UNIVERSITÉ DU QUÉBEC

VARIABILITÉ DU TAUX DE CROISSANCE CHEZ L'OMBLE CHEVALIER (*Salvelinus alpinus*) : EFFETS GÉNÉTIQUES ET PHYSIOLOGIQUES

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RÉSUMÉ

Le potentiel de deux souches d'omble chevalier disponibles au Québec a été évalué dans le cadre de la mise en place éventuelle d'un programme de sélection visant à améliorer le taux de croissance. La variabilité génétique a été estimée pour chacune des souches et s'est avérée non seulement inférieure à celle de populations sauvages mais aussi à celles d'autres populations aquacoles de la même espèce. Puisqu'une trop faible variabilité génétique peut rapidement amener à l'apparition de consanguinité il a été suggéré d'augmenter la variabilité de ces souches avant la mise en place de tout programme de sélection. Les marqueurs microsatellites se sont révélés être des outils efficaces pour estimer le degré de parenté des géniteurs quand leur pedigree n'est pas connu et leur utilisation devrait être considérée afin de minimiser les croisements consanguins et la diminution rapide de la variabilité génétique. Des familles à fort et à faible potentiel de croissance ont été identifiées pour chacune des souches évaluées et une corrélation négative a été observée entre les performances de croissance des familles et le degré de parenté des géniteurs (estimé à l'aide de marqueurs microsatellites), les familles les plus proches génétiquement présentant des performances de croissance diminuées. Il a donc été suggéré d'utiliser les informations de parentés dans un programme de sélection puisqu'elles peuvent favoriser la sélection de familles performantes tout en évitant les effets de la consanguinité.

Finalement les voies physiologiques impliquées dans le processus de croissance ont été investiguées. Les poissons présentant les taux de croissance les plus élevés avaient des capacités digestives et métaboliques plus importantes au niveau des caeca pyloriques et du muscle blanc respectivement. La sélection des individus les plus performants en ce qui a trait à la croissance favoriserait donc la sélection des individus présentant les meilleures capacités digestives et métaboliques.

TABLE DES MATIERES

REMERCIEMENTS ii

RÉSUMÉ iv

TABLE DES MATIERE v

LISTE DES TABLEAUX vii

LISTE DES FIGURES ix

INTRODUCTION GÉNÉRALE 1

CHAPITRE I 11

Applying microsatellites in two commercial strains of Arctic charr (*Salvelinus alpinus*): potential for a selective breeding program. 12

- Abstract 13
- Introduction 14
- Material and methods 17
- Results 19
- Discussion 21
- Acknowledgements 25
- Figure caption 27

CHAPITRE II 30

Growth potential of two commercial strains of arctic charr (*Salvelinus alpinus*): Genetic and physiological approach 31

- Abstract 32

Introduction..33
Material and methods..38
Results..43
Discussion..47
Conclusion..53
Acknowledgements..54
Figure caption..64
CONCLUSION GÉNÉRALE..68
BIBLIOGRAPHIE..72

LISTE DES TABLEAUX

Table 1. Summary statistics of variation detected at 7 microsatellite loci showing allele ranges, number of individuals (N), number of alleles (A), number of private alleles (PA), Observed heterozygosity (H_O), gene diversity (H_E), fixation index (F_{IS}) and tests for probability of departure from Hardy-Weinberg expectations (HW) in two arctic charr strains 26

Table 2. Allele frequencies at the four loci used for assignment for the overall breeder population, and for each strain depending on hatching date........ 55

Table 3. Enzymes investigated in each tissue. CS= citrate synthase; AAT= Aspartate aminotransferase, GDH= Glutamate dehydrogenase; PK= Pyruvate kinase and LDH= Lactate dehydrogenase. 56

Table 4- Substrates and pH used for enzyme analysis........ 57

Table 5- Growth information's summary showing growth coefficients (Gc), cube root of initial weight ($Wi^{1/3}$) and coefficient of determination of growth (r^2) for each tank in each experimental location. A.S.E corresponds to Asymptotic Standard Error. 58

Table 6 - Mean weight in grams of the upper (W_{Fast}) and lower (W_{Slow}) tails for each tank in each experimental location. SD corresponds to standard deviation. 59

Table 7- Relatedness coefficient (R_C) estimated for each pair of breeders used to produce families of the Fraser and Buteux strains........ 60

Table 8- Families classified as Fast or Slow growers for each group in each experimental location........ 61

Table 9: Sample size (N), Weight, Lenght, Pyloric caeca somatic (PCI) and Hepatosomatic (HSI) indexes (mean ± standard deviation) of Fast and Slow growers for each group. 62

Table 10- Enzyme activities in Units per gram of tissue (mean ± SD) for each hatching group and each strain 63

LISTE DES FIGURES

Figure 1. Percentage of full-sibs and unrelated offsprings and of Fraser and Buteux breeder pairs per r-value group. 28

Figure 2. Mean number of alleles (A) per loci in 5 domesticated Fraser populations. Fraser Qc (n=46) corresponds to the fraser strain evaluated in this study. LAl (n=40), LRoX (n=50), LRoY (n=20) and LRoW (n=50) correspond to four other Canadian captive populations of the Fraser strain (Lundrigan et al., 2005) 29

Figure 3- Experimental design at commercial and research facilities (F1...F12 and B1...B14 are Fraser and Buteux families respectively 65

Figure 4- Relatedness coefficients (R_C) of families classified as fast or slow growers in each experimental stations. 66

Figure 5- Relatedness coefficients (R_C) of individuals from the upper and from the lower tails in each experimental place. 67

INTRODUCTION GÉNÉRALE

« Le gouvernement du Québec considère qu'un secteur des pêches et de l'aquaculture moderne, composé d'entreprises dynamiques, orientées vers la croissance et disposées à créer des liens d'affaires avec de nouveaux investisseurs est une composante essentielle au développement économique du Québec »

