UNIVERSITÉ DU QUÉBEC

ÉVALUATION D'UN PROGRAMME DE SÉLECTION ET IDENTIFICATION DES TRAITS PHYSIOLOGIQUES LIÉS À L'ANADROMIE CHEZ L'OMBLE DE FONTAINE (*SALVELINUS FONTINALIS*)

THÈSE

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ANDRÉANE BASTIEN

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À mes deux petits amours, Émeric et Adèle

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AVANT-PROPOS

L'omble de fontaine est une espèce d'intérêt pour l'aquaculture au Québec. Il existe actuellement plusieurs piscicultures qui concentrent leurs efforts sur l'élevage de cette espèce et leur production est, pour la plupart, essentiellement dédiée à l'ensemencement. L'étude des changements liés à la sélection et à la domestication est importante dans le but de produire des poissons répondant aux critères de l'industrie. Les deux premiers chapitres sont en lien avec cette problématique.

De façon plus précise, le Chapitre 1 décrit les gains obtenus suite à deux générations de sélection visant l'amélioration de la croissance et la diminution de l'incidence de maturité sexuelle précoce. Présenté sous la forme d'un article intitulé *Genetic gain for growth and delayed sexual maturation using a feral strain of anadromous brook trout (Salvelinus fontinalis)*, ce chapitre résulte d'une collaboration avec le Dr Guy Perry, un spécialiste de la génétique quantitative, et de Jean-Yves Savaria qui a travaillé, au cours de sa maîtrise, au démarrage d'un programme de sélection dans le cadre de travaux financés par la Société de recherche et développement en aquaculture continentale (SORDAC). Cet article a été soumis au printemps 2009 et est actuellement en correction pour la revue *North American Journal of Aquaculture*.

Le Chapitre 2 est consacré aux effets indirects de la sélection sur la réponse au stress et sur l'étude de la possibilité d'inclure la réponse au stress parmi les traits de sélection. L'article intitulé *Effects of selection for growth and late sexual maturity on*

other performance traits in brook charr (Salvelinus fontinalis mitchill) est prêt à être soumis.

Étant donné le déclin des populations de saumon atlantique, l'industrie de la pêche sportive se tourne vers l'exploitation des ombles de fontaine anadromes. C'est pourquoi, une bonne connaissance de l'évolution des stratégies de vie de cette espèce, qui dans le cas présent concerne l'anadromie, favoriserait l'efficacité des politiques de conservation. Comme les animaux traités dans les deux premiers chapitres sont issus d'une population anadrome de la Côte-Nord, nos travaux ont tout naturellement évolué vers cette problématique. Le troisième chapitre vise donc l'amélioration des connaissances de la physiologie et de la génétique des formes anadrome et résidente de l'omble de fontaine. Nous y avons comparé des traits physiologiques liés à l'adaptation à l'eau de mer et avons estimé l'héritablité de ces traits. Un article ayant pour titre *Quantitative genetic basis for physiological divergence between sympatric anadromous and resident brook charr (Salvelinus fontinalis)* est actuellement en préparation pour soumission. Cet article résulte d'une collaboration avec Mathieu Caron dont les résultats sur l'expression de l'IGF-1 ont été utilisés et aussi de la participation du Dr Guy Perry pour le design expérimental des croisements et pour l'analyse génétique des résultats.

Des données recueillies chez les ombles issus des croisements utilisés pour l'expérience présentée au Chapitre 3, ont également permis une collaboration avec le Dr Marylène Boulet, pour la préparation d'un article s'intitulant *Comparative*

transcriptomics of sympatric anadromous and resident brook charr (Salvelinus fontinalis) before their first salt water transition. Cet article est en préparation.

À noter que dans les trois manuscrits (Chapitres 1, 2 et 3), le style de présentation utilisé pour la publication originale a été conservé.

RÉSUMÉ

L'omble de fontaine est une espèce combattante et appréciée des adeptes de la pêche sportive. Comme pour toute espèce exploitée, l'efficacité des politiques de conservation est liée à une bonne connaissance de l'évolution des stratégies de vie, qui dans le cas présent concerne l'anadromie. L'amélioration des connaissances sur la biologie de l'espèce est essentielle à la gestion et la protection des populations d'ombles de fontaine d'autant plus que sa production aquacole au Québec est dédiée au marché des ensemencements qui, de ce fait, peut avoir une influence sur les populations en place. D'ailleurs, des travaux d'amélioration des performances ont été mis de l'avant afin de fournir aux producteurs une souche indigène répondant aux critères de l'industrie.

Le premier objectif de cette thèse était d'évaluer la réponse d'ombles de fontaine anadromes soumis à un programme de sélection dès la troisième génération et d'en dissocier les effets liés au processus de domestication. Après une génération, le gain en poids est similaire entre les poissons issus de la lignée sélectionnée (23%) et de la lignée contrôle (35%). Par contre, suite à une seconde génération de sélection, on a observé chez les F_3 un gain de 32% alors qu'un gain négligeable a été observé chez la lignée contrôle. De plus, la proportion de poissons immatures est passée de 32% chez la F_1 à plus de 60% chez la génération F_3 issue du programme de sélection. La proportion d'immatures chez la lignée contrôle est demeurée inchangée et indique que le changement dans la proportion d'immatures chez les sélectionnés est véritablement dû à la sélection appliquée. En plus des gains obtenus pour le poids et la diminution de poissons immatures à l'âge de 1^+, les résultats indiquent également que la sélection visant à améliorer la croissance et l'absence de maturité sexuelle précoce sont des traits compatibles pour la sélection chez l'omble de fontaine.

Le deuxième objectif était de vérifier si la sélection appliquée sur des critères de croissance et d'absence de maturité sexuelle précoce avait un effet indirect sur la réponse au stress et à quel stade du développement il était possible d'identifier les familles résistantes. Trois types de stress ont été appliqués à des âges différents : l'exposition précoce à l'eau salée, un stress aigu (maintien hors de l'eau) et la susceptibilité à une infection opportuniste. Les résultats indiquent que le programme de sélection a occasionné une diminution générale de la réponse primaire au stress mesurée par le taux de cortisol et une diminution de la susceptibilité à l'infection *Flexibacter maritimus*. Les réponses aux trois agents de stress utilisés indiquent qu'il est possible d'identifier des familles ayant des sensibilités au stress différentes. La présence d'effets familiaux importants suggère la présence d'une composante génétique additive qui permettrait, en principe, la sélection basée sur la réponse au stress.

Le troisième objectif visait l'amélioration des connaissances de la physiologie et de la génétique des formes anadrome (A) et résidente (R) de l'omble de fontaine. Nous avons croisé des ombles de fontaine anadromes et résidents pour former quatre types de croisement (♀A♂A, ♀A♂R, ♀R♂A et ♀R♂R). Des traits physiologiques liés à

l'adaptation à l'eau de mer ont été comparés chez ces groupes élevés en conditions identiques et l'héritabilité de ces traits a été estimée. Les résultats indiquent que les résidents ont à fournir un effort physiologique plus important pour l'adaptation à l'eau salée. En effet, ils présentent une osmolalité plasmatique plus grande après deux semaines d'exposition, une diminution de l'indice hépato-somatique, une concentration plus élevée en triiodothyronine, une activité plus faible de la Na^+/K^+ATPase branchiale et une augmentation d'expression de l'IGF-1. Comme ces traits se sont avérés être héritables soit chez les deux formes, soit chez l'une ou l'autre des deux formes (AA ou RR), cela suppose que ces performances physiologiques se transmettent aux générations subséquentes, favorisant ainsi une divergence génétique. De plus, les croisements hybrides entre anadromes et résidents ont permis d'observer la présence d'une composante génétique additive sur le facteur de condition ainsi que la présence d'un effet de la lignée paternelle sur le poids et sur le niveau d'expression de l'IGF-1 après 14 jours en eau salée. Comme cette étude confirme que les ombles anadromes et les résidents de la rivière Laval constituent deux populations différentes, on envisage que les résultats présentés ici auront des implications pour la gestion des stocks d'omble de fontaine. La présence d'une ou de deux populations pourrait, par exemple, influencer le choix de la forme d'omble de fontaine à privilégier lors d'ensemencements, ou influencer la gestion de la pêche en estuaire sur la base de l'existence de deux populations.

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INTRODUCTION GÉNÉRALE

L'aquaculture au niveau mondial et au Québec

Actuellement, les dix espèces les plus prélevées, représentant 30% de la production totale des pêches de capture, sont soit pleinement exploitées ou surexploitées. Cette situation démontre l'importance de produire le poisson nécessaire pour soutenir la croissance démographique. D'ailleurs, au cours des quarante dernières années, le secteur de l'aquaculture a connu une croissance soutenue et devrait, sous peu, produire la moitié du poisson consommé dans le monde (FAO 2009). Au début des années cinquante, la production représentait moins d'un million de tonnes par an, alors qu'en 2006 la production totalisait 51,7 millions de tonnes. Ce changement s'explique notamment par le dynamisme de ce secteur, mais aussi par la croissance économique mondiale. Les pêches et l'aquaculture ont produit en 2006 environ 110 millions de tonnes de poissons destinés à l'alimentation. De ce nombre, l'aquaculture représentait 47% du total de production.

La production aquacole dans le monde varie beaucoup d'un continent à l'autre. L'Amérique du Nord compte pour 1,2% de la production. L'aquaculture mondiale est largement dominée par la région Asie-Pacifique, qui représente 89% de la production en volume. En ce qui concerne la production des salmonidés, la Norvège et le Chili sont les deux plus gros joueurs dans ce domaine avec, respectivement, 33% et 31% de la production mondiale (FAO, 2009).

Au Québec, c'est avant le 20^{e} siècle qu'ont débuté les premières activités d'aquaculture en eau douce avec l'élevage du saumon atlantique et de l'omble de fontaine. D'abord gouvernementale, cette production a démarré en entreprise privée à partir des années cinquante et la majorité de la production était alors destinée à l'ensemencement de plans d'eau.

De nos jours, les ventes issues de la production aquacole s'élèvent à environ 13 millions de dollars (MAPAQ, 2007). Les espèces d'eau douce représentent 94% du secteur de l'aquaculture et les principales espèces piscicoles sont l'omble de fontaine (63%) et la truite arc-en-ciel (33%). Du côté de la production marine, les ventes de moules représentent plus de 90% des ventes de produits issus de l'aquaculture marine.

Malgré ces chiffres, il s'avère que le Québec se taille difficilement une place dans ce secteur mondialement important. De toute évidence, les conditions environnementales hivernales extrêmes rendent difficile l'aquaculture côtière, nous n'avons qu'à penser à la présence des glaces en hiver et à la température de l'eau trop froide pour une bonne croissance chez les salmonidés. De plus, le développement de l'industrie piscicole au Québec est actuellement défavorisé par sa dépendance en approvisionnements en eau de qualité et en la réduction de la pollution par le phosphore (Archer, 2008). Or, différentes stratégies ont été mises de l'avant. Des travaux de recherches s'effectuent abordant différentes tactiques pour contourner ces difficultés, notamment concernant le phosphore,

par le traitement des eaux piscicoles et l'amélioration des moulées. Des recherches visent aussi l'utilisation d'espèces adaptées aux conditions froides (par exemple, la plie rouge et l'omble chevalier) ou l'utilisation de la sélection génétique pour favoriser la croissance de souches indigènes et adaptées aux températures locales.

Amélioration des performances

Sélection génétique

La sélection génétique est définie par Falconer et Mackay (1996) comme étant l'action de croiser des individus choisis parmi une population en fonction de différents critères de performance. L'effet de la sélection pouvant être observé est le changement de la valeur moyenne de la population pour le caractère en question. Ce changement dans la moyenne de la population est ce qu'on appelle la réponse à la sélection ($R=h^2S$; Roff, 1997; Falconer et Mackay, 1996). Dans le cadre d'un programme de sélection la première étape consiste à établir les critères de sélection qui permettront de rencontrer les besoins de l'industrie en termes de rentabilité. Un fort taux de croissance et l'absence d'une maturité sexuelle précoce sont des critères connus favorisant la production (Nilsson 1992; Winkelman et Peterson 1994; Gjedrem, 1997, 2000; Kause *et al.* 2003). Les poissons à plus forte croissance permettent une rotation plus rapide des cohortes dans les pisciculture ce qui diminue les coûts de production (Winkelman et Peterson, 1994; Gjedrem, 1997). L'absence de maturité sexuelle précoce permet au poisson d'atteindre une taille commerciale plus rapidement, puisque l'énergie est davantage investie pour la croissance

plutôt que vers la production de gamètes (Aksnes *et al.* 1986; Gjerde, 1986). De plus, on associe l'absence de maturité sexuelle précoce à un taux réduit de mortalité et une meilleure qualité de chair (Nilsson, 1992; Crandell et Gall, 1993).

Héritabilité

L'efficacité d'un programme de sélection (réponse à la sélection), dépend principalement d'une variance génétique élevée dans la population pour le ou les traits choisis. En effet, la réponse à la sélection est fonction de l'héritabilité du caractère, soit de la variance génétique additive et de l'intensité de sélection appliquée (pourcentage d'individus retenus). D'une génération à l'autre, l'amélioration s'effectue à mesure que les traits se transmettent mais est conditionnelle au maintien d'un écart-type à la moyenne élevé et tout en sélectionnant une faible proportion d'individus sans occasionner une perte génétique (Falconer et Mackay, 1996).

L'héritabilité (h^2) d'un trait exprime la contribution relative des différences génétiques et des différences environnementales à la variation du phénotype. Cette mesure est spécifique à la population. L'héritabilité est définie comme étant le ratio de la variance génétique additive à la variance phénotypique (Falconer et Mackay, 1996):

$$h^2 = V_a / V_p$$

Un estimé d'héritabilité de 1 signifie que le caractère est totalement héritable et que la réponse à la sélection sera rapide, alors qu'un estimé de 0 dénote un caractère non héritable et implique donc qu'il est impossible de sélectionner pour ce trait.

Chez les salmonidés, l'héritabilité pour le poids est généralement de modérée à élevée (h^2>0.2) (Thériault *et al.*, 2007b, omble de fontaine, milieu naturel; Chevassus *et al.*, 2004, truite brune, sélection individuelle; Hershberger *et al.*, 1990; Neira *et al.*, 2006, saumon coho *Oncorhynchus kisutch*, sélection familiale; Nilsson, 1992, omble chevalier, population non-sélectionnée, et revu par Carlson et Seamons, 2008). Ceci confirme que le poids peut être amélioré via un programme de sélection.

De la même façon, l'héritabilité pour la maturation sexuelle chez les salmonidés a aussi été étudiée et s'est avérée être beaucoup plus variable mais avec aussi des valeurs modérées à élevées (h^2=0.19-0.45, omble chevalier, Nilsson, 1992; 0.21-0.39, truite arc-en-ciel, Gjerde et Gjedrem, 1984). Il y a cependant un bémol à la sélection simultanée de ces deux traits. Certains auteurs ont observé une corrélation entre la maturité sexuelle et le poids (Gjerde et Gjedrem, 1984; Martyniuk *et al.*, 2003). Ceci implique donc qu'en sélectionnant pour des individus plus gros, il y a un risque de sélectionner par le fait même le phénotype montrant une maturité sexuelle précoce. D'autres études effectuées chez des salmonidés ont montré de faibles corrélations entre l'âge où le poisson devient mature et la croissance, notamment chez le saumon atlantique et la truite arc-en-ciel (Gjerde, 1986; Naevdal, 1983; Crandell et Gall, 1993).

Méthodes de sélection

Il existe plusieurs méthodes de sélection (Falconer et Mackay, 1996). Tout d'abord, la sélection individuelle permet de sélectionner les individus performants en se basant sur leur déviation par rapport à la moyenne de l'ensemble de la population. Cette méthode est la plus simple à utiliser. Par contre, il est possible que certaines familles peu performantes soient éliminées du processus de sélection ce qui a pour incidence de diminuer la diversité génétique. La sélection familiale quant à elle fait intervenir uniquement la composante familiale. Les familles sont comparées entre elles et les familles plus performantes sont sélectionnées entièrement. Cette méthode permet une réponse efficace à la sélection mais peut aussi occasionner une perte de variabilité génétique liée à la réduction du nombre de familles. La sélection de type intra-familiale sélectionne les individus performants à l'intérieur de leur famille. Ils sont choisis de par leur supériorité de performance en comparaison avec la moyenne familiale. Il s'agit de la méthode conservant un maximum de diversité génétique. Cependant, il faut s'attendre à une réponse moins efficace puisque même des individus de familles peu performantes sont également conservés. La sélection combinée fait intervenir la composante familiale et intra-familiale. Elle prend en compte la supériorité d'un individu par rapport à sa famille mais aussi la supériorité de la famille par rapport aux autres familles. Ainsi les familles plus performantes sont plus représentées en termes de nombre d'individus sélectionnés. Elle permet donc de conserver le maximum de variabilité génétique tout en étant relativement efficace. Dans le cas de cette étude, il s'est avéré que la sélection combinée était un meilleur choix. La sélection familiale ou

individuelle aurait occasionné trop de perte génétique étant donné le nombre limité de familles disponibles pour ce projet (Savaria, 1998).

Domestication

Les gains obtenus apparaissent suite à la réponse à la sélection expérimentale mais aussi à la suite d'une sélection attribuable à l'environnement d'élevage (Ruzzante, 1994). Les individus ne pouvant s'adapter aux pratiques et conditions d'élevage peuvent mourir et sont donc exclus par défaut. Comme ils ne contribuent pas aux générations ultérieures, on observe dans ce cas une sélection appelée domestication. Il est difficile de distinguer les changements attribuables à la sélection dirigée des changements résultant de la domestication. En produisant une lignée de référence n'ayant pas subi la sélection mais ayant été soumise aux mêmes conditions environnementales, il est donc possible de distinguer les effets de la sélection versus ceux de la domestication en comparant les deux lignées.

Plusieurs études ont mesuré des réponses à la sélection en comparaison avec un groupe témoin ou avec la population d'origine. Fleming et collaborateurs (2002) ont observé qu'après sept générations de sélection chez le saumon atlantique domestiqué, le poids des poissons atteignait trois fois le poids des poissons non sélectionnés à la fin de l'expérimentation. Hershberger et collaborateurs (1990) ont trouvé qu'une lignée contrôle de saumon coho montrait une réponse comparable à celle observée chez des poissons issus d'une sélection dirigée pour le poids alors que les poissons sauvages restaient relativement inchangés. En comparant un groupe sélectionné et un groupe contrôle, Roberge et

collaborateurs (2006) ont démontré que seulement cinq à sept générations de sélection artificielle pouvaient modifier de façon significative l'expression des gènes. Étant donné les gains observés chez les salmonidés suite à une sélection artificielle, les résultats attendus suite à une sélection devraient se manifester assez rapidement chez l'omble de fontaine.

Le stress

Le stress est défini comme une perturbation biologique ou physique d'un organisme qui est perçue comme une agression. Les poissons dans les installations aquacoles sont soumis à plusieurs manipulations stressantes et récurrentes (tri, transport, vaccination), qui dans certaines conditions peuvent initier des réponses sévères au stress (Specker et Schreck, 1980; Schreck *et al.*, 1989; Barton et Iwama, 1991; Portz *et al.*, 2006). L'exposition chronique à un stress peut interférer avec la croissance, les capacités immunes, le métabolisme, le succès reproducteur et la tolérance à l'eau de mer (Pickering, 1981; Adams, 1990; Barton et Iwama, 1991; Iversen *et al.*, 1998) (Figure 1). La résistance au stress est donc également un trait d'intérêt en production aquacole (Øverli *et al.*, 2006; Trenzado *et al.*, 2006). Comme pour la sélection dirigée vers une meilleure croissance, une variabilité dans l'intensité de réponse est nécessaire afin d'identifier des phénotypes plus résistants ou plus faibles parmi la population (Refstie, 1986; Fevolden *et al.*, 1991; Pottinger *et al.*, 1992). Chez la truite arc-en-ciel, la réponse au stress est une caractéristique individuelle stable et ayant une héritabilité modérée à élevée (Pottinger *et al.*, 1992; Pottinger *et al.*, 1994; Fevolden *et al.*, 1999).

Ces caractéristiques, permettent d'envisager une amélioration de ce critère. L'identification précoce des individus sensibles au stress dans le cadre d'un programme de

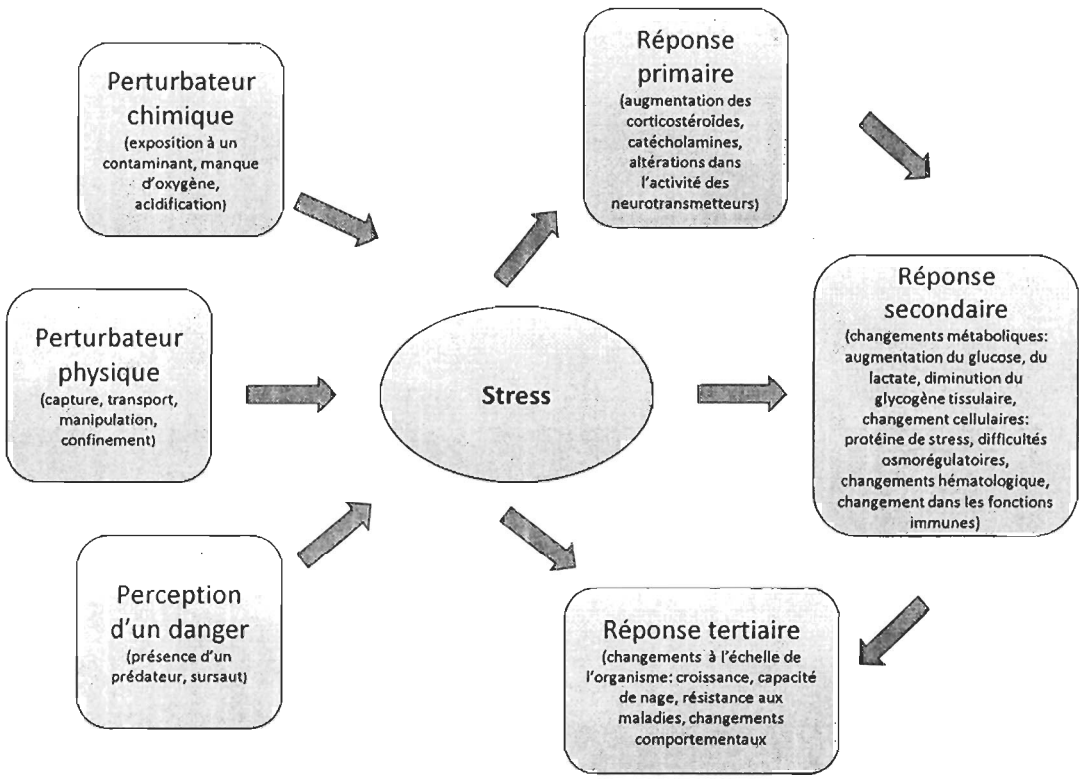

Figure 1. Les différents agents de stress et les différents niveaux de réponse (inspiré de Barton, 2002).

sélection permettrait de réduire les coûts de production par l'élimination de ceux-ci. Un des défis d'une sélection liée à la performance face aux perturbations est d'identifier des indicateurs de stress qui sont fiables et reproductibles. La concentration en cortisol, un indicateur primaire, est l'un des indicateurs les plus étudiés et permet une bonne

approximation de l'intensité de l'agent stressant (Barton et Iwama, 1991; Fevolden *et al.*, 2002). Des études précédentes ont montré que le niveau de cortisol atteint une heure après le stress n'avait pas d'influence sur la croissance (Fevolden *et al.*, 1991; Pottinger *et al.*, 1994). C'est pourquoi, il s'est avéré que la vitesse à laquelle la concentration plasmatique en cortisol diminue suite à un stress aigu est possiblement un meilleur indicateur que la concentration plasmatique maximale atteinte peu de temps après la perturbation (Weil *et al.*, 2001).

