CHAPITRE 2
RÉPONSES PHYSIOLOGIQUES ET HÉRITABILITÉ DE TRAITS LIÉS À LA REPRODUCTION CHEZ DES OMBLES DE FONTAINE (*SALVELINUS FONTINALIS*) ANADROMES, RÉSIDENTS ET HYBRIDES

2.1 RÉSUMÉ

L'objectif de cette étude était de déterminer si des ombles de fontaine anadromes, résidents et hybrides réciproques présentaient des différences physiologiques et génétiques pour des traits liés à la reproduction. Des ombles de fontaine anadromes (A) et résidents (R) provenant du même bassin hydrographique ont été utilisés pour produire quatre types de croisements (♀A♂A, ♀A♂R, ♀R♂A, et ♀R♂R). Les conditions d'élevage étaient identiques, de l'incubation des oeufs jusqu'à l'âge de 22 mois. Aucune différence pour le pourcentage de poissons atteignant la maturité sexuelle à 1+ n'a été observée entre les croisements, mais ce pourcentage était plus élevé chez les mâles que chez les femelles. Chez les femelles, des différences entre les anadromes et les résidents au chapitre de l'accumulation des réserves énergétiques hépatiques (principalement le glycogène), de l'indice hépato-somatique et des concentrations hépatiques de vitellogénine entre juin et novembre suggèrent un rythme de maturation et un investissement reproducteur différents pour les deux souches pures. Chez les mâles, aucune différence d'accumulation de glycogène hépatique n'a été observée en novembre, vraisemblablement en raison du coût relativement faible de la reproduction chez les mâles, indépendamment de la stratégie migratrice utilisée. Des effets de croisement ont été observés en juin pour le contenu protéique hépatique, et en novembre pour le contenu glycogénique hépatique chez les femelles. Les hybrides ont souvent présenté des valeurs similaires à celles des poissons d'au moins une des deux souches pures pour les réserves hépatiques et d'autres traits. Plusieurs traits se sont

avérés héritables chez au moins une des deux souches pures. Chez les anadromes, seuls la masse corporelle et le glycogène hépatique étaient hautement héritables (h^2 = 0,60 et 0,87). Les valeurs d'héritabilité des résidents étaient élevées pour l'IHS et le KF (h^2 = 0,65 et 0,56, respectivement), modérées pour la masse corporelle (h^2 = 0,40), et nulles pour toutes les réserves énergétiques hépatiques (h^2 = 0,00). Les variances des EBV (variances génétiques des valeurs reproductives estimées, c'est-à-dire variances additives estimées) étaient élevées pour la masse corporelle chez les anadromes et les résidents (σ^2 = 114,26 et 75,82, respectivement) et pour le glycogène hépatique chez les anadromes (σ^2 = 242,16), mais faibles pour tous les autres traits chez les deux souches pures. Ainsi, les ombles de fontaines anadromes et résidents sympatriques provenant de la rivière Laval ont présenté des différences phénotypiques et des héritabilités différentes pour plusieurs traits liés à la reproduction. Globalement, ces résultats indiquent une base génétique significative pour certains des traits reliés à la reproduction. Enfin, les valeurs de Q_{ST} (différenciation génétique quantitative de traits phénotypiques entre populations), à l'exception du KF, étaient élevées (de 0,31 à 0,82) et généralement supérieures à l'attendu neutre (F_{ST} = 0,15). Ceci suggère que des pressions sélectives divergentes ont contribué à maintenir des adaptations locales associées aux stratégies anadrome et résidente dans la rivière Laval.

2.2 PHYSIOLOGICAL RESPONSES AND HERITABILITY OF ANADROMOUS, RESIDENT AND HYBRID BROOK CHARR (*SALVELINUS FONTINALIS*) FOR TRAITS RELATED TO REPRODUCTION

INTRODUCTION

Utilization of alternative life history strategies, namely anadromy or freshwater residency, is relatively common in salmonids (Power, 1980; Gross, 1987; Thorpe, 1989; Thorpe, 1994; Arndt, 2000). The choice of alternative strategies presumably occurs because salmonids have high energetic requirements for growth and reproduction, that have to be obtained in environments where food is scarce and where competition is high (Thorpe, 1994). North temperate regions, where salmonids usually live, also have marked seasonal variations in biotic and abiotic variables, to which the fish must adapt to survive and ensure successful reproduction (Berg and Bremset, 1998). The alternative strategies are different behavioural solutions to obtain sufficient food while avoiding being eaten (Thorpe, 1994).

In brook charr, the utilization of contrasting habitats by individuals may lead to alternative life history strategies (anadromy or freshwater residence) (Boula *et al.*, 2002; Castric and Bernatchez, 2003) between populations, or alternative life history tactics within populations, in coastal areas (Thériault and Dodson, 2003; Thériault *et al.*, 2007a; Homel *et*

al., 2008). Such a differentiation is not only a consequence of a high phenotypic plasticity (Hutchings, 1996), but also presumably the result of different selective pressures leading to an evolutionary divergence of populations into contrasting niches (Schluter, 2000; Bernatchez, 2004; Perry *et al.*, 2005).

Differences in migratory behaviour may have an impact on reproductive physiology. In ecotypes issued from the same population, body growth is one of the most critical factors in determining the onset of sexual maturation; as such, early maturation is very likely to be positively correlated with rapid growth (Thériault and Dodson, 2003; Unwin *et al.*, 2004; Thériault *et al.*, 2007a). Comparing resident and anadromous females issued from two sympatric populations, Perry *et al.* (2005) found that, at the late juvenile stage, life history traits were characterized by divergent selection. Independantly of size, anadromous dams produced more eggs and had more surviving fertilized eggs than resident dams (Perry *et al.*, 2005).

In males, sexual maturation generally occurs at younger age in resident than in anadromous fish, whether they originate from a single genetic population (Thériault *et al.*, 2007b) or from two sympatric ones (Boula *et al.*, 2002). Morinville and Rasmussen (2003) demonstrated that growth efficiency (the ratio of growth to food consumption) was higher in resident than in anadromous brook charr of the Ste. Marguerite River, suggesting a more rapid body growth in the former. Also, as it was demonstrated in other salmonid species,

higher growth rate and larger body size can be achieved by migrating to sea water, where richer feeding areas are available (Gross, 1987). Better feeding opportunities and the resulting higher lipid body reserves may be particularly critical for sustaining the physiological processes involved in sexual maturation, such as vitellogenesis, which requires the incorporation of a large amount of proteins and lipids into yolk precursors (Wiegand, 1996).

Vitellogenin (VTG) is a large glycolipophosphoprotein that serves as the major yolk precursor in oviparous vertebrates (Tyler and Sumpter, 1996; Buisine *et al.*, 2002). In fish, VTG is synthesized in hepatocytes, mainly under estrogenic control, and transported in the bloodstream to developing oocytes (Jalabert, 2005; Moussavi *et al.*, 2009). In salmonids, proteins and lipids of the yolk material are stored until the late stages of oogenesis and in the embryo (Kwon *et al.*, 2001); they constitute an important proportion of the final egg size (80% in rainbow trout, *Oncorhynchus mykiss*; Kwon *et al.*, 2001). This yolk material is thus of primary importance for the embryos, which must rely entirely on this internal food source for their growth and nutrition for several weeks after fertilization until first feeding (Kwon *et al.*, 2001; Lim *et al.*, 2001). In brook charr as well as in other salmonid species, vitellogenesis begins several months prior to spawning (Tyler and Sumpter, 1996). Despite this early increase in VTG levels, the most intensive phase of vitellogenesis, which is characterized by pronounced oocyte growth (Tyler and Sumpter, 1996) and a several-fold increase of the gonadosomatic index (GSI) (Tyler and Sumpter, 1996; Berg *et al.*, 2004),

takes place from July to September. The period of vitellogenesis may be subject to temporal variation within and between species, as there is evidence that growth rate is critical in determining the onset and extent of gonadal development, thus vitellogenesis, in salmonid fish (Adams and Huntingford, 1997).

Vitellogenesis in salmonids is relatively well known (e.g. Mellinger, 2002), but whether this physiological process, as well as the other aforementioned traits related to sexual maturation (hepatic reserves, HSI), varies in function of life history strategies remains unclear.

Using a common garden experimental design (same rearing and experimental environmental conditions), we had three aims. We tested whether phenotypic differences in terms of energy accumulation during summer and fall, age at first sexual maturation, and vitellogenesis exist between freshwater resident and anadromous brook charr originating from two distinct genetic pools (Boula *et al.*, 2002; Perry *et al.*, 2005). We also tested whether phenotypic differences in reproductive traits observed in the field and related to life history strategy have a significant genetic basis (selection versus neutral genetic processes, additive genetic effects and parental effects). Finally, we verified whether the differences between both populations could have been maintained by selective pressures related to local adaptations.