Politique de développement des pêches et de l'aquaculture

Face à la demande croissante de produits halieutiques pour la consommation humaine et à la chute des stocks naturels mondiaux, les productions aquacoles sont passées de 20,8 millions de tonnes métriques en 1994 à 37,8 millions de tonnes métriques en 2001 faisant de l'aquaculture l'industrie de production animale qui se développe le plus rapidement actuellement (FAO, 2000). Sur ces 37,8 millions, le Canada est à l'origine de seulement 152 milliers de tonnes, soit 0,4 % des productions mondiales (FAO, 2003). Le Québec, lui, a produit moins de 2% du tonnage canadien. L'aquaculture devient donc une avenue de développement économique intéressante pour le Québec. Actuellement, plus de 88% des productions aquacoles québécoises se concentrent sur les mollusques (moules, pétoncles…) et les poissons d'eau douce (truite arc-en-ciel et Omble de fontaine). Concernant les poissons, les productions sont en majeure partie destinées au réensemencement. Aucune espèce de poissons n'est vraiment cultivée en mariculture pour la consommation humaine (Institut de la statistique Québec, 2003). Le choix des espèces destinées à l'aquaculture doit se faire avec beaucoup d'attention et actuellement, une des meilleures stratégies repose sur le choix d'espèces indigènes d'eau froide qui permettent l'ouverture de marchés de niches (Funge-Smith et Phillips, 2001). En 2002, Le François *et al.* publient les résultats d'une étude destinée à mettre en avant de nouvelles espèces de poisson à fort potentiel en

mariculture au Québec. Quarante-cinq espèces indigènes ont été examinées pour différents critères aquacoles (degré de connaissance du cycle de vie, potentiel de croissance et d'optimisation du stock). L'Omble chevalier (*Salvelinus alpinus*) est ressortie comme étant la deuxième espèce à plus fort potentiel après le loup Atlantique (*Anarhichas lupus*) et comme la seule espèce anadrome bien classée. Le taux de croissance, la température optimale de croissance, la durée de la période de sevrage, la densité d'élevage, la taille des larves et l'alimentation ont été les principaux critères décisifs. Au Canada, l'intérêt porté à l'Omble chevalier par l'industrie aquacole remonte aux années 80s. La plupart des connaissances de l'époque reposaient sur les travaux effectués au Rockwood Aquaculture Research Centre (RARC) de Gunton, Manitoba (Tabachek et March, 1991). Ce centre distribuait également la quasi totalité des œufs d'Omble chevalier à travers le Canada. En 1991, Pêche et Océan publie un rapport concernant l'état de situation de l'Omble chevalier au Canada (Tabachek et March, 1991). Outre les nombreux aspects positifs relevés (croissance intéressante à basse température, à forte densité, bon prix de ventes en comparaison d'autres salmonidés...), deux désavantages majeurs limitaient le développement de cette industrie : la grande variabilité des taux de croissances au sein d'une même population (les individus les plus lents n'atteignent jamais une taille commerciale) et les problèmes d'approvisionnement en juvéniles de qualité (bonne survie à l'éclosion et à la première alimentation). Dix ans plus tard, Rogers et Davidson (2001) effectuent une enquête similaire : « État de situation : Élevage de l'Omble chevalier (*Salvelinus alpinus*) ». De nouveau, la non disponibilité en juvéniles de qualité a été identifiée comme l'une des principales raisons de la stagnation de l'élevage de l'Omble

chevalier. Les participants à l'étude ont défini l'amélioration génétique comme l'une des voies pouvant amener à résoudre ce problème. Actuellement, trois établissements travaillent sur l'amélioration génétique de l'Omble chevalier au Canada:

- l'Université Simon Fraser, en Colombie Britannique,
- l'Université de Guelph, en Ontario, en collaboration avec
- le Centre Marin de Shippagan, au Nouveau-Brunswick.

Aucun programme n'a encore été mis en place au Québec où l'Omble chevalier est pourtant la deuxième espèce à plus fort potentiel en ce qui concerne la mariculture.

Cette étude se veut une exploration du potentiel de deux souches commerciales d'Omble chevalier disponibles au Québec pour la mise en place d'un programme de sélection. Par souche nous entendons une population qui a évolué isolée d'autres populations, développant ainsi ses propres caractéristiques génétiques. La première souche évaluée est la souche Fraser. Elle a été développée par la pisciculture des Alléghanys, Qc, Canada, à partir d'œufs fournis par le Rockwood Aquaculture Research Center dans les années 80s. Les ancêtres de cette souche proviennent de la rivière Fraser, au Labrador. La seconde est la souche Buteux. Cette souche a été développée à partir de croisements entre mâles du lac Buteux, au nord du Saint Laurent, et femelles de la première souche.

Les objectifs de cette étude sont triples :

1- Estimer la variabilité génétique des souches commerciales Fraser et Buteux,
2- Évaluer les performances de croissance intra- et inter-souches,

3- Identifier des indicateurs physiologiques associés au potentiel de croissance chez cette espèce.

La sélection et l'hybridation ont joué un rôle important dans la domestication des espèces terrestres augmentant le rendement, le taux de survie, et améliorant la qualité des produits (Gjedrem, 1983). Contrairement au milieu agricole où les forts rendements de production sont en majeure partie dus à l'utilisation de géniteurs et de variétés améliorés génétiquement, moins de 10% des productions aquacoles sont basées sur l'utilisation de souches améliorées. Sur le plan génétique, les stocks d'aquaculture sont bien plus proches de leurs congénères sauvages que les animaux ou les cultures terrestres (Gjerde et Villanueva, 2003). Actuellement, les gains de croissance le plus souvent rapportés dus à la sélection génétique en aquaculture sont d'environ 10% par génération (Gjerdrem, 2000). Des études antérieures effectuées sur plusieurs espèces de salmonidés indiquent que l'héritabilité du taux de croissance (degré par lequel l'expression de la croissance dans une descendance est contrôlée par la contribution génétique des parents), est suffisamment élevée pour permettre un programme de sélection (Gjedrem, 1983; Kinghorn, 1983). Ceci a également été vérifié chez l'Omble chevalier (Nilsson, 1994). D'autres caractéristiques propres aux poissons comme la fécondité élevée ou la possibilité de récolter séparément les œufs et le sperme permettent une multitude de designs de croisements et offrent ainsi la possibilité d'obtenir très rapidement des gains de croissance en sélectionnant les meilleurs croisements (Gjerde et Villanueva, 2003). Le succès d'un programme de sélection dépend largement de la variabilité génétique qui existe dans le stock de géniteurs pour le trait que l'on souhaite mettre en valeur (croissance, survie, texture de la chaire...). Plus cette

