with the compensation mechanisms involved (Kirschner, 1993). Gill epithelium is qualified as a tissue with high energy needs and this is further supported by the abundance of mitochondria in the chloride cells (CC) (Perry and Walsh, 1989), high protein turnover (Haschemeyer and Smith, 1979; Lyndon and Houlihan, 1998) and protein breakdown (Somero and Doyle, 1973). This is made particularly obvious by its ability to adapt in response to changing environmental conditions (Laurent and Perry, 1991) by adjusting the number, orientation and/or mitochondrial content of the CC or mitochondrial enzyme activities after exposure to natural disturbances or environmental pollutants (Utida et al., 1971; Pärt and Bergström, 1995; Gagnon and Holdway, 1999). During SW adaptation, the gills, kidney and intestine are known to increase energy consumption (Kirschner, 1993). The energy reserves of the gills in SW conditions have been qualified by Perry and Walsh (1989) and Soengas et al. (1995a) as insufficient to cover the increased demand in easily oxidizable fuels.

Both Na⁺K⁺ATPase activity (Madsen and Korsgaard, 1991; Schmitz and Mayer, 1993; Madsen et al., 1997) and CC number and size (Miwa and Inui, 1986) were reduced after steroid hormone treatment. Gonadal development similarly affects the level of activity of this enzyme in two species of charr (*S. alpinus*: Staurnes et al., 1994; *S. fontinalis*: Le François and Blier, 2000); immature or sterile fish SW displayed significantly higher Na⁺K⁺ATPase activity during the reproduction period. Uchida et al. (1997) observed a reduction of filament CC density, Na⁺K⁺ATPase activity and increased osmolality in maturing chum salmon (*Oncorhynchus keta*) both in SW and FW. Since Na⁺K⁺ATPase activity is an energy demanding process, and gill tissue has a high aerobic capacity, it is possible that the osmoregulatory function of this tissue could be partly limited by its metabolic capacity.

Changes in the cost of osmoregulation are not easily linked to salinity-related changes at the whole-body metabolism level (Morgan and Iwama, 1991; Kirschner, 1993). In order to relate energy metabolism to osmoregulation performances, we propose to evaluate changes in the activity of key metabolic enzymes at the gill level based on the widely

accepted assumption that enzyme activity levels measured *in vitro* are directly related to enzyme quantity and thus enables the detection of metabolic adjustments in a given tissue (Simpkins, 2000; Hochacka and Somero, 2002). Studies aimed at the elucidation of the relationship between osmoregulation and metabolic capacity in salmonids are few (Langdon and Thorpe, 1984; Langdon et al., 1984; McCormick and Saunders, 1987; Soengas et al., 1995a, b) and to our knowledge, this is the first investigation involving gill energy metabolism and the observed decline in SWA linked to sexual maturation in an anadromous species. The activities of key metabolic enzymes: cytochrome *C* oxidase (COX), citrate synthase (CS), pyruvate kinase (PK) and lactate dehydrogenase (LDH) were measured. Mitochondrial enzyme activity (CS and COX) is indicative of tissue aerobic capacity, and gill CS activity is indicative of CC number and Na⁺K⁺ATPase activity (Perry and Walsh, 1989). PK and LDH measurements are being used as indicators of the glycolytic capacity of the gill tissue and of lactate oxidation capacity.

IR technology provides a useful tool for examining metabolic changes in the presence or absence of gonadal growth. This fish sterilization method (Donaldson et al., 1993) is based on the high radiosensitivity of germ cells (Konno, 1980; Konno and Tashiro, 1982). An interesting characteristic is that IR applied at various stages and at different doses (Konno and Tashiro, 1982; Le François et al., 1997) results in varying yields of sterility (i.e. both mature and sterile fish are produced for a given exposure). The respective effects of the IR treatment (i.e. the possible side effects of the IR exposures) and gonadal growth inhibition on gill glycolytic and mitochondrial metabolic capacity can then be properly evaluated.