MATERIALS AND METHODS

Experimental design, rearing conditions, and sampling

The reader is referred to chapter 1 for description of breeding design, rearing conditions and samplings description. However, in the present chapter, an additional sampling time was added, i. e. in November, in fresh water at the time of reproduction. Fish were also sampled in June, while sill in FW, and in August, once returned in fresh water after 50 days spent in salt water.

Energetic reserve assays

Total hepatic protein content was assayed using the Bradford method (1976) with minor modifications. Biorad reagent (Biorad) was used, and the assays were performed as specified in the manufacturer's protocol. Protein concentrations were read at a wavelength of 595 nm. Hepatic glycogen was measured according to the Carr & Neff method (1984), with glucose being measured using o-toluidine reagent (Sigma-Aldrich) and glucose standards from the Quantichrom glucose assay kit (BioAssays). Glucose concentrations were read at a wavelength of 630 nm. The Frings method (1972) was used to assay total hepatic lipid content.

Vitellogenin enzyme-linked immunosorbent assays

Liver samples were homogenized in 0.1 M phosphate buffer at pH 7.0 to which 50 µl/ml protease inhibitor cocktail (Sigma-Aldrich) had been added to inhibit vitellogenin proteolysis; samples were stored at –80°C until analysis. Crude homogenates were centrifuged at 12,000 rpm for 10 min before the vitellogenin assays, which were performed using rainbow trout vitellogenin ELISA kits (Cayman Chemical) as described in the manufacturer's protocol. Vitellogenin was assayed in the livers of females only, since males do not produce this lipoprotein in a significant amount under normal physiological conditions.

Data analyses

Fulton's condition factor (KF) was calculated as $KF = (W\ L^{-3}) \times 100$, where W is fish body weight in grams and L is the fork length in centimetres (Pennell and Barton, 1996). The gonadosomatic index was calculated as $GSI = GW\ (W\text{-}GW)^{-1} \times 100$, where GW is gonad weight in grams. Gonad weight has a direct effect on body weight, thus gonad weight was subtracted from body weight to eliminate biases linked to gonad weight fluctuations. The hepatosomatic index was calculated as $HSI = LW\ (W\text{-}LW)^{-1} \times 100$, where LW is liver weight in grams. Kolmogorov-Smirnov and Brown-Forsythe tests were applied to assess data normality (K-S) and homogeneity of variance (B-F). In males and females, physiological differences among cross-types (CT), time (T), and maturation stages (MS) were analyzed by multi-ways analysis of variance (ANOVA). The family factor was nested

in cross-type and the following interactions were tested: CT × T, .MS × T, CT × MS, and CT × MS × T. Analyses of covariance (ANCOVA), with body weight as a covariate, were performed to analyze hepatic energy reserve results. Appropriate a posteriori tests (Tukey HSD) were applied when needed. Differences at $p < 0.05$ were considered to be significant. All the statistical analyses were performed with Statistica version 6.0.

Heritability estimates were calculated for phenotypic traits. However, it was not calculated for vitellogenin concentrations, because of insufficient numbers of individuals within each family. Heritability estimates were obtained using the ASREML software 2.0 (VSN International Ltd., UK) using the model $Y_i = \mu + A_i + e$, where Y is the response variable, μ is the mean, A is the random additive effect, and e is the vector associated with random error. Heritability values were quantified on pure cross-types only, and separately for each pure cross-type. Heritability calculations were done on data obtained in August. ASREML was also used to determine estimated breeding values (EBV, additive genetic merit; Mrode 2005) and parental effects (maternals and paternals). These effects were estimated by the ratio of parental (dam and sire) additive genetic variance on phenotypic variance. Parental effects were determined using the model $Y_i = \mu + A_i + M_i + P_i + e$, where Y is the response variable, μ is the mean, A is the random additive effect, M is the maternal effect, P is the paternal effect and e is the vector associated with random error. Parental effects were measured on all sampled individuals in August (anadromous, residents and reciprocal hybrids).

The extent of quantitative genetic variation in phenotype (Q_{ST}) between anadromous and resident brook charr for the studied physiological traits was calculated as $Q_{ST} = \sigma^2_{bw}/(2\sigma^2_{wn} + \sigma^2_{bw})$, where σ^2_{bw} was the phenotypic variance between populations (anadromous and residents), and σ^2_{wn} the within-population quantitative genetic variance, according to Perry *et al.* (2005). All the variance components used in the formula were obtained using the ASREML software 2.0 (VSN International Ltd., UK). The F_{ST} value used in the present study was the one calculated by Perry *et al.* (2005) between anadromous and resident brook charr of the Laval River (e.g. $F_{ST} = 0.153$, 95% CI = 0.071-0.214).

RESULTS

Females

Percentage of early sexual maturation and gonadosomatic index

Significantly less females than males experienced early sexual maturation at 1+ ($df = 1$, $F = 50.3$, $p < 0.001$). The percentage of early sexual maturation in females did not differ among cross-types ($df = 3$, $F = 0.53$, $p > 0.05$; Table 2.1) and occurrence of maturation was not correlated either with mass (ANCOVA, $df = 1$, $F = 1.03$, $p > 0.05$) or length (ANCOVA, $df = 1$, $F = 0.47$, $p > 0.05$) as determined from data obtained in November (Fig. 2.1). In August and November, GSI was similar among cross-types in both mature and immature females (Table 2.2). There was no significant interaction between cross-types and maturation stages (Table 2.2).

Vitellogenin

In June (all sexually immature fish), hepatic vitellogenin concentrations were low (0.02 - 5.06 mg g liver^{-1}), and did not differ between pure crosses but were significantly higher in the RR cross-type compared to AR females (Fig. 2.2A). In August, hepatic vitellogenin concentrations in females with gonadal maturation was more than 10 fold higher than concentrations measured in June (0.04 - 227.46 mg g liver^{-1}) but no cross-type

effect was present (Fig. 2.2B). However, large variations were observed among RR females. In November, hepatic vitellogenin concentrations were 10 fold higher than in August (5.85 - 2472.90 mg g liver^{-1}) and significantly higher in anadromous females than in resident ones (Fig. 2.2C). The two hybrid cross-types showed intermediate concentrations. In August and November, hepatic vitellogenin concentrations were significantly different between immature females and females with gonad development (maturation stage (MS), df =1, $F = 75.0$, $p < 0.001$; MS × CT, df = 3, $F = 0.30$, $p > 0.05$), immature females having similar vitellogenin concentrations than the ones measured in June (data not shown).

Table 2.1. Percentage of gonadal maturation in 1+ female and male brook charr.

Group	Females	Males
Anadromous (AA)	25.5 ± 5.6	69.7 ± 12.1
	[10.5 - 46.6]	[23.1 - 100.0]
Anadromous/resident hybrids (AR)	32.6 ± 6.3	69.7 ± 11.7
	[11.8 - 50.5]	[20.0 - 94.4]
Resident/anadromous hybrids (RA)	19.0 ± 6.8	76.4 ± 9.1
	[9.0 - 38.8]	[50.0 - 90.9]
Residents (RR)	25.5 ± 6.3	80.0 ± 6.6
	[5.5 - 41.9]	[63.6 - 100.0]

Mean ± SE. The familial range within each group is given in brackets.

Hepatosomatic index

Concomittant to vitellogenin production, HSI increased from August to November. In August, there was no difference among cross-types in HSI, which was significantly larger in females undergoing sexual maturation than in immature females (Table 2.2). In November, HSI was larger in mature anadromous females than in resident ones (Table 2.2). HSI in AA and AR females undergoing sexual maturation was similar (Table 2.2). Interestingly, HSI in RR immature females was larger than in immature females from the other cross-types (Table 2.2).

Fig. 2.1 A. Body weight of anadromous, resident, and reciprocal hybrid female brook charr from June to November. **B.** Body weight of anadromous, resident, and reciprocal hybrid male brook charr from June to November. AA: anadromous brook charr; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids; RR: resident fish. Results are expressed as mean ± standard error. Black symbols indicate sexually immature fish and white symbols mature fish. June: number of females varied from 56 to 78, and number of males from 64 to 79; August: number of females varied from 8 to 18, and number of males from 8 to 16; November: number of females varied from 14 to 37, and number of males from 8 to 49.