variabilité est faible et plus la réponse à la sélection va être limitée. Au Canada, les données de pedigree n'ont pas été relevées au cours des générations. Il est donc difficile de connaître l'état de la variabilité génétique de chaque stock. De plus, à cause du faible nombre de géniteurs à l'origine des stocks actuels d'Omble chevalier, il se pourrait que la variabilité génétique soit devenue trop faible pour établir un programme de sélection (Rogers et Davidson, 2001). Utiliser les stocks de géniteurs actuels sans évaluer leur variabilité génétique pourrait amener après quelques générations à une dépression consanguine. Ce phénomène est dû à des croisements entre individus proches génétiquement (parce qu'ils ont un ancêtre commun) et résulte le plus souvent en une diminution des performances du trait quantitatif sous sélection (Gjerde *et al.*, 1983; Kincaid, 1983; Kinghorn, 1983; Su *et al.*, 1996). L'évaluation de la variabilité génétique de tout stock susceptible d'être utilisé dans un programme de sélection est donc nécessaire. Une étude de Lundrigan *et al.* (2005) constitue le premier effort pour évaluer la variabilité génétique chez l'Omble chevalier au Canada. Ces auteurs ont constaté une diminution de la variabilité génétique des populations aquacoles par rapport à celles trouvées dans des populations sauvages. La variabilité génétique peut être estimée grâce à des marqueurs moléculaires. Les marqueurs microsatellites, qui correspondent à de courtes séquences répétitives d'ADN (Tautz et Renz, 1984), ont un polymorphisme très élevé et une transmission mendélienne (Jarne et Lagoda, 1996). Ces caractéristiques les rendent très utiles pour les questions liées à la structure génétique des populations. Plusieurs marqueurs microsatellites ont été isolés chez les salmonidés, notamment chez la truite arc-en-ciel, et ils semblent être applicables à la plupart des espèces de salmonidés (Morris *et al.*, 1996). Plusieurs de ces marqueurs ont

donc servi à de nombreuses études sur la structure génétique de populations d'Omble chevalier (Bernatchez et al., 1998, Brunner et al., 1998, Gislason *et al.*, 1999, Hansen *et al.*, 2000, Lundrigan *et al.*, 2005). Le premier objectif de notre étude est donc d'évaluer la variabilité génétique des deux souches commerciales québécoises à l'aide de huit marqueurs microsatellites. La variabilité génétique de nos souches commerciales devrait être inférieure à celles des populations sauvages et comparable à celles trouvées dans d'autres populations aquacoles de la même espèce (Chapitre I).

Il existe une forte variabilité du taux de croissance chez l'Omble chevalier. Celle-ci s'observe aussi bien entre individus du même âge appartenant à la même souche (Jobling *et al.*, 1989) qu'entre individus de souches différentes (Jobling *et al.*, 1993). Ces variations sont très utiles dans le milieu naturel où elles leur permettent d'occuper des niches écologiques très diversifiées. En revanche, dans un contexte aquacole, ces variations deviennent problématiques puisque certains poissons n'atteignent jamais une taille commerciale (Rogers et Davidson, 2001). Une étude portant sur la souche Harmmerfest a démontré que, même en les isolant, les avortons n'atteignaient jamais un taux de croissance intéressant suggérant que leur taux de croissance faible était déterminé génétiquement (Rogers et Davidson, 2001). Pouvoir sélectionner les poissons en fonction de leur potentiel de croissance devrait donc amener à une amélioration de la croissance et à une diminution des écarts de taille. L'approche de sélection de masse a été utilisée dans de nombreuses industries aquacoles (Herbinger *et al.*, 1995; Romana-Eguia *et al.*, 2005). Par cette méthode, les individus présentant les meilleures caractéristiques de croissance sont retenus et utilisés comme géniteurs pour les générations suivantes. La limite majeure de cette

méthode est que les individus sélectionnés sont plus susceptibles d'être proches génétiquement que s'ils avaient été sélectionnés au hasard (Rodzen *et al.*, 2004), augmentant les risques de perte de variabilité par l'apparition de consanguinité. Sekino *et al.*, (2003) ont observé lors d'une étude sur le flet japonais (*Paralichtys olivaceus*) que la contribution d'une famille donnée aux individus sélectionnés pour la taille était très significative par rapport à celle des autres. Dans ce cas, la sélection de masse favoriserait une seule famille et amènerait très rapidement à une diminution de la variabilité génétique de départ. Une approche plus sophistiquée requiert la connaissance du pedigree du stock de géniteurs (Herbinger *et al.*, 1995). Connaître le degré de parenté de chacun des individus présentant de bonnes performances de croissance offre la possibilité de faire des croisements ciblés en évitant les croisements entre individus de même parenté. Les informations concernant le pedigree des populations sont rarement connues. Elles nécessiteraient de pouvoir élever chaque famille séparément jusqu'à ce que les poissons aient une taille suffisante pour être marqués (puce électronique ou autres) ce qui n'est pas envisageable dans un contexte de production commerciale tant au niveau des coûts qu'au niveau des infrastructures (Herbinger *et al.*, 1995). L'essor des techniques moléculaires et la découverte de marqueurs hypervariables comme les microsatellites ont apporté une solution à ce verrou technologique et permettent de mélanger des alevins appartenant à différentes familles à un stade précoce tout en permettant leur identification ultérieure. Ces marqueurs permettent aussi d'estimer le degré de parenté entre deux individus quand aucune donnée de pedigree n'est connue. Les marqueurs microsatellites se sont révélés déjà très efficaces dans plusieurs problématiques de ce genre en aquaculture (Mallet, 1995;

Estoup *et al.*, 1998; Hara et Sekino, 2003; Sekino *et al.*, 2003; Borrell *et al.*, 2004). Ces derniers seront donc utilisés pour remplir notre deuxième objectif qui est de comparer les performances de croissances intra- et inter-souches des souches Buteux et Fraser. Un effet de la famille sur la croissance a déjà été reporté chez la truite arc-en-ciel et le saumon atlantique (Herbinger *et al.*, 1995; Herbinger *et al.*, 1999) ce qui nous laisse supposer que les individus génotypés devraient se regrouper en famille en fonction de leur potentiel de croissance. Dans leur étude sur la truite arc-en-ciel, Herbinger *et al.* (1995) ont également observé des indications de l'effet négatif des croisements consanguins sur les performances de croissance. Nous nous proposons donc de tester l'hypothèse selon laquelle le coefficient de parenté, estimable pour chaque couple de géniteurs à l'aide des marqueurs microsatellites, puisse être un bon indicateur des performances de croissance des familles; Les familles les plus performantes devraient ainsi exhiber des degrés de parenté moindres (Chapitre II).

Les coûts dus à l'alimentation sont très élevés en aquaculture. L'optimisation de l'efficacité de conversion des aliments est également à prendre en compte dans la mise en place d'un programme de sélection. La croissance est un processus complexe qui a un coût énergétique élevé (Blier *et al.*, 1997). L'organisme doit assurer la synthèse d'enzymes digestives et métaboliques (Blier *et al.*, 1997) qui permettront respectivement la digestion de la nourriture absorbée et la synthèse de protéines (Houlihan *et al.*, 1995). Le développement des capacités digestives et métaboliques pourrait donc être un des facteurs limitants du potentiel de croissance. De nombreuses études ont été effectuées dans le but de mieux comprendre les voies physiologiques impliquées dans les processus de croissance.