In a previous experiment (Le François and Blier, 2000) in SW, sterile brook charr displayed a significantly higher Na⁺K⁺ATPase activity during the reproductive season than maturing females. This suggests that iono- and osmoregulatory impairment during sexual maturation is related to a decreased capacity of ion transportation. However, impairment could also be due to the impact of sexual maturation on gill metabolic capacity since Na⁺K⁺ATPase activity depends on the tissue capacity to generate ATP. In this case, the impact of maturation on the activity of the key energy metabolic enzymes should be higher or equivalent to the observed impact on Na⁺K⁺ATPase activity.

2. Materials and methods

2.1. Origin of fish

A domestic strain of *Salvelinus fontinalis* (Baldwin strain) was used and maintained at the Aquaculture Station in Pointe-au-Père (Québec, Canada). Sterility was induced by exposure to increasing levels of IR (6.2, 7.8 and 11.4 gray). One gray (Gy) dose is equivalent to 1 J of deposited energy per kilogram of tissue. The IR treatment was applied on

newly hatched fry as previously described in Le François et al. (1997). Using three separate groups at increasing IR exposures made it possible to determine the effect of irradiation *per se* on the physiological characteristics investigated. Initial growth in FW (Le François et al., 1999), survival (Le François et al., 1997) and level of activity of gill Na⁺K⁺ATPase (Le François and Blier, 2000) following transfer to SW conditions revealed no persistent negative side effects of the irradiation treatment.

Sterility was successfully induced in females only and thus intergroup and mature/sterile comparisons will only consider females in our results and discussion. Early in the summer (June and July samplings), gonadal status and sex could not be assessed accurately and thus no distinction was made regarding gender or gonadal status. The number of mature and sterile fish within the different treatments are reported in the appropriate figures and tables. The proportion of sterile fish within the four experimental treatments was found related positively to the level of IR exposure (0% in the control group, 27% in the 6.2 and 7.8 Gy and 60% in the 11.4 Gy). Mortality in SW only began occurring at the onset of the spawning season (September) and at the last sampling was found to be significantly higher in the maturing fish than in the sterile fish (65.9% compared to 0%) (Le François et al., 1997).

2.2. Experiment design

The experiment was initiated in the spring when osmoregulatory capacities are at their highest level (Besner and Pelletier, 1991) and the fish are outside the period of sexual maturation. On 24 June 1997, 25 brook charr per treatment measuring at least 19 cm were taken at random from the FW tanks. The fish were weighed (g), measured (cm) and assigned to four 200-l tanks (general mean weight and length: 140.8 ± 32.2 g and 22.1 ± 1.5 cm, respectively). Prior to SW transfer, the fish were acclimated in order to minimize the stress caused by the transfer procedures. The FW supply was then directly replaced by natural SW (flow-through system, salinity of 27.6). Temperature and salinity followed environmental variations and were recorded daily. The natural photoperiod was in effect. Fish were hand-fed to satiety on a commercial feed every 2 d. The mean temperature during the SW phase was 6.8 ± 1.7 °C (minimum = 2.2 °C, 29 November, maximum = 11.2 °C, 8 August), and mean salinity was 28.2 ± 1.3 (minimum = 24.6, maximum = 31.5).

2.3. Sampling procedure

Fish sampled to determine enzyme activity were killed by a blow to the head, their length (cm) and weight (g) measured. Sterility was assessed by an anatomical observation of the gonads, coupled with a gonadosomatic calculation as previously described (Le François et al., 1997). More than 80% of the fish considered sterile had a GSI of less than 1%. No sterile or immature fish were found in the control group during the reproductive season.

Three samplings were carried out: the first prior to the direct transfer (late June; $n = 5$ per treatment), the second 1 month after the introductions (late July; $n = 5$ per treatment) and the third on the last day of the experiment on 29 November ($n = 5, 8, 9$ and 10 for the control, 6.2, 7.8 and 11.4 Gy groups, respectively, depending on the number of mortalities within each experimental group). An additional sampling was done in October in FW conditions (control and 11.4 Gy) ($n = 10$) as a means of evaluating the potential effects of the IR exposures and gonadal status of brook charr females held in a hypoosmotic environment.