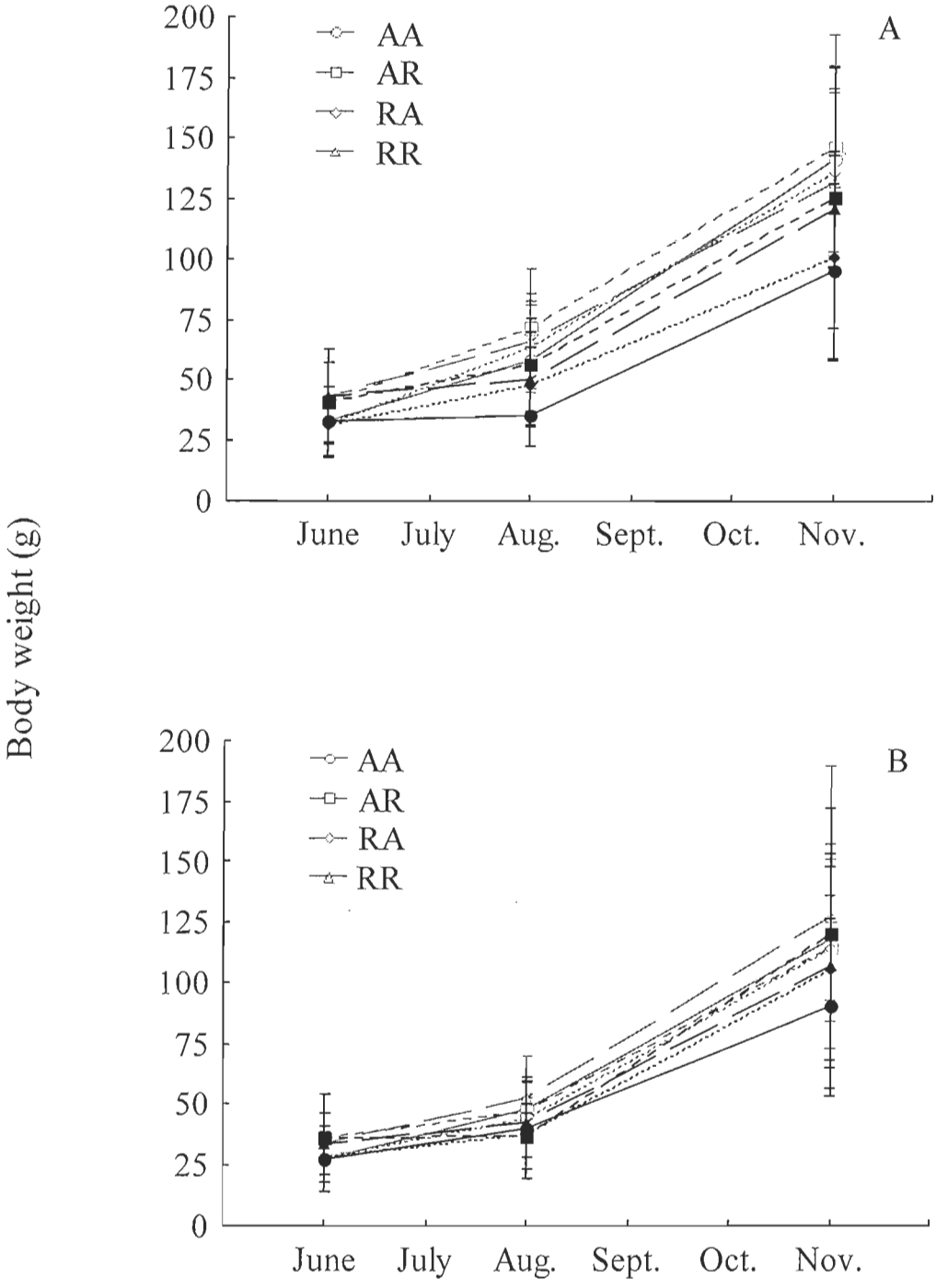
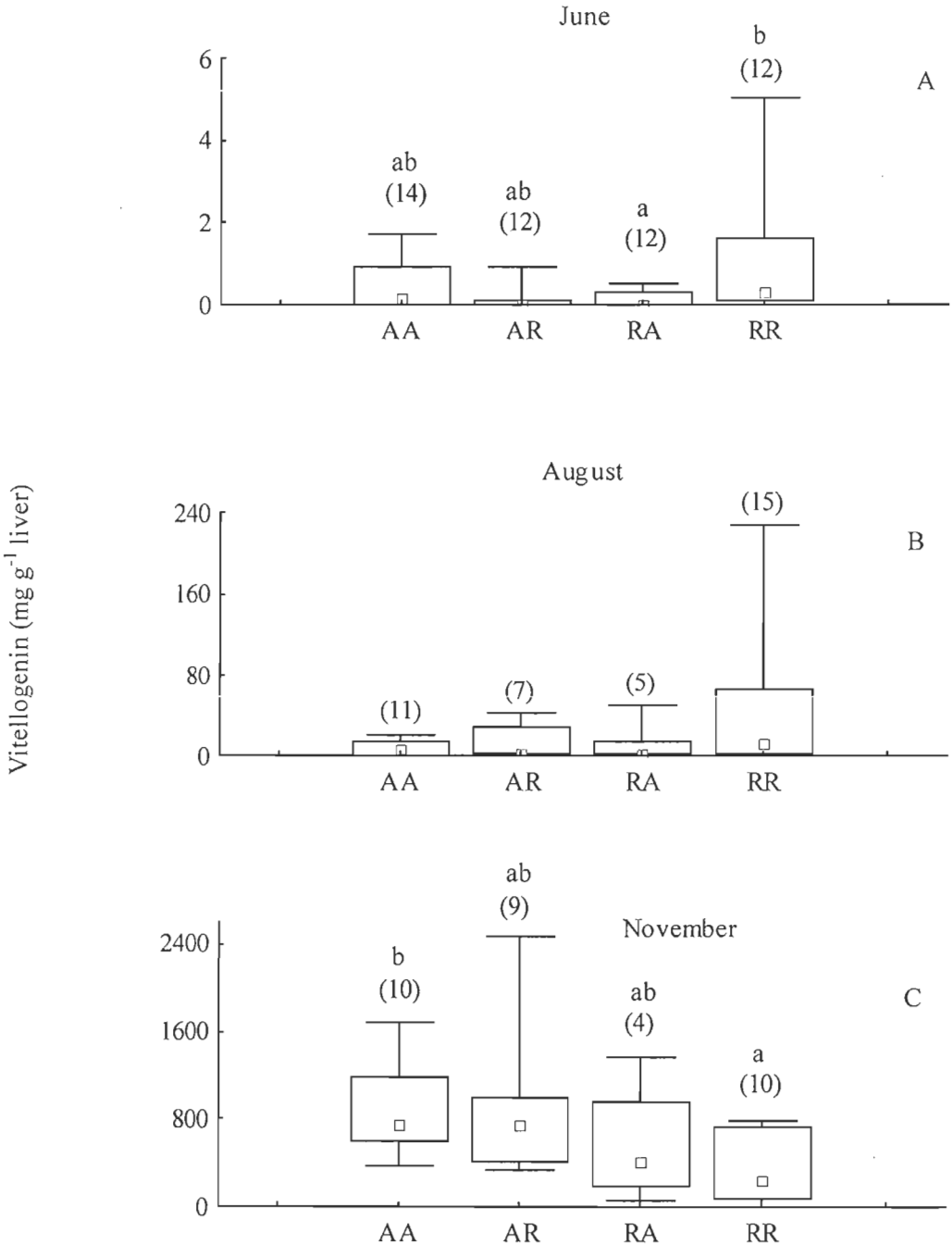

Body weight (g)
200
175
150
125
100
75
50
25
0
AA
AR
RA
RR
A
June
July
Aug.
Sept.
Oct.
Nov.
B

Table 2.2. Condition factor (KF), gonadosomatic index (GSI), and hepatosomatic index (HSI) in 1+ female brook charr (mean ± SE, n).