La plupart de ces études sont basées sur des différences de taux de croissance provoquées; en utilisant différents régimes alimentaires (Couture *et al.*, 1998; Le François *et al.*, 2000), différents traitements environnementaux comme la température (Pelletier *et al*,. 1993a, b, 1994, 1995; Overnell et Batty, 2000), des restrictions de nourriture suivi d'une période réalimentation (Bélanger *et al.*, 2002), ou encore l'insert de gène codant pour une hormone de croissance (Stevens *et al.*, 1999; Blier *et al.*, 2002; Stevens et Devlin, 2005). Toutes ces approches peuvent avoir un effet confondant puisque les traitements peuvent agir directement ou indirectement sur l'expression des enzymes digestives et métaboliques ce qui peut expliquer la variabilité des résultats. L'Omble chevalier est un excellent modèle pour les études de croissance puisqu'il exhibe une forte variabilité des performances de croissances non seulement entre souches mais aussi au sein d'une même souche. Lemieux *et al.* (2003) ont utilisé deux souches d'Omble chevalier connues pour avoir des taux de croissance différents pour investiguer les liens entre capacités digestives et métaboliques et les performances de croissance chez les jeunes stades. Les résultats suggèrent que la souche présentant le taux de croissance le plus élevé a des capacités métaboliques réduites. Notre troisième objectif est d'examiner les capacités digestives et métaboliques au niveau de tissus et organes impliqués dans la croissance. Ainsi, nous évaluerons les capacités digestives d'Ombles chevalier de la souche Fraser et Buteux présentant des taux de croissance différents au niveau des caeca pyloriques. Les capacités métaboliques seront évaluées au niveau du foie qui est un des tissus ayant une activité énergétique importante et au niveau du muscle blanc puisqu'il s'agit du tissu le plus important chez les poissons. Notre hypothèse est que les performances de croissance sont contrôlées par des voies

physiologiques détectables au niveau d'activités enzymatiques qui pourraient servir d'indicateurs dans un éventuel programme de sélection (Chapitre II).

CHAPITRE I

UTILISATION DES MARQUEURS MICROSATELLITES CHEZ DEUX SOUCHES COMMERCIALES D'OMBLE CHEVALIER (*SALVELINUS ALPINUS*): POTENTIEL POUR LA MISE EN PLACE D'UN PROGRAMME DE SÉLECTION

Applying microsatellites in two commercial strains of Arctic charr (*Salvelinus alpinus*): potential for a selective breeding program.

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Abstract

Genetic variability was estimated at eight microsatellite loci in two strains of Arctic charr (Salvelinus alpinus) available in Québec (Buteux and Fraser) to evaluate their potential in a selective breeding program. Both strains showed restricted genetic diversity with a mean number of alleles per locus of 6.28 and 4.28 for the Buteux and the Fraser strains respectively. The two strains had significantly different allelic and genotypic distribution Genetic variability was lower in the Quebec domesticated strains than in wild populations of the same species (Kruskall-Wallis H = 15.227; df=4 and P=0.0042) and in other broodstock of the same strain (Kruskall-Wallis H = 12.60; df=4 and P=0.0134). The Fraser strain appeared more inbred than the Buteux with mean relatedness values (r-value) of -0.082 and -0.160 respectively. Surprisingly even with a restricted genetic diversity, r-value estimates showed that a high percentage of all possible crosses are not a risk for inbreeding in both strains (61 and 71% for the Fraser and the Buteux strains respectively). These results have shown that genetic variability assessed by microsatellites was low in both strains. However, genotyping microsatellites could minimize inbreeding risks by crossing most genetically distinct breeders.

Keywords: Salvelinus alpinus, genetic variation, microsatellites, relatedness coefficients, selective breeding program

Introduction

Arctic charr (Salvelinus alpinus) is a species with a high potential for cold-water aquaculture in eastern Québec, Canada. Arctic charr displays higher growth rate, and survival, and a lower food conversion ratio at low temperatures than Salvelinus fontinalis another species of commercial interest. In addition, Arctic charr can be reared at much higher densities (Le François et al., 2002), commands a higher market price and is considered as a more refined product on the high-end restaurant market (Johnston, 2002). Interest for Arctic charr culture in Canada arose in the 1980s, but the emergence of a sustainable industry based on this species is still experiencing drawbacks due to variability in growth rate and limited supply of eyed eggs and fry (Tabachek and March, 1991; Rogers and Davidson, 2001). A recent report on the state of this industry in Canada came up with several recommendations including 1) the development of breeding programs focused on increased and constant growth, 2) the pedigree determination and assessment of genetic variability available in commercial broodstocks, and 3) the development of the Arctic charr genetic map (Rogers and Davidson, 2001).

Heritabilities for economically important traits such as growth rate, body weight, and diseases resistance have been estimated for various salmonid species. Heritability estimates for growth rates are typically above 0.20 and show high variation for all species, suggesting the possibility of a rapid response to selection (Gjedrem, 2000). Selection response to growth rates have been estimated to be above 10% per year in several breeding programs and cost-benefits ratio of 1:15 have been calculated by the Norwegian Breeding Program

for Atlantic salmon (Gjedrem, 1997; 2000). A selective breeding program for Arctic charr should lead to increased growth rate and a reduction of the observed size variation actually experienced by commercial growers.

One way to improve performance in a trait is to cull out individuals with poor characteristics. The drawback of this approach is the potential loss in genetic diversity arising from restricting the number of breeders (Norris et al., 1999, Silverstein et al., 2004). Sustainability of a breeding program depends largely on the genetic variability existing in the original broodstock and maintained across subsequent generations for traits of interest. If variability is low at the beginning or decreases across generations it can lead to inbreeding depression and thus to a decrease in the response to selection (Gjerde et al., 1983, Kincaid, 1983, Kinghorn, 1983, Su et al., 1996).

Arctic charr from the Fraser River, Labrador, were used to create the first aquaculture strain in Canada by the Rockwood Aquaculture Research Center, Manitoba. This center was the major egg supplier across Canada. It has been reported earlier that the original broodstock could have originated from no more than two families (Rogers and Davidson, 2001). However, others studies have revealed that genetic diversity currently observed in aquaculture strains is too high to be the result of only two families (Lundrigan et al., 2005; De March, 1991; De March and Baker, 1990). The exact number is difficult to pinpoint but it remains clear that very few families have contributed to the actual stocks. Furthermore, eggs shipped to various facilities often originated from a few parents and many hatcheries developed all their charr stocks from a single egg shipment (Rogers and Davidson, 2001).

Pedigree information has not been maintained from generation to generation resulting in a general lack of information needed to establish a viable selective program.