2.4. Tissue extraction

At each sampling, the second gill arches on the left side were dissected out, carefully washed with ice-cold imidazole buffer (pH 7.4), dried softly and quickly frozen at -80°C in SEI buffer (300 mM sucrose, 20 mM Na_2EDTA and 100 mM imidazole, pH 7.1) until analysis. Enzymatic contamination in remaining blood in the branchial tissue (Ferguson and Storey, 1991) was evaluated ($n = 6$ fish with or without perfusion of parts of the same gill arch and indicated a stable 5 % and 11% contribution for CS and LDH activity, respectively, and less than 2% contribution was measured for PK and COX). Gill samples were thawed on ice and carefully blotted dry. Gill filaments were then carefully separated from the gill arch, delicately sponged up and kept on ice between manipulations. The samples used to measure enzyme activity were then immediately weighed, rinsed and homogenized in 10 volumes of ice-cold 50 mM imidazole-HCl buffer (pH 7.4). All homogenates were kept on ice prior to measurement.

2.5. Gill enzyme activity measurement

To maximize our capacity to detect changes in enzyme activity levels, the assays were performed at 20°C on replicates by continuous spectrophotometry using an UV/Vis spectrophotometer (Perkin Elmer, Lambda 11) with a temperature-controlled cell holder. The assays for PK and LDH followed the disappearance of nicotinamide adenine dinucleotide, reduced form (NADH) at 340 nm. COX was assayed at 550 nm to follow the oxidation of reduced cytochrome *C*. CS was monitored at 412 nm to detect the transfer of sulfhydryl groups of reduced acetyl-CoA to 5,5' dithiobis-2-nitrobenzoic acid (DTNB). The micromolar extinction coefficients for NADH, DTNB and cytochrome *C* were 6.22, 13.5 and $19.1 \text{ ml } \mu\text{mol}^{-1} \text{ cm}^{-1}$, respectively. The concentration of the extract was adjusted to provide a linear response for at least 2 min and assessed in a final volume of 1 ml. Multisubstrate reactions were started by adding the substrate which was omitted from the control. Activity is expressed in units ($\mu\text{mol mg}^{-1} \text{ protein min}^{-1}$). The protein content in the crude gill homogenate was measured (Smith et al., 1985). The assay conditions were optimized for gill brook charr tissue prior to measurement.

2.5.1. Mitochondrial enzymes

Cytochrome *C* oxidase (EC 1.9.3.1): 50 mM imidazole-HCl, 50 μM reduced cytochrome *C*, pH 8.0. Reactions were run against a reference of 50 μM cytochrome *C* oxidized with 0.33% (w/v) potassium ferricyanide.

Citrate synthase (EC 4.1.3.7): 50 mM imidazole-HCl, 0.1 mM DTNB, 0.1 mM acetyl-CoA, 0.15 mM oxaloacetate (omitted from the control), pH 8.0.

2.5.2. Glycolytic enzymes

Pyruvate kinase (EC 2.7.1.40): 50 mM imidazole-HCl, 10 mM MgCl_2 , 100 mM KCl, 5 mM ADP, 0.16 mM NADH and 7 mM phosphoenol pyruvate (omitted from the control), LDH was provided in excess, pH 7.4.

Lactate dehydrogenase (EC 1.1.1.27): 50 mM imidazole-HCl, 0.16 mM NADH, 0.8 mM pyruvate (omitted from the control), pH 7.4.

2.5.3. Iono-osmoregulatory enzyme

Sodium-potassium adenosinetriphosphatase, $\text{Na}^+\text{K}^+\text{ATPase}$ (EC 3.6.1.37): was measured in the gills of same experimental fish using the method of Zaugg (1982), modified according to Heinonen and Lahti (1981) (in Le François and Blier, 2000).

2.6. Calculations and statistical analyses

Differences in enzyme activity values amongst the experimental groups and sampling times were detected using the one-factor ANOVA procedure. Whenever there were significant differences, a Tukey multiple-comparisons test was conducted to identify the groups that differed. The decision to pool the maturing and the sterile fish among the three levels of IR exposure (6.2, 7.8 and 11.4 Gy) for mature vs. sterile comparison was made after testing for possible differences among mature and sterile females originating from the different groups. Mature/sterile comparisons and the experimental groups in the October FW control sampling were analyzed using the Student's *t*-test (Bonferonni correction applied). A stepwise simple linear regression analysis was used to detect the significance of a functional dependence between levels of $\text{Na}^+\text{K}^+\text{ATPase}$ and metabolic enzyme activity. Normality (Kolmogorov-Smirnov) and homogeneity of the variance was assessed and all enzyme activity data were log transformed prior to statistical analysis. A probability level of $P < 0.05$ was set to indicate significant differences between sample means (Zar, 1984).