GSI	Maturation stage	AA	AR	RA	RR	Cross-type effects
June		0.22 ± 0.02, 25	0.21 ± 0.02, 26	0.22 ± 0.01, 21	0.24 ± 0.02, 23	
August	Immature	0.24 ± 0.01, 15	0.24 ± 0.02, 14	0.20 ± 0.02, 8	0.24 ± 0.01, 11	
	Mature	**0.40 ± 0.02,** 11	**0.42 ± 0.02,** 10	**0.41 ± 0.03,** 8	**0.87 ± 0.19,** 18	
November	Immature	0.41 ± 0.03, 32	0.33 ± 0.02, 35	0.42 ± 0.05, 28	0.45 ± 0.04, 32	
	Mature	**10.02 ± 0.38,** 31	**8.32 ± 0.93,** 37	**7.81 ± 0.99,** 14	**10.71 ± 1.08,** 21	
KF						
June		0.98 ± 0.01^{a}, 73	1.03 ± 0.01^{b}, 71	1.02 ± 0.01^{b}, 56	1.09 ± 0.01^{c}, 78	
August	Immature	1.05 ± 0.03, 16	1.10 ± 0.02, 14	0.98 ± 0.05, 8	1.12 ± 0.02, 10	AA^{a}AR^{ab}RA^{a}RR^{b}
	Mature	**1.06 ± 0.02, 10**	**1.10 ± 0.02, 10**	**1.11 ± 0.02, 8**	**1.15 ± 0.02, 18**	
November	Immature	1.00 ± 0.01, 32	1.07 ± 0.01, 35	1.06 ± 0.02, 28	1.17 ± 0.02, 32	AA^{a}AR^{b}RA^{ab}RR^{c}
	Mature	**1.08 ± 0.01, 31**	**1.12 ± 0.01, 36**	**1.07 ± 0.02, 14**	**1.22 ± 0.04, 21**	
HSI						
June		1.42 ± 0.04, 73	1.46 ± 0.03, 71	1.37 ± 0.05, 56	1.46 ± 0.03, 78	
August	Immature	1.35 ± 0.05, 16	1.52 ± 0.07, 14	1.38 ± 0.10, 8	1.43 ± 0.06, 11	
	Mature	**1.59 ± 0.10, 10**	**1.45 ± 0.05, 10**	**1.46 ± 0.08, 7**	**1.70 ± 0.08, 18**	
November	Immature	2.01 ± 0.06^{a}, 32	2.07 ± 0.06^{a}, 35	1.96 ± 0.07^{a}, 28	2.44 ± 0.09^{b}, 32	
	Mature	2.95 ± 0.11^{b}, 31	3.11 ± 0.17^{b}, 37	2.60 ± 0.19^{ab}, 14	1.95 ± 0.11^{a}, 21	

AA: anadromous; RR: freshwater residents; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids. Bold characters: significant maturation stage effect ($p < 0.05$) and absence of significant interaction between cross-type and maturation stage ($p > 0.05$). Different letters indicate significant difference among cross-types for data presented on the same line. GSI data were log-transformed for statistical analysis, but untransformed data are presented in the table.

Fig. 2.2. Vitellogenin concentrations in mature female brook charr from June to November. AA: anadromous brook charr; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids; RR: resident fish. Each box covers the middle 50 % of the data values (between the lower and upper quartiles) and the whiskers extend up to the minimum and maximum values, while the central point is at the median. Different letters indicate significant differences among cross-types ($p < 0.05$). Numbers of fish (n) are indicated above each data point.


June
A
b
(12)
ab
(14)
ab
(12)
a
(12)
6
4
2
0
AA
AR
RA
RR
August
B
(15)
(11)
(7)
(5)
240
160
80
0
November
C
ab
(9)
b
(10)
ab
(4)
a
(10)
2400
1600
800
0
Vitellogenin (mg g-1 liver)

Hepatic glycogen

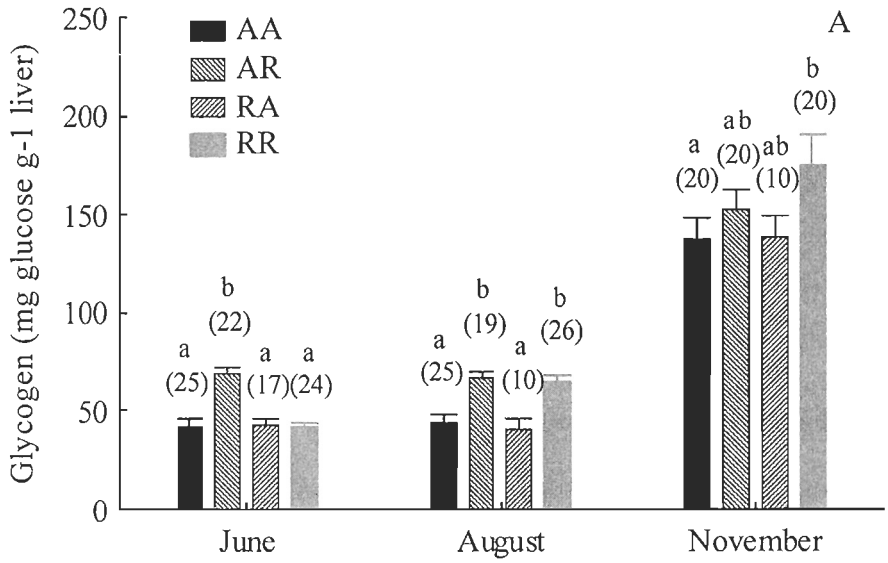
Overall, in August and November, the relative liver glycogen content was more elevated in resident than in anadromous females (Fig. 2.3A), hybrids having either intermediate (November) or similar (August) glycogen content than their paternal line. In August, relative liver glycogen content was significantly lower in immature females (50.8 ± 3.4 mg g liver $^{-1}$, n = 41) than in females undergoing gonad development (61.0 ± 2.8 mg g liver $^{-1}$, n = 39; MS: df = 1, F = 4.4, $p < 0.05$; MS × CT: df = 3, F = 1.0, $p > 0.05$). On the contrary, in November, glycogen was lower in sexually mature females (114.8 ± 6.3 mg g liver $^{-1}$, n = 34) than in immature females (187.9 ± 6.8 mg g liver $^{-1}$, n = 36; MS: df =1, F = 60.2, $p < 0.001$; MS × CT: df = 3, F = 1.28, $p > 0.05$).

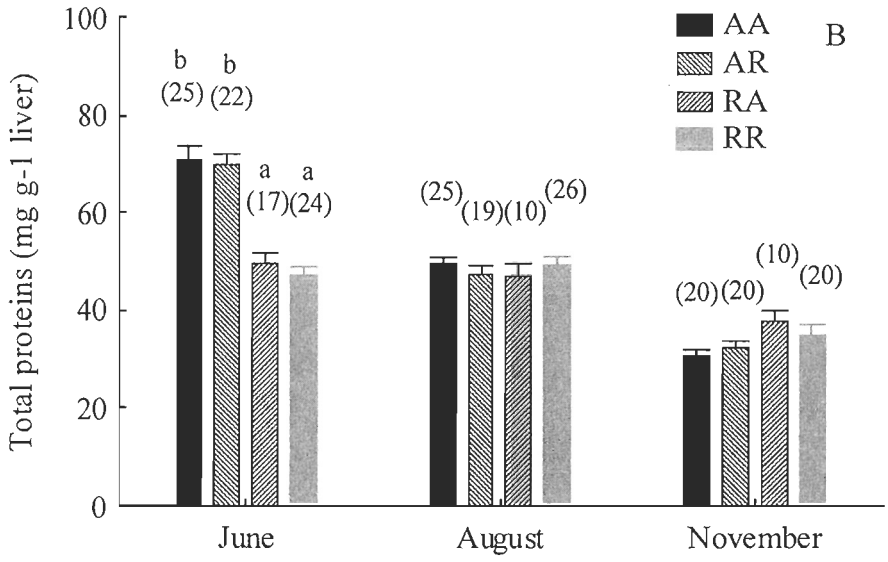
Hepatic proteins

In June, resident females had significantly lower hepatic protein content than their anadromous counterparts (Fig. 2.3B). In August, relative hepatic protein content did not differ among cross-types (CT: df = 3, F = 0.6, $p > 0.05$) or between immature females and those undergoing gonad development (MS: df = 1, F = 2.0, $p > 0.05$; MS × CT: df = 3, F = 2.5, $p > 0.05$). Again, no cross-type difference was observed in November (Fig. 2.3b). However, at this time of the year, relative protein content was significantly lower in immature (30.5 ± 1.0 mg g liver $^{-1}$, n = 36) than in sexually mature females (36.4 ± 1.3 mg g liver $^{-1}$, n = 34; MS: df = 1, F = 13.2, $p < 0.001$; MS × CT: df = 3, F = 0.8, $p > 0.05$). Hepatic lipid content varied similarly between males and females from June (22.8 ± 0.3

mg/g, n = 189) to November (28.4 ± 0.6 mg/g, n = 110), and increased by 1.25 times regardless of cross-type (CT × T × S: $F = 1.27$, $p > 0.05$). Overall, hepatic lipid contents were similar between anadromous and residents. RR livers contained significantly lower amounts of lipids (24.2 ± 0.5 mg/g, n = 121) than those of RA hybrids (26.2 ± 0.6 mg/g, n = 84). There was a significant positive correlation between body mass and hepatic lipid content (Fig. 2.4).

Fig. 2.3 A. Hepatic glycogen content of female brook charr from June to November. **B.** Hepatic protein content of female brook charr from June to November. AA: anadromous brook charr; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids; RR: resident fish. Results are expressed as mean ± standard error. Different letters indicate significant differences among cross-types for each specific sampling time ($p < 0.05$). Numbers of fish (n) are indicated above each data point.