Les infections opportunistes peuvent se manifester et sont davantage présentes si les poissons sont immunosupprimés. Ainsi en améliorant les performances face aux stress on s'attend à avoir des poissons plus résistants aux infections opportunistes. Des travaux effectués dans le même laboratoire que la présente étude ont identifié une bactérie responsable (*Flexibacter maritimus*) de l'infection observée chez les ombles de fontaine lorsque transférés en eau salée avant l'âge de deux ans (Lefrant, 2006). Comme il existe une grande variabilité inter-familiale quant à la résistance à cette infection, il s'avère donc possible d'identifier des poissons plus performants face à cette agression.

Caractéristiques générales de l'omble de fontaine

L'omble de fontaine (*Salvelinus fontinalis*) fait partie de la famille des salmonidés dans laquelle nous retrouvons notamment au Québec le saumon atlantique (*Salmo salar*), la

truite brune (*Salmo trutta*), le touladi (*Salvelinus namaycush*), l'omble chevalier (*Salvelinus alpinus*), la truite arc-en-ciel (*Oncorhynchus mykiss*) et le grand corégone (*Coregonus clupeaformis*) (Bernatchez et Giroux 2000). L'omble de fontaine est aussi appelé truite mouchetée ou truite de mer. Ce poisson habite les eaux oxygénées fraîches et claires en rivière ou en ruisseau et, pour les populations anadromes, les eaux estuariennes et côtières. Dans l'est du Canada, nous retrouvons l'omble de fontaine sous deux formes. La première forme est appelée résidente, c'est-à-dire qu'elle demeure en eau douce tout au long de l'année. La seconde forme est dite anadrome, c'est-à-dire qu'elle effectue des migrations vers l'eau salée (McCormick *et al.*, 1985). Au printemps, les migrants effectuent une migration trophique vers les eaux côtières où ils profitent d'une disponibilité alimentaire accrue pendant quelques semaines ou mois pour ensuite remonter en rivière où ils se reproduisent (Castonguay *et al.*, 1982; Doyon *et al.*, 1991). Il existe aussi, chez d'autres espèces de salmonidés, l'existence de stratégies de vie différentes notamment chez l'omble chevalier (Arnesen *et al.*, 1995), la truite arc-en-ciel (Narum *et al.*, 2004; 2008), la truite brune (Jonsson *et al.*, 2001), et chez le saumon Atlantique et sokeye (*Salmo salar*, Nilsen *et al.*, 2008; *Oncorhynchus nerka*, Wood et Foote, 1996). Dans plusieurs rivières, les deux formes d'omble de fontaine (écotypes) vivent en sympatrie (Dutil et Power, 1980; Boula *et al.*, 2002; Thériault et Dodson, 2003).

Migration en eau de mer

La migration implique des comportements spécifiques issus de la sélection naturelle (Dingle et Drake, 2007). En effet, on doit s'attendre à observer chez les migrants une optimisation du fitness par le changement approprié d'habitat (Krebs et Davies, 1993). Le poisson migre si la croissance et la survie sont favorisées, améliorant ainsi leur succès reproducteur, par l'utilisation d'un deuxième environnement et ce malgré le coût que ce déplacement engendre en comparaison des avantages de demeurer dans le même habitat pour la même période déterminée (Gross, 1987). Suite à leurs travaux sur le terrain chez l'omble de fontaine, Morinville et Rasmussen (2003) ont conclu que les poissons migrants adoptaient cette stratégie possiblement à cause d'une limitation énergétique. Les résidents seraient donc les plus performants au niveau de l'efficacité en eau douce, mais ultimement les anadromes au retour de leur séjour en mer seraient plus gros et plus féconds. Ainsi, les œufs et la progéniture seraient plus gros et auraient de meilleures chances de survie. Une étude sur les effets maternels a d'ailleurs démontré que, chez l'omble de fontaine, le poids de la femelle était fortement corrélé avec la taille de l'embryon, du sac vitellin et de la longueur de l'alevin (Perry *et al.*, 2004).

Parmi les salmonidés, le genre *Salvelinus* (à l'exception des corégones) est celui qui présente l'anadromie la moins prononcée et une résidence en eau salée de courte durée (Power, 1980). Comparativement au saumon atlantique, l'omble de fontaine présente une smoltification peu développée (McCormick *et al.*, 1985). La migration s'effectue davantage

vers les abords de la rivière en eau saumâtre, ou bien dans le cas d'une migration en milieu marin elle nécessite une stabulation en milieu estuarien pour permettre l'acclimatation à l'eau salée (Dutil et Power, 1980; McCormick *et al.*, 1985). Les déplacements et la durée du séjour en eau saumâtre semblent être contrôlés par la salinité, la température et la maturation sexuelle (Dutil et Power, 1980). Dans la rivière Laval, les travaux de Curry et collaborateurs (2006) ont démontré que les ombles de fontaine migraient principalement à l'intérieur de la baie Laval et que rares étaient les déplacements en dehors de la baie. Les ombles ont été observés à des salinités variant de 1 à 34 ppm, mais majoritairement entre 26 et 30 ppm.

Chez les ombles de fontaine anadromes, la dévalaison se fait au printemps et la durée passée en milieu estuarien peut varier en fonction de divers facteurs abiotiques, tels la température et la salinité et par des facteurs biologiques, notamment la maturation sexuelle (Dutil et Power, 1980; Castonguay *et al.*, 1982). Par ailleurs, on sait que la capacité d'adaptation en milieu estuarien varie en fonction de la période de l'année (Claireaux et Audet, 2000). En milieu naturel, au Québec et au nord du Nouveau-Brunswick, les migrations en milieu salé durent de deux à trois mois au printemps et les ombles anadromes remontent les rivières (montaison) du milieu de l'été jusqu'à la période de reproduction. La disponibilité alimentaire en eau salée étant plus élevée, cette migration offre par le fait même la possibilité d'une meilleure croissance (Whoriskey *et al.*, 1981; Doyon *et al.*, 1991). Différents patrons de migration ont cependant été rapportés démontrant des

migrations après la période de reproduction vers l'estuaire supérieur, ce milieu servant alors de refuge hivernal (Castonguay *et al.*, 1982; Lenormand *et al.*, 2004).

La smoltification est une étape importante du développement chez les salmonidés. Elle prépare leur entrée en eau de mer. Il semblerait que le synchronisme entre cette préparation et le mouvement migratoire soit régulé par des facteurs environnementaux. Chez les salmonidés migrants, il a été établi que la smoltification et la maturation sexuelle étaient influencées par les changements photopériodiques du printemps ou de l'automne (Hoar 1988; Randall et Bromage, 1998; Stefansson *et al.*, 2007). De plus, l'atteinte d'une certaine taille semble être liée au déclenchement du processus chez l'omble de fontaine (Morinville et Rasmussen, 2003; Thériault et Dodson, 2003). La smoltification implique des séquences spécifiques d'évènements impliquant des changements au niveau cérébral, endocrinien, structurel, physiologique et comportemental (Hoar, 1988; Bœuf, 1993; McCormick *et al.*, 1998; Ebbesson *et al.* 2008). Bon nombre de fonctions physiologiques telles que la circulation, l'excrétion, la respiration, la croissance et l'osmorégulation sont impliquées et affectées lors du processus, signe que la smoltification nécessite l'action de plusieurs systèmes (Bœuf, 1993).

Les branchies

Chez les poissons téléostéens, le maintien de la concentration plasmatique en ions nécessite l'absorption ou l'excrétion d'ions à l'encontre du gradient du milieu dans lequel ils se trouvent. En eau douce, le poisson doit compenser le gain passif d'eau et la perte

d'ions par la production d'urine diluée et le transport actif d'ions par les branchies. Inversement, en eau salée, le poisson absorbe de l'eau et des ions par le système digestif et secrète l'excès d'ions par les branchies ou les reins (McCormick, 2001). Les branchies sont reconnues comme étant le principal lieu d'échanges ioniques permettant la régulation osmotique de l'organisme (Figure 2).

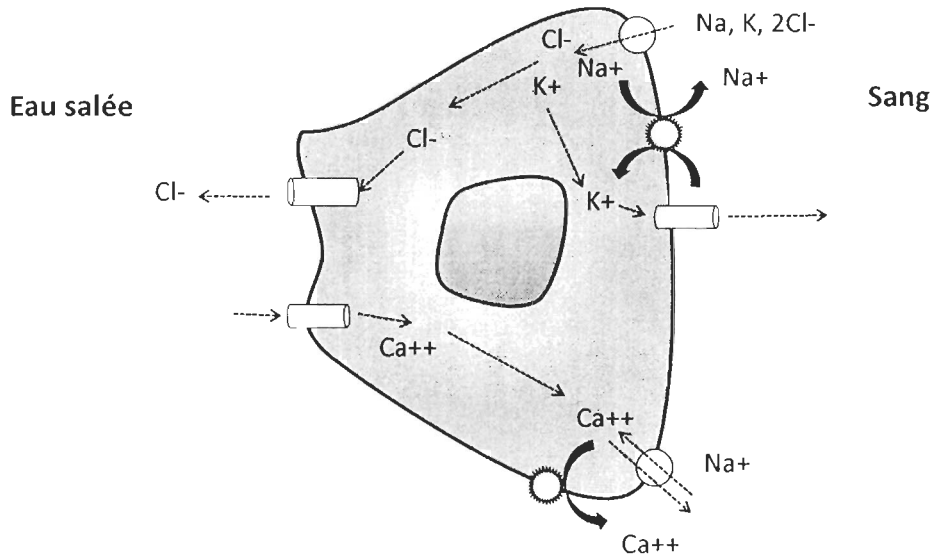

Figure 2. Schéma démontrant le transport d'ions entre le sang et l'eau de mer dans une cellule branchiale (inspiré de Marshall et Grosell, 2006).

Dans le tissu branchial, les cellules à chlorure sont responsables de l'excrétion des ions monovalents lors du transfert en milieu hyper-osmotique. Cette excrétion est liée à une activité enzymatique à l'intérieur des cellules à chlorure. La Na^+/K^+ATPase est un enzyme

membranaire dont l'activité régule de nombreux type d'échanges ioniques et qui permet le transport actif des ions à l'encontre du gradient de concentration cellulaire (Marshall et Grosell, 2006). Chez les salmonidés en général, l'augmentation de l'activité Na^+/K^+ATPasique branchiale qui précède ou qui se produit durant la dévalaison est considérée comme l'un des principaux indicateurs de smoltification (McCormick, 1993).

Les hormones impliquées

Plusieurs hormones sont impliquées dans le contrôle endocrinien de l'osmorégulation lors du passage de l'eau douce vers l'eau salée. La prolactine est reconnue comme étant l'hormone d'adaptation à l'eau douce. L'hormone de croissance (GH), le facteur de croissance de type insulinique (IGF-1) et le cortisol seraient plus spécifiquement impliqués dans l'acclimatation à l'eau salée (Sakamoto *et al.*, 1993; McCormick, 1996; McCormick, 2001). Chez le saumon atlantique, un traitement d'hormone de croissance (GH) augmente le nombre de récepteurs au cortisol dans les branchies (Shrimpton et McCormick, 1998), ce qui a pour effet de stimuler l'activité de la Na^+/K^+ATPase (Shrimpton et McCormick, 1999).

Le rôle des hormones thyroïdiennes en lien avec la smoltification a aussi été étudié. Certains rôles de la glande thyroïde sont bien définis, notamment pour l'empreinte olfactive (Hoar, 1976) et la livrée argentée (Hoar, 1988). Plusieurs études ont pu démontrer des profils saisonniers des concentrations d'hormones thyroïdiennes. Les valeurs maximales étaient atteintes au printemps chez le saumon coho (*Oncorhynchus kisutch*) (Dickoff *et al.*,

1982) et chez l'omble de fontaine (Audet et Claireaux, 1992). Comme ce moment coïncide avec la migration, le développement des mécanismes osmorégulatoires semble nécessiter une activité de la glande thyroïdienne puisqu'une augmentation de la concentration plasmatique en T_3 (triiodothyronine) et du ratio T_3/T_4 (thyronine) suite à un transfert en eau salée est observée chez la truite brune et la truite arc-en-ciel (Leloup et Lebel, 1993). D'autres études ont montré des variations de concentration en lien avec l'adaptabilité à l'eau de mer chez l'omble de fontaine (Boula *et al.*, 2002; Lavallée, 2004). Par contre, le lien direct entre les hormones thyroïdiennes et les mécanismes d'osmorégulation n'est pas encore clair (Leatherland, 1994; Ebbesson *et al.*, 2008).

Plasticité phénotypique ou divergence génétique?

D'un point de vue évolutif, Curry et collaborateurs (sous presse), ont récemment émis l'hypothèse que le comportement de résidence aurait émergé des individus anadromes était la plus probable pour expliquer la présence des deux formes. D'ailleurs, lorsque que l'environnement le permet, le comportement anadrome est exprimé. La présence simultanée des deux formes est donc le résultat d'une sélection naturelle sur l'adoption d'une stratégie menant vers une divergence génétique. La présence d'une plasticité phénotypique montre que les deux formes seraient actuellement moins différenciées que lorsqu'une divergence génétique est achevée. La plasticité phénotypique se définie comme étant un changement dans les caractéristiques d'un organisme en réponse à un signal environnemental (Schlichting et Smith, 2002). Ainsi, ces différences de stratégie de vie peuvent être

interprétées comme découlant d'une grande plasticité phénotypique, les ombles pouvant adapter leur physiologie et leur comportement à une vaste étendue de conditions environnementales (Hutchings, 1996). Des études ont montré des différences physiologiques, principalement liées à l'allocation d'énergie (Morinville et Rasmussen, 2003) et des différences morphologiques (Morinville et Rasmussen, 2008), soit l'existence d'une relation entre la taille et la tactique utilisée (Thériault et Dodson 2003; Thériault *et al.*, 2007b) chez l'omble de fontaine. Signe de la présence d'une plasticité phénotypique, Thériault et collaborateurs (2007a) ont observé que les deux formes se reproduisaient entre elles et que la progéniture en résultant était viable. Ainsi, sur la rivière Ste-Marguerite (un tributaire du Saguenay), les ombles de fontaine anadromes et résidents feraient partie du même pool génétique. Sur la rivière Laval (Côte-Nord), la situation est différente de celle de la rivière Ste-Marguerite. Dans cette rivière (Laval) l'étude des ombles de fontaine anadromes et résidents a établi que les deux formes constituaient deux populations distinctes (Boula *et al.*, 2002). Chez les jeunes stades, Perry et collaborateurs (2005a) ont mis en évidence que la différenciation entre les anadromes et les résidents de la rivière Laval pouvait être attribuable à la sélection naturelle et serait donc adaptative. Nous savons aussi qu'en milieu naturel ces deux populations montrent des différences physiologiques dans un même environnement, notamment au niveau de l'activité de la Na^+/K^+ATPase branchiale et des hormones thyroïdiennes (Boula *et al.*, 2002; Lavallée, 2004).

Le contexte de l'étude

Importance de l'omble de fontaine au Québec

Au Québec, l'omble de fontaine est une ressource importante. Étant donné le déclin des populations de saumon atlantique dans les rivières de l'est du Canada, la pêche sportive a dû se tourner vers une autre espèce combattante et appréciée des adeptes : l'omble de fontaine anadrome. Ainsi, l'exploitation de l'omble de fontaine pour la pêche sportive a été envisagée comme une solution à la baisse de la rentabilité des ZEC (zone d'exploitation contrôlée) occasionnée par la diminution des stocks de saumon atlantique (Lejeune, 1987). L'efficacité des politiques de conservation est directement liée à une bonne connaissance de l'évolution des stratégies de vie, qui dans le cas présent concerne l'anadromie. Par exemple, advenant une surpêche des anadromes, il est possible que les différences entre anadromes et résidents ne permettent pas le recrutement d'anadromes au sein de la progéniture résidente. L'amélioration des connaissances sur la biologie de l'espèce est essentielle à la gestion et la protection des ombles de fontaine anadromes (Dutil et Power, 1980) et les caractéristiques génétiques et biologiques des populations anadromes ont été beaucoup étudiées récemment démontrant clairement l'intérêt d'augmenter nos connaissances afin de mieux gérer cette ressource naturelle (Boula *et al.*, 2002; Morinville et Rasmussen, 2003; 2008, Thériault et Dodson, 2003; Thériault *et al.*, 2007a; 2007b, 2008; Fraser et Bernatchez, 2005; Perry *et al.*, 2004; 2005a; Mavarez *et al.*, 2009).

L'omble de fontaine est aussi une espèce d'intérêt pour l'aquaculture. Il existe actuellement au Québec plusieurs pisciculture en activité et leur production d'omble de fontaine est principalement dédiée à l'ensemencement des plans d'eau. Afin d'éviter l'introduction en milieu naturel de souches domestiques pouvant être génétiquement différentes des souches sauvages (Martin *et al.* 1997), il s'est avéré qu'une souche indigène pourrait être utilisée. De plus, l'apport d'une nouvelle souche permettrait aussi d'augmenter le bagage génétique des productions existantes. Cependant, chez le saumon atlantique, il a été observé que des changements phénotypiques et génétiques peuvent être observés après quelques mois d'élevage en milieu contrôlé (Blanchet *et al.*, 2008). C'est pourquoi l'étude des changements liés à la sélection et à la domestication doit être poursuivie afin de produire des poissons répondant aux critères spécifiques du marché visé (ensemencement, conservation ou alimentation).

Utilisation d'une souche indigène pour l'aquaculture

Afin de permettre à cette industrie d'occuper une place plus importante sur le marché, il est essentiel de rendre disponible aux producteurs une souche répondant aux critères de performance de l'industrie. Ainsi, des travaux de recherche sur deux souches sauvages (Laval et Rupert) ont été entamés au début des années 90 (Savaria, 1998). L'idée de l'apport de souches sauvages dans l'industrie avait aussi pour but d'élargir le bagage génétique. Il avait été démontré par Martin et collaborateurs (1997) que les souches domestiques utilisées étaient rapprochées génétiquement entre elles. La même étude indiquait également que les deux souches sauvages concernées étaient génétiquement

éloignées des souches domestiques. Sachant que la distance génétique favorise l'hétérozygotie diminuant ainsi les risques de voir apparaître des désavantages phénotypiques attribuables à la présence d'allèles récessifs (Fjalestad, 2005; Sonesson *et al.*, 2005; Falconer et Mackay, 1996), il s'est avéré que l'utilisation des souches indigènes Laval et Rupert pourrait être appropriée pour augmenter la variation génétique des souches domestiques.

Dans le but de mieux comprendre la biologie de cette espèce et d'investiguer la possibilité d'utiliser l'omble de fontaine anadrome dans un programme de sélection, des géniteurs issus de ces populations indigènes ont été capturés. La rivière Laval est située sur la côte-nord du St-Laurent et la rivière Rupert se déverse dans la baie de Rupert dans la région de la Baie James. Ces deux souches ont été choisies pour débuter un programme de sélection (Savaria, 1998). Le programme de sélection a été effectué de façon séparée pour les deux espèces : celui sur la souche Laval se déroule principalement à la station aquicole de l'ISMER, alors que celui de la souche Rupert a été initié au Laboratoire régional des sciences aquatiques (LARSA) et se poursuit au Centre de transfert et de sélection des salmonidés (CTSS) situé dans la Baie-des-Chaleurs.

Objectifs de l'étude

Premier volet : évaluation du programme de sélection

Le premier volet avait pour objectif général d'évaluer la performance des ombles de fontaine issus d'un programme de sélection après deux générations de sélection chez les ombles de la lignée Laval (Savaria, 1998). Plus précisément, le premier objectif était d'évaluer le gain génétique attribuable à une sélection dirigée en comparaison avec celui lié au phénomène de domestication pour la croissance et l'absence de maturité sexuelle précoce.

Dans un deuxième temps, l'objectif était de vérifier si la sélection dirigée vers les deux traits mentionnés pouvait avoir eu un effet indirect sur d'autres traits d'importance, notamment la réponse au stress. Cette expérience avait aussi pour but d'identifier la faisabilité d'inclure des traits liés à la réponse au stress dans un éventuel programme de sélection pour la résistance au stress chez l'omble de fontaine.

Second volet : l'anadromie chez l'omble de fontaine

Le second volet avait pour objectif général d'améliorer les connaissances sur les bases génétiques de l'anadromie chez l'omble de fontaine. Plus spécifiquement, l'objectif était de comparer la physiologie et l'héritabilité de variables physiologiques entre des ombles de fontaine anadromes et résidents en milieu contrôlé afin d'éliminer les réponses liées à l'exposition à des environnements différents.

CHAPITRE I. GENETIC GAIN FOR GROWTH AND DELAYED SEXUAL MATURATION USING A FERAL STRAIN OF ANADROMOUS BROOK TROUT (*SALVELINUS FONTINALIS*)

I.1 ABSTRACT

A selective breeding program was initiated using a wild anadromous brook trout *Salvelinus fontinalis* population from the Laval River (Québec). The objective was to develop a new strain characterized by improved growth and reduced precocious sexual maturation. A control line was maintained using random within- family selection. Length and weight were measured and sexual maturity was determined as mature or non-mature at 22 months. In the selected line, phenotypic variance, additive genetic variance and heritability for weight within the selected families were reduced. A comparison between generations at the end of the second year of growth (22 months) showed that in the selected line fish weight increased by 23.1% from F_1 to F_2 and by 32.1% from F_2 to F_3. The control line increased similarly in weight from F_1-F_2 (34.7%) but not thereafter. The proportion of immature fish was 32.2% in the first generation and increased to 61.4% by the F_3 generation in the selected line; it did not change significantly after two generations in the control line (27.5%). Our results showed that simultaneous selection for growth and late sexual maturation are compatible goals in brook trout.

I.2 INTRODUCTION

With the decline of Atlantic salmon *Salmo salar* populations in rivers of eastern Canada, sport fishing activities increasingly focus on anadromous brook trout *Salvelinus fontinalis* populations. The genetic and biological characteristics of anadromous populations are largely unknown, but in the wild they present many traits that are attractive for fish producers. In an attempt to better understand the biology of these populations and to investigate how anadromous brook trout could be included in fish production programs, breeders from a feral population were captured in the Laval River (north shore of the St. Lawrence estuary, Québec, Canada). The actual production of brook trout in Québec is mainly designed for fish stock enhancement.