Efforts have been made to follow the three recommendations made in 2001. A genetic linkage map of Arctic charr has been published (Woram et al., 2004) and the amount of genetic variability has been estimated in different captive and wild strains of arctic charr in Canada (Lundrigan et al., 2005). No other breeding programs than those initiated in 1996 in New Brunswick have been established in Canada. The amount of genetic diversity is likely to be contingent upon aquaculture practices. Therefore it remains an important step prior to the development of a selective breeding program of a particular broodstock.

The objective of this study was to analyze polymorphism at eight microsatellite loci in order to evaluate genetic diversity in two strains of Arctic charr available in Québec. The first strain is the Fraser strain developed from eggs provided by the Rockwood Aquaculture Research Center and grown at the Pisciculture des Alléghanys since the 1980s. Ancestors of this strain originated from the Fraser River, Labrador. The second strain (Buteux) is the third generation of a hybrid between domesticated Fraser females and six males of the Lake Buteux population on the north of the St Lawrence, Quebec (Yves Boulanger, pisciculture des Alléghanis, personal communication). This study aims to determine the potential of using these strains for the initiation of a breeding program in Québec, Canada.

Material and methods

Fish and sampling

Access to breeders of both strains was granted by the Pisciculture des Alléghanys Inc., Ste Émélie-de-l'Énergie, Qc, Canada. The adipose fins of 46 and 49 breeders (Male:Female ratio were 24:22 and 27:22) from Fraser and Buteux strains respectively were sampled and preserved in 100% ethanol. A simple mating design was used in order to estimate the reliability of the relatedness coefficients values estimated later between pairs of breeders: 7 females and 7 males were individually mated to randomly produce 7 families of full sibs from which 10 larvae per family were genotyped.

Microsatellite Analysis

DNA was extracted with a standard phenol-chloroform extraction method modified according to Hillis et al. (1996). All samples were assayed for allelic variation at eight microsatellite loci known to be polymorphic in *Salvelinus alpinus*: Sco-19 (Taylor et al. 2001), Cocl-3 (Bernatchez, 1996), Mst-85 (Presa and Guyomard, 1996), Sfo-8, Sfo-18 & Sfo-23 (Angers et al. 1995), Ogo-8 (Olsen et al., 1998) and Ssa-85 (O'Reilly et al. 1996). One primer for each locus was 5' labeled with TET or FAM dye. Polymerase chain reaction (PCR) amplifications were run on a TouchGene®Gradient thermal cycler (Techne) in 15 µl reactions containing 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5mM $MgCl_2$, 0.2 mM each dNTP, 30 pmol of each primer, 50 ng genomic DNA and 1 Unit *Taq* Polymerase. Amplification conditions were the following: initial denaturation for 3 minutes at 94°C, 35 cycles of 30 seconds at 94°C, 30 seconds at annealing temperature, 30 seconds at 72°C, and

final extension of 20 minutes at 72°C. PCR products were electrophoresed on 6 % polyacrylamide gels and scanned with a FMBio III scanner (Hitachi). Allele sizes were assigned using a Fluorescent Ladder (Promega).

Genetic variability analysis

The number of alleles at each locus (A), observed (H_O) and expected (H_E) heterozygosities were estimated for each strain using Genetix software (Belkhir, 2001). The number of private alleles (PA) (those confined to a single strain) was also estimated for each locus. Statistical differences between measures of genetic diversity of each broodstock (A, PA and H_O) were assessed using the Kruskall-Wallis non parametric test of SYSTAT 10.0 (SPSS Inc.).

Hardy-Weinberg departure (HW) and allelic and genotypic distributions between strains were estimated using Genepop 3.3 (Raymond and Rousset, 1995). Inbreeding of individuals within each strain was estimated as F_{IS} according to Weir and Cockerham (1984) using F_{STAT} 2.9.3 (Goudet, 2000; updated from Goudet, 1995). Significance of the F_{IS} values was estimated using the same program. Genetic differentiation between strains was estimated using F_{ST} according to Weir and Cockerham (1984) in Arlequin 2.0. One thousand permutations were generated to assess significance of the value according to Excoffier et al. (1992).

Relatedness estimation

Relatedness coefficients (r-values) between all possible pairs of breeders were estimated using KINSHIP 1.3.1 (Queller and Goodnight, 1989). The calculation is based on frequency of the alleles in the population, in the current individual and in the current

individual's partner. This measure of relatedness range from -1 to +1, where a positive value indicates that two individuals share more alleles that are identical by descent than expected by chance. Theoretically, first-degree relatives such as parents and offspring or full siblings should have a R_C of 0.5 whereas pairs of unrelated individuals should have a R_C of 0. Statistical differences between r-values distribution of both strains were assessed using the Kruskall-Wallis test in SYSTAT 10.0 (SPSS Inc.). We evaluated the reliability of our estimated r-values among pairs of offspring by comparing results obtained using individuals with known genetic relationships (Borrell et al., 2004).

Results

Genetic variability

Cocl-3 locus appeared to be monomorphic in both strains but since it was impossible to determine exactly if this result was due to a PCR problem or to a real monomorphic condition, this locus was eliminated of the following analyses.

Summary statistics are presented in Table 1. The seven loci examined showed a restricted number of alleles. The locus Sco-19 had the greatest number of alleles (A=11 and 8 for Buteux and Fraser broodstock respectively). Sfo-18 had the least number of alleles (A=2 for both broodstocks). Allele range was similar for both broodstocks. The number of private allele per locus was the only parameter significantly different between Buteux and Fraser Broodstocks (Mann-Whithney U test statistic = 43.5; df=1, p=0.0109), Buteux having more private alleles than Fraser (16, 2 respectively). No differences were detected for the number of alleles per locus, or the observed or expected heterozygosities. Observed

heterozygosities varied from 0.4348 to 0.8980 with a mean value of 0.5824 ±0.142 and 0.6715 ±0.167 for Fraser and Buteux respectively. All private alleles were detected at frequencies less than 0.03 (results not shown). Significant departure from Hardy-Weinberg equilibrium was detected for four and three loci respectively for Buteux and Fraser respectively (Table 1). Mean F_{IS} values were not significantly different from 0 for both strains. Pairwise analysis based on F_{ST} values did not reveal significant differences between the Buteux and Fraser broodstocks but both strains appeared genetically different for allelic and genotypic distribution (χ^2=24.608 and 26.991, p= 0.03864 and 0.01930 respectively).

Relatedness degree

Reliability of the r-values estimation system

The percentage of individuals classified as full-sibs or as unrelated per r-value group is shown in Figure 1. Full-sibs showed higher r-values than unrelated individuals with mean r-values of 0.374± 0.0162 and -0.017± 0.00559 respectively. This was relatively close to the expected values of 0.5 and 0. If we consider that r-values ≤ 0 correspond to unrelated individuals, 97.11% of the possible pairs that exhibited r-values ≤ 0 were really unrelated (i.e. from two different families). In contrast to full-sib pairs, the numbers of unrelated pairs decreased when r-values increased.