A
AA
AR
RA
RR
Glycogen (mg glucose g-1 liver)
250
200
150
100
50
0
a (25)
b (22)
a (17)
a (24)
a (25)
b (19)
a (10)
b (26)
a (20)
ab (20)
ab (10)
b (20)
June
August
November


B
AA
AR
RA
RR
Total proteins (mg g-1 liver)
100
80
60
40
20
0
b (25)
b (22)
a (17)
a (24)
(25)
(19)
(10)
(26)
(20)
(20)
(10)
(20)
June
August
November

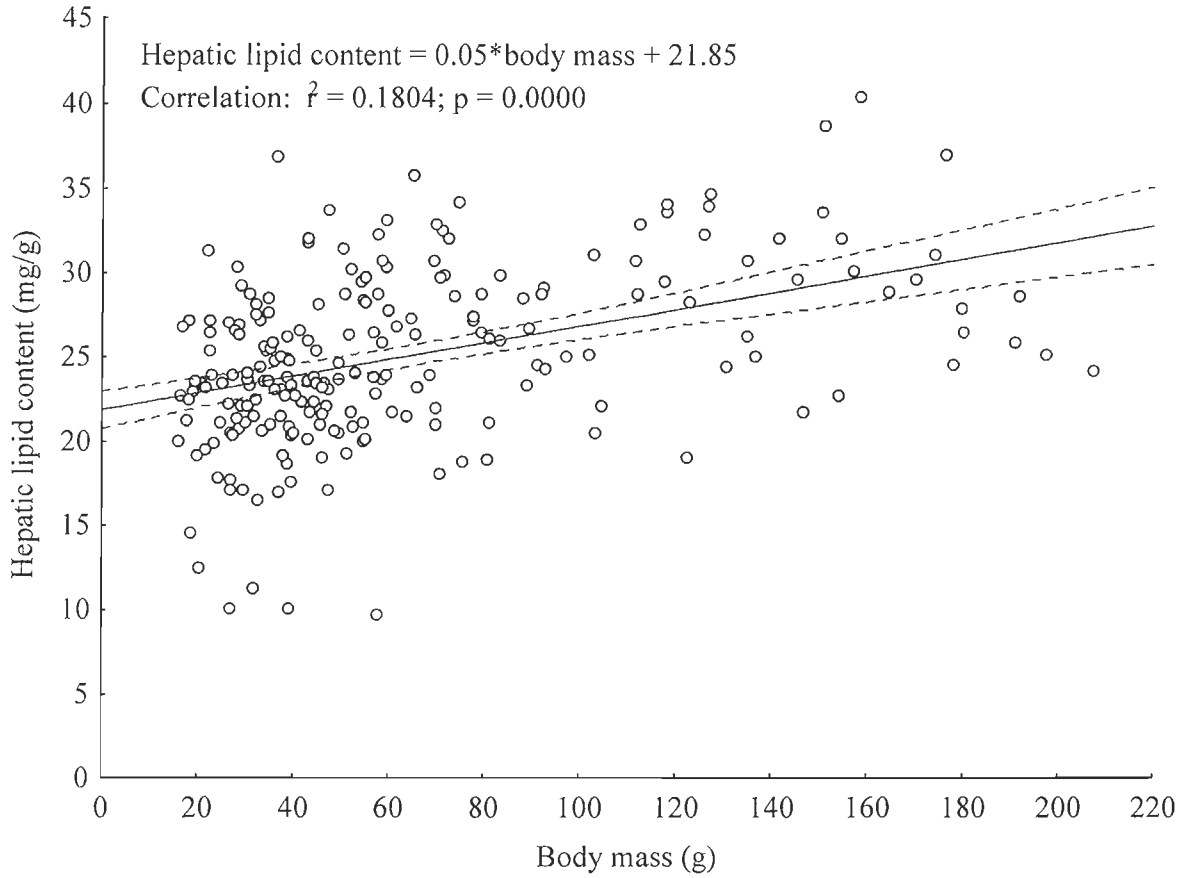


Fig. 2.4. Correlation between body mass and hepatic lipid content in brook charr.

Males

Percentage of early sexual maturation, condition factor and gonadosomatic index

In November, all the males sampled were sexually mature. The percentage of early sexual maturation was 2.8 fold higher in males than in females (Table 2.1). As in females, there was no cross-type effect (CT: df = 3, F = 0.23, p > 0.05) and data obtained at the time of sexual maturation did not show correlation either with mass (ANCOVA, df = 1, F = 0.05, p > 0.05) or length (ANCOVA, df = 1, F = 0.98, p > 0.05; Fig. 2.1 and Table 2.3).

In June, KF was higher in freshwater resident males than in males from other cross-types (CT: $df = 3$, $F = 10.7$, $p < 0.001$; Table 2.3). In August and November, KF was higher in males that were undergoing gonad maturation (Table 2.3). In November, KF was higher in freshwater resident than in anadromous males, regardless of maturation stage (CT: $df = 3$, $F = 34.9$, $p < 0.001$; Table 2.3). KF values of both reciprocal hybrids were intermediate (Table 2.3).

In June, GSI values were very low and similar among cross-types (Table 2.3). In August, the GSI of mature and immature males varied differently among cross-types (CT: $df = 3$, $F = 5.5$, $p < 0.01$; MS: $df = 1$, $F = 182.0$, $p < 0.001$; CT × MS: $df = 3$, $F = 4.8$, $p < 0.01$; Table 2.3). In immature males, there was no difference between cross-types, while in mature males, GSI was markedly higher in residents than in other cross-types (Table 2.3). In November, the GSI of mature and immature males varied differently among cross-types (CT: $df = 3$, $F = 8.1$, $p < 0.001$; MS: $df = 1$, $F = 1692.9$, $p < 0.001$; CT × MS: $df = 3$, $F = 7.0$, $p < 0.01$; Table 2.3). GSI was similar in all immature males (Table 2.3). In mature males, both reciprocal hybrids had the highest GSI, while anadromous had higher GSI than freshwater residents (Table 2.3).

Table 2.3. Condition factor (KF), gonadosomatic index (GSI), and hepatosomatic index (HSI) of 1+ male brook charr (mean ± se, n).

GSI	Maturation stage	AA	AR	RA	RR	Cross-type effects
June		0.09 ± 0.01, 25	0.15 ± 0.02, 24	0.16 ± 0.03, 18	0.12 ± 0.01, 27	
August	Immature	0.08 ± 0.02, 10	0.07 ± 0.01, 10	0.11 ± 0.02, 8	0.10 ± 0.02, 5	
	Mature	0.79 ± 0.09^{a}, 14	0.98 ± 0.11^{a}, 16	1.08 ± 0.11^{a}, 16	2.37 ± 0.37^{b}, 16	
November	Immature	0.21 ± 0.15, 18	0.09 ± 0.02, 22	0.08 ± 0.01, 8	0.07 ± 0.02, 17	
	Mature	2.98 ± 0.16^{b}, 27	3.60 ± 0.09^{c}, 49	3.36 ± 0.27^{bc}, 32	2.29 ± 0.10^{a}, 47	
KF						
June		1.01 ± 0.01^{a}, 73	1.05 ± 0.01^{a}, 79	1.03 ± 0.01^{a}, 64	1.10 ± 0.02^{b}, 72	
August	Immature	1.05 ± 0.03, 10	1.12 ± 0.03, 10	1.14 ± 0.03, 8	1.14 ± 0.03, 5	
	Mature	**1.15 ± 0.02**, 14	**1.14 ± 0.02**, 16	**1.11 ± 0.01**, 16	**1.21 ± 0.03**, 16	
November	Immature	1.04 ± 0.01, 17	1.09 ± 0.01, 22	1.07 ± 0.03, 8	1.24 ± 0.03, 17	AA^{a} AR^{b} RA^{ab} RR^{c}
	Mature	**1.13 ± 0.01**, 27	**1.17 ± 0.01**, 49	**1.12 ± 0.03**, 32	**1.32 ± 0.02**, 47	
HSI						
June		1.48 ± 0.03^{b}, 74	1.48 ± 0.04^{b}, 78	1.35 ± 0.03^{a}, 64	1.40 ± 0.03^{ab},	
August	Immature	1.48 ± 0.06, 10	1.41 ± 0.08, 10	1.33 ± 0.06, 8	1.34 ± 0.09, 5	
	Mature	1.50 ± 0.04, 14	1.41 ± 0.05, 16	1.32 ± 0.05, 16	1.42 ± 0.06, 16	
November	Immature	1.84 ± 0.09^{a}, 17	2.03 ± 0.05^{a}, 22	1.95 ± 0.14^{a}, 8	2.52 ± 0.09^{b}, 17	
	Mature	1.48 ± 0.08, 27	1.68 ± 0.05, 48	1.60 ± 0.09, 32	1.58 ± 0.05, 47	

AA: anadromous; RR: freshwater residents; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids. Bold characters: significant maturation stage effect ($p < 0.05$) and absence of significant interaction between cross-type and maturation stage ($p > 0.05$). Different letters indicate significant differences among cross-types for data presented on the same line. GSI data were log-transformed for statistical analysis, but untransformed data are presented in the table.