High growth rate and reduced incidence of precocious sexual maturation are standard breeding goals (Nilsson 1992; Winkelman and Peterson 1994; Gjedrem 2000; Kause et al. 2003). Fast-growing fish allow faster turnover at fish farms, which decreases production costs (Winkelman and Peterson 1994). Late sexual maturity allows fish to reach commercial size more rapidly since fish invest energy in growth instead of gametogenesis (Aksnes et al. 1986; Gjerde 1986); it is also associated with reduced mortality and improved flesh quality (Nilsson 1992; Crandell and Gall 1993). A number of major commercial enterprises continue to suffer from a high incidence of precocious maturation (e.g. Glebe et al. 2003). In salmonid fishes, heritability for growth and size-at-age tends to be moderate-high (h^2>0.2) (Chevassus et al. 2004; Hershberger et al. 1990; Neira et al.

2006; Nilsson 1992; Perry et al. 2005a; Rye and Refstie 1995) and so genetic gain is often considerable (Gjedrem 2000). The heritability of precocious maturation is also moderate-high (h^2=0.19-0.45, Nilsson 1992; 0.21-0.39, Gjerde and Gjedrem 1984), suggesting that similar selective improvement is possible.

Growth rate and late maturity may be conflicting traits for selection in salmonids; body weight and the incidence of sexual maturation are positively genetically correlated in rainbow trout and Atlantic salmon (Thorpe et al. 1983; Gjerde and Gjedrem 1984; Martyniuk et al. 2003). However, genetic covariance is a function of underlying population-level genetic variation for an association between traits from pleiotropy and or linkage (Lynch and Walsh 1998), requiring case-by-case examination. Strong linkage, at least, might be circumvented in highly fecund species, where it may be possible to choose genotypes with advantageous breeding values for both sexual maturity and growth among thousands of individuals (Kause et al. 2003).

Genetic change is realized by genetic shifts that occur in response to experimental selection as well as selection against individuals that fail to adapt to the aquacultural environment (Ruzzante 1994). Without a comparison to unselected controls, it is not possible to differentiate effects of the selection program from those of domestication, resulting in false interpretation of gain (see Fleming et al. 2002; Hershberger et al. 1990). Several studies of salmonid evolution in new environments indicate very rapid progress towards a locally adapted state (Hendry et al. 2000; Quinn et al. 2000; Hendry 2001; Quinn

et al. 2001). Roberge et al. (2006) showed that only five to seven generations of artificial selection could lead to significant changes in gene expression between selection and control groups, the average magnitude of the observed differences being approximately 20% for at least 1.4 and 1.7% of genes expressed at the juvenile stage. It might be reasonably surmised that selective gains in salmonid breeding programs should be relatively rapid.

The objectives of this study were to examine the early stages of selection and to distinguish the resulting genetic gain due to selection from that of incidental gain via domestication. Since the low occurrence of early sexual maturation is a trait of commercial interest, a second objective was to test the heritability of this characteristic in the Laval strain. Finally, we estimated the relative changes in the quantitative genetic architecture of the above traits, including changes in the genetic correlation between growth and early sexual maturation resulting from selection.

I.3 METHODS

A selective breeding program was initiated using an anadromous strain of brook trout from the Laval River (Martin et al. 1997; Savaria 1998). Wild breeders (F_0) were captured in the Laval River near Forestville (Québec) (48°44'N, 68°05'W) from 1991 to 1993 and brought to the Station aquicole de l'Institut des sciences de la mer de Rimouski (Québec) (48°31'N, 68°28'W). In the fall 1994, 12 crosses using six dams and eight sires were made to produce an F_1 generation. Eggs from each female were separated into two or three aliquots per female and each aliquot was fertilized by a different male. Since the size of the wild population is unknown, the Ministère québécois des ressources naturelles et de la faune restricted the capture of breeders for resource conservation reasons. However, microsatellite data confirmed the absence of inbreeding and showed relatively high heterozygosity in river brook charr (Martin et al. 1997) and on F2 control line (Boula et al. 2002; Perry et al. 2005b).

The first selection of fish began in 1996 on 1+ fish (22 months) (generation F_1). In order to maximize genetic gain, a combined between- and within-family selection protocol was used (Falconer and Mackay 1996) based on the absence of precocious sexual maturation and growth. Fish immature at age 22 months in the autumn were retained and the largest of these were used as breeders for the next generation. The number of fish selected by family (NS_i) was determined according to:

$$NS_i = [(x_i - X)\, X^{-1} * (N\, K^{-1})] + (N\, K^{-1})$$

where x_i is the mean of weight for family i, X is the general mean for the population, N is the number of breeders considered necessary, and K is the number of families (Dubé and Blanc 1992). In the F_1 generation, 4.1% to 14.2% of fish were selected from the different families based on the specific family performance. In the second generation (F_2), 11 full-sib families were produced from the selected line with 14.3% of the population that was selected to produce the F_3 generation. In November 2001, 10 full-sib F_3 families were produced from the F_2 selected line breeders. To differentiate between the effects of selection and domestication, a control group was maintained using 10 randomly selected fish from each family in the F_1 population before the selection to create an F_2 control line (nine families from F_1 breeders). This pattern was repeated and an F_2 and F_3 control generations were formed with 10 random crosses (full-sibs) from control F_1 and F_2 breeders. Breeders for the F_2 and F_3 generations were only used once.

The rearing protocol was identical over all generations. Fertilized eggs were incubated in darkness. Each family was incubated separately in individual clays, and each incubation tank contained 11 clays. During incubation, heaters were used to maintain the temperature at 4°C. At hatching, the temperature was gradually increased (1°C per week) to 8°C until natural temperature conditions reached the same level (beginning of June). Each family was maintained in its individual clay until exogenous feeding. Heaters were then removed, and fish were raised in fresh water under natural photoperiod and temperature conditions (minimal temperature [1.2°C] reached in February; maximal temperature [15°C] reached in September). When fish reached 17 months of age, they were gradually (2 g/L

per day) transferred to estuarine sea water (final salinity 20 g/L) for the summer (June to August). In August, fish were returned to fresh water. Except for 0+ fish, all other age groups spent the summer at 20 g/L as described above. Fish were fed commercial pellets with the percent weight per day adjusted according to fish age and water temperature (Savaria 1998).

When exogenous feeding was well established (March-April), fish from all families were randomly transferred from individual clays into sections in evenly divided 0.03 m^3 tanks with separators (three families per tank, one family per section). Family sets were randomly selected. At this stage, fish were too small for being marked. Each family was therefore kept in a tank section until fish reached approximately 1.5 g. At this point (June-July), the pelvic fins of fish were clipped for familial identification (right, left, or both fins, or unmarked) and fish were by groups of four families to larger tanks (0.5 m^3) for the rest of the study period. Again, family sets were randomly selected. Fin markings were verified every 3-4 months and unidentifiable fish were discarded. In the F_3 generation, length and weight were measured monthly from May to September 2002 (20 fish per family), in January 2003 (20 fish per family), and in April 2003 (100 fish per family) to monitor growth. All fish were anaesthetized (3-aminobenzoic acid ethyl ester, 0.16 g/L) before measurements. Weight was measured to the nearest 0.1 g and fork length to the nearest 0.1 cm. Fulton's condition factor (K) (Barton 1996) was calculated as:

$K = (W L^{-3})\ 100$

where W is the weight in grams and L is the fork length in cm. In November 2003, when fish were 22 months old, length and weight were measured in all individuals for each control and selected family (approximately 70 fish per family for the F_3) and the presence or absence of sexual maturation was determined via presence of milt or eggs following gentle pressure on the abdomen. Sexual maturation was treated as a binary variable, with 1 for mature males or females and 0 for immature fish. Mature males and females were grouped together since early maturation causes a diversion of energetic resources and reductions in flesh quality in both sexes (Aksnes et al. 1986). Sampling of fish at sexual maturity from each line and each generation was made as already described in detail for the F_3. The number of fish differed among generations since the number of families was different.

Throughout the study, fish were healthy and we encountered no problem in maintaining all families, lines, or generations. Once fish reached the exogenous feeding stage, the number of fish was standardized among families (1000 individuals). Regular random culls within family were used to maintain appropriate stocking conditions in the rearing facility (< 30 kg fish per m^3).

Normality of data was verified by Kolmogorov-Smirnov tests (Sokal and Rohlf 1995). When data were not normally distributed, a suitable exponential transformation was obtained via a Box-Cox macro (M. Friendly, York University, Canada). We tested for tank

effects on the experimental groups using a two-way ANOVA (PROC GLM; SAS 1998) including full-sib family and rearing tank.

Realized heritability in this population was estimated from phenotypic gain using the breeder's equation $h^2 = R/S$ where R is the response to selection and S is the selection differential (Falconer and Mackay 1996). Genetic variance components were estimated separately within the F_3 control and selected groups using restricted maximum likelihood (REML) (PEST3.0/VCE4.2; Groeneveld et al. 1990; Groeneveld 1994). Genetic variances and genetic covariance were estimated using PEST3.1 (Groeneveld et al. 1990) and VCE4.2 (Groeneveld 1994). Estimated breeding values (EBV), variances and covariances, heritabilities (h^2), and genetic correlations (r_a) were calculated using VCE. Genetic variance parameters were estimated for weight (W), condition factor (K) and precocious maturation using a bivariate animal model of the form

$$y_i = (X_i b_i) + (Z_i a_i) + e_i$$

where y is the vector of phenotypic observations on trait i (W or K), X is the incidence matrix for trait i, b is the vector of fixed effects (rearing tank), Z is the incidence matrix of random (animal) effects for trait i, a is the vector of random animal effects (breeding values) for trait i, and e is the vector of random error for trait i.

Relationships among animals was limited to parent-progeny relationship between the F_2 and F_3 generations, since pedigree records beyond this immediate point were not available. Heritability estimates and standard errors for maturation were estimated for the

control and selected populations on the observed binary scale and then transformed to the liability scale using the transform of Roff (1997):

$$h^2 = h_{0,1}^2 p(1-p)/z^2,$$

where $h_{(0,1)}^2$ is heritability on the binary scale in each population, h^2 is the heritability on the underlying liability scale, p is the proportion of mature individuals and z is the point on the normal curve corresponding p, where

$$z = \exp(-\frac{1}{2}x^2)/\sqrt{2\pi},$$

where

$$x = (sign(0.5-p))(1.238c(1+0.0262c))$$

and

$$c = \sqrt{-\ln(4p(1-p))}.$$

Heritability values for the complete set of traits (*BW, K*, and precocious maturity) were compared using a t-test (Satterthwait approximation for unequal variances, SAS 1998) to test for general trends in genetic variance between selected and control lines.

Analyses of variance (ANOVAs) were used to compare the phenotypic values of lines and families within lines (selected or control) for traits in the F_2 and F_3 generations (Sokal and Rohlf 1995). A Tukey test was used to compare post-hoc differences among means when variances were homogenous and a Games and Howell test was used when variances were heterogeneous ($P < 0.05$; see (Sokal and Rohlf 1995).

I.4 RESULTS

Mean weight increased through the generations for both the control and selected groups. The entire F_1 cohort was considered 'control' since no selection had been imposed prior to this point. In the control line, weight increased by 36 g from the F_1 to F_2 generations (34.7%) but only by 6 g from F_2 to F_3 (4.0%), for a total increase of 42 g (41%) (Table I.1). In the F_2, the phenotypic variance was significantly greater in the control than in the selected line (Fig. I.1). In the F_3, no difference in weight was observed between the selected and control groups in the first months. As fish reached 15-16 months, however, the selected fish were significantly heavier than the control ones (Table I.2). Significant family effects were present in both the selected and control groups in September, January and April ($P < 0.001$). As in the F_2, phenotypic variability among families was higher in the controls (Fig. I.2), in which both heavier and lighter families were found. The average weight observed in the different families at the age of 15-16 months (April) followed the pattern observed when fish were eight months old (previous September). Tank effects were negligible for all traits.

There were no apparent changes in early sexual maturity after two generations in the control line. However, selection led to a decrease in the proportion of early sexual maturity at the age of 1+ (22 months) over generations: in the F_1 generation, the proportion of immature fish was 32.2% at the age of 1+ while in the F_3 generation, 27.5% of the control group and 61.4% of the selected group were immature. Weight comparisons at the time of

Table I.1 Mean weight and number of fish per generation and number of fish selected to contribute to the next generation for control and selected group at the age of sexual maturation (22 months).

Generation	Weight (g) (SD)	n	immature n	immature %	♂ n	♀ n	n randomly selected in population	N selected in immature fish
Control line								
F1	103.8 (45.9)	2106	679	32.2	786	641	120	201
F2	139.8 (68.5)	491	199	40.5	169	123	90	
F3	145.8 (54.4)	546	150	27.5	221	175		
Selected line								
F2	127.8 (54.9) ($P = 0.02$)	524	283 ($P < 0.001$)	54.0	106	135		75
F3	168.9 (55.2) ($P < 0.001$)	632	388 ($P < 0.001$)	61.4	123	121		

P values for weight and maturity indicate comparisons between control and selected lines calculated with ANOVA. 'N random selected' and 'N immature selected' refer to the number of individuals selected for production of the succeeding generation and the number of immature individuals in each generation, respectively.

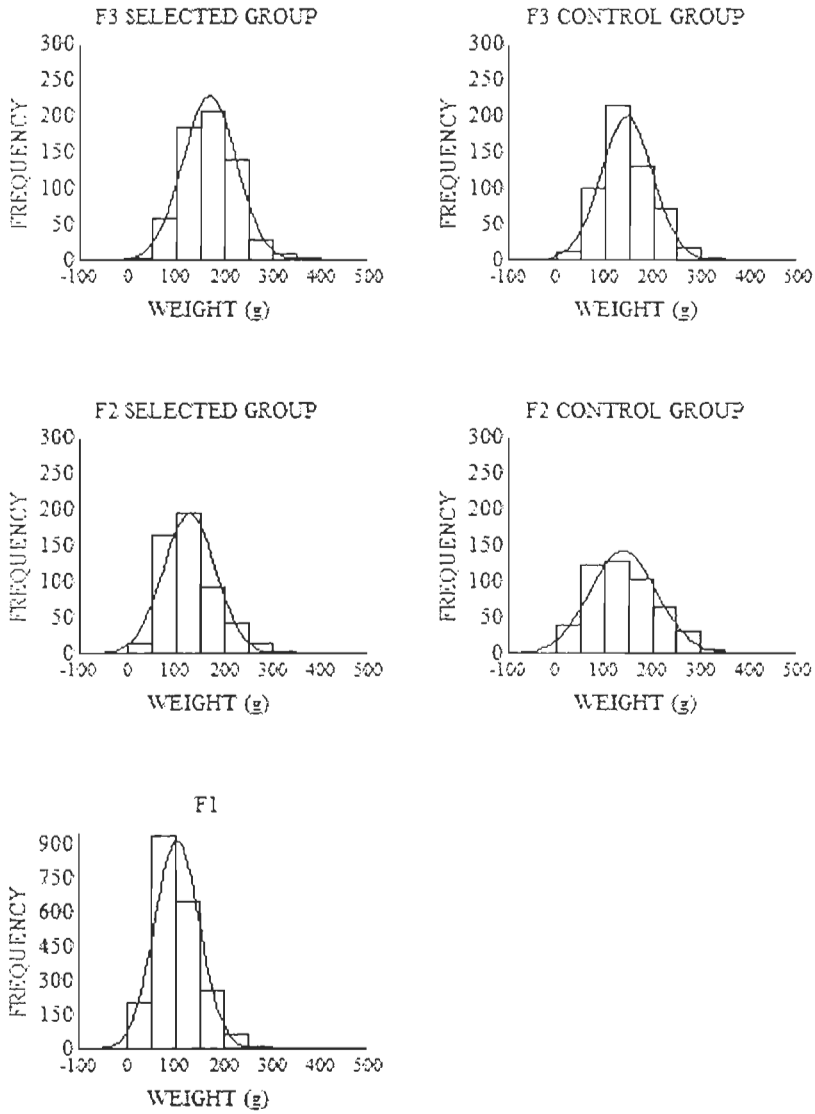

Figure I.1 Weight distribution of F_1, F_2, and F_3 brook trout at 22 months.

Table I.2 Mean weight, condition factor (Kf) and standard deviation (SD) for F_3 selected and control groups of brook trout over the sampling year.

Group	Date	Mean weight (g)	SD	P	Mean Kf	SD	P
Selected	09/2002	11.1	3.1	0.576	1.058	0.090	0.334
Control		11.3	4.6		1.051	0.073	
Selected	01/2003	30.4	13.7	0.187	0.926	0.071	0.006
Control		29.0	15.4		0.950	0.101	
Selected	04/2003	33.7	15.0	0.005	0.935	0.404	0.920
Control		32.2	16.5		0.937	0.084	
Selected	11/ 2003	168.9	55.2	0.000	1.081	0.096	0.024
Control		146.8	54.4		1.093	0.091	

Probabilities (*P*) calculated using ANOVA associated with differentiation between control and selected lines are indicated.

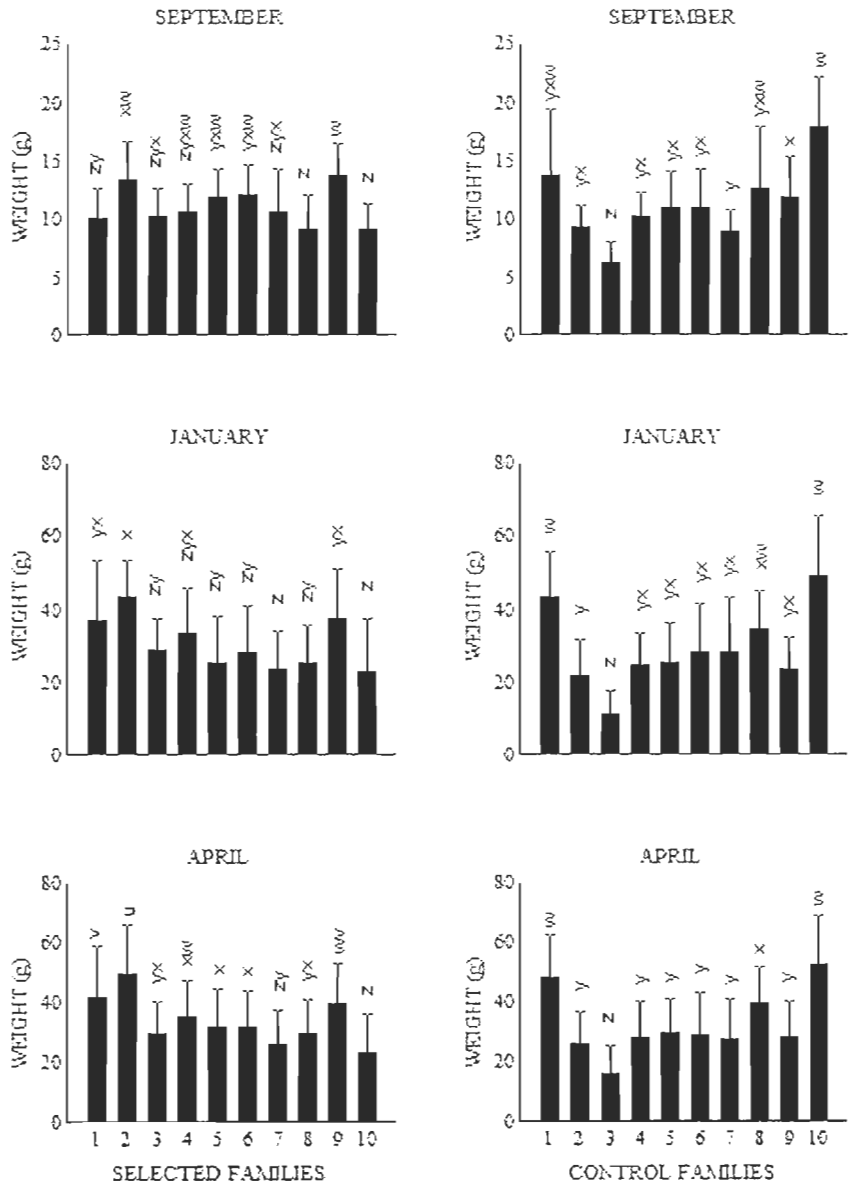

Figure I.2 Mean weight (g) (± S.D.) of 0^+ (September 2002, January 2003) and 1^+ (April 2003) brook trout in F_3 control families (right panels) and families from a selection program (left panels). The phenotypic standard deviation per family is given. Different letters (a-f) indicate significant differences ($P < 0.05$) among families.

selection (age 1+; 22 months, November) showed an increase of 24 g from F_1 to F_2 (23.1%) and of 41 g (32.1%) from F_2 to F_3 with a total increase of 65 g (62.7%) from the F_1 generation (Table I.1).

Additive genetic variance and heritability among selected and control lines after two generations of selection

Realized heritability (F_2 to F_3) for November weight was estimated as $h^2 = 0.83$ for the F_3 selected group, and heritability for body weight at 22 months in the control line compared favourably with the realized heritability from the selection line. Realized heritability for percentage of immature fish was estimated as $h^2 = 0.16$ for the F_3 selected group. Heritability estimates calculated using REML in the F_3 generation were generally high ($h^2 > 0.4$; Table I.3). Genetic variance for weight and condition factor was higher in the control group than in the selected line (Table I.3). Genetic correlation between weight and condition factor was high and positive (> 0.8) except in April, when genetic correlation was significantly lower (non-overlap of r_a estimates including standard error) in the F_3 selected line than in the F_3 control group.

The analysis of genetic variance–covariance of precocious maturation and weight at 22 months in the F_3 population indicated high heritability for both characters ($h^2 > 0.3$) in both the selected and control lines. However, the heritability of weight appeared to be considerably higher in the control group than in the selected line (Table I.3). In contrast, estimates of heritability for precocious maturation, while nearly overlapping, were actually

Table I.3 Heritability estimates (h^2), genetic correlations (r_a), and associated standard error (SE) for weight and condition factor calculated using REML for F_3 selected and control brook trout.

Character	Date	Age	Selected families h^2 (SE)	Selected families r_a (SE) with weight	Control families h^2 (SE)	Control families r_a (SE)
Weight	09/2002	10	0.494 (0.179)	-	0.679 (0.175)	-
Condition factor			0.490 (0.178)	0.858 (0.128)	0.827 (0.168)	0.921 (0.064)
Weight	01/2003	14	0.589 (0.190)	-	0.830 (0.242)	-
Condition factor			0.433 (0.167)	0.980 (0.055)	0.610 (0.209)	0.985 (0.035)
Weight	04/2003	17	0.660 (0.192)	-	0.651 (0.185)	-
Condition factor			0.671 (0.200)	0.689 (0.170)	0.582 (0.180)	0.964 (0.027)
Weight	11/2003	22	0.497 (0.019)	-	0.860 (0.093)	-
Precocious maturation			0.482 (0.018)	0.054 (0.029)	0.306 (0.160)	-0.940 (0.140)

higher in the selected line than in the controls ($h^2_s \approx 0.8$; $h^2_c \approx 0.5$). All h^2 estimates may be partially biased upwards due to dominant genetic variance (Falconer and Mackay 1996). Estimates of genetic correlation between precocious maturation and weight were also highly divergent: genetic correlation was highly negative in the controls ($r_a \approx$ -0.9), indicating that precociously mature fish had poor genetic value for growth, but was marginally positive in the selected line ($r_a \approx 0.05 \pm 0.03$; Table 3). Heritability values for the complete set of traits (*BW, K*, and precocious maturity) were marginally lower in the selected line than in the control line ($t = 2.01$, $P = 0.0638$).