3. Results

At the time of the spring in hypoosmotic conditions and during a period of little gonadal growth, no significant differences in the level of measured metabolic enzyme activity was observed amongst the experimental groups ($P > 0.05$). The overall mean activity levels were 0.05 ± 0.03 , 0.12 ± 0.04 ,

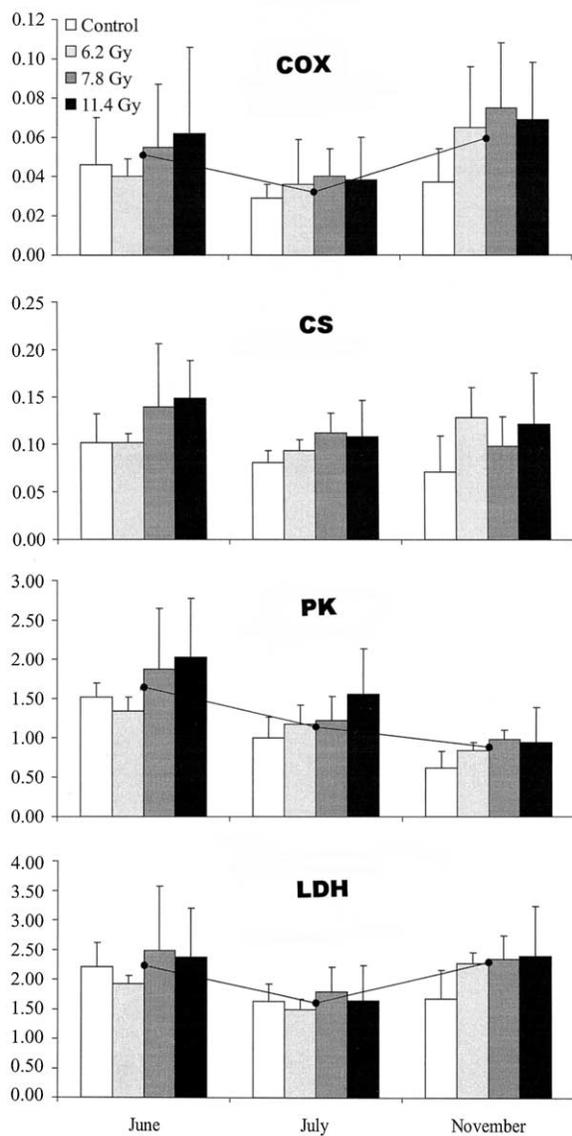


Fig. 1. Branchial metabolic enzyme specific activities of COX, CS, PK and LDH ($\mu\text{mol mg}^{-1}\text{protein min}^{-1}$) of all ionizing exposures at the three sampling intervals (June-July-November) (June and July, $n = 5$ per experimental group; in November, $n = 5, 8, 9$ and 10 for the control, $6.2, 7.8$ and 11.4 Gy, respectively). Significant differences among sampling periods for a given enzyme are indicated by the presence of a line joining their mean activity values.

Table 2

FW and SW enzymatic activities ($\mu\text{mol mg}^{-1}\text{protein min}^{-1} \pm \text{S.D.}$) measured during the reproductive season¹ and used for the ratio calculations of $\text{Na}^+\text{K}^+\text{ATPase}:\text{metabolic enzyme}$ (0 and 11.4 Gy in FW, $n = 10$; mature and sterile in FW, $n = 8$ and 7 , respectively, and in SW, $n = 12$ and 15 , respectively)