Hepatosomatic index

In June, HSI values in anadromous and resident males were similar (Table 2.3) and HSI value in RA hybrids was the lowest. In August, no cross-type effect was present. In November, immature resident males had significantly higher HSI than immature fish from other cross-types, but no cross-type effect was present in mature fish (Table 2.3).

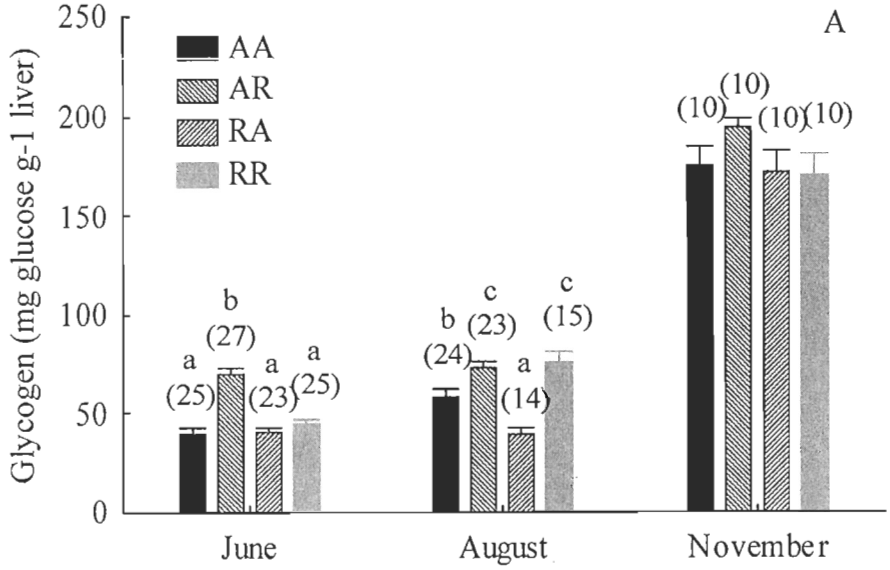
Hepatic glycogen and proteins

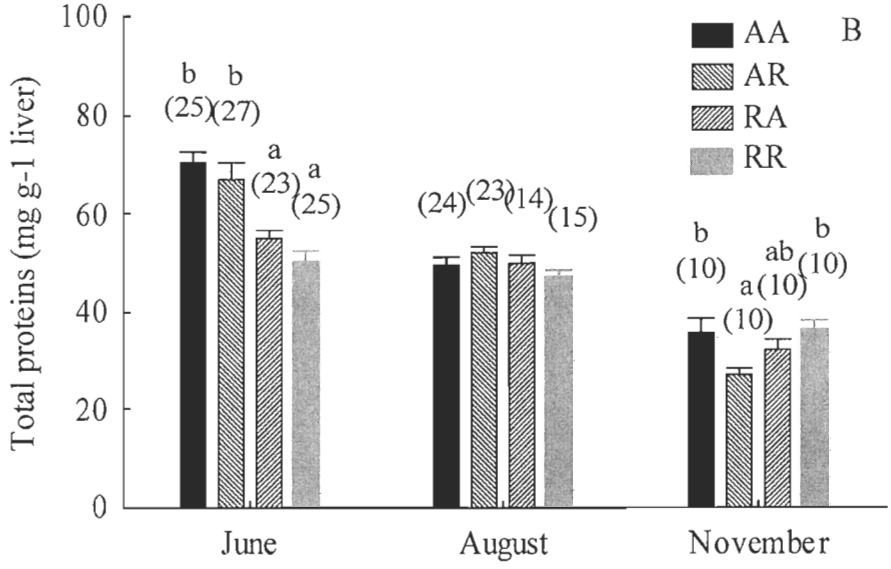
In June and November, hepatic glycogen content was similar between anadromous and resident males (Fig. 2.5A). However, resident males had higher glycogen content than anadromous ones in August (Fig. 2.5A). In August, the livers of immature males contained 1.28 times less glycogen (53.6 ± 3.5 mg g^{-1}, n = 29) than those with developing gonads (68.4 ± 2.9 mg g^{-1}, n = 47) (MS: df =1, F = 14.2, $p < 0.001$). A large increase in hepatic glycogen content occurred in all males from August to November. No cross-type effect was present in November. Hybrids differed from one another except in November.

In males, hepatic protein content decreased from June to November (Fig. 2.5B). In June, residents had significantly lower hepatic protein content than their anadromous counterparts, with hybrids showing values similar to their corresponding maternal line (CT: df = 3, F = 13.4, $p < 0.001$; Fig. 2.5B). These cross-type effects were no longer present in

August. In November, anadromous and freshwater resident males had similar hepatic protein content, both higher than in AR hybrids (CT: $df = 3$, $F = 4.0$, $p < 0.05$; Fig. 2.5B).

Fig. 2.5 A. Hepatic glycogen content of male brook charr from June to November. **B.** Hepatic protein content of male brook charr from June to November. AA: anadromous brook charr; AR: anadromous/resident hybrids; RA: resident/anadromous hybrids; RR: resident fish. Results are expressed as mean ± standard error. Different letters indicate significant differences among cross-types for each specific sampling time ($p < 0.05$). Numbers of fish (n) are indicated above each data point.


A
AA
AR
RA
RR
Glycogen (mg glucose g-1 liver)
250
200
150
100
50
0
a (25)
b (27)
a (23)
a (25)
b (24)
c (23)
a (14)
c (15)
(10)
(10)
(10)
(10)
June
August
November


B
AA
AR
RA
RR
Total proteins (mg g-1 liver)
100
80
60
40
20
0
b (25)
b (27)
a (23)
a (25)
(24)
(23)
(14)
(15)
b (10)
a (10)
ab (10)
b (10)
June
August
November

Heritability, parental effects and Q_{ST} estimates

Heritability estimates for body mass were relatively high both for anadromous and for resident fish, albeit being lower in the latter while the EBV variance was very high in both pure lines (Table 2.4). KF heritability was different between the pure lines, with a low and non significant value in anadromous fish and a much higher value in residents (Table 2.4). The EBV variance was very low in both lines (Table 2.4). Heritability estimates for HSI were high in residents but low in anadromous fish, and the EBV variance was very low in both pure lines (Table 2.4).

Heritability estimates for hepatic lipid content were low in anadromous fish and null in residents (Table 2.4). The same trend occurred for EBV variance in both cross-types (Table 2.4). In contrast, heritability estimates and EBV variance for hepatic glycogen content were high in anadromous but null in residents (Table 2.4). Heritability estimates and EBV variance for hepatic protein content were very low in both anadromous and residents (Table 2.4).

Ratios of parental (dam and sire) variance on phenotypic variance for body mass were low, suggesting that there was no significant parental effect for this trait (Table 2.5). There was no significant maternal effect for KF and HSI, as indicated by the low ratios of maternal variance on phenotypic variance for these traits (Table 2.5). However, the ratio of

sire variance suggests the occurrence of a slight paternal effect for KF (Table 2.5). Ratios of parental (dam and sire) variance on phenotypic variance indicate a high sire effect on hepatic glycogen content, but no other significant parental effect on hepatic reserves (Table 2.5).

Table 2.4. Heritability estimates (h^2 ± se) and variance of breeding values (σ^2 EBV) of physiological traits measured in anadromous (AA) and resident (RR) 1+ brook charr during summer.

Trait	AA h^2	RR h^2	AA σ^2 EBV	RR σ^2 EBV
Body mass	0.60 ± 0.35	0.40 ± 0.28	114.259	75.819
KF	0.07 ± 0.12	0.56 ± 0.33	0.000	0.005
HSI	0.21 ± 0.20	0.65 ± 0.35	0.011	0.037
Hepatic glycogen	0.87 ± 0.38	0.00 ± 0.00	242.160	0.000
Hepatic lipids	0.18 ± 0.18	0.00 ± 0.00	1.499	0.000
Hepatic proteins	0.06 ± 0.12	0.00 ± 0.08	2.683	0.000

Table 2.5. Ratios of parental (dam and sire) variance on phenotypic variance in anadromous (AA) and resident (RR) 1+ brook charr during summer.

Trait	Dam	Sire
Body mass	0.00 ± 0.00	0.00 ± 0.00
KF	0.05 ± 0.10	0.11 ± 0.18
HSI	0.00 ± 0.00	0.00 ± 0.00
Hepatic glycogen	0.00 ± 0.00	0.44 ± 0.39
Hepatic lipids	0.06 ± 0.13	0.00 ± 0.00
Hepatic proteins	0.00 ± 0.00	0.00 ± 0.00

Q_{ST} estimates for body weight and physiological indices were globally higher than the estimate of neutral genetic variance (F_{ST} = 0.153, 95% CI = 0.071-0.214), except for KF (Table 2.6). Q_{ST} estimates for hepatic lipids, glycogen and proteins were higher than the estimate of neutral genetic variance (F_{ST} = 0.153, 95% CI = 0.071-0.214; Table 2.6).