Finally, we also compared the observed values of gains in growth and the predicted efficacy of the selection scheme given the intensity of selection and the above estimates of heritability variance using the derived breeders' equation $R_p = i\ h^2\ \sigma_p$, where R_p is the response, i is the intensity of selection, and σ_p is the phenotypic standard deviation (Falconer and Mackay 1996). For weight at an approximate selection intensity of 10%, R_p in the F_2 and F_3 generations was (1.282) (0.86) (45.9) = 50.6 g and (1.282) (0.86) (54) = 59.0 g, for a realized improvement of approximately 47.4% and 69.0% of R_p. These values are higher than those actually observed, that is an increase of 24 g from F_1 to F_2 (23.1%) and of 41 g (32.1%) from F_2 to F_3. Thus, while we obtained very good response to selection, its full potential was apparently not achieved based on those predictions. We calculated expected gain in reducing maturation (*i.e.* increasing immaturity), also using the derived breeder's equation, since linear methodologies appear to generally confer equivalent power in analysis. Treating p as 0.01 (i = 3.960) in standard truncation tables to approximate the intended intensity of complete selection (s = 1.0) against precociously mature individuals and using σ^2_p and h^2 from the REML analysis in the control line (see above), we estimated a predicted R_p = (3.96) (0.482) (0.455) = 0.55. Realized gain, using the simple linear interpretation (see Lopes et al. 2000) was calculated at $G_R = p_{F1} - p_{F2}$ = 0.22 between F_1 and F_2 and 0.074 from F_2-F_3, for a realized gain of 40% and 13% in the reduction of precocious maturation, respectively, for a total of 53%. So both predicted and realised responses to selection for precocious maturation were very similar.

I.5 DISCUSSION

Selection effects at younger ages (F_3 families)

There was little difference in weight between selected and control fish until 15 months post-hatch and no pattern before seven months. Although the best performing families could be detected as early as eight months posthatch, Silverstein and Hershberger (1994) found that egg size still had a significant effect on size after 10 months of age in coho salmon. Early identification of superior families would be very useful for reducing production costs, minimizing time to market and improving homogeneity (Vandeputte et al. 2002; see Winkelman and Peterson (1994).

Gain per generation

Gain for weight and reduction of precocious maturation was different for the selected and control groups. Mean weight increased in the selected line by 23.1% after one generation and by 32.1% after the second. In comparison, Charo-Karisa et al. (2006) observed a selection response for growth of 34.7% from F_0 to F_1 and 14.9% from F_1 to F_2 in Nile tilapia. In coho salmon, a 60% increase in weight was observed after four generations of family selection (Hershberger et al. 1990). Friars et al. (1995) observed cumulative gains in the market size of Atlantic salmon using mass and index selection over two generations in a high-grilse stock. Most of the genetically controlled gain in weight from the F_1 to the F_2 generations may have resulted from incidental domestication selection or adaptation, since there was a large increase in weight from F_1 to F_2 (34.7%) that occurred

in the control line but almost none from F_2 to F_3 (4.0%). The large difference in weight from F_1 and F_2 in both groups might partially be due to improvements in feeding protocol. The wild strain used to initiate this experiment had different feeding behaviour that prompted us to adjust first-feeding protocol and to switch from floating to sinking pellets in older animals.

Phenotypic variance for weight was generally lower in the selected line compared to the controls: standard deviation steadily decreased from F_1 to F_3. Glover et al. (2001) suggested that selection via mortality during first-feeding might play a role in an inadvertent domestication selection scheme. Moreover, Hershberger et al. (1990) also observed a significant domestication effect in coho salmon, which continued for four generations. The response to the selection for age at first sexual maturation was also clearly present by the F_3 generation. The proportion of immature fish increased from 32.2% in the F_1 to 61.4% in F_3, demonstrating the accuracy of the combined selection scheme for this trait. There was no concomitant increase in the proportion of immature fish occurring in the control line, suggesting that selection for this trait was effective.

Heritability

Estimates of the heritability of morphological traits tend to be high ($h^2 > 0.3$) in the salmonidae (Winkelman and Peterson 1994; Gjedrem 2000; Kause et al. 2003; Martyniuk et al. 2003; Perry et al. 2005a). Estimates in the control and selection lines were also very high ($h^2 = 0.40$-0.85) possibly representing the partial effects of dominance and/or residual

maternal variance in the full-sib families (Lynch and Walsh 1998). However, the lack of clear trends in variance/covariance parameters among traits during ontogeny suggests that dominance or maternal overestimation was itself unbiased with respect to line. Notably, heritability estimates for weight in the selected and control lines differed more in September and January but less in April (age 15 months), perhaps suggesting a decrease of the maternal effect during the first year of growth. Overall, heritability values for the complete set of traits (*BW*, *K*, and precocious maturity) were marginally lower in the selected line than in the control line. However, our high heritability estimates might also have been partially affected by over-estimation related to the small number of full-sib families used or unknown relationships between parents higher in the pedigree. Nevertheless, since the number of families used both for selected and control lines were identical, this cannot explain the differences in heritability values observed between lines, which are therefore most likely to reflect the effect of selection.

Two generations of selection appear to be sufficient to produce significant differences in the proportion of fish showing early sexual maturation. Our estimates of heritability for precocious maturation were roughly in the range of those in other species of salmonids (0.19-0.45 for Arctic charr, Nilsson 1992; 0.21-0.39 for rainbow trout, Gjerde and Gjedrem 1984), but notably estimates of genetic variance for precocious maturation appeared to be actually lower in the control line than in the selected group, although standard error associated with the estimate of heritability for precocious maturation in the F_3 control group was unusually high compared to other estimates. Genetic variance for the

trait in the two groups may be closer than is immediately apparent. There are several explanations for these findings. Firstly, selection may have culled out specific dominant genotypes (leaving others intact) rather than having increased additive genetic variance; our full-sib variance estimates were not capable of discriminating dominant and additive genetic variance. Secondly, phenotypic differentiation between the lines may have resulted from the apparent deterioration of genetic associations between weight and precocious sexual maturation resulting from, or coupled with, selection for weight (Lynch and Walsh 1998).

Interaction between traits

The high genetic correlation between weight and condition factor detected in our study showed that the gene expressions of weight and condition factor are strongly associated, which has also been found elsewhere (Su et al. 2002; Martyniuk et al. 2003; but see Neira et al. 2004).

Coupled selection for high growth and late sexual maturation is typically presumed to be incompatible because of negative genetic and phenotypic associations between growth rate/size-at-age and age at sexual maturity (Thorpe et al. 1983; Gjerde and Gjedrem 1984; Rye and Gjerde 1996; Quinton et al. 2002; Martyniuk et al. 2003). However, such relationships are not uniformly observed (Huang and Gall 1990; Crandell and Gall 1993) and we detected radical sign changes in genetic correlation in the selection line compared to the controls. Part of this deviation might be related to the relatively small numbers of

families; however, our genetic variance component estimates were fairly consistent by trait within groups. Inbreeding might cause such radical changes (Phillips et al. 2001) but Martin et al. (1997) and Boula et al. (2002) observed no significant reduction in genetic diversity in the F_1 or F_2 generations relative to wild fish, suggesting that the number of breeders used was sufficient to avoid significant inbreeding effects. The narrow demographic passes at the F_1-F_2 and F_2-F_3 junctures could have caused drift at functional loci instead of markers, fixing alleles with antagonistic or neutral effects on weight and maturation. Our estimates of genetic correlation between body weight and precocious maturation in the control group, presumably representative of their association in the absence of selection, were generally in line with negative genetic correlations between age at maturation and growth rate in other salmonid systems.

Our selective breeding program resulted in reductions in precocious sexual maturity and improvements in growth as well as in genetic coupling between these traits relative to unselected controls although its full potential was apparently not reached based on observed and predicted response. Despite this, genetic gain was substantial, more than double seen in other salmonids (Kincaid et al. 1977; Gjedrem 1979; Gjedrem 2000; Hershberger et al. 1990; Neira et al. 2006; Charo-Karisa et al. 2006). Domestication effects on growth were high in the first generation of fish reared at our facility, suggesting maternal effects on offspring size. Indeed, responses in salmonid selection schemes average 15% which is much less than observed here (Gjedrem 2000). Yet, there are currently no comprehensive selective schemes for the commercial aquaculture of brook trout and few

genetically improved lines of any salmonid species in use anywhere in the world today (1-2% globally) despite the potential for massive economic returns (Gjedrem 2000). The combined improvement in growth and precocious maturation — coupled with the apparently rapid attenuation of domestication effects — may suggest greater amenability to commercial rearing in Laval brook trout than other salmonid populations, and our results suggest ample opportunity for bidirectional or simultaneous genetic improvement.

CHAPITRE II. EFFECTS OF SELECTION FOR GROWTH AND LATE SEXUAL MATURITY ON OTHER PERFORMANCE TRAITS IN BROOK CHARR (*SALVELINUS FONTINALIS* MITCHILL)

II.1 ABSTRACT

The objectives of this study were to verify if selection for growth and late sexual maturity in anadromous brook charr had indirect effects on the way fish were able to respond to different challenges: early exposure to salt water (7-month-old fish) and acute stress response and sensitivity to *Flexibacter maritimus* infection in 15-month-old animals (correlated selected trait). Experiments were done on 10 control families (no selection applied) and 10 selected families. In contrast to our expectations, young-of-the-year fish showed no distress after saltwater transfer. However, different familial patterns of gill Na^+/K^+ ATPase were present. Combined selection for late sexual maturity and high growth resulted in a general decrease in cortisol responsiveness and less susceptibility to the opportunistic infection. Strong family-related effects suggest the presence of additive genetic components that would allow selection based on stress response.

II.2 INTRODUCTION

High growth rate and reduced incidence of precocious sexual maturation are standard breeding goals for aquaculture (Nilsson, 1992; Winkelman & Peterson, 1994; Gjedrem, 2000; Kause *et al.*, 2003). In 1996, a selective breeding program for coastal production was initiated using an aquaculture stock of brook charr derived from a wild anadromous population from the Laval River, Quebec, Canada. A comparison between generations showed that the mass of fish in the selected line increased by more than 20% from F_1 to F_2 and by more than 30% from F_2 to F_3 (Chapitre I). The control line increased similarly in mass from F_1 to F_2 but not thereafter. The proportion of immature 1+ fish was about 30% in F_1 fish. This proportion increased to more than 60% in selected F_3 fish while it did not change significantly in the control line. One question that may arise from such genetic changes is whether selection for these traits could have indirect effects on general fish performance (correlated selected traits).

In brook charr, saltwater exposure may represent a great challenge when it occurs early in the development process (Sutterlin *et al.*, 1976). Gill Na^+/K^+ ATPase activity, which is known to increase following saltwater transfer in salmonids (McCormick *et al.*, 1985), can be used as a indicator of saltwater adaptation. Older (1^+) animals acclimate easily to salt water (Besner & Pelletier, 1991; Claireaux & Audet, 2000; Hiroi & McCormick, 2007). However, fish younger than 2 years old remain susceptible to infection by *Flexibacter maritimus* (Lefrant, 2006) while in salt water, and pronounced variations in

infection susceptibility among families have been observed with this opportunistic infection.

Fish in aquaculture facilities are submitted to many stressful manipulations (handling, sorting, transportation, vaccination), all of which have the potential to initiate a severe stress response (Specker & Schreck, 1980; Schreck *et al.*, 1989; Barton & Iwama, 1991; Portz *et al.*, 2006). Recent studies found that stress resistance may be an important trait of interest for improving fish performance in aquaculture (Øverli *et al.*, 2006; Trenzado *et al.*, 2006). Since the magnitude of the stress response varies among individuals, fish with stronger or weaker responses may be identified within a population (Fevolden *et al.*, 1991; Pottinger *et al.*, 1992). Heritability of the stress response has been studied, notably in rainbow trout (*Oncorhynchus mykiss* Walbaum), in which the magnitude of the stress cortisol response is a stable individual characteristic with a moderate to high degree of heritability (Pottinger *et al.*, 1992; Pottinger *et al.*, 1994; Fevolden *et al.*, 1999). Weber & Silverstein (2007) suggested that because of the complexity of the stress response, the genetic basis of the phenotypes of high-responding and low-responding lines would probably differ among strains, families, and individuals. The early identification of stress-sensitive families in a breeding program should reduce production costs by eliminating sensitive families early in the production process.

The objectives of this study were to verify if selection for growth and late sexual maturity had indirect effects on the performance of other traits and to verify if resistant

families could be identified early in development. To do so, it was tested if families resistant to different challenges could be easily identified and if this sensitivity could be linked to a typical stress response pattern. Three challenges were used to quantify fish performance: 1) transfer to salt water in 0^+ fish for a 7-day period, 2) acute handling stress exposure in 1^+ fish, and 3) sensitivity to *Flexibacter maritimus* infection in 1^+ fish raised in salt water during summer. Responses were evaluated according to the expected result: 1) survival and gill Na^+/K^+ ATPase activity in 0^+ fish transferred to salt water, 2) cortisol response following acute handling stress, and 3) bacterial infection susceptibility and cumulative mortalities in 1^+ fish transferred to salt water.

II.3 MATERIALS AND METHODS

From 1991 to 1993, wild breeders were captured in the Laval River near Forestville (Quebec) (48°44'N, 68°05'W) and brought to the Station aquicole de l'ISMER (Quebec, Canada) (48°31'N, 68°28'W). In fall 1994, 13 crosses using six dams and eight sires were made to produce an F_1 generation. Eggs from each female were separated into two or three aliquots and each aliquot was fertilized by a different male. The selection program began in 1996 on 1^+ fish (generation F_1). Selection was based on two criteria: state of sexual maturity at 1^+ (22 months) and growth. Immature 1^+ fish were selected and the largest of these individuals were kept as breeders. To differentiate between the effects of the selection program and the effects of domestication, a control line (random crosses) was maintained over generations. In fall 2001, ten F_3 full-sib families were produced from the selected fish (F_2) and ten families from the control line (F_2).

The rearing protocol was identical from one generation to the next. Fertilized eggs were incubated in darkness. Each family was incubated separately in individual clays, and each incubation tank contained 11 clays. During incubation, heaters were used to maintain the temperature at 4°C. At hatching, the temperature was gradually increased (1°C per week) to 8°C until natural temperature conditions reached the same level (beginning of June). Heaters were then removed, and fish were raised in fresh water under natural photoperiod and temperature conditions (the minimum temperature [1.2°C] occurred in February; the maximum [15°C] in September). Throughout the study, fish were healthy and

no problems were encountered in maintaining families, lines, or generations. Once fish reached the exogenous feeding stage, the number of fish was standardized among families to 1000 individuals. Regular random culls within a family were used to maintain the density at < 30 kg fish per m^3. Fish were fed with commercial pellets with the percent mass per day adjusted according to fish age and water temperature. Each F_3 family was kept separate until fish reached approximately 1.5 g. In June–July, the pelvic fins of fish were clipped for identification (right, left, or both pelvic fins clipped, or unmarked) and fish were pooled by groups of four families into 0.5 m^3 tanks. Each experiment described below was conducted on F_3 fish.

Early exposure to salt water

In August, 50 0^+ fish (7 months old) per family were gradually transferred to salt water (20) for a 7-day period. Experimental fish (eight families per tank) were placed in 0.3 m^3 tanks three to six days before the experiment. On day 0, fish were in fresh water; salinity was raised to 13 on day 1, to 16 on day 2, and was maintained at 20 from day 3 to 7. Water temperature varied from 13.5°C (fresh water) to 12.0°C (20 salt water). We sampled eight fish per family on day 0 (prior to saltwater transfer), day 3 (20), and day 7 (20); tanks were checked for mortalities every day. The mean masses of fish used in this experiment are presented in Table II.1.

At sampling, fish were randomly and rapidly caught by net and placed in an anaesthetic solution (3-aminobenzoic acid ethyl ester, 0.16 g/L) prior to measurements. Because fish were too small for the blood sampling necessary for cortisol measurements,

we used gill Na^+/K^+ ATPase activity as a measure of saltwater acclimation and saltwater exposure response at this life stage. Mass was measured to the nearest 0.01 g and gill tissue was sampled according to Siegler *et al.* (1996). In brief, filaments from the left gill arches were cut and directly transferred to 100 µL of SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole) and stored at -80°C until Na^+/K^+ ATPase activity measurements. Determination of gill Na^+/K^+ ATPase activity was done according to Siegler *et al.* (1996).

Table II.1. Early saltwater exposure experiment on 7-month-old (0^+) brook charr: mean mass (± SD) of sampled fish.

Selected families	Mass (g)	Control families	Mass (g)
S1	5.86 ± 1.42	**C1**	8.85 ± 2.2
S2	5.45 ± 1.63	**C2**	6.15 ± 1.45
S3	7.21 ± 1.07	**C3**	4.29 ± 1.38
S4	6.43 ± 1.76	**C4**	4.91 ± 0.97
S5	5.00 ± 0.99	**C5**	7.08 ± 2.26
S6	4.24 ± 0.80	**C6**	4.50 ± 0.95
S7	6.57 ± 2.04	**C7**	5.77 ± 1.60
S8	5.56 ± 1.63	**C8**	8.42 ± 1.36
S9	7.58 ± 2.08	**C9**	5.29 ± 1.15
S10	4.27 ± 0.99	**C10**	5.49 ± 1.36

Handling stress

This experiment was done when fish were 15 months old. The mean masses of fish used for the acute stress experiment are presented in Table II.2. Twenty-five fish per family were individually stressed by 1 minute of handling out of water in a small net. After the

handling stress, fish were placed back in fresh water in three different tanks for later blood sampling. Blood samples were taken on six fish per family captured in rearing tanks before handling and on six handled fish per family 1 h, 3 h, and 24 h after the handling stress (for a total of 24 fish per family and 120 fish per sampling time). All sampling was done between 1000 hours and 1300 hours to avoid diel variation effects on plasma cortisol concentration. Fish were caught, immediately placed in an anaesthetic solution (3-aminobenzoic acid ethyl ester, 0.16 g L^{-1}), and weighed before caudal puncture; fish were sacrificed immediately after caudal puncture according to regulations of the Canadian Council of Animal Care. Blood was centrifuged at 7200*g* for 3 min. All manipulations were done quickly so that blood was obtained within 2 to 3 min following transfer into the anaesthetic solution. Plasma was collected and immediately frozen at -80°C. Cortisol concentrations were measured using a radioimmunoassay commercial kit (Cortisol ^{125}I RIA kit, Immuchem™ Inc, Biomedicals, USA).

Table II.2. Handling stress experiment on 15-month-old (1^{+}) brook charr: mean mass (± SD) of sampled fish.

Selected families	Mass (g)	Control families	Mass (g)
S1	46.6 ± 18.1	**C1**	60.9 ± 17.5
S2	55.1 ± 14.3	**C2**	32.3 ± 14.4
S3	37.1 ± 11.4	**C3**	16.0 ± 10.0
S4	40.0 ± 18.2	**C4**	33.6 ± 18.1
S5	32.6 ± 12.9	**C5**	34.8 ± 13.8
S6	37.3 ± 16.7	**C6**	33.3 ± 17.4
S7	33.4 ± 13.4	**C7**	31.3 ± 18.9
S8	29.7 ± 15.3	**C8**	46.1 ± 15.3
S9	45.0 ± 14.6	**C9**	33.0 ± 14.5
S10	29.7 ± 17.0	**C10**	56.9 ± 15.1

Challenge with Flexibacter maritimus

Sub-adult brook charr are susceptible to opportunistic infection when raised in salt water. The causative agent has been identified as *Flexibacter maritimus* based on culture on selective media and PCR analysis (Lefrant, 2006). When fish reached 16 months of age, they were transferred to salt water for the summer period. Saltwater rearing began on 9 June, when better performance for saltwater adaptation has been observed (Besner & Pelletier, 1991; Claireaux & Audet, 2000), with salinity increasing in increments of 2 per day to reach 20 after ten days. Each experimental 0.5 m^3 tank contained four families, with 70 fish per family. The freshwater temperature was 9.0°C at the beginning of the experiment, and temperature gradually rose to 12.0°C by the end of the summer (natural summer conditions). Mortalities attributable to *Flexibacter maritimus* were noted each day. Mass and length measurements were done on all fish in April (prior to transfer), at the beginning of July (25 fish per family), and at the end of August (30 fish per family). The saltwater rearing experiment ended on 29 August, when all fish were returned to fresh water.

Statistics

Normality of data was verified by Kolmogorov-Smirnov tests. Fish masses and plasma cortisol concentrations were square-root transformed to obtain normality. Levene tests were used to verify the homogeneity of variance and then Tukey or Games-Howell tests were performed for post-hoc analysis. Two-way ANOVAs (Line, Day) were used with the family factor being nested in the line factor (Control or Selected). The influence of fish

mass on Na^+/K^+ ATPase activity and on cortisol concentrations was examined by adding mass as a covariate in the model (ANCOVA). The daily growth coefficient for summer growth in salt water was calculated as 100 (ln W_2 - ln W_1) nb of $days^{-1}$.

II.4 RESULTS

Early exposure to saltwater

No mortality was observed in any of the 20 families during the seven days of saltwater exposure. The pattern of the Na^+/K^+ ATPase activity response differed between control and selected lines (Line: d.f. = 1, $P = 0.602$; Line x Day: d.f. = 2, $P = 0.007$) and among families (Family (Line): d.f. = 18, $P < 0.001$). No change in Na^+/K^+ ATPase activity occurred in three selected families (4, 5, and 7) and two control families (5 and 6), whereas four selected families (1, 3, 8, and 9) and five controls (1, 2, 7, 8, and 10) showed an increase in Na^+/K^+ ATPase activity on day 3 with a return to control levels on day 7 (Table II.3). Selected families 2, 6, and 10 and controls 3, 4, and 9 all showed different patterns. In the ANOVA model, the mass co-factor was significant (d.f. = 1, $P = 0.046$) but with a weak relationship (Fig. II.1).

Handling stress

The response to handling stress differed significantly among the selected and control lines (Line: d.f. = 1, $P = 0.044$; Line x Time: d.f. = 1, $P = 0.588$). In the control line, no response difference was observed among families (Family x Time: d.f. = 27, $P = 0.112$): plasma cortisol was significantly higher after 3 h and all fish recovered rapidly, with cortisol levels being back to the initial concentration after 24 h (Fig. II.2a). However, the cortisol response pattern differed among families in the selected lines (Family x Time: d.f. = 27, $P < 0.001$). Six out of ten families showed no significant cortisol response to handling

Table II.3. Mean (± SD) gill Na^+-K^+-ATPase activity (μg Pi mg $protein^{-1}$ $hour^{-1}$) for each family. Different letters indicate significant differences through time for a specific family. Families with similar response patterns are grouped.