Distribution of r-values for both strains

The distribution of r-values among pairs of breeders for each broodstock varied from -0.778 to 0.718 and from -0.775 to 0.722 with means of -0.160± 0.01131 and -0.082± 0.01239 for Buteux and Fraser group respectively. Fraser broodstock was significatively more related than Buteux (Mann Whitney U test statistic= 1.33550E+05, df=1, p= 0.0000)

since more individuals exhibited r-values above 0 (Figure 1). Our results show that 71% of the possible crosses for Buteux were classified as unrelated thus only 29% of the possible crosses represent a high risk of inbreeding. The corresponding value for the Fraser strain was 39%.

Discussion

Genetic variability within both broodstocks was limited with a mean number of alleles per locus of 4.28±1.989 and 6.28±2.75 for Fraser and Buteux stocks respectively. Surprisingly, H_O values were relatively high considering the low number of founders in these strains.. As reported in other studies, allelic diversity appears to be a more sensitive measure of genetic variability than observed heterozygosities (H_O) (Norris et al., 1999; Amos and Balmford, 2001; Lundrigan et al., 2005). As only a few families are suspected to be the origin of most charr aquaculture broodstocks in Canada, the limited genetic variability within the Fraser strain revealed by this study is not surprising. In contrast, genetic variability within the Buteux strain was expected to be greater as hybridization often leads to an increase in the genetic variability, but this was not the case. Most of the private alleles present in the Buteux strain are rare alleles (frequency < 0.01). The presence of rare alleles could be indicative of a small number of effective breeders from Lake Buteux used to develop this strain. Indeed only six males from Lake Buteux were used to develop the first generation of Buteux broodstock in the 1990s. All sperm were pooled and used to fertilize domesticated females of the Fraser strain. Details on the number of females used are not known. The broodstock used in this study may be the third generation (Yves Boulanger, Pisciculture des

Alléghanis, personal communication). The number of breeders from the Lake Buteux at the origin of the Buteux population was small and by pooling all sperm, all males could have contributed unequally to the first generation. Cocl-3 locus was found to be monomorphic in our strains whereas it was variable in all arctic charr populations studied in Lundrigan et al. (2005). One hypothesis to explain this result is a problem with the PCR conditions that lead to non specific products. PCR reactions have been carried out several times under different conditions but results were always the same. Alternatively, it is possible that this locus is really monomorphic in our strains. This being the case, it would reflect severe bottleneck events in our strains.

Lundrigan et al. (2005) estimated genetic variability among different domesticated and wild Arctic charr populations in Canada. This study was the first carried out in Canada and highlighted a global loss of genetic variability in domesticated compared to wild populations. Ten aquaculture and three wild populations were assessed at six microsatellite loci (Sco-19; Sfo-23; Sfo-8; Ogo-8; Cocl-3 and Mst-85). Since the same six microsatellites were also used in this study, a comparison of our results is possible with Lundrigan and al. (2005) but since we were unable to conclude with certainty on Cocl-3 results we decided to use the five other loci in the following comparison. The Buteux and Fraser broodstocks from our study had less alleles than the wild populations (Kruskall-Wallis $H = 15.227$; $df=4$ and $p=0.0042$), as is typically found in the literature. Many studies have already revealed the extent to which genetic variability in hatchery stocks differed from wild populations as a result of domestication (Perez-Enriquez et al., 1999; Koljonen et al., 2002; Sekino et al., 2002). Surprisingly, the degree of genetic diversity in our Fraser strain was lower than what

was found in the four other domesticated Fraser populations (Kruskall-Wallis H = 12.60; df=4 and p=0.0134) (Figure 2). This could be a cause of concern as a decrease in levels of genetic variability often leads to an increase in homozygosity and inbreeding in the successive selected generations as fewer and genetically less diverse individuals contribute to the next breeding generation (Romano-Eguia et al., 2005). Loss of homozygosity at hyper-variable loci like microsatellites must be avoided since it can be indicative of the loss of genetic variability at less variable loci like important coding loci. The probability of loosing an allele is related to its frequency. Alleles with low frequencies will be lost more rapidly than common ones (Simialer, 2005). Genetic variability within both strains studied is already low and if we consider that all private alleles (2 and 16 for Fraser and Buteux strains, respectively) are represented in very small frequencies, risks of increased homozygosity are high. However, the rate of this decline can be limited with good management practices. Even in the absence of pedigree information, relatedness between breeders can be evaluated using molecular markers. The use of relatedness coefficients as a tool for the appropriate selection of breeding pairs in hatcheries would contribute to inbreeding avoidance (Norris and al., 2000). Similar to Borrell et al. (2004), we show the reliability of this tool to discriminate relatives from unrelated individuals. Full-sibs and unrelated individuals showed mean r-values of 0.374 and -0.017 respectively which is in agreement with the theoretical values (Queller and Goodnight, 1989) but an important variation of the r-values was observed among full-sib and unrelated group (results not shown). However, more than 97.0% of individuals classified as unrelated were correctly assigned and displayed r-values lower than 0. The high variation in our results was

observed for r-values higher than 0, where an important number of unrelated individuals were wrongly assigned. Breeding pairs classified as related using this method of calculation could be individuals of the same family or with a common ancestor or individuals from different families but having similar genotypes. Individuals displaying r-values lower than 0 are the most genetically distinct and since 97% of the individuals were well assigned in this group of primary interest, pairwise relatedness estimation can be considered as an easy and informative method to make the best choice in breeding pairs selection.

Mean of relatedness coefficients for Buteux and Fraser groups were -0.160 and -0.082, respectively. This is quite surprising given the low genetic variability present in both strains. The Fraser strain seems more inbred than the Buteux, which is consistent with our results and with the history of both strains. Twenty-nine% and 39% of all possible breeder pairs in the Buteux and Fraser strains, respectively have a high risk of inbreeding. These results are encouraging if we consider that Borrell et al. (2004) estimated that a risk of inbreeding as high as 45% is acceptable for the initiation of a selective breeding program in turbot.

Considering the low genetic variability observed in the Fraser and in the Buteux strains, we urge that the development of a sustainable selective breeding program in Québec be conducted only after application of measures aimed at enhancing genetic variation (through breeders exchange with other producers or sampling of wild breeders) and that genetic information be used to alleviate inbreeding risks.