Table 2.6. Quantitative genetic variation in phenotype (Q_{ST}) of physiological traits measured in anadromous (AA) and resident (RR) 1+ brook charr after 14 days in salt water. σ^2_{bw} is the phenotypic variance between populations, and σ^2_{wn} the within-population quantitative genetic variance.

Trait	σ^2_{bw}	σ^2_{wn}	Q_{ST}
Body weight	258.300	510.100	0.336
KF	0.019	0.100	0.163
HSI	0.002	0.002	0.500
Hepatic glycogen	139.600	317.600	0.305
Hepatic lipids	2.440	4.077	0.374
Hepatic proteins	64.070	14.390	0.817

DISCUSSION

The main aim of this study was to assess whether gonad development, storage and use of hepatic energy reserves varied in different ways between anadromous and freshwater resident brook charr during transition from the juvenile to the adult stage. We found that vitellogenin production, gonad development (GSI), and variations in hepatosomatic index (HSI) indicate differences between the two forms (Table 2.7), differences that seem to be at least partly explained by quantitative genetic differences between resident and anadromous populations.

Divergence of traits linked to life history in anadromous and resident brook charr

Occurrence of early maturation was similar in 1+ females of both forms. However, the higher vitellogenin values in August in mature resident females compared to anadromous ones, and the reverse situation observed in November indicates different rates of sexual maturation between the two forms even in presence of similar environmental rearing conditions. This confirms results obtained in the field (Thériault *et al.*, 2007a) and suggests a strong genetic basis underlying reproduction timing in females. The lowest HSI values observed in resident maturing females compared to anadromous ones may suggest lower costs related to vitellogenesis in the former, since vitellogenesis occurs in the liver and usually peaks during sexual maturation (Kwon *et al.*, 2001). Indeed, the lower hepatic

Table 2.7. Summary of differences between anadromous and resident brook charr for all the studied phenotypic traits.

Trait	Sampling	Difference between AA and RR	
		Females	Males
Percentage of maturation		No	No
GSI	June	No	No
	August	No	AA < RR (M)
	November	No	AA > RR (M)
KF	June	AA < RR	AA < RR
	August	AA < RR	No
	November	AA < RR	AA < RR
HSI	June	No	No
	August	No	No
	November	AA < RR (I) AA > RR (M)	AA < RR
Vitellogenin	June	No	-
	August	No	-
	November	AA > RR	-
Hepatic glycogen	June	No	No
	August	AA < RR	AA < RR
	November	AA < RR	No
Hepatic lipids	June	No	No
	August	No	No
	November	No	No
Hepatic proteins	June	AA > RR	AA > RR
	August	No	No
	November	No	No

I: immature stage; M: mature stage

glycogen content in mature compared to immature females in November supports the notion that glycogen has been utilized to sustain development and growth of gonadal

tissues and oocytes. The important role of hepatic glycogen, not only in gonadal development, but also in other physiological functions such as fish metabolism, have been demonstrated in several studies on other teleost species (Rios *et al.*, 2006; Heermann *et al.*, 2009).

Interestingly, the heritability estimates of hepatic glycogen content as well as the EBV variance of this trait were very high in anadromous fish, which implies that this trait could be efficiently selected in this cross-type. Moreover, the high level of temporal variance in anadromous charr suggested that glycogen could also vary significantly in function of the environmental conditions they would experience. This could represent an adaptative strategy of energy storage and metabolism to deal with seasonal changes and local patterns (Collins and Anderson, 1995; Hurst, 2007; Finstad *et al.*, 2010). A high level of flexibility in energy storage would also be useful to overcome the periods of low food production and risk of starvation occurring in seasonal climates (Huss *et al.*, 2008). On the other hand, the very low heritability of hepatic glycogen content in resident fish strongly suggests that its expression depends almost entirely on non additive genetic variance in this cross-type. The relatively high Q_{ST} value for this trait also suggests that it has been differentially selected in anadromous and resident brook charr of the Laval River, and as such may have played an important role in adaptive divergence of both forms. Crespel *et al.* (2011) observed that anadromous brook charr from the Laval River accumulated low energy reserves by the onset of winter, while domestic brook charr accumulated a high

amount of energy reserves before winter. The maintenance of relatively low energy reserves prior to winter season in anadromous brook charr from the Laval River was likely a consequence of the utilization of these reserves to sustain the physiological processes related to sexual maturation. Since the domestic and Laval strains are also genetically very distinct, their different strategies in reserve allocation may also reflect adaptive responses to distinct living conditions (Collins and Anderson, 1995; Hurst, 2007; Finstad *et al.*, 2010).

In males, hepatic glycogen content increased in residents but not in anadromous fish and GSI values were higher in the former during summer. This would suggest a greater reproductive investment by maturing resident males. On the other hand, no difference in hepatic glycogen content was found between anadromous and resident males in November, which could be related to the relatively low cost of reproduction in males (Berg *et al.*, 1998), regardless of life history strategy. Indeed, while females have to invest massive amounts of reserves to produce their eggs and vitellogenin to ensure endogenous feeding of their embryos, male gonadal and gametic development only require a minor part of their energetic reserves (Berg *et al.*, 1998). In the field, male salmonids normally invest considerable amounts of energy in their fighting behaviour during the spawning period, with an increase in their metabolic rate while on the spawning grounds (Brett, 1995). However, since fish of the present study were all reared in the same controlled conditions, there were no spawning grounds to reach or defend, thus no need for males to spend energy in fighting.

In both forms, the percentage of maturing males at 1+ was similar and high (70.0 to 80.0 %), which is not surprising since high occurrence of early sexual maturation is a common feature in male salmonids (brown trout (*Salmo trutta*): Dellefors and Faremo, 1988; Atlantic salmon (*Salmo salar*): Fleming, 1996; brook charr: Thériault and Dodson, 2003).

The significant differences observed between anadromous and resident fish for KF, along with significant heritability values for the resident fish (but not for anadromous fish) suggest that differences for this trait between populations are partly genetically based. However, the fact that Qst estimate for this trait did not differ from neutral expectation suggests that these differences could be explained by neutral processes (e.g. genetic drift) only. On the other hand, in some fish species, KF is strongly correlated with the amount of energetic reserves (Herbinger and Friars, 1991; Neff and Cargnelli, 2004; Wysujack *et al.*, 2009) but it also confers a general morphological shape that is an important factor for swimming efficiency in brook charr (Morinville and Rasmussen, 2008). The lower KF of anadromous fish confer a more streamlined body shape and Fraser and Bernatchez (2005) showed that migrant brook charr swim more efficiently than their resident (or sedentary) counterparts. Indeed, a more streamlined morphology in fast water reduces swimming costs by minimizing the effects of drag (Pettersson and Brönmark, 1999). Yet, despite potential swimming advantage associated with a lower KF, and as mentioned above, the Q_{ST} value of KF was similar to the F_{ST} value calculated by Perry *et al.* (2005) for brook charr from the

Laval River. In this context, a variation in KF among individuals adopting different life history strategies or at different maturation stages could be a consequence of divergent selective pressures on other physiological traits highly correlated with KF, such as endogenous body reserves but not on KF per se.

Except for KF, Q_{ST} values for all traits also considered in the present study were globally higher than the F_{ST} calculated by Perry *et al.* (2005) for resident and anadromous brook charr of the Laval River, suggesting that the effect of divergent selection was important in driving observed differences for these traits. The F_{ST} value from this river was high, indicating an important level of neutral genetic divergence between anadromous and residents (Whitlock, 1999; Merilä and Crnokrak, 2001). This F_{ST} was not only higher than the values measured in other brook charr populations (Castric and Bernatchez, 2003), but also than the values measured in other salmonid species with sympatric anadromous and resident individuals, such as bull trout (*Salvelinus confluentus*; Homel *et al.*, 2008) and rainbow trout (*Oncorhynchus mykiss*; Heath *et al.*, 2008). The fact that the Q_{ST} values of all traits, except KF, were significantly higher than the F_{ST} value suggests that divergent natural selection, rather than neutral genetic processes such as genetic drift, migration and mutation, has potentially played an important role in the genetic differentiation between anadromous and residents originating from the Laval River for the physiological traits measured in the present study. Such divergent selective pressures may have favored the evolution of intrinsic mechanisms for the maintenance of reproductive isolation and

restriction to gene flow between anadromous and resident populations (Perry *et al.*, 2005). If divergent selection pressures actually shaped the genetic differentiation between both forms for those traits, it is possible that those pressures eventually lead to speciation events if they are maintained. Values of Q_{ST} exceeding those of F_{ST} have been reported in several species on a variety of traits, especially morphological ones (Merilä and Crnokrak, 2001).