Response patterns	Fam	Na^+-K^+-ATPase activity		
		Day 0	Day 3	Day 7
No response	S4	0.474 ± 0.119	0.623 ± 0.115	0.581 ± 0.237
	S5	0.703 ± 0.173	0.579 ± 0.089	0.510 ± 0.200
	S7	0.578 ± 0.156	0.552 ± 0.162	0.668 ± 0.172
	C5	0.732 ± 0.101	0.591 ± 0.106	0.589 ± 0.274
	C6	0.475 ± 0.077	0.613 ± 0.299	0.607 ± 0.152
Transient response on day 3	S1	0.140 ± 0.072 [a]	0.567 ± 0.149 [b]	0.276 ± 0.127 [a]
	S3	0.128 ± 0.080 [a]	0.518 ± 0.092 [b]	0.176 ± 0.102 [a]
	S8	0.062 ± 0.063 [a]	0.619 ± 0.141 [b]	0.128 ± 0.094 [a]
	S9	0.118 ± 0.070 [a]	0.549 ± 0.057 [b]	0.266 ± 0.220 [a]
	C1	0.144 ± 0.087 [a]	0.494 ± 0.256 [b]	0.117 ± 0.146 [a]
	C2	0.444 ± 0.098 [a]	0.714 ± 0.110 [b]	0.504 ± 0.221 [a]
	C7	0.101 ± 0.049 [a]	0.620 ± 0.159 [b]	0.194 ± 0.113 [a]
	C8	0.203 ± 0.077 [a]	0.518 ± 0.183 [b]	0.142 ± 0.134 [a]
	C10	0.083 ± 0.068 [a]	0.576 ± 0.111 [b]	0.132 ± 0.076 [a]
Variable patterns	S2	0.541 ± 0.150 [ab]	0.419 ± 0.300 [a]	0.830 ± 0.256 [b]
	S6	0.882 ± 0.367 [b]	0.475 ± 0.052 [a]	0.393 ± 0.225 [a]
	S10	n/a	0.324 ± 0.290 [a]	0.724 ± 0.187 [b]
	C3	0.628 ± 0.140 [b]	0.386 ± 0.245 [a]	0.578 ± 0.091 [ab]
	C4	0.546 ± 0.130 [a]	0.660 ± 0.081 [a]	0.821 ± 0.121 [b]
	C9	0.444 ± 0.107 [b]	0.873 ± 0.802 [ab]	0.171 ± 0.171 [a]

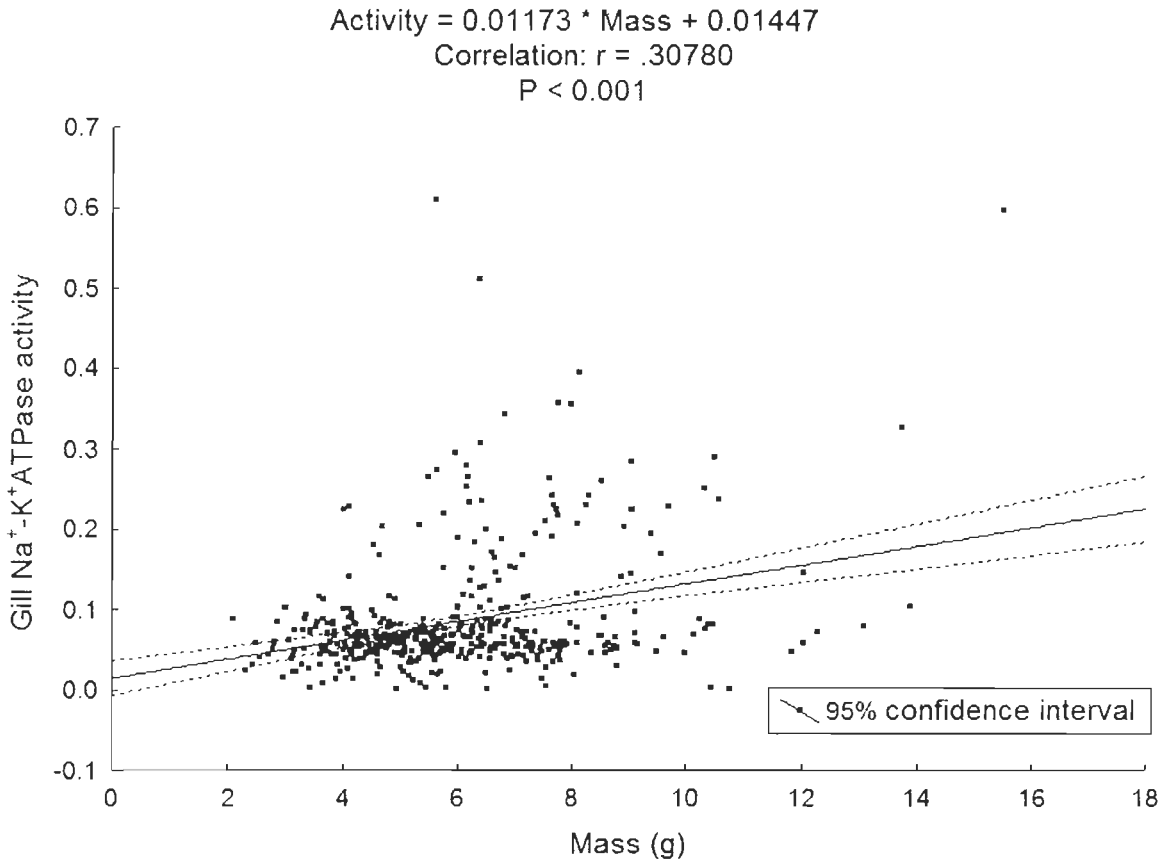

Figure II.1. Correlation of gill Na^+-K^+-ATPase activity (µg Pi mg protein^{-1} hour^{-1}) and mass for sampled fish (7-month-old brook charr) used for the early saltwater experiment.

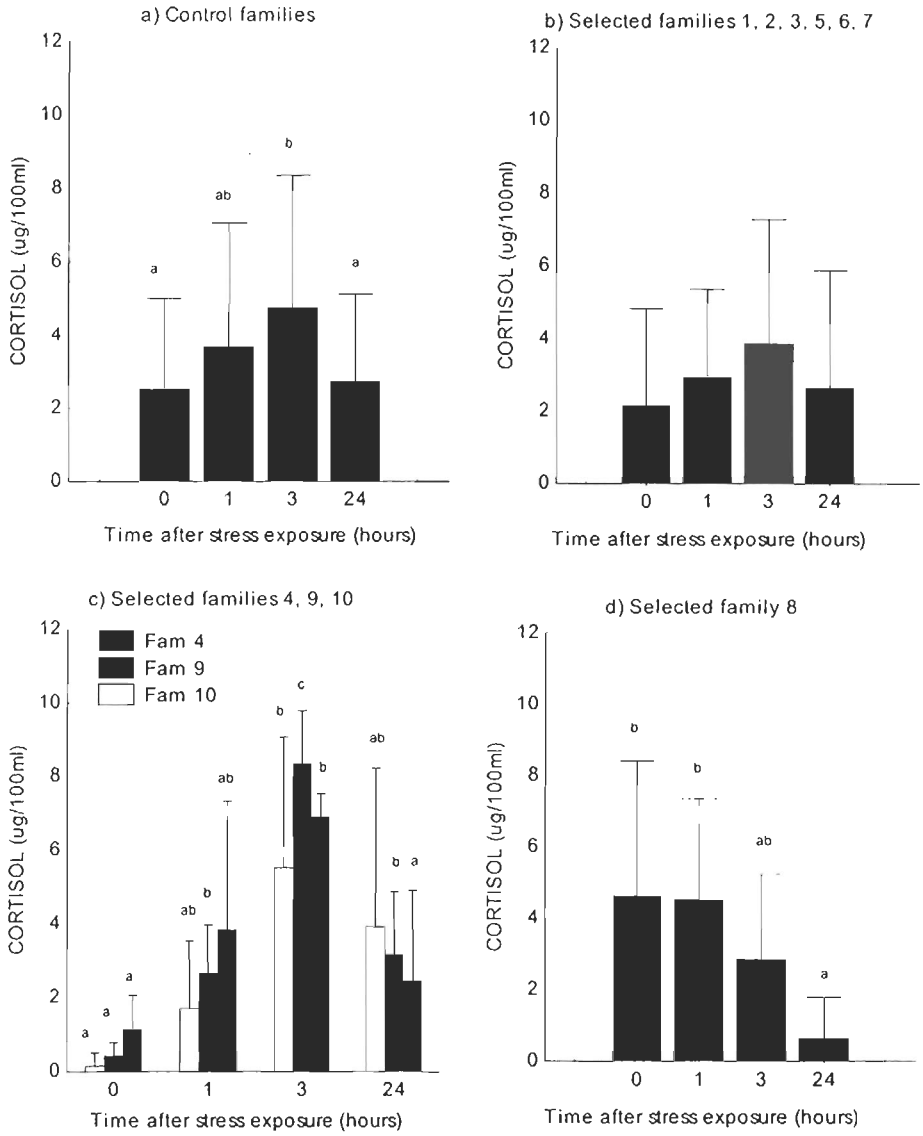

Figure II.2. Plasma cortisol concentration in stress-handled 1^{+} fish in a) control lines, b) selected families showing no response, c) selected families with a transient increase and d) selected family 8. Statistical analysis was performed on transformed data. Different letters indicate significant differences among sampling times.

stress (1, 2, 3, 5, 6, and 7; Fig. II.2b), but the trend was similar to controls. In families 9 and 10, a significant transient cortisol increase was observed after 1 or 3 hours, but cortisol was back to the control level after 24 h for family 9 and was at an intermediate level in family 10 (Fig. II.2c). Fish from family 4 exhibited the same pattern, but cortisol was still significantly elevated after 24 hours. Family 8 displayed a decrease in cortisol concentration over time (Fig. II.2d), but plasma cortisol was particularly elevated prior to handling stress, suggesting the presence of another source of stress in this case. Mass had no significant effect on the cortisol response (d.f. = 1, $P = 0.414$).

Susceptibility to Flexibacter maritimus *and growth in salt water*

Lesions attributable to *Flexibacter maritimus* were present in both the selected and control lines. Sensitivity was evaluated based on mortality since no cure is available except for a return to freshwater conditions. Large family differences were present, and there was a greater number of resistant families in the selected line. Families with very low sensitivity (no mortality or cumulative mortalities less than 10%) were present both in the selected (S1, S2, S3, S5, and S10) and the control (C3 and C6) lines. Among the families exhibiting cumulative mortalities ≥10% (Fig. II.3), two selected (S4 and S9) and two control (C2 and C4) families were returned to fresh water in mid-July because of high mortalities and animal care considerations. During saltwater rearing, the daily growth coefficient (specific growth rate) did not differ between selected and control lines (ANOVA, d.f. = 18; $P < 0.05$); we found an average gain of 195.9% (Table II.4).

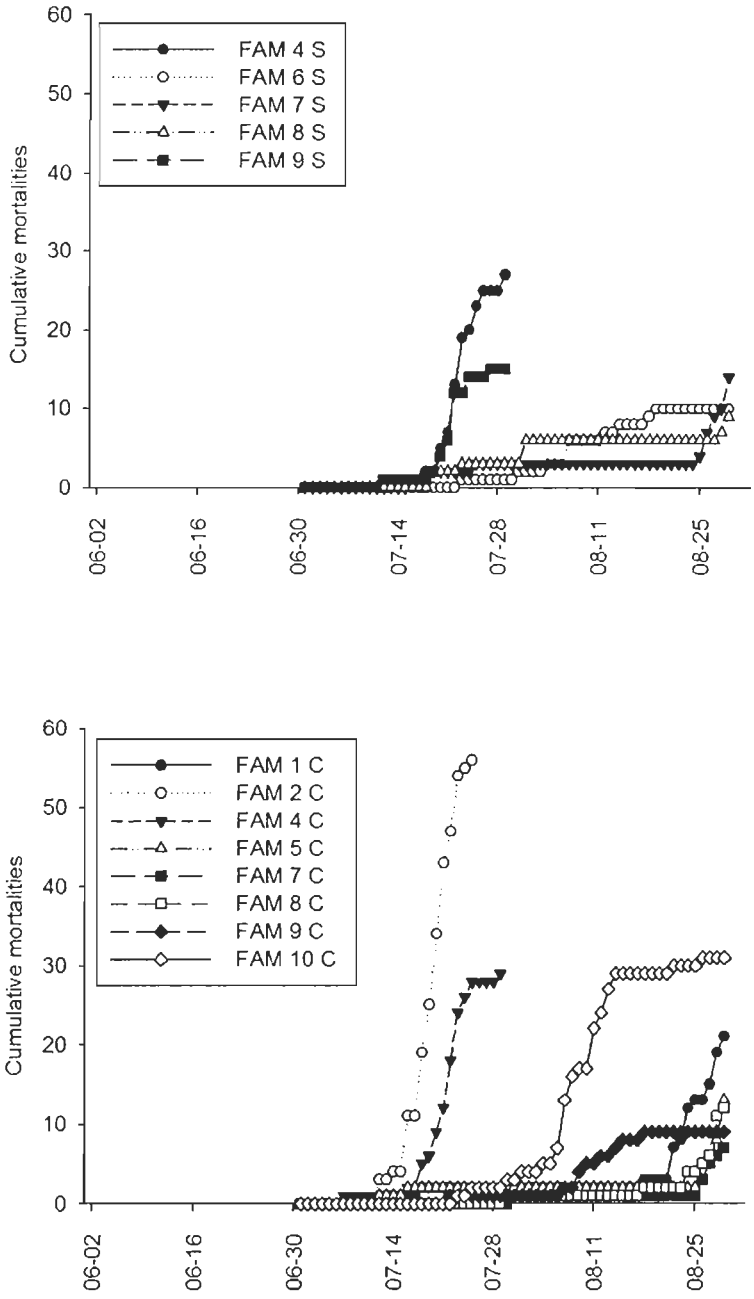

Figure II.3. Cumulative mortality by *Flexibacter maritimus* infection during saltwater rearing (from 9 June to 29 August). Only families with mortalities ≥10% are presented.

Table II.4. Mean mass per family during salt water rearing and daily growth coefficient reached at the end of the summer. Specific growth rate (SGR) was calculated by family since families, not individuals, were identified.

Family	April	July	August	Daily growth coefficient (SGR)
S1	41.7 ± 16.6	78.8 ± 25.2	124.5 ± 36.8	0.776
S2	49.2 ± 16.2	83.9 ± 31.0	120.7 ± 29.1	0.680
S3	29.3 ± 10.7	53.8 ± 23.6	85.2 ± 26.6	0.731
S4	34.9 ± 12.1	67.1 ± 15.1	102.1 ± 25.4	0.761
S5	31.7 ± 12.7	58.9 ± 22.1	95.5 ± 29.5	0.897
S6	31.8 ± 11.9	52.4 ± 11.8	94.3 ± 25.8	0.877
S7	25.9 ± 11.5	50.7 ± 21.7	75.6 ± 41.2	0.760
S8	29.5 ± 11.6	49.5 ± 15.3	88.2 ± 24.2	0.777
S9	39.5 ± 13.5	66.4 ± 23.7	113.8 ± 26.9	0.750
S10	23.2 ± 12.4	38.3 ± 16.9	67.2 ± 30.9	0.812
C1	47.8 ± 14.7	86.9 ± 29.6	118.9 ± 35.1	0.642
C2	26.1 ± 10.1	49.2 ± 17.6	86.4 ± 35.5	0.849
C3	15.8 ± 9.1	29.6 ± 15.1	51.7 ± 21.9	0.898
C4	28.2 ± 11.5	55.6 ± 19.9	99.5 ± 41.7	0.894
C5	29.2 ± 11.2	51.9 ± 15.6	93.2 ± 31.5	0.823
C6	28.5 ± 14.7	57.6 ± 23.4	72.1 ± 40.3	0.703
C7	26.9 ± 13.8	55.0 ± 24.8	96.4 ± 40.0	0.905
C8	39.1 ± 12.6	68.2 ± 19.5	110.4 ± 37.1	0.711
C9	27.8 ± 12.1	44.8 ± 16.0	80.0 ± 29.9	0.852
C10	52.4 ± 16.5	90.4 ± 25.4	139.4 ± 28.9	0.795

II.5 DISCUSSION

The main objective of the present study was to verify for the first time if selection for growth and late sexual maturity had indirect effects on different performance traits in brook charr. The second objective was to verify the feasibility of selecting for resistant fish early in life to decrease production costs. The most interesting results were those obtained in 1^+ animals. Control families all presented a typical stress response with a transient increase in cortisol while diverse response patterns, with either attenuation or an increase in the cortisol response to handling stress, were observed among the selected families. In addition, more selected families appeared resistant to opportunistic infections compared to the control ones. The most sensitive 1^+ families (S4, S9, C2, and C4) were among those showing the highest cortisol peak at the 3 h sampling. However, the most resistant ones in the selected line (S1, S2, S3, S5, and S10) had very low cortisol responses.

The identification of stress-resistant fish needs to be based on stress indicators that are reliable and reproducible. In fish, the elevation of plasma cortisol levels in response to stressors is very well demonstrated (Barton & Iwama, 1991; Ackerman *et al.*, 2000; Liebert & Schreck, 2006; Fernandes-de-Castilho *et al.*, 2008), and both the duration and intensity of the plasma cortisol response provides a good approximation of stress intensity (Barton & Iwama, 1991; Fevolden *et al.*, 2002). However, Weil *et al.* (2001) suggested that the speed at which the cortisol concentration decreases after an acute stress exposure may be a better trait for selecting fish than the 1-h peak concentration. Indeed, earlier studies had shown

that a high response 1 h after stress is not correlated to growth performance (Fevolden *et al.*, 1991; Pottinger *et al.*, 1994). Moreover, it is not clear whether fish that display a high or low corticosteroid stress response are more or less stressed relative to one another or if they simply exhibit different capacities to respond to stressors (see review by Barton, 2002). Because stress may affect the whole animal's performance (Adams, 1990; Barton, 2002), stress resistance may represent an important trait of interest because stressful manipulations during production may result in unwanted traits such as immunosuppression, reduced growth, reduced reproductive capacity, or death (Barton & Iwama, 1991; Barton, 2002). Results from the present study clearly indicate that a selection process could be applied based on this type of trait performance measurement. Correlations between acute stress response and susceptibility of 1^+ fish to *Flexibacter maritimus* should also be investigated in the future both in terms of correlated trait selection and in terms of stress-resistant fish lines.

In contrast to the handling experiment, no selection effect was observed for the response to early saltwater exposure; we had predicted differences in survival of lines and/or families because this experiment occurred early in development. Indeed, Höglund *et al.* (2008) suggested that the behavioural response to environmental stress may be inherited, and they observed that early stages of rainbow trout originating from strains selected for high stress response were more sensitive. Unexpectedly, the twenty families showed very good adaptation to saltwater and no mortality occurred. It was generally observed either an absence of change or a transient increase in gill Na^+/K^+ ATPase activity.

Nevertheless, families with different sentivities to *Flexibacter* or selected families with different acute stress response exhibited very different gill Na^+/K^+ ATPase activity patterns when brook charr were transferred to salt water at age 0^+.

The gill Na^+/K^+ ATPase activity in the "no response" families was similar to the activity measured on day 3 in families that showed a transient increase. Day 0 fish were still in fresh water. Thus, high activities cannot be related solely to a salinity response but could be also be function of stress levels (e.g. Nolan *et al.* 1999). On the other hand, even yearling anadromous salmonids may experience a spring seasonal increase in gill Na^+/K^+ ATPase activity while still in fresh water (Madsen & Naamansen 1989). Boula *et al.* (2002), showed that seasonal changes in gill Na^+/K^+ ATPase activity were similar between anadromous wild and laboratory-reared animals (the same control line than the one used in the present study) and suggested that the propensity of fish to acclimate to seawater should be heritable. Even though the mechanisms underlying the occurrence of different gill Na^+/K^+ ATPase activity patterns cannot be identified, the variable response among families (e.g., absence or presence of response) suggests that an additive genetic component is present in the expression of gill Na^+/K^+ ATPase activity.

Lankford & Weber (2006) observed significant genetic variation among broodstock rainbow trout families in the cortisol response to a 3-h confinement stressor and a positive correlation between body mass and the cortisol responsive. In the present study, there was no correlation between fish mass and cortisol level. However, selected fish were heavier

than controls, an expected outcome of the selective breeding program. This difference was maintained following the summer saltwater rearing in 1^{+} fish. It should be noted that the association between the stress response and other production traits such as growth have been observed in many studies, but results differed. Pottinger & Carrick (1999) found a positive correlation between cortisol response and body size in rainbow trout. They suggested that differences in size may indicate that fish showing strong responses adapted more rapidly to changes in environmental and social factors. Fevolden *et al.* (2002) observed better growth performance in fish with low responses while Hemre & Krogdahl (1996) did not find any significant relationship between the cortisol response resulting from mild handling and size in Atlantic salmon.

To conclude, combined selection for late sexual maturity and high growth rate resulted in a general decrease in cortisol responsiveness and lower sensitivity to opportunistic infection. Yet, family effects (independent of the selected vs control groups) were more pronounced than correlated selection effects for all three challenges. This suggests the existence of an additive genetic basis underlying the expression of the three performance traits examined here, which could allow their incorporation in the ongoing selective breeding program on brook charr.

CHAPITRE III. QUANTITATIVE GENETIC BASIS FOR PHYSIOLOGICAL DIVERGENCE BETWEEN SYMPATRIC ANADROMOUS AND RESIDENT BROOK CHARR (*SALVELINUS FONTINALIS*)

III.1 ABSTRACT

In our study, anadromous and resident brook charr originating from the same river system were mated and four cross-types produced (♀A♂A, ♀A♂R, ♀R♂A, and ♀R♂R) that were raised in identical environmental conditions. At 17 months of age, they were gradually transferred to estuarine salt water for the summer (salinity increased by 2 per day: final salinity 20). Fish were sampled for physiological traits associated with saltwater migration including weight, condition factor (K_f), plasma osmolality, percent muscle water content, hepatosomatic index (HSI), Na^+/K^+ATPase activity, insulin-like growth factor-1 (IGF-1) and the thyroid hormones T_4 and T_3, just prior to saltwater transfer, at 14 days following transfer, and at the end of the summer when they were returned to fresh water. Resident fish clearly required a higher physiological effort for saltwater adaptation. Physiological and morphological differences were observed between anadromous and resident fish. Plasma osmolality was higher in resident fish following salt water transfer; this coupled with lower Na^+/K^+ATPase activity and higher IGF-1 expression indicated that resident fish were less adapted to saltwater conditions. The condition factor was higher in resident than in anadromous fish with hybrids being intermediate, suggesting an additive component for that trait. These traits were heritable, with $h^2>0.2$ in at least one of the forms, indicating that the physiological states of anadromous and resident fish are transmitted to succeeding generations.