	$\text{Na}^+\text{K}^+\text{ATPase}^2$		CS		COX		PK		LDH	
	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW
0 Gy	1.1 ± 0.5		0.09 ± 0.03		0.06 ± 0.03		0.93 ± 0.32		1.21 ± 0.34	
11.4 Gy	1.5 ± 0.3		0.11 ± 0.03		0.09 ± 0.05		0.88 ± 0.12		1.52 ± 0.34	
<i>P</i> -value	0.4594		0.4882		0.9071		0.6290		0.4826	
Mature	$0.9 \pm 0.4^*$	4.0 ± 1.5^a	0.07 ± 0.03	0.08 ± 0.03^a	0.07 ± 0.04	0.05 ± 0.03	0.94 ± 0.20	0.79 ± 0.21	1.39 ± 0.30	1.88 ± 0.35^a
Sterile	$1.3 \pm 0.3^*$	7.2 ± 1.9^b	0.10 ± 0.03	0.13 ± 0.02^b	0.08 ± 0.03	0.07 ± 0.03	0.91 ± 0.09	0.95 ± 0.37	$1.47 \pm 0.25^*$	2.57 ± 0.36^b

¹Enzymes presenting significantly different levels of activity (mature-sterile comparisons) are identified with different superscript letters (vertical axis). Significantly different levels of activity within a given group (FW-SW comparisons) are identified by the presence of an asterisk (horizontal axis).

²Data from Le François and Blier (2000) in $\mu\text{mol mg}^{-1}\text{protein h}^{-1}$ measured at 37°C (Zaugg, 1982 modified according to Heinonen and Lahti, 1981).

Table 1

Comparison of mean branchial metabolic enzyme values: COX, CS, PK, and LDH of mature and sterile brook charr after 5 months in hyperosmotic conditions (June-July-November) ($n = 12$ and 15 , respectively)

	Enzyme activities ($\mu\text{mol mg}^{-1}\text{protein min}^{-1}$)		<i>P</i> -value
	Mature	Sterile	
COX	0.056 ± 0.030	0.069 ± 0.030	0.290
CS	0.082 ± 0.034	0.124 ± 0.045	0.035
PK	0.799 ± 0.261	0.935 ± 0.350	0.289
LDH	1.86 ± 0.82	2.44 ± 0.60	0.034

1.64 ± 0.48 and $2.25 \pm 0.61 \mu\text{mol mg}^{-1}\text{protein min}^{-1}$ for COX, CS, PK and LDH, respectively (Fig. 1).

One month after SW transfer (in July), no significant differences in the activity level of the four enzymes were observed amongst the groups ($P > 0.05$). At the last sampling in November, when gonadal status was considered as a grouping criterion (mature vs. sterile), significant differences in CS ($P = 0.024$) and LDH ($P = 0.0188$) ($\text{CS} = 0.08 \pm 0.03$ and 0.13 ± 0.03 and $\text{LDH} = 1.88 \pm 0.35$ and $2.57 \pm 0.36 \mu\text{mol mg}^{-1}\text{protein min}^{-1}$, respectively, 1.6 and 1.4 times higher in sterile fish) activity between the mature and sterile females were revealed (Table 1). In general, a significant reduction of COX ($P = 0.001$), and LDH ($P = 0.003$) activity was observed 1 month after the SW transfer, followed by an increase in activity levels in November. PK displayed a steady reduction in mean specific activity (June > July > November, $P = 0.029$). In hypoosmotic conditions (October FW control sampling), neither gonadal status nor IR treatment (11.4 Gy) were found to significantly alter gill metabolic capacity ($P > 0.05$) (Table 2).

We evaluated the respective adjustments in the metabolic enzyme activity of mature and sterile fish in relation to their increase in $\text{Na}^+\text{K}^+\text{ATPase}$ activity levels (as measured previously by Le François and Blier, 2000) following SW introductions by comparing their metabolic enzyme activity values with those of the October FW control sampling (Table 2). After 5 months in SW conditions, the $\text{Na}^+\text{K}^+\text{ATPase}$ activity of the maturing fish was $4.0 \pm 1.5 \mu\text{mol Pi mg}^{-1}\text{protein h}^{-1}$ compared to the value of $1.1 \pm 0.3 \mu\text{mol Pi mg}^{-1}\text{protein h}^{-1}$ measured in the October FW control (a 264% increase in SW). However, at the same period, sterile fish maintained in SW displayed an 80% higher $\text{Na}^+\text{K}^+\text{ATPase}$ activity than the

maturing fish (7.2 ± 1.9 compared to $4.0 \pm 1.5 \mu\text{mol Pi mg}^{-1}$ protein h^{-1} , $P = 0.0002$) (increase of 454% compared to FW values: $1.3 \pm 0.3 \mu\text{mol Pi mg}^{-1}$ protein h^{-1}). However, no clear indication of a positive relationship was found using regression analysis involving $\text{Na}^+\text{K}^+\text{ATPase}$ and CS, COX, PK or LDH activity levels.