Acknowledgements

This work was supported by funds from the Conseil des Recherches en Pêche et en Agroalimentaire du Québec (CORPAQ) and the Fondation de l'Université du Québec à Rimouski (FUQAR) to France Dufresne, Pierre Blier, and Nathalie Le François. We want to express our thanks to Yves Boulanger for providing us with his broodstocks and to the personal at the Pisciculture des Alléghanys Inc., Ste Émélie-de-l'Énergie, Qc, Canada, for their help in sample collection.

Table 1. Summary statistics of variation detected at 7 microsatellite loci showing allele ranges, number of individuals (N), number of alleles (A), number of private alleles (PA), Observed heterozygosity (H_O), gene diversity (H_E), fixation index (F_{IS}) and tests for probability of departure from Hardy-Weinberg expectations (HW) in two arctic charr strains

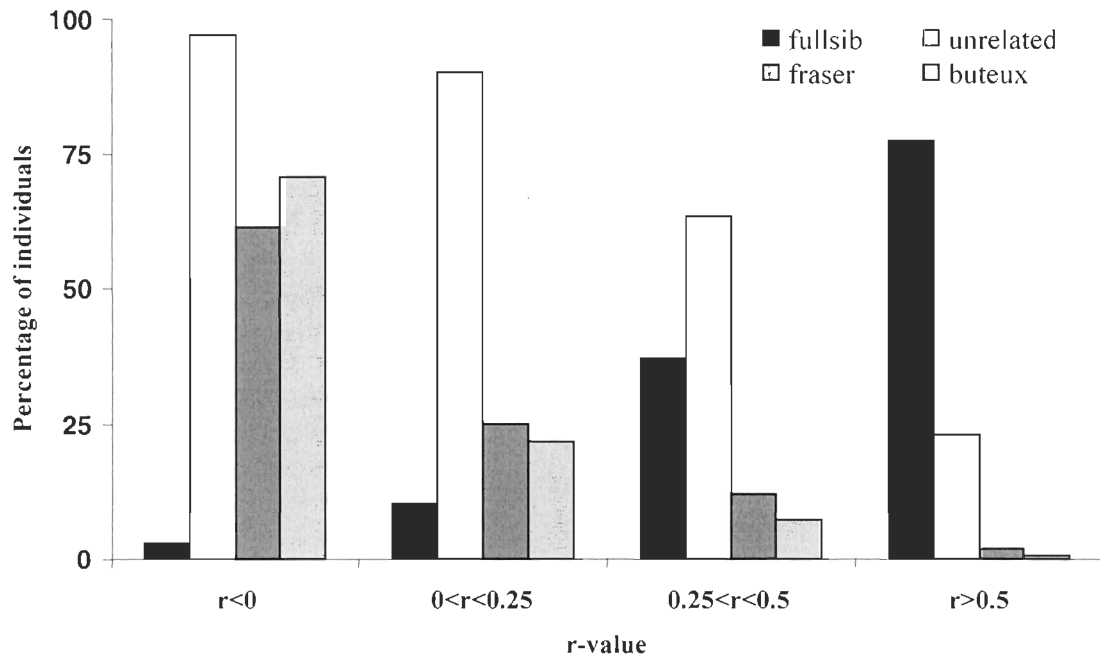
		Sfo-23	Sfo-8	Sfo-18	Ogo-8	Sco-19	Ssa-85	Mst-85	Mean values
FRASER	Allele Range	175-209	281-297	162-164	99-125	191-245	183-251	205-211	
	N	46	46	46	46	46	46	46	
	A	4	4	2	4	8	5	3	4.2857
	PA	0	0	0	1	1	0	0	0.2857
	H_O	0.8478	0.4783	0.5435	0.4348	0.6957	0.5435	0.5333	0.5824
	H_E	0.6882	0.4838	0.4651	0.6178	0.7652	0.5905	0.5405	0.5939
	F_{IS}	-0.2350	0.0110	-0.1710	0.2990	0.0920	0.0810	0.0240	0.0195
	HW	0.0003	ns	ns	0.0445	ns	ns	0.0141	
BUTEUX	Allele Range	171-227	255-297	162-164	97-129	179-245	183-251	203-211	
	N	49	49	49	49	49	49	49	
	A	8	6	2	6	11	6	5	6.2857
	PA	4	2	0	3	4	1	2	2.2857
	H_O	0.8980	0.4761	0.5510	0.5714	0.8776	0.5918	0.7347	0.6715
	H_E	0.7633	0.4969	0.4210	0.6495	0.7679	0.6183	0.6118	0.6193
	F_{IS}	-0.1790	-0.2350	-0.3130	0.1210	-0.1440	0.0430	-0.1910	-0.1171
	HW	0.0000	0.0240	0.0384	ns	0.0034	ns	ns	

Figure caption

Figure 1. Percentage of full-sibs and unrelated offsprings and of Fraser and Buteux breeder pairs per r-value group.

Figure 2. Mean number of alleles (A) per loci in 5 domesticated Fraser populations. Fraser Qc (n=46) corresponds to the fraser strain evaluated in this study. LAl (n=40), LRoX (n=50), LRoY (n=20) and LRoW (n=50) correspond to four other Canadian captive populations of the Fraser strain (Lundrigan et al., 2005)

Figure 1. Percentage of full-sibs and unrelated offsprings and of Fraser and Buteux breeder pairs per r-value group.



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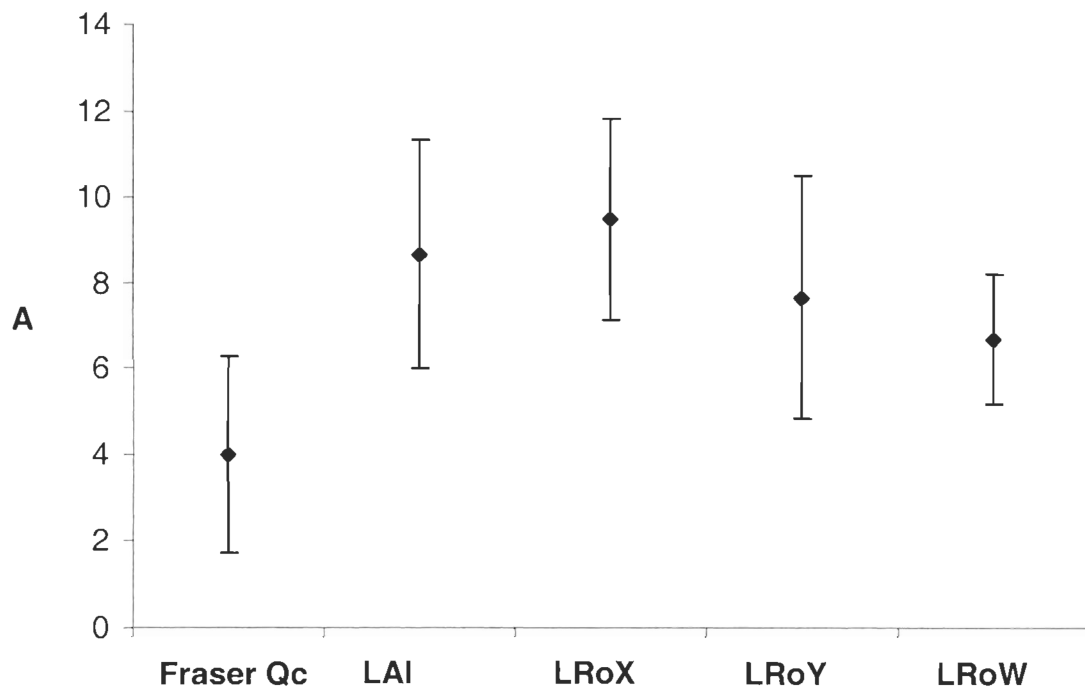