III.2 INTRODUCTION

Phenotypic plasticity is defined as the change in the average phenotype expressed by a genotype in different environments. Individuals that are above the threshold develop into one form whereas those below the threshold are recognized as the alternate form. The resulting differentiation is thus cued by the environment and this plasticity can be observed at the organism level using various methods: biochemistry, morphology, behaviour, and physiology (see Schlichting and Smith, 2002; Roff, 1997; Falconer and Mackay, 1996). It is generally thought that plasticity will respond to directional selection. The adaptive differentiation may be more obvious in the evolutionary divergence of populations into different niches (Bernatchez, 2004). To understand the evolution and maintenance of two alternative strategies, a comparison of their performance under a controlled environment may help to identify factors that influenced the differentiation.

Anadromous and nonanadromous (resident) brook charr live in sympatry in numerous rivers throughout eastern North America (Boula *et al.*, 2002; Morinville and Rasmussen, 2003; Thériault and Dodson, 2003). Anadromous brook charr undergo seasonal migrations to the estuarine environment from May to September to take advantage of the increased feeding opportunities and then return to fresh water for reproduction while residents remain in fresh water. Different migration patterns are observed in brook charr and recently Curry *et al.* (2006) characterized the habitat of anadromous brook charr in the Laval River. They observed that fish entered the marine environment (Laval Bay) in June

and that they were found in salinities up to 34 with a preference for 26-30, which is higher than previously believed.

Physiological differences related to these life history strategies have been reported in salmonids, but most of these studies were performed on fish captured from the wild and thus could not exclude the effects of the natural environment on life-history (Jonsson and Jonsson, 1993; Boula *et al.*, 2002; Morinville and Rasmussen, 2003; Thériault and Dodson, 2003; Amstutz *et al.*, 2006; Thériault *et al.*, 2007b; Morinville and Rasmussen, 2008).

Variations in life history strategies in brook charr may reveal extremes of phenotypic plasticity. For instance, in the Ste. Marguerite River (a tributary of the Saguenay River), resident and anadromous fish are part of the same breeding population, with reproduction and viable offspring between the two forms having been observed (Thériault *et al.*, 2007a). In this population, Morinville and Rasmussen (2008) observed differences in morphology between the two forms: fish expressing resident behaviour had a morphology more appropriate for a slow-moving riverine systems and anadromous fish a morphology suited to limiting drag, suggesting the importance of energetic demands on life history strategy. Thériault *et al.* (2007b) established a relationship between body size and life history strategy, with anadromous brook charr being larger at age 1. Migrants might simply adopt anadromy as a consequence of energetic limitations in the resident environment: migrating fish consume more food but still have lower growth efficiencies compared to resident fish before seawater migration (Morinville and Rasmussen 2003;

2006). However, post-migration, anadromous fish grow faster than residents of the same age (Lenormand *et al.*, 2004).

In contrast, resident and anadromous fish from the Laval River appear to represent genetically distinct populations based on microsatellite polymorphisms and physiological differences associated with their hypo-osmoregulatory ability, rather than a stable polymorphism within a single gene pool (Boula *et al.*, 2002; Perry *et al.*, 2005a). Phenotypic divergence between resident and anadromous Laval River brook charr in embryonic/alevin morphology has also been described and may be driven by adaptive directional selection (Perry *et al.*, 2005a). This study by Perry *et al.* provided some of the earliest evidence that the relative importance of selective differentiation may be context-specific; it also found that differences in phenotype vary at specific periods during ontogeny according to the sire's or dam's genetic contributions to the phenotype. Perry and collaborators (2005a) suggested that there are intrinsic mechanisms operating to maintain reproductive isolation and restrict gene flow between the anadromous and resident populations following selection pressure. Field studies done in the Laval River (north shore of the St. Lawrence estuary, Québec) showed higher gill Na^+-K^+ATPase activity in anadromous brook charr compared with resident fish in spring (Boula *et al.*, 2002). A higher level of T_4 (thyroxine) was observed in anadromous brook charr (Boula *et al.*, 2002) and, using the same wild populations, Lavallée (2004) observed an elevation of T_4 when wild resident brook charr were maintained in the main section of the river where anadromous fish are captured in the spring (salinity from 1 to 16). Evidence from other

salmonids also suggests ontogenetic changes in genetic variation for morphological traits that stabilize only later on (Fishback *et al.*, 2002; Su *et al.*, 2002).

In salmonids, including brook charr, somatic growth is controlled mainly by growth hormone (GH) (Sakamoto and Hirano, 1993; Kopchick and Andry, 2000; Calduch-Giner *et al.*, 2001; Fukada *et al.*, 2004; Larsen *et al.*, 2004; Picha *et al.*, 2006) and insulin-like growth factors I and II (IGF-1 and IGF-2) (Palamarchuk *et al.*, 1997; Shimizu *et al.*, 1999; Beckman *et al.*, 2004; Volkoff *et al.*, 2005). IGF-1, along with GH and cortisol, plays a pivotal role in the regulation of smoltification by stimulating the proliferation of seawater-type chloride cells in the gills and increasing gill Na^+-K^+-ATPase activity (McCormick, 2001; Sakamoto *et al.*, 2001; Jørgensen *et al.*, 2007; Nilsen *et al.*, 2008). In brook charr, Côté *et al.* (2007) showed that environment and sex had high impacts on the expression of mRNA for the GHR and IGF genes. The GHR mRNA transcription level was higher in saltwater than in fresh water. One of the most intriguing questions about the expression of the IGF-1 gene is whether it varies in relation to life history strategy (anadromy vs. freshwater residency), and if so, to what extent.

In this study, we were interested in the potential adaptive physiological divergence between freshwater-resident and seawater-migrant brook charr populations. As in other salmonids, physiological differences between anadromous and resident brook charr are difficult to assess in the field because of the potential for wide ranges in relatedness among individuals and the complex spectrum of unknown environmental history and individual

condition. Our experiment allowed us to eliminate confounding environmental factors and investigate genetic variation for the physiological response to summer saltwater transfer and growth in older fish from parapatric (approx. 10km distant) populations of anadromous and resident brook charr. We hypothesized that if divergence in saltwater adaptation is present, resident brook charr should have a less pronounced physiological response in common characteristics of seawater acclimation than anadromous fish during saltwater exposure.

III.3 MATERIALS AND METHODS

Fish collection, experimental design, and rearing conditions

The Laval River system runs approximately 20 km from the Laval Bay inlet to Lake Jacques (roughly 3 km in length), then continues for an additional 10 km to the Adams Brook tributary branch. Adams Brook brook charr are predominantly resident types while fish in the main Laval River system are mostly anadromous (Boula *et al.*, 2002). Adams Brook individuals were line-fished directly from the brook at a single sampling point (48° 54' 44" N / 69° 12' 55" W) in the summers of 2001 and 2002. Laval River brook charr were line-fished from several points in the Laval River within approximately 10 km of the Adams Brook spawning site between 1991 and 1993. These two populations are thus technically parapatric but have a very limited geographic separation given the absence of physical barriers and the migratory capabilities of this species compared to the average distances between different allopatric forms (Castric and Bernatchez, 2003; Castric and Bernatchez, 2004). Captured breeders were brought to our experimental facility of the Station aquicole de Pointe-au-Père, Université du Québec à Rimouski.

Six males and 12 females from the second generation of a fish stock descended from anadromous Laval River fish were maintained at the Station aquicole. Six males and 12 females were also collected from the resident Adams Brook population in 2001 and 2002 and maintained at the same facility. From mid November until early January, each sire was bred with one female from each population (anadromous and resident) to create 12

full-sib families, for a total of six pure anadromous families, six pure resident families and 12 hybrid full-sib families. Only one full-sib family was available for one sire since eggs from one of the females did not develop properly, thus resulting in a total of 23 full-sib families. The progeny of each dam were reared in separate units, but the environment was rigorously controlled within the overall experimental system (12:12 L:D photoperiod at 4°C during until after hatching, then ambient temperatures [7–8°C] starting at the first external feeding onward).

Once fish reached the exogenous feeding stage (in May), the number of fish was standardized among families (700 individuals). Each family was isolated from the others using separators in juvenile tanks until fish reached approximately 4.0 g. Families were then randomly pooled in 500 L circular tanks, after having been identifed by different combinations of pelvic and/or adipose fin clippings. All families were regularly culled to maintain good stocking conditions in the rearing tanks (< 30 kg fish per m^3; Pennell and Barton 1996). Fin markings were checked every 3–4 months and unidentifiable fish were removed.

Fish were reared in fresh water under natural photoperiod and temperature conditions (minimal temperature [2.0°C] was reached in February; maximal temperature [15°C] in September). Length and weight were measured periodically to monitor growth. Fish were fed on commercial pellets and the daily ration was adjusted according to fish size and water temperature. When fish reached 17 months of age, they were gradually (2‰ day^{-1}

$^{-1}$) transferred to estuarine salt water (final salinity 20) for the summer (June to August). Ten fish per family were sampled on the last day spent in fresh water (Day 0). Then, three days after exposure to 20 salt water (that is, 14 days after the initial introduction of saltwater to the freshwater tanks), 30 fish per family were sampled. After two months of growth in saltwater conditions, fish were returned to fresh water and 10 additional fish per family were sampled.

Measurements of physiological traits

Fish were randomly captured and anaesthetized in 3-aminobenzoic acid ethyl ester (0.12 g L^{-1}). All samplings were done between 10:00 and 16:00 to limit the effect of diurnal variations in plasma hormone concentrations. Weight was measured to the nearest 0.1 g and fork length to the nearest 0.1 cm. Blood was sampled by caudal puncture using ammonium-heparinized syringes and then centrifuged at 7200 g for 3 minutes. Plasma was collected and immediately frozen at -80°C. All filaments from the left gill arch were removed, transferred to 100 µL of SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole), and stored at -80°C until Na^+-K^+-ATPase activity measurements. Fish liver and gonads were rapidly weighed (to the nearest 0.0001 g) and stored at -80°C until further analysis. A piece of epaxial muscle was excised for measurement of percent tissue water content.

Plasma osmolality was measured using a 3MO micro-osmometer (Advanced Instruments Inc., Norwood, MA, USA.). Muscle samples were weighed before and after dessication in a drying oven (70°C for 72 h). Plasma concentrations of thyroid hormones

(T_3 and T_4) were measured using an enzymatic immunoassay method (T_3-EIA and T_4-EIA kits, Immunocorp Inc., Montréal QC). Determination of gill Na^+-K^+-ATPase activity was done according to Siegler *et al.* (1996). All fish used in this study were handled in accordance with the principles and guidelines of the Canadian Council on Animal Care.

RNA extraction

Liver samples (10 fish per family, 19 families) weighing 0.025 g were ground and mixed with 1.0 ml of trizol reagent (Invitrogen) using a tissuelyser. Chloroform (200 µl) (Invitrogen) was added to each sample prior to centrifugation at 4°C and 12,000 rpm for 15 minutes. A 1.0 ml volume of isopropanol (Sigma-Aldrich) was added to the aqueous phase. Samples were stored overnight at -80°C. Samples were centrifuged at 4°C and 13,000 rpm for 60 minutes. The supernatant was removed with a vacuum pump and the pellet washed with 1.0 ml of 70% ethyl alcohol (Sigma-Aldrich). The ethyl alcohol was removed and the pellet dried for 15 minutes at room temperature. The dried pellet was resuspended in 40.0 µl of RNase-free water (Ambion) and then spiked with 1.0 µl of superase in Ambion.

RNA purification and DNase I treatment

RNA samples were purified using micro-columns (microcons) (Fisher Scientific). The microcons were centrifuged with 100 µl of RNase-free water at 14,000 g for 15 minutes. Then, 35.0 µl of each RNA sample was transferred to the microcon, eluted with 170 µl of RNase-free water, and centrifuged at 14,000 g for 20 minutes; the elution step was repeated. The filter within each microcon was subsequently transferred to another

microcon along with 30 µl of RNase-free water and centrifuged at 4,000 g for four minutes. All RNA samples were incubated for 35 minutes at room temperature with 2.0 µl of deoxyribonuclease I (Invitrogen) and 3.0 µl of 10X deoxyribonuclease I buffer (200 mM Tris-HCl pH 8.4, 20 mM $MgCl_2$, and 500 mM KCl) to remove any contamination by genomic DNA. Samples were incubated with 3.0 µl of 25 mM EDTA for 10 minutes at 65°C to stop the deoxyribonuclease reaction.

Reverse transcription and real-time PCR

The RNA samples were diluted to a concentration of 0.1 µg RNA/µl with nuclease-free water in a volume of 20 µl. A volume of 20 µl of master mix (4 µl of 10X reverse transcription buffer, 1.6 µl of 25X dNTPs, 4 µl of 10X random primers, 2 µl of MultiScribe™ reverse transcriptase 50 U/µl, and 8.4 µl of nuclease free H_20) from a cDNA reverse transcription kit (Applied Biosystems) was added to each sample, giving a final reaction volume of 40 µl. All reverse transcription reactions were carried out on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) with the following thermal profiles: 10 min at 25°C, 120 min at 37°C, and 5 sec at 85°C. All samples were kept at -20°C until the real-time PCR experiments. Eukaryotic 18S rRNA (Applied Biosystems) was used as the endogenous control (reference gene) for relative quantification by real-time PCR. TaqMan™ MGB probes and primers for the IGF-1 gene were designed and manufactured by Applied Biosystems. All reactions were carried out on an ABI Prism 7500 Sequence Detection System (Applied Biosystems). Primers of brook charr IGF-1 mRNA were designed by Côté *et al.* (2007). Gene expression profiles were measured with the

relative quantification method of the $2^{-\Delta\Delta C}{}_T$, as described in Côté *et al.* (2007) with minor modifications. Each reaction (25 µl) was run in triplicate and contained 5 µl of cDNA (diluted 1:5 for the target gene and 1:5000 for the 18S rRNA gene), 12.5 µl of Taqman Universal PCR master mix (Applied Biosystems), and 0.9 µM F/R primers. The thermocycling profile used was the default from the SDS 3.1.3 software (50°C for 2 min, 95°C for 10 min, then 40 cycles at 95°C for 15 sec and at 60°C for 1 min). For all primer/probe sets, amplification efficiencies were calculated following the manufacturer's instructions, and all the values were sufficient to allow direct comparison of amplification plots (see Sequence Detection Systems Quantitative Assay Design and Optimization, Applied Biosystems).

Data analysis

Fulton's condition factor (K_f) was calculated as $K_f = (W \bullet L^{-3}) \times 100$, where W is fish weight in grams and L is the fork length in centimetres (Pennell and Barton, 1996). The specific growth rate (SGR) was calculated as $SGR = ((\ln W_f - \ln W_i) \bullet days^{-1}) \times 100$, with family being the statistical unit. Kolmogorov-Smirnov and Brown-Forsythe tests were applied to assess data normality (K-S) and homogeneity of variance (B-F). To obtain normality, fish weight and plasma thyroid hormone concentrations were log-transformed (log [x+1]). Physiological variables were analyzed with three-way nested ANOVAs (cross-type, sampling time (before saltwater transfer, 14 days after transfer, and after two months in salt water), and family, with the family factor being nested in cross-type; familial effects are not presented). Fish weight was added as a covariable in the model to test for possible

weight bias. Differences in IGF-1 expression among groups, families, sexes, and samplings were analyzed by multivariate nested analysis of variance (ANOVA) followed by an appropriate *a posteriori* analysis. When ANOVAs indicated a significant treatment effect, Tukey or Games-Howell tests were performed for post-hoc analysis ($\alpha = 0.05$). All statistical analyses were performed with Statistica version 6.0 for Windows (StatSoft, Tulsa, OK, USA). T-tests were used to compare the effect of parental line. Heritability estimates for all traits were obtained using the ASREML software 2.0 (VSN International Ltd., UK) with 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. ASREML was also used to determine estimated breeding values (EBV, additive genetic merit; Mrode 2005). These estimates were verified using the same model estimated using Parameter ESTimator (PEST) 3.0 (Groeneveld *et al.*, 1990) and Variance Component Estimator (VCE) 5.12 (Kovac *et al.*, 2002), a program set used for heritability analysis using animal, sire and dam models. Heritability estimates were analyzed separately on day 14 after saltwater transfer measurements in pure anadromous and pure resident fish.

III.4 RESULTS

Physiological variables related to osmoregulation

Prior to saltwater transfer, plasma osmolality was similar among all cross-types and increased with different intensities in all cross-types after saltwater transfer (Fig. III.1; Cross-type x Time: $F = 3.8$; $P = 0.01$). The intensity of the response was higher in RR (3.9% increase) than in AA (2.6%), with significantly higher osmolality after 14 days in salt water. The intensity of the response in both hybrid groups was the same as in AA, with the osmolality level being similar after transfer. Weight was weakly but significantly correlated with osmolality ($F = 3.9$; $P = 0.048$; osmolality = 0.08898*weight + 309.74; $r = 0.128$). Heritability estimates for osmolality were very different between resident and anadromous fish, approaching 1.0 in RR animals but effectively zero in AA animals (Table III.1). Variance of EBV was 0.0 in AA and 168.7 in the resident fish. Dam origin had a significant effect on osmolality, with the progeny of AA dams having significantly lower plasma osmolality than the progeny of RR dams (Table III.2).

The different increase in osmolality among cross-types was not accompanied by a differential decrease in percent muscle water content, although muscle water content was lower in all groups at the end of the summer (77.5 ± 0.7%) (Cross-type: $P > 0.05$; Cross-type x Time: $P > 0.05$; Time $F = 21$; $P < 0.001$). Percent muscle water content had high heritability in both lines (AA, $h^2 = 0.75 \pm 0.33$; RR, $h^2 = 0.97 \pm 0.30$) although the variance

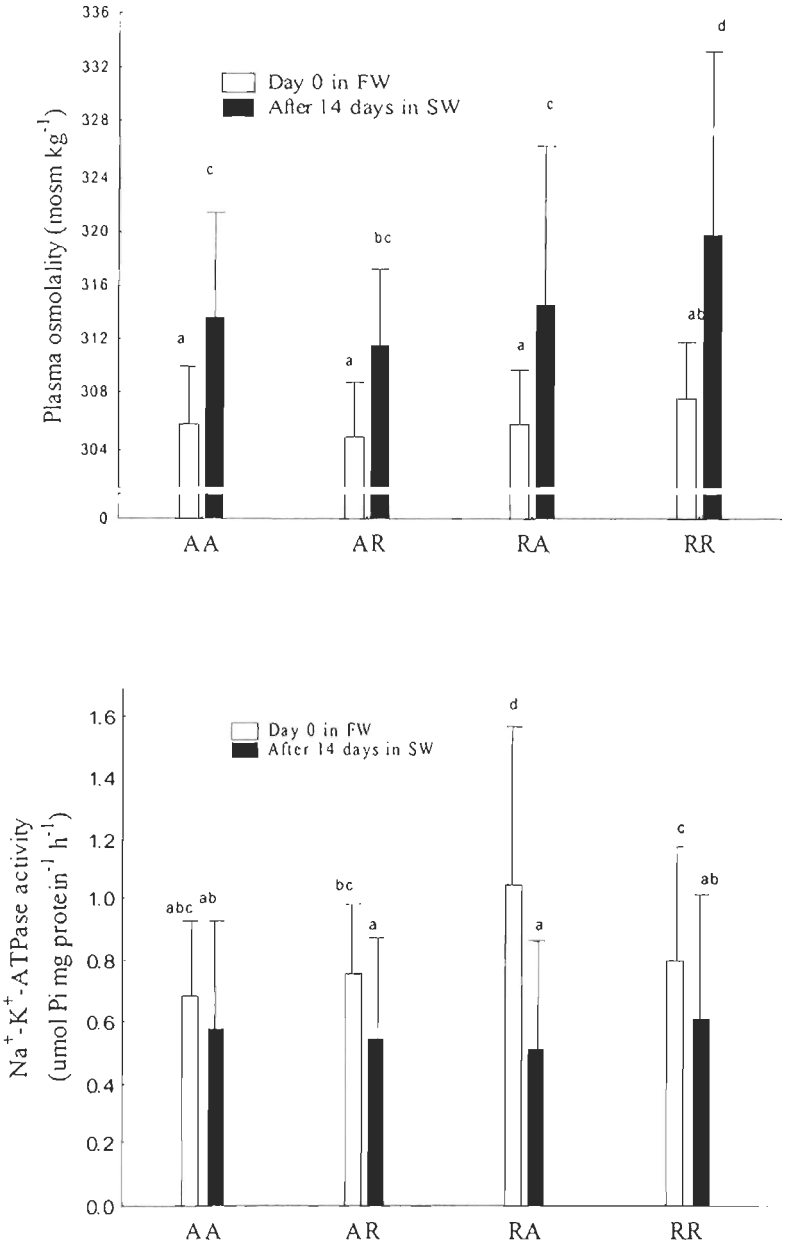

Figure III.1. Plasma osmolality and gill Na^+-K^+-ATPase activity in anadromous (AA), resident (RR), and hybrid (AR, RA) 1^+ brook charr prior to saltwater transfer and after 14 days in salt water. Values are means ± standard deviation (SD). Different letters indicate significant differences.

Table III.1. Heritability estimates (h^2 ± SE) and variance of estimated breeding values (σ^2 EBV) of physiological traits measured in anadromous (AA) and resident (RR) 1^+ fish after 14 days in salt water.

Trait	AA h^2	RR h^2	AA σ^2 EBV	RR σ^2 EBV
Weight (g)	0.910±0.338	0.780±0.321	137.911	159.721
K_f	0.098±0.100	0.401±0.233	0.001	0.003
Hepato-somatic index	0.359±0.222	0.124±0.113	0.031	0.004
Muscle water %	0.751±0.325	0.974±0.303	0.297	1.265
Osmolality (mosm kg^{-1})	0.000±0.000	0.970±0.100	0.000	168.722
Na+-K+-ATPase (μmol Pi mg $protein^{-1}$ h^{-1})	0.704±0.319	0.572±0.284	0.055	0.057
T_4 (ng ml^{-1})	0.572±0.294	0.325±0.206	0.043	0.026
T_3 (ng ml^{-1})	0.215±0.164	0.394±0.232	0.362	0.501

Table III.2. Means (± SD) of physiological traits measured after 14 days in salt water shown by dam and sire origin. Anadromous, resident, and hybrid fish were included in the analysis. The significance of parental origin comparisons are provided.

Trait	DAM			SIRE		
	AA	RR	*P*	AA	RR	*P*
Weight (g)	33.9±15.6	33.9±15.0	0.65	30.4±12.7	36.8±16.7	<0.001
K_f	1.02±0.12	1.07±0.11	<0.001	1.00±0.12	1.07±0.10	<0.001
Hepato-somatic index	1.48±0.42	1.42±0.31	0.047	1.40±0.39	1.50±0.35	<0.001
Muscle water %	78.4±0.9	78.4±1.1	0.57	78.5±0.8	78.3±1.1	0.001
Osmolality (mosm kg^{-1})	312.6±6.7	317.7±13.0	<0.001	314.1±9.4	315.7±11.2	0.06
Na^+/K^+ATPase (μmol Pi mg $protein^{-1}$ h^{-1})	0.562±0.342	0.576±0.383	0.62	0.555±0.351	0.578±0.368	0.43
T_4 (ng ml^{-1})	0.684±0.363	0.761±0.438	0.01	0.757±0.419	0.689±0.383	0.03
T_3 (ng ml^{-1})	2.287±2.022	1.783±1.593	0.001	2.264±1.975	1.886±1.736	0.02
IGF-1	1.254±0.822	1.445±0.810	0.888	1.597±1.034	1.123±0.467	<0.001

of EBV was higher in RR than AA (AA 0.3; RR 1.3). Muscle water percentage was significantly higher for progeny of RR males (Table III.1).