4. Discussion

Our results corroborate earlier findings that sexual development, i.e. gonadal growth, impairs SWA. The present study links the detrimental effect of gonadal growth on $\text{Na}^+\text{K}^+\text{ATPase}$ activity to gill metabolic capacity modifications involving CS and LDH. CS and COX are indicators of the tissue mitochondrial content and these enzymes are widely used as indicators of respiratory tissue capacity (McCormick et al., 1989; Pelletier et al., 1994). Perry and Walsh (1989) attributed the differences in metabolic rate levels observed in gill cell suspensions between SW and FW adapted fish to the abundance of gill epithelial CC in correlation with CS and $\text{Na}^+\text{K}^+\text{ATPase}$ activity. Administration of steroids has been shown to decrease CC size and number (Miwa and Inui, 1986; Madsen et al., 1997) and lower $\text{Na}^+\text{K}^+\text{ATPase}$ activity (Ikuta et al., 1987; Miwa and Inui, 1986; Schmitz and Mayer, 1993; Madsen et al., 1997). Hence, the lower gill $\text{Na}^+\text{K}^+\text{ATPase}$ and CS activity of maturing fish could partly be related to the known effects of sexual hormones on mitochondria-rich CC abundance and/or size of the gill epithelium. Variations in the level of gill COX activity were for the most part similar to those of CS, but of a lesser magnitude. Gill COX did not, however, at any time differ significantly between mature and sterile fish, while CS did present significantly different levels of activity. Greater variation in COX activity among individuals may have obscured statistically significant differences or as suggested by numerous studies, COX could be regulated differently (McCormick et al., 1989; Thibault et al., 1997; Bélanger et al., 2003).

The general decrease in metabolic enzyme activity observed 1 month after the estuarine introductions could suggest tissue degradation following direct introduction to hyperosmotic conditions in the spring. The one cell-layer design of the branchial tissue makes it particularly susceptible to environmental stressors or damage. However, since no apparent differences in protein concentrations were observed amongst the experimental groups at any time, a loss of functionality rather than a general structural degradation seems more likely.

$\text{Na}^+\text{K}^+\text{ATPase}$ activity rose gradually after transfer from FW to SW, until late November (Le François and Blier, 2000). However, and in accordance with McCormick et al. (1989), we did not detect significant concurrent changes in gill CS and COX activity as significant increases of $\text{Na}^+\text{K}^+\text{ATPase}$ activity occurred. Increases from 150% to 400% in the $\text{Na}^+\text{K}^+\text{ATPase}$ activity of Atlantic salmon transferred from an isoosmotic milieu to salinities of 10 and 30

were reported with not more than a 20% variation in CS or COX activity levels. Our results indicate variations of 17.7% for CS and 20.5% for COX between FW and SW acclimated fish, despite an increase in $\text{Na}^+\text{K}^+\text{ATPase}$ activity of the order of 400–500%. Kelly et al. (1999) also reported a mismatch between $\text{Na}^+\text{K}^+\text{ATPase}$ variations and metabolic enzyme activities in black seabream (*Mylio macrocephalus*) at various salinities. McCormick et al. (1989) concluded that gill respiratory capacity was probably maintained in excess and that the increased $\text{Na}^+\text{K}^+\text{ATPase}$ activity in hyperosmotic environments represented a negligible component of the energy requirements of the whole gill tissue. Interestingly, our results indicate a 75% higher LDH activity level in SW than in FW in sterile fish and only a 35% increase in activity in maturing fish (a 40% discrepancy). These results suggest that the capacity of gills to mobilize and oxidize lactate is impaired during sexual maturation. As reported by Mommson (1984), gill tissue appears to rely on glucose and lactate, obtained either through gill gluconeogenesis or via the bloodstream (Soengas et al., 1995a), as their main fuel to perform its osmoregulatory task. Increases in the level of glucose in the gills of rainbow trout (*O. mykiss*) with increasing salinity were linked to an increase in gill glycolytic capacity (Soengas et al., 1995a).