Several field studies (Silverstein and Hershberger, 1992; Heath *et al.*, 1994; Wild *et al.*, 1994; Mousseau *et al.*, 1998; Heath *et al.*, 2002; Thrower *et al.*, 2004) provided heritability estimates on threshold behavioural or morphological quantitative traits, while the present study focused more on providing heritability estimates for physiological quantitative traits under controlled laboratory conditions. Thériault *et al.* (2007a) also calculated heritability estimates of life-history tactics (anadromy and residency), but in brook charr under natural conditions and originating from the same genetic pool. The heritability values obtained in these studies, as in the present study, were highly variable depending on the trait (i.e. from null to high). Heritability depends on the magnitude of all components of variance and a change in any one of these components will affect it (Falconer and Mackay, 1996). Heritability also depends on the amount of selection applied for a given trait. Life-history traits under continuous natural selection are predicted to have reduced additive genetic variance, thus lowered heritability (Falconer and Mackay, 1996). Moreover, heritability values are not only a property of the traits themselves, but also of the population under study and the environmental conditions in which they are measured

(Stearns, 1992; Falconer and Mackay, 1996; Thériault *et al.*, 2007a; Visscher *et al.*, 2008). So, relatively uniform environmental conditions—as in our study in controlled conditions—should increase heritability values by reducing the environmental component of the variance (Falconer and MacKay, 1996), even though levels of heritability typically measured under laboratory conditions are comparable to those that occur in the natural environment (Weigensberg and Roff, 1996).

Traits that varied in function of factors other than life history strategy

Some physiological traits did not show significant divergence between anadromous and freshwater resident brook charr, such as hepatic lipid and protein contents. Hepatic lipid content clearly increased, though slightly, from June to November, regardless of cross-type or sex. The replenishment of stored lipid is important for the maintenance of maturation (Rowe *et al.*, 1991; Kadri *et al.*, 1996), and it is the primary energy source that sustains reproduction in some salmonids (Kadri *et al.*, 1996; Adams and Huntingford, 1997). Hepatic lipid content does not have a strong genetic basis, at least in resident fish, as reflected by low heritability and EBV values for this trait. Thus, hepatic lipid content apparently varies mainly as a function of environmental conditions or physiological requirements for metabolism and reproduction.

Except in June, when it was higher in anadromous than in male and female freshwater resident brook charr, hepatic protein content did not show any divergence between both pure cross-types. These results indicate that proteins, especially in anadromous fish, were mainly utilized during summer and other reserves during autumn. This delay in resource utilization could be related to the roles of different energetic reserves. It is known that proteins play a role in sustaining metabolism and also act as building materials for the cellular machinery involved in gonad cell mitosis and the formation of cell junctions between those cells (Mommsen, 2004). Proteins also play a pivotal role in vitellogenesis (Wiegand, 1996).

Physiological responses of hybrid brook charr

Our initial hypothesis related to hybrids was that they would show intermediate (or additive) values for the physiological traits studied. However, few additive physiological responses were observed, namely for vitellogenin, hepatic glycogen concentrations, and HSI in females in November, as well as for KF at all sampling periods, and most of the results obtained in this study rather suggest that other types of genetic controls predominate in the hybrid background. Traits for which only nonadditive physiological responses were observed, namely hepatic lipid and protein concentrations, generally had low heritability values. No significant parental effects were found for those two traits either, suggesting that they are regulated by other types of genetic mechanisms, possibly dominance or epistasis, as it seems to usually happen with nonadditive responses (Falconer and Mackay, 1996).

Another laboratory experiment (Bougas *et al.*, 2010) have shown a majority of nonadditive responses in transcription regulation inheritance of hybrids resulting from crosses between the Rupert and Laval populations of brook charr. These authors have also found a positive correlation between the extent of nonadditive transcription regulation inheritance in hybrids and the genetic distance between parental populations. Thus, hybrids resulting from crosses between the most genetically distant parental populations (Rupert and Laval) showed more nonadditive transcripts than any other hybrid crosses (Bougas *et al.*, 2010). The majority of nonadditive physiological responses in hybrids observed in the present study could also be related with genetic distance between both pure cross types, since the level of neutral genetic divergence between anadromous and freshwater resident brook charr of the Laval River, as expressed by the F_{ST} value, was found to be high (Perry *et al.*, 2005).

KF was one of the only traits where values of both hybrids were intermediate to those of pure crosses over the course of the experiment, except for males in June and August, which implies that this trait was likely maintained by additive genetic mechanisms.

The high GSI values observed in AR maturing males in November suggest a higher relative energetic investment in gonadal tissue in this hybrid. This was one of the only cases of heterosis (i. e. a phenotypic trait for which the mean was significantly higher in hybrids than in both parents or in parental populations) in the present study. In June, low HSI in RA males relative to pure crosses suggests a lower hepatic activity, and presumably

a lower production of hepatic reserves or structural components, in this hybrid. This could be reflected in the lower hepatic protein concentration of RA hybrids compared to residents in June. A maternal effect could explain the hepatic protein contents measured in June in male and female hybrids since they were similar to their corresponding maternal line. The maternal genetic variance on physiological traits associated with energy reserve accumulation appears to be lower in adults than at the alevin stage, prior to yolk sac resorption (Heath *et al.*, 1999; Perry *et al.*, 2004; 2005). The fact that the hepatic protein content was globally lower in November than in August further emphasizes the main utilization of this energy source during summer, in the early stages of sexual maturation. The accumulation of reserves in the liver was generally neither lower nor higher in hybrids than in pure crosses. Vitellogenin concentrations of hybrids were intermediate between those of pure strains in November, which suggests an additive genetic component for this physiological trait. These results, along with the concentrations of reserves, also suggest an intermediate reproduction cost for hybrids relative to pure crosses.

Physiological responses of brook charr in the ecological context of the Laval River

Hepatic glycogen concentrations, along with HSI results, suggest that resident female brook charr of the Laval River spawned sooner than their anadromous counterparts, probably because they mainly use their hepatic glycogen reserves to fuel their sexual maturation during autumn. Indeed, they do not have to spend energy to migrate and overcome hurdles linked to changing environmental conditions like their anadromous

counterparts. In the latter, the shortening photoperiod of late summer induces sexual maturation that must be completed in freshwater (Curry *et al.*, 2006). Consequently, anadromous charr are forced to move from rich feeding areas into suboptimal thermal and feeding habitats of the river, where feeding is reported to be reduced (Naiman *et al.*, 1987; Curry *et al.*, 2006). They also have to go through unfavourable, warm waters to reach these poorer feeding habitats before spawning (Curry *et al.*, 2006).

As indicated by their high heritability values hepatic glycogen reserves of anadromous brook charr seem to have a strong additive genetic basis. In the ecological context of the Laval River, high heritability values for this trait in anadromous fish could have been maintained by balancing selection associated with the use of contrasting habitats (freshwater and coastal marine waters) by those fish. Resident brook charr of the Laval River do not have to experience such fluctuations in their environmental conditions during their sexual maturation period. It is possible that the patterns of hepatic glycogen accumulation and mobilization of anadromous brook charr that were observed in this study were representative of the physiological responses that would be observed in the ecological context of their native system, namely the Laval River, even though they were not exposed to the same environmental conditions, because levels of heritability measured under laboratory conditions are usually comparable to those that occur in the natural environment (Weigensberg and Roff, 1996).

Conclusions

In summary, our results provided further evidence for the occurrence of genetically based differences in physiological traits related to reproduction between resident and anadromous brook charr from the Laval River. Such differences, along with the values of Q_{ST} generally higher than the level of neutral genetic variation, may reflect the outcomes of divergent selective pressures that maintained the local adaptations associated with both life history strategies within the Laval River system (Perry *et al.*, 2005). In the present study, one of the first to provide heritability estimates for physiological quantitative traits in brook charr, traits related to reproduction were generally more heritable in anadromous than in resident fish, but heritability and variance of EBV were sometimes low in both cross-types. Consequently, these results suggest that the potential of brook charr to respond to selective pressures would vary according to specific traits and life history strategies, and that external or internal environmental cues would also be very important to determine physiological responses for traits related to reproduction. Producing hybrids did not seem to have any significant advantage or disadvantage for reproductive investment, because there were no clear cases of inbreeding depression or heterosis in our results. Future research on energy allocation in sympatric anadromous and freshwater resident brook charr should focus on tissue-specific responses of several metabolic enzymes and expression of the corresponding genes in the field, from the juvenile stage to spawning time.