In AA, gill Na^+-K^+-ATPase activity was not modified following saltwater transfer (Fig. III.1), but a significant 23.7% decrease was observed in RR (Cross-type x Time: F = 10.2; $P < 0.001$). Na^+-K^+-ATPase activity also decreased in both hybrids, but the intensity of the response was higher in the RA cross-type because gill Na^+-K^+-ATPase activity in RA was higher than in the other cross-types prior to transfer (Fig. III.1). Weight appeared to be a significant co-factor explaining gill Na^+-K^+-ATPase activity (F = 13.6; $P < 0.001$), but the correlation was not significant (Na^+-K^+-ATPase activity = 0.002 × weight + 0.566; r = 0.05; $P = 0.142$). Heritability estimates were high in AA ($h^2 = 0.70$) and RR ($h^2 = 0.57$) for gill Na^+-K^+-ATPase activity, and both lines presented the same variance in EBV (0.06) (Table III.1). There was no effect of parental population (Table III.2).

IGF-1 expression

In June (day 0), the relative expression of IGF-1 was not significantly different between cross-types (Fig. III.2). After 14 days in salt water, IGF-1 expression was significantly higher in RR fish compared to freshwater levels, but not in AA fish (Cross-type x Time: F = 7.03; $P < 0.001$). The response of hybrids was similar to that of their maternal line (Fig. III.2). At the end of the summer, the relative expression of IGF-1 was

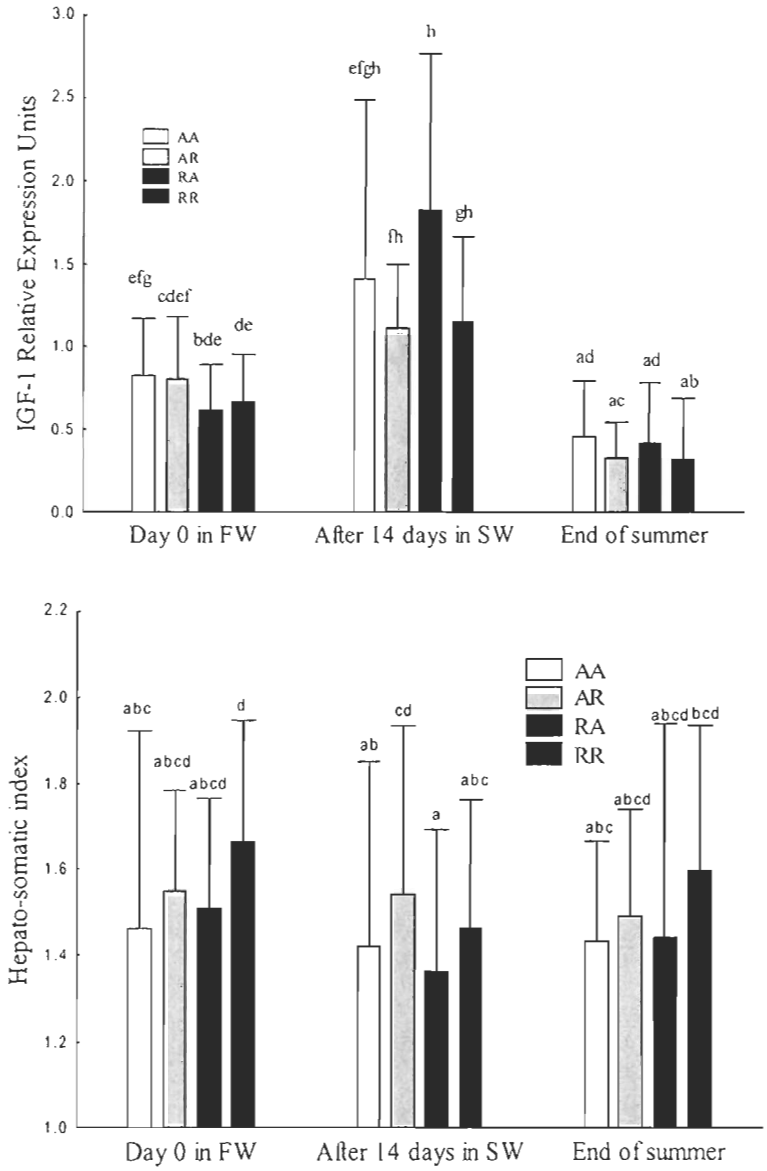

Figure III.2. IGF-1 expression and hepato-somatic index in anadromous (AA), resident (RR), and hybrid (AR, RA) 1^{+} brook charr prior to saltwater transfer, after 14 days in salt water, and at the end of summer. Values are means ± sd. Different letters indicate the presence of significant differences.

low in all cross-types and even lower than in early June in pure cross-types. Significant familial effects were observed ($F = 4.37$; $P < 0.001$). For each cross-type, the familial response pattern was similar to the cross-type pattern, but the intensity of the response differed among families. Expression was 1.3 fold higher in males than in females ($P < 0.01$). The dam origin had no effect on IGF-1 expression on day 14, but the progeny of anadromous sires had higher IGF-1 expression than those of resident sires (Fig. III.2; Table III.2).

Thyroid hormones

Prior to saltwater transfer, T_4 and T_3 concentrations were similar among cross-types and there was no change in the concentration of either thyroid hormone with saltwater exposure in fish of the anadromous population (Fig. III.3). Fourteen days later, plasma T_4 was similar among AA and RR fish, although RA hybrids had higher T_4 than AR fish. Once fish were returned to fresh water, AA fish had a higher T_4 level than RR or AR fish (Fig. III.3). Plasma T_4 concentrations were not influenced by fish weight ($P = 0.40$).

Plasma T_3 concentration was higher in AA than in RR 14 days after transfer (Cross-type x Time: $F = 4.14$; $P < 0.001$) (Fig III.3). A significant decrease in T_3 was observed in the two hybrids between day 0 and day 14. There was no apparent difference among the four cross-types for T_3 prior to saltwater transfer, but T_3 concentration in AA fish was higher after 14 days in salt water than in the other groups (Fig. III.3). At the end of

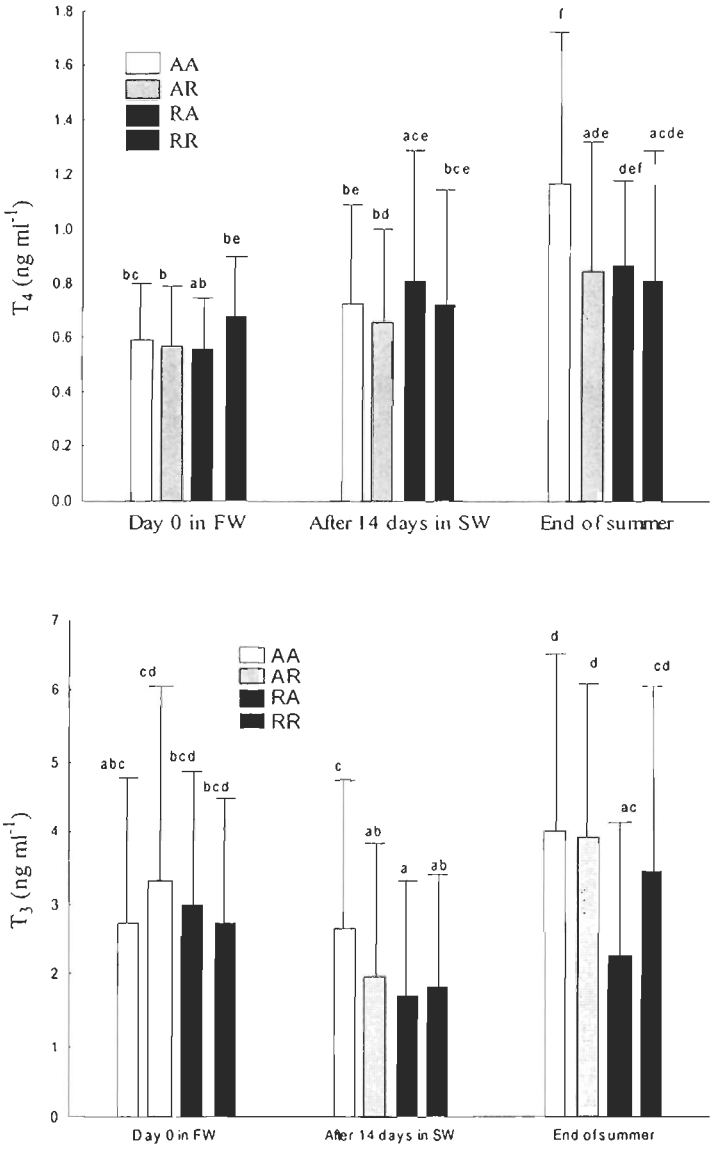

Figure III.3. Plasma concentrations of thyroid hormones (T_3, T_4) in anadromous (AA), resident (RR), and hybrid (AR, RA) 1^+ brook charr prior to saltwater transfer, after 14 days in salt water, and at the end of summer. Statistical analyses were performed on transformed data. Values are means ± sd. Different letters indicate the presence of significant differences.

summer, T_3 concentration was low in RA relative to the AA and AR groups, and the T_3 concentration was more elevated than in June for all groups except RA (day 14). Weight was significantly but weakly correlated with T_3 concentrations (T_3: $F = 23.4$; $P < 0.001$; $T_3 = 0.0094 \times \text{Weight} + 0.257$; $r = 0.157$). Both thyroid hormones had moderate heritability in AA and RR fish (T_4 $h^2 = 0.325$–0.572, T_3 $h^2 = 0.2$—0.4; Table III.1). Variance in EBV was < 0.05 for T_4 and 0.4—0.5 for T_3. The progeny of anadromous dams had lower T_4 but higher T_3 than those of resident dams ($P < 0.05$), but both T_4 and T_3 plasma concentrations were higher in fish bred from anadromous sires ($P < 0.05$) (Fig III.3; Table III.2).

Weight

Familial specific growth rates were similar among groups, with an average value of 0.89 ± 0.34 g day^{-1} (Cross-type: $F = 1.39$; $P = 0.279$). The summer weight pattern was not significantly different among cross-types (Fig. III.4; Time; $F = 139.6$; $P < 0.001$; Cross-type x Time: $F = 1.80$; $P = 0.10$). RR fish were heavier than AA fish, and continued to be so throughout the summer. The average weight of AR hybrids was similar to RR and RA hybrids were similar to AA (Cross-type: $F = 10.9$; $P < 0.001$). Heritability estimates for weight were high for AA (0.91) and for RR (0.78) (Table III.1). The variance of EBV was 137.9 and 159.7 for AA and RR, respectively. The progeny of resident sires were heavier than those bred from anadromous sires ($P < 0.001$; Table III.2).

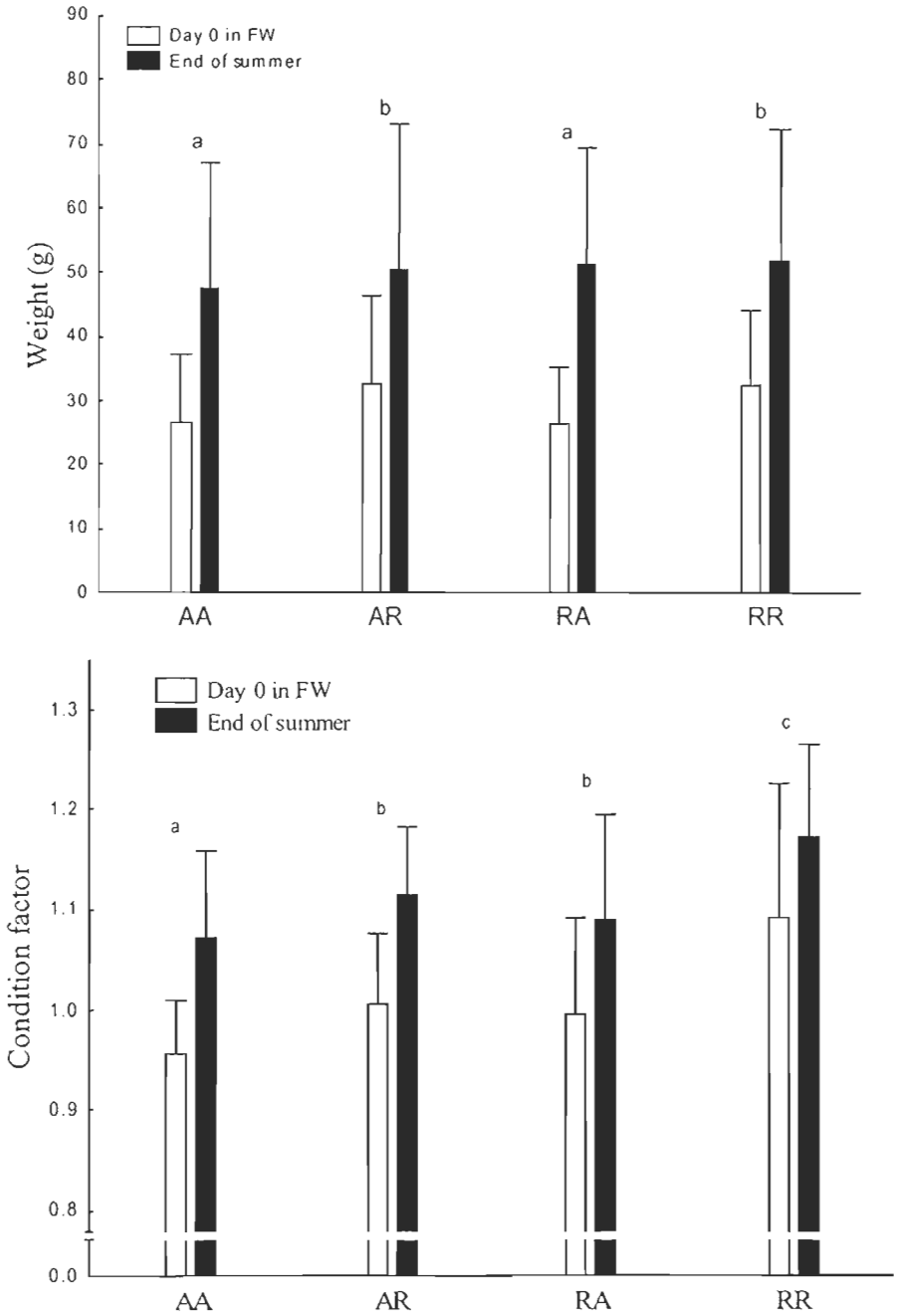

Figure III.4. Weight and condition factor presented for anadromous (AA), resident (RR), and hybrid (AR, RA) 1^{+} brook charr prior to saltwater transfer and at the end of summer. Statistical analyses were performed on transformed data for weight. Values are means ± sd. Different letters indicate the presence of significant differences among cross-types.

In June, the hepato-somatic index was higher in RR than in AA with intermediate values in both hybrids (Fig. III.2). The hepato-somatic index decreased in RR fish after 14 days in salt water (Fig. III.2; Cross-type x Time; F = 2.25; P = 0.04), but all groups were similar at the end of the summer. Heritability estimates were low in RR and moderate in AA (0.124 and 0.359 respectively). The variance of EBV was weak in both lines (0.03 and 0.004). The progeny of resident sires had higher hepato-somatic index than those bred from anadromous sires and the progeny of anadromous dams had slightly higher hepato-somatic index than those from resident ones (Table III. 2).

AA fish had a lower condition factor than RR throughout the summer (Fig. III.4; Cross-type: F = 56.4; $P < 0.001$) and the two hybrids were intermediate; this pattern remained until the end of saltwater rearing (Time: F = 61.4; $P < 0.001$; Cross-type x Time: F = 0.56; $P = 0.77$). Condition factor heritability was different between the two lines, with AA fish showing a lower heritability (0.098) than residents (0.401) for that trait. The variance around EBV was similar (0.001 and 0.003) in both lines. Sire and dam origin effects were significant (Table III.2).

III.5 DISCUSSION

To our knowledge, this study presents the first heritability estimates of physiological traits directly related to saltwater adaptation in brook charr. To date, other studies have estimated heritability for other traits linked to anadromy or residency in salmonids also under experimental conditions (*i.e.*, maturity, smolting) (Wild *et al.*, 1994; Thrower *et al.*, 2004). In addition, Carlson and Seamons (2008) reviewed the quantitative genetic components of salmonids and reported that parameter estimates for migratory behaviour in salmonids were nearly absent from the published data. We observed differences in physiological responses to saltwater transfer between anadromous and resident brook charr. Physiological differences in condition factor, plasma osmolality, HSI, gill Na^+/K^+ATPase activity, and IGF-1 expression occurred between anadromous and resident fish, confirming both that these two parapatric populations significantly differ for many physiological traits involved with anadromy and that there is a significant underlying genetic component to this divergence in life history strategy in the Laval River system. It should be noted that heritability was measured only 14 days after saltwater transfer (30 individuals per family) to highlight differences in physiology related to saltwater adaptation even when reared under identical conditions. This suggests that a divergent natural selection may be involved in differentiating between the two populations. In agreement with our hypothesis, resident fish appeared less adapted to saltwater, with higher plasma osmolality, lower gill Na^+-K^+ATPase activity, lower hepato-somatic index and elevated IGF-1 expression, suggesting physiological differences related to saltwater adaptation.

These traits had heritability greater than zero in at least one of the two forms, indicating that they could evolve under the effect of natural selection.

Phenotypic differences in saltwater adaptation

A higher plasma osmolality response was observed in resident fish compared to anadromous fish 14 days after saltwater transfer, even though the level reached did not indicate an inability for saltwater acclimation. In comparison, other studies have shown unsuccessful acclimation following saltwater with higher osmolality levels (sockeye salmon, Franklin *et al.*, 1992; brook charr, Claireaux and Audet, 2000). Along with higher plasma osmolality, HSI decreased in resident fish possibly indicating a higher energy demand during saltwater acclimation. The use of hepatic energy reserves may indicate that the slower saltwater acclimation could have initiated a stress response. Stress in known to increase glucose demand (Pickering, 1981; Barton, 2002), since glucose demand increases liver glycogenolysis, this could explain the decrease in HSI.

The response of gill Na^+/K^+ATPase activity also varied between cross-types, with anadromous fish showing no change in activity while residents and hybrids had lower activities 14 days after transfer. A recent study on arctic charr showed that the hypo-osmoregulatory ability (gill Na^+/K^+ATPase activity) of landlocked charr was generally weaker than that of anadromous charr (Ojima *et al.* 2009). Differences in seawater tolerance between anadromous and landlocked Atlantic salmon has also been reported (Nilsen *et al.* 2003) Because an increase is expected to be related either to the

osmoregulatory response (McCormick and Saunders, 1987; Mackie *et al.*, 2003; McCormick *et al.*, 2008) or to the stress response (Nolan *et al.*, 1999), this suggests that the Na^+/K^+ATPase activation may have occurred between the first and second samplings. Therefore, the values measured on day 14 suggest a homeostatic recovery. A similar return to control levels of gill Na^+/K^+ATPase activity post-stress was found to occur after seven days in a saltwater challenge test (Chapter 2) and after 10 days in stress conditions induced by infection (Nolan *et al.*, 1999).

In the literature, elevated IGF-1 expression is known to increase osmoregulatory capacity by increasing gill Na^+/K^+ATPase activity in salmonids (Madsen and Bern, 1993; McCormick, 2001). In the present study, anadromous fish were well acclimated to salt water after 14 days. Resident fish had a more elevated IGF-1 expression after saltwater transfer, indicating a greater hormonal response than anadromous fish for the change in salinity, this may indicate that the physiological response was not completed. However, this could be related to other physiological adjustments in the osmoregulatory response, such as gill Na^+/K^+ATPase activity. Our results also show that gill Na^+/K^+ATPase activity decreased in resident fish. This supports the hypothesis that resident fish require a greater physiological effort for osmoregulation than anadromous fish. Moreover, in a study by Côté *et al.*, (2007), brook charr from the Rupert River (which drains into the James-Hudson Bay, Québec) did not show any change in hepatic IGF-I expression between the freshwater and saltwater environments. These authors suggested that the genetic origin of the fish is a critical factor for gene expression. In agreement with this, our results suggested the

presence of two genetic origins for anadromous and freshwater residents from the Laval River and may explain why we observed differences in expression.

Differences in morphology

Phenotypic differences between anadromous and resident fish were also observed in morphological traits. Condition factor was higher in residents, which is in agreement with reports on the shape of anadromous and resident fish (Morinville and Rasmussen, 2008). In the present study, fish were reared under the same conditions, including feeding. Resident fish were heavier than anadromous fish, suggesting that different metabolic strategies were expressed under controlled conditions. A study on fish from the Ste. Marguerite River, showed differences in metabolic activity between anadromous and resident brook charr (Morinville and Rasmussen, 2003). They demonstrated a link between metabolic costs and life-history strategy: migrants consumed more food than residents in the year prior to migration, suggesting that anadromous fish require more food than residents to grow the same amount. Mavarez *et al.* (2009) also worked on the Laval River populations and observed differences between anadromous and resident fish at the expression level of genes related to biological functions associated with energy metabolism (e.g., the aldehyde dehydrogenase 9 family, NADH-ubiquinone oxidoreductase chain 1, pyruvate kinase).

Because no difference in specific growth rate was observed, it is not surprising that IGF-1 expression did not differ among groups for each sampling time. Strong positive correlations between plasma IGF-1 and growth rate have been observed in chinook and

coho salmon (Duan *et al.*, 1995; Beckman *et al.*, 1998; Shimizu *et al.*, 2000; Beckman *et al.*, 2001; Pierce *et al.*, 2001) as well as in other teleost species (Perez-Sanchez *et al.*, 1995; Kajimura *et al.*, 2001). Different patterns of gene expression between individuals using different life history strategies have been documented in brown trout (*Salmo trutta*) (Amstutz *et al.*, 2006). In this case, transaldolase 1 transcript levels were significantly lower in migratory trout at the onset of migration compared to sedentary trout of the same age. These results imply that specific expression profiles are associated with migratory behaviour (Giger *et al.*, 2006), probably as a result of the distinct selective pressures experienced by the anadromous fish and their freshwater-resident counterparts.

Thyroid hormones

Seasonal patterns in thyroid hormones presumably related to smoltification and seawater adaptation have been observed in salmonids (Atlantic salmon, Ebbesson *et al.* 2008; brown trout and rainbow trout, Leloup and Lebel, 1993; brook charr, Audet and Claireaux, 1992). We therefore expected a difference between the two forms, but no difference in T_3 concentrations was observed prior to or after transfer. On the other hand, anadromous fish showed a higher T_3 concentration at 14 days post-transfer. This is in accordance with the findings of Boula *et al.* (2002), who observed that anadromous fish had higher levels of both gill Na^+-K^+-ATPase and T_3. Our findings and theirs therefore suggest that differences in T_3 concentration related to a migratory life strategy occur even under controlled conditions and are likely to be important in divergence.