Despite twofold increases in the $\text{Na}^+\text{K}^+\text{ATPase}$ activity of the fish exposed to estuarine conditions after 1 month, an initial decrease in the activity levels of most metabolic enzymes was measured in July. This sharp decrease in activity levels was not related to any mortality. This suggests that the catalytic capacity of the brook charr in hyperosmotic conditions did not accompany the increase in energy requirements imposed by enhanced $\text{Na}^+\text{K}^+\text{ATPase}$ activity. However, activity levels of LDH, CS and COX for the maturing fish were similar to initial FW levels at the last sampling (November) while sterile fish experienced higher levels of activity for LDH and CS. In hyperosmotic conditions, the effect of gonadal development on metabolic organization could be linked to the potential effect of sexual hormones on the regulation of the protein synthesis of osmoregulatory tissues (Pickering and Dockray, 1972). If ionic and osmotic regulation utilizes a higher proportion of the gill's available aerobic capacity in hyperosmotic conditions, a lower flux of ATP is left to support other tasks like cell maintenance. The structural and functional integrity of the tissue could have been impaired. If we consider the possible competition for energy between the demanding process of protein turnover, the maintenance of the structural and functional integrity of the gill epithelium could be challenged in hyperosmotic conditions. In this respect, Houlihan et al. (1986) reported for rainbow trout that compared to muscle, where 76% of protein synthesis translates into growth, in gills only 4% of the synthesized protein will contribute to the growth process.

Furthermore, the calculation of the ratios of energy-consuming $\text{Na}^+\text{K}^+\text{ATPase}$ activity and the activity of key metabolic enzymes (CS and LDH), allowed the observation of an important bias in hyperosmotic conditions in favor of

$\text{Na}^+\text{K}^+\text{ATPase}$ (data extracted from Table 2). For instance, mature fish have an $\text{Na}^+\text{K}^+\text{ATPase}/\text{CS}$ ratio in FW (October control) of 12, while this ratio is 50 for fish in SW. For the same fish, the $\text{Na}^+\text{K}^+\text{ATPase}/\text{LDH}$ ratio is 0.65 in FW and 2.13 in SW. This indicates that the response of $\text{Na}^+\text{K}^+\text{ATPase}$ largely exceeds the response of the energy metabolism apparatus in hyperosmotic conditions. However, when we compare mature and sterile fish, it seems that this discrepancy in enzyme responses to SW does not explain the weaker SWA of maturing fish. When we compare $\text{Na}^+\text{K}^+\text{ATPase}/\text{CS}$ and $\text{Na}^+\text{K}^+\text{ATPase}/\text{LDH}$ ratios of sterile and maturing fish in SW, the ratios are even higher in sterile fish (55 compared to 50 for $\text{Na}^+\text{K}^+\text{ATPase}/\text{CS}$ and 2.80 compared to 2.13 for $\text{Na}^+\text{K}^+\text{ATPase}/\text{LDH}$). Therefore, it appears that a key response during SW adaptation is the adjustments of $\text{Na}^+\text{K}^+\text{ATPase}$, and, that the energy metabolism capacity can, in most conditions, fulfill the energy requirements set by the osmoregulatory processes.

5. Conclusion

SW exposure prior to the reproductive events exerts some degree of alteration on the energetic metabolism of the gill tissue of brook charr. However, the increases in $\text{Na}^+\text{K}^+\text{ATPase}$ activity following SW introduction were not accompanied by an equivalent increase in the aerobic capacity of the tissue. Our results indicate that the loss of SWA in hyperosmotic conditions due to sexual development is mainly attributable to $\text{Na}^+\text{K}^+\text{ATPase}$ decreases rather than the inability of the metabolic capacity of the gill tissue to meet the energy requirements. Furthermore, additional evidence of the uncoupled nature of $\text{Na}^+\text{K}^+\text{ATPase}$ activity variations and key metabolic enzyme in anadromous species is provided.

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