No difference was observed in T_4 concentrations prior to or after saltwater transfer although field studies show physiological differences between sympatric anadromous and resident brook charr. T_4 was higher during smoltification (spring), and different seasonal patterns of plasma thyroid hormones were observed between anadromous and resident charr (Boula *et al.*, 2002). However, in the same river system (Laval River), elevated T_4 was observed in resident brook charr brought to the estuarine portion of the river (Lavallée, 2004). With a chum salmon fry release experiment, Ojima and Iwata (2007) studied the T_4 surge and suggested that it was a result of new environmental stimuli including changes in water quality (e.g., turbidity). They thus concluded that even though thyroid hormones play a pivotal role in the preparation of migration, they were not the sole factors implicated in the onset of migration. These studies, in conjunction with our own experiment suggest that environmental factors are keys to explaining thyroid hormone patterns.

Genetic basis

Strong sire effects on progeny size suggest the importance of paternal the genotype in energetic allocation, which may influence life history strategies. The weight in the AR cross-type was similar to RR while RA was similar to AA, suggesting that fish weight was determined by the sire for that period. Other studies suggested the presence of paternal effects on fish weight at different moments in ontogeny (masu salmon, Yamamoto and Reinhardt, 2003; brook charr, Perry *et al.*, 2005a). Paternal effects on growth performance were found to be present during young stages and seem to influence survival in freshwater

fishes (reviewed by Fraboulet *et al.* 2010). In agreement with the observation of paternal effect on weight, IGF-1 expression on day 14 was also influenced by sire origin. Heritability estimates for weight were very high in both anadromous and resident brook charr, and both lines showed high variance among EBV, which confirmed that weight is a highly heritable trait (Carlson and Seamons, 2008) that could be modified by selective pressure in brook charr (Chapter 1). Thériault *et al.* (2007b) found a genetic correlation between life history strategy and body size in the Ste. Marguerite River population at age 1, with sampling from mid-May to mid-June. They thus concluded that strategies have the potential to evolve in response to selection acting directly on the strategy or indirectly via body size.

The heritability of the condition factor was lower in anadromous fish than in resident fish and AR and RA hybrids had intermediate condition factors; this evidence suggests additive genetic control of that trait. We previously observed a higher heritability estimate in younger fish (two months younger) of the same anadromous line for condition factor (Chapter 1). These findings suggest an ontogenetic variation in the genetic variation for this trait, presumably related to the hydrodynamic shape suited to their future migratory needs. Other work supports this contention of ontogenetic variability in genetic control (Perry *et al.*, 2005a), suggesting that these lines have achieved adapted states tailored to the requirements of their life-history strategy.

Gill Na^+-K^+-ATPase activity appeared highly heritable in both anadromous and resident brook charr. The level observed on day 14 was not affected by the parental line, suggesting that the transmission of this trait is independent of the parental type. However, it appeared highly heritable in both lines. Mackie *et al.* (2003) observed familial differences in iono-regulatory ability related to gill Na^+-K^+-ATPase activity in cold sea water in Atlantic salmon smolts. This suggests that this ability has a genetic basis. Muscle water percentage also appeared to be highly heritable in both anadromous and resident fish, with the progeny of anadromous sires having different percentages of muscle water content than resident sires, although there was no effect of cross-type independent of sire. As for weight, the finding of only a significant sire effects suggests a sex-specific relevance for the genetic control of this trait. Muscle water content may be an important indicator of difficulties in attaining homeostasis (dehydration) (Sigholt and Finstad, 1990; Finstad *et al.*, 1989). Heritabilities for both T_3 and T_4 were non-zero but relatively low. Moreover, sire and dam origin effect had relatively little significance, with crossovers in means for sire and dam origin for T_4 plasma concentration. Thyroid hormones may be more likely to be affected by increased residual variation since they are associated with behaviour, metabolism, and reproduction (Cyr and Eales, 1996; Leatherhead 1994).

The comparison of heritability estimates for osmolality indicates strong differences between anadromous and resident brook charr for this trait. This trait appears highly heritable in residents but not in anadromous fish. The extreme values obtained for osmolarity heritability estimates may be attributed to the structure of the data. All values

were in a very small range with no extreme values, and all fish had attained homeostasis at the moment of sampling. We hypothesized that this indicated successful acclimation of fish to the saltwater conditions after 14 days. The high variance around EBV in resident fish showed that the genetic contribution of individual fish to the population is highly variable.

No saltwater acclimation disability in resident fish?

Despite differences in heritabilities, cross-type means and sire- and dam-population effects on physiological and morphological characteristics associated with saltwater acclimation, none of the fish showed extreme difficulties in osmoregulation. This suggests a certain level of plasticity in resident fish for persistence at higher physiological demands without reaching a damaging level of osmolarity or gill Na^+/K^+ATPase activity. Moreover, at the end of the summer in salt water, all cross-types had the same hepato-somatic index and resident fish had hepato-somatic index levels similar to those measured at the beginning of summer, which suggests a generally successful acclimation after the stress of exposure. Similarly, other studies have found that the internal salinity of salmon is similar to that of brackish water, and thus less energy is required to maintain homeostasis than in fresh or seawater (Webster and Dill, 2006; Morgan and Iwama, 1998). A recent study made on Laval River anadromous brook charr characterized the movement of anadromous brook charr and observed that anadromous fish were found in the Laval Bay at salinities up to 34 with preference for 26—30 (Curry *et al.*, 2006) These salinities are higher than what was used here. Thus, it suggests that the challenge of 20 may not have been sufficient to

provoke difficulties in osmorégulation, which could have revealed stronger differences between anadromous and resident fish.

Overall, the results of this study indicate that the genetic control of anadromous physiology that differs between anadromous and resident fish is independent of environmental cues. However, the possibility of a genetic–environmental interaction cannot be excluded, and further research in several controlled environments could provide more information on the genetic basis of anadromy in brook charr.

DISCUSSION GÉNÉRALE

La première étude effectuée dans le cadre de cette thèse avait pour objectif d'évaluer les performances d'une sélection dirigée vers l'amélioration de la croissance et l'augmentation de la proportion de poissons immatures à l'âge de 1^+ chez l'omble de fontaine originaire de la souche anadrome de la rivière Laval. Un autre trait d'intérêt pour l'industrie aquacole est la résistance au stress, trait que nous avions étudié plus en détail dans le deuxième chapitre de la thèse. La souche Laval était, au début de ces travaux, utilisée pour la première fois dans un contexte aquicole et avait été choisie sur la base de certaines caractéristiques observées dans la population sauvage. La question demeurait toutefois à savoir si ces caractéristiques étaient déterminées génétiquement où si elles reflétaient divers facteurs environnementaux influençant le développement et la physiologie des animaux depuis leur plus jeune âge. Comme on retrouve dans la rivière Laval des formes résidente (les animaux passent leur vie en eau douce) et anadrome (les animaux effectuent des migrations saisonnières en eau salée) d'omble de fontaine, nous disposions donc du modèle biologique nous permettant d'approfondir nos connaissances sur la divergence génétique et sur l'héritabilité de différents caractères. Étant donné que par définition ce qui différencie le plus le cycle vital des ombles résidents des ombles anadromes, ce sont les migrations en milieu salé, je me suis penchée en premier sur les caractères liés à l'adaptabilité à l'eau de mer. Mes différents échantillonnages m'ont cependant permis de suivre les animaux jusqu'à 22 mois, soit jusqu'à la première maturation sexuelle chez les animaux à maturation sexuelle précoce. L'étude génétique des

aspects liés au métabolisme et à la maturité sexuelle présentait toutefois une somme additionnelle de travail beaucoup trop importante dans le cadre d'un doctorat et fera donc l'objet de publications ultérieures. Comme les éléments précis de chacun des chapitres ont déjà été discutés, la discussion générale reprendra les idées générales dans le but de faire le lien entre les diverses parties de l'étude et de démontrer les implications éventuelles et perspectives de recherche que cette thèse aura permis d'apporter.

Les contributions de l'étude

Le programme de sélection mis de l'avant chez l'omble de fontaine de la souche Laval a permis de démontrer qu'il était possible d'améliorer les performances pour des traits d'importance économique dans un contexte aquacole. Nos travaux démontrent clairement qu'il est possible à la fois d'améliorer la croissance tout en réduisant l'incidence de la maturité sexuelle précoce (Chapitre 1). Les résultats obtenus montrent qu'il est possible d'identifier les familles plus performantes pour la croissance dès l'âge de huit mois, âge à partir duquel un patron comparatif du poids s'est installé de façon stable entre les familles. Il semblerait cependant qu'il faille attendre l'âge de 15 mois pour voir apparaître des différences significatives entre les différents groupes. Chez les salmonidés la présence d'effets maternels est importante chez les jeunes stades ce qui suggère qu'il faille attendre l'atteinte d'un certain âge pour permettre l'identification précoce d'un phénotype performant pour la production. D'ailleurs, Silverstein et Hershberger (1994) ont montré que la taille des œufs pouvait avoir un effet sur la taille jusqu'à 10 mois chez le saumon coho.

Les gains rapidement obtenus (suite à deux générations de sélection) laissent envisager un avenir prometteur à ce type de programme de sélection relativement simple à appliquer. Le poids moyen des poissons issus du programme de sélection a augmenté de plus de 20% après une génération de sélection et de plus de 30% entre la génération F_2 et F_3. Dans la littérature, on retrouve aussi des gains de poids importants suite à une sélection génétique notamment chez le tilapia (Charo-Karisa *et al.*, 2006), le saumon coho (Hershberger *et al.*, 1990) et chez le saumon atlantique (Friars *et al.*, 1995). L'originalité de la présente étude est très certainement d'avoir maintenu une lignée contrôle n'ayant subi aucune sélection dirigée, ce qui nous a permis de quantifier la sélection naturelle attribuable à l'environnement d'élevage (domestication). Ainsi, l'utilisation de cette lignée contrôle a permis de montrer que la domestication avait, à elle seule, engendré un gain de plus de 30% du poids moyen après une seule génération, mais un gain négligeable (< 5%) à la génération suivante. Nos résultats indiquent également qu'il est possible d'augmenter la proportion de poissons immatures (de 32% à 60%) dans un programme de sélection combinant ces deux traits (croissances et absence de maturation), alors que ces deux traits sont réputés comme étant typiquement incompatibles (Gjerde et Gjedrem, 1984; Quinton *et al.*, 2002; Martyniuk *et al.*, 2003). En effet, les études précédentes ont démontré que les poissons à croissance rapide sont plus susceptibles de présenter une maturité sexuelle plus précoce.

En sélectionnant pour une meilleure croissance et l'absence de maturité sexuelle précoce, il est possible qu'une sélection indirecte soit observée. Ce phénomène se produit

lorsque la sélection appliquée pour le trait voulu (directe), occasionne l'amélioration d'un autre trait (indirect) puisque ces deux traits sont liés par une corrélation génétique positive. (Falconer and Mackay, 1996; Roff, 1997). Plus la population est grande, plus il est possible de sélectionner des individus présentant ou non certains traits indirects souhaitable ou pas.

Comme certains auteurs ont montré une corrélation positive entre le poids et le niveau de cortisol observé suite à un stress chez la truite arc-en-ciel (Pottinger et Carrick, 1999; Lankford et Weber, 2006), il s'est avéré utile de vérifier si la sélection dirigée vers une plus grande croissance avait indirectement influencé la réponse au stress chez les poissons issus du programme de sélection. Effectivement, la sélection semble avoir eu un effet sur la réponse au stress et sur la résistance à l'infection *F. maritimus* (Chapitre 2). Les principales différences observées se situent au niveau de la réponse primaire, mesurée dans ce cas-ci par la concentration en cortisol plasmatique, puisque des trois tests utilisés, le test aigu est celui pour lequel la différence entre les deux lignées a été la plus marquée. Suite à l'application d'un stress de capture, les différentes familles de la lignée contrôle ont démontré une réponse homogène alors que dans la lignée issue du programme de sélection, différents patrons ont été observés. Ainsi les poissons issus de la lignée contrôle ont montré une hausse de la concentration plasmatique en cortisol trois heures après le stress et un retour au niveau initial après 24 heures. Parmi les familles issues de la sélection, six d'entre elles n'ont montré aucune hausse significative du taux de cortisol mais présentaient le même profil que le patron observé chez les contrôles. Cependant, on ne peut totalement exclure la possibilité qu'une hausse ait eu lieu entre les pas d'échantillonnage. Deux

familles ont montré une hausse semblable à celle des contrôles et une seule famille a montré un niveau encore élevé après 24 heures. Il s'est avéré que dans notre étude, le poids des poissons n'avait pas d'influence sur le taux de cortisol observé. Ceci a aussi été observé chez le saumon atlantique (Hemre et Krogdahl, 1996).

Il existe donc un potentiel de sélection pour la résistance au stress. On note d'ailleurs dans la littérature, un intérêt grandissant pour l'amélioration des connaissances concernant l'utilisation de la réponse au stress dans le cadre de programmes de sélection (par exemple, chez la truite arc-en-ciel : Fevolden *et al.*, 2002; Øverli *et al.*, 2006; Pottinger, 2006; Trenzado *et al.*, 2006; Weber et Silverstein, 2007; Weber *et al.*, 2008). En effet, le stress occasionné par les pratiques d'élevages, peut avoir des effets néfastes sur la santé des poissons lors d'une exposition chronique; il peut interférer avec la croissance, la capacité immunitaire, la reproduction et la tolérance à l'eau salée (e.g. Pickering, 1981; Adams, 1990; Vijayan *et al.*, 1990; Barton et Iwama, 1991; Iversen *et al.*, 1998).

Dans les deux autres expériences susceptibles d'induire un stress important chez les poissons, i.e. transfert de juvéniles en eau de mer, susceptibilité à l'infection par *Flexibacter maritimus* à l'âge de 1^{+}, nous avons observé que la sélection avait eu aussi un effet sur la sensibilité à *F. maritimus* puisqu'un nombre plus grand de familles contrôles présentaient des mortalités supérieures à 10%. Par contre, on peut dire que nous n'avons pas dénoté de stress notable dans le cas de l'expérience chez les juvéniles. Les effets familiaux observés en regard de la sensibilité à *F. maritimus* indiquent la présence d'une

composante additive qui laisse envisager la possibilité d'inclure un trait lié à la résistance aux infections opportunistes dans un programme de sélection.

Dans l'ensemble, nos résultats indiquent qu'il est possible d'envisager une sélection basée sur un critère de réponse au stress. Cependant, il nous est difficile d'établir sur quel critère « objectif » devrait être basé un éventuel programme de sélection afin d'inclure ce caractère. Il va sans dire que la possibilité d'identifier précocement les familles ou individus plus performants, tant pour la croissance que pour la résistance au stress, permettrait de réduire des coûts de production en éliminant plus tôt les familles ou individus non rentables et en favorisant l'homogénéité. Winkelman et Peterson (1994) ont comparé l'utilisation des mesures obtenues lors des deux premiers hivers comme trait de sélection avec la sélection sur le poids au moment de la récolte (poids final) chez le saumon chinook. Il s'est avéré que la sélection indirecte (hivers) pour le poids des poissons au moment de la récolte serait, au mieux, efficace à 74% comparée à la sélection directe effectuée à ce moment précis.

La comparaison entre individus issus de parents anadromes et ceux issus de parents résidents montre clairement que les résidents et les anadromes diffèrent sur plusieurs traits et ce, malgré des conditions d'élevage identiques (Chapitre 3). En effet, nos résultats semblent indiquer que l'effort physiologique consenti en période d'adaptation à l'eau salée est plus grand chez les résidents que chez les anadromes. Plusieurs éléments contribuent à cette conclusion. Ainsi, les résidents présentaient une osmolalité plasmatique plus grande

après deux semaines d'exposition, une diminution de l'indice hépato-somatique, une concentration plus élevée en triiodothyronine, une activité plus faible de la Na^+/K^+ATPase branchiale alors que l'osmolarité n'est pas encore revenue à la normale et que l'expression de l'IGF-1 est demeurée élevée.

Ces résultats indiquent clairement la présence d'une composante génétique puisque des différences physiologiques ont été observées et ce, malgré des conditions d'élevage identiques. Ceci suggère que le processus de différenciation entre ces deux populations attribuable à la sélection naturelle est en cours. D'ailleurs, Curry et collaborateurs (sous presse) ont identifié la théorie de l'ancêtre anadrome comme la plus probable pour expliquer la présence de ces deux formes. Effectivement, les estimés d'héritabilité pour la majeure partie des traits mesurés, indiquent un niveau modéré à élevé d'héritabilité pour au moins une des deux formes, indiquant que les traits sont héritables et que les différences physiologiques observées sont transmissibles. D'ailleurs Thériault et collaborateurs (2007b) ont observé que la stratégie était héritable et que la sélection naturelle pouvait agir indirectement sur la taille puisqu'une corrélation entre ces deux traits a été observée. L'ensemble des résultats obtenus suggère la présence d'une divergence génétique entre ces deux formes sur la rivière Laval et que les différences de performance lors de l'adaptation à l'eau de mer peuvent être impliquées dans la différenciation de ces deux formes.

Suite et perspectives

Suite aux résultats obtenus, il serait intéressant d'étudier les corrélations génétiques entre l'ensemble des traits d'intérêt identifiés. En effet, nous avons vu qu'il était possible de sélectionner pour la maturité sexuelle et pour la croissance, mais les résultats obtenus au niveau de la réponse primaire au stress laissent envisager la présence de corrélations possibles entre les traits utilisés et la réponse au stress. Aussi, la réponse au stress est complexe et les bases génétiques des phénotypes présentant une forte ou faible réponse au stress varient en fonction des familles, des souches et des individus. Dans la littérature on retrouve des résultats contradictoires chez la truite arc-en-ciel. Par exemple, Pottinger et Carrick (1999) ont observé que les poissons ayant une réponse plus intense étaient plus gros, mais n'ont pas vu de différence de croissance entre les poissons à faible ou forte réponse. Puis, Fevolden et collaborateurs (2002) on vu une meilleure performance de croissance chez des poissons montrant une réponse au stress de faible intensité. Weber et Silverstein (2007) ont observé des différences importantes entre les familles et les individus au niveau de la concentration en cortisol et en glucose chez la truite arc-en-ciel, mais dans leur étude la taille des poissons ne semble pas avoir affecté la réponse au stress de confinement. Pottinger (2006) a suggéré qu'il pouvait y avoir un lien entre le comportement (compétitivité) et la croissance plutôt qu'un lien direct avec la réponse au stress lorsque les lignées de truite arc-en-ciel avec forte ou faible réponse étaient élevées en co-culture. C'est pourquoi, d'un point de vue plus fondamental, il serait intéressant d'utiliser un suivi individuel chez l'omble de fontaine pour améliorer les connaissances sur

la réponse à différents agents stresseurs. Évidemment, le travail devrait être effectué sur des poissons plus gros afin de pouvoir effectuer des prélèvements sanguins non létaux. Comme plusieurs auteurs ont démontré le potentiel de transmission (h^2 = 0.4-0.56) de la réponse au stress d'une génération à l'autre chez la truite arc-en-ciel (Höglund *et al.*, 2008; Weber *et al.*, 2008; Fevolden *et al.*, 2002), ceci laisse envisager la possibilité de sélectionner chez les adultes les phénotypes performants et on pourrait s'attendre à retrouver une réponse similaire chez la progéniture. D'un point de vue différent, il serait aussi intéressant d'investiguer l'application de méthodes récentes et non-létales de mesure du cortisol (travaux de Ellis *et al.*, 2007). En effet, ces auteurs ont utilisé le cortisol présent dans l'eau comme indicateur de stress chez le saumon atlantique. Dans un contexte d'élevage, on pourrait ainsi penser effectuer un suivi comparatif entre des bassins, des groupes ou des familles.

La sélection a indubitablement eu un effet sur la croissance et la diminution du pourcentage de poisson à maturation sexuelle précoce de l'omble de fontaine, mais son impact sur le succès reproducteur suscite un questionnement. En effet, il est clair qu'un effet indirect de la sélection diminuant le succès reproducteur n'est pas souhaitable dans un contexte d'optimisation de la production. Ainsi d'autres travaux sont actuellement en cours au laboratoire afin de comparer le succès reproducteur des géniteurs issus du programme de sélection avec ceux de la lignée témoin (contrôle) permettant d'évaluer l'impact de la sélection pour l'absence de maturité sexuelle précoce. Différents indicateurs pourront être utilisés tels, le suivi des hormones sexuelles chez les géniteurs, la qualité de la laitance, la

qualité des œufs (taille, réserves), le taux de fécondation et les taux de survie aux différents stades (éclosion, première alimentation). Selon les résultats qui seront obtenus, il est possible qu'un trait favorisant le succès reproducteur soit identifié et applicable à un éventuel programme de sélection basé sur plusieurs traits.

En ce qui concerne l'étude des différences entre anadromes et résidents de la rivière Laval, des travaux se poursuivent également en regard d'autres traits davantage liés à l'investissement énergétique pour la croissance et la reproduction. Comme ces travaux sont effectués sur les tissus récoltés des mêmes animaux qui ont servi à l'étude présente au Chapitre 3, il serait éventuellement intéressant de mettre en relation les traits liés aux performances osmorégulatoires, à la croissance et à l'investissement en énergie afin de mieux connaître la physiologie et les bases génétiques de ces deux populations.

Dans l'optique de mieux connaître la physiologie des ombles anadromes, il serait intéressant de comparer les résultats obtenus à ceux de poissons ayant déjà été en contact avec l'eau de mer. Ceci permettrait de voir si la réponse diffère suite à un premier contact ou bien si elle est liée à d'autres signaux environnementaux printaniers présents en milieu naturel. De plus, il a été démontré chez plusieurs études chez les salmonidés que quelques générations de sélection ou encore l'élevage en milieu contrôlé suffisent à occasionner des changements phénotypiques et génétiques (saumon coho, Hershberger *et al.*, 1990; saumon atlantique, Roberge *et al.* 2006; Blanchet *et al.*, 2008. Éventuellement, il serait également

intéressant de vérifier si des ombles issus d'une production pourraient effectuer la migration attendue une fois ensemencés en rivière.

Conclusion

En conclusion, les résultats obtenus au cours de l'ensemble des expériences menées, ont des implications tant pour le développement de l'industrie aquacole québécoise que pour la gestion des stocks d'omble de fontaine. Mais au-delà des applications, cette thèse aura démontré que la maturité précoce est un trait héritable et compatible avec la sélection pour une meilleure croissance ce qui est contraire à ce que laissaient supposer plusieurs études précédentes. De plus, elle aura permis de montrer le potentiel d'insérer un trait lié à la réponse au stress dans le cadre d'un programme de sélection. Elle permet également de corroborer l'hypothèse d'une divergence génétique entre ombles de fontaine anadromes et résidents. De plus, cette étude a permis d'établir les premiers estimés d'héritabilité de plusieurs traits physiologiques liés à l'adaptabilité à l'eau de mer.

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