CHAPITRE II

PERFORMANCES DE CROISSANCE CHEZ DEUX SOUCHES D'OMBLE CHEVALIER (*SALVELINUS ALPINUS*): EFFETS GÉNÉTIQUES ET PHYSIOLOGIQUES

Growth potential of two commercial strains of arctic charr (*Salvelinus alpinus*): Genetic and physiological approaches

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Abstract

Genetic and Physiological approaches were used to examine growth performance of two commercial strains of arctic charr (*Salvelinus alpinus*). Stains did not show differences in growth rate but significant differences among families were detected. A relatedness coefficient was attributed to each family using genotypes of the parental couples. Inbred crosses were characterized by high relatedness coefficients. Families categorized as Fast growers exhibited low relatedness coefficients suggesting that the latter are good indicators of growth performance in these strains. Fish with high growth rate were characterized by higher trypsin activity suggesting their higher digestive capacities compared to fish with lower growth rates. Metabolic capacities were lower in the liver but higher in the white muscle of fast growing fishes suggesting different functional implications of these tissues in growth rate performance. The liver may play a role in catabolism allowing slow growing fishes to adjust their lower supply in nutrients through digestion. Higher glycolytic capacities of the white muscle may reflect the higher burst swimming capacities of Fast fish favoring food access and attack avoidance. The selection of fish with higher growth performances will favor the selection of individuals with higher digestive and metabolic capacities.

Keywords

Salvelinus alpinus, growth potential, family, relatedness coefficients, digestive and metabolic capacities and selective breeding program

Introduction

Heterogeneity of individual growth is a common phenomenon in cultured fish (Dou *et al.*, 2004) and is particularly problematic in arctic charr *Salvelinus alpinus* as some fish never attain a commercial size (Rogers and Davidson, 2001). Size variation in this species is an important limitation to the development of an industry based on its culture and, since the potential of this species in aquaculture is high in Canada, the reduction of the observed variability is of primary importance for commercial growers (Tabachek and March, 1991; Rogers and Davidson, 2001). Heritability estimates for growth rate in salmonids are typically above 0.20 and show high variation suggesting the possibility of a rapid response to selection (Gjedrem, 2000). Different selection methods are available to improve growth. Mass selection has often been used since it was the easiest way for regular fish farms (Gjerde and Rye, 1998; Herbinger *et al.*, 1995; Romana-Eguia *et al.*, 2005). This method involves culling individuals with the poorest phenotypic values in the breeding stock. The draw-back of mass selection is that individuals selected as broodstock based on superior phenotypes are more likely to be genetically related than if they were selected at random (Rodzen *et al.*, 2004). Such practices increase the risks of inbreeding (Herbinger *et al.*, 1995; Norris *et al.*, 1999, Silverstein *et al.*, 2004). Inbreeding is a natural consequence of selection but it is important to limit it since it can reduce future selection response through loss of genetic variation and inbreeding depression. Inbreeding depression is the effect of inbreeding measured as the decline in performance of a quantitative trait (Su *et al.*, 1996). Increased homozygosity, reduction of genetic variability, growth, survival, disease

resistance, and occurrence of abnormalities are some inbreeding consequences reported in the literature (Gjerde *et al.*, 1983; Kincaid, 1983; Kinghorn, 1983; Su *et al.*, 1996). More sophisticated selection approaches require the knowledge of pedigrees of the selected populations (Herbinger *et al.*, 1995). The lack of pedigree information currently observed in many aquaculture strains is partly due to the difficulties of maintaining this information because it requires that each family be grown separately until fish had attained sufficient size to allow their tagging (Herbinger *et al.*, 1995). The emergence of hypervariable molecular markers such as microsatellites has alleviated this logistic problem. Microsatellites are short tandem repeats inherited in a Mendelian way making them useful in parentage assignment problematics (Tautz and Renz, 1984; Jarne and Lagola; 1996). Larvae of different families can thus be pooled at hatching and assigned to their respective families later by genotyping. Microsatellites have proved useful in parentage assignment when potential parents are known (Herbinger *et al.*, 1995; Herbinger *et al.*, 1999) but also in relatedness estimation when pedigree is unknown (Norris *et al.*, 2000; Borrell *et al.*, 2004). Selection can then be made on individuals, on families or on mix between individual and family scale. The combination of individual and family selection is considered the optimal selection method when applicable (Gjerde and Rye, 1998). Family effects on growth have been documented in rainbow trout (*Onchorynchus mykiss*) and in Atlantic salmon (*Salmo salar*) (Herbinger *et al.*, 1995; Herbinger *et al.*, 1999) but it remains difficult to attribute representative breeding values since environmental conditions can be very confounding (Herbinger *et al.*, 1999). Furthermore, large scale genotyping is required for significant detection of families with high performance and is thus inaccessible for

commercial growers. An indicator of growth potential prior to families' creation is thus highly relevant. In a study initiated to assess the usefulness of microsatellites for establishing pedigrees in mixed aquaculture populations, Herbinger *et al.* (1995) found indications that progeny of inbred crosses had depressed growth performances. Inbreeding consequences on growth performances have already been documented for different species (Gjerde *et al.*,1983; Kincaid, 1983; Su *et al.*, 1996) and since a significant diminution of genetic variability has been documented in different farmed populations of arctic charr compared to their wild relatives in Canada (Lundrigan *et al.*, 2005; Ditlecadet *et al.*, Ms. accepted), inbreeding could be sufficient to have a measurable effect on growth performances. Risk of inbreeding increases with the relatedness of the breeders. The genetic software Kinship allows the estimation of relatedness coefficient (R_C) of each breeder pair using microsatellites information (Queller and Goodnight, 1989). The reliability of these coefficients in estimating relatedness has been shown in turbot (*scophthalmus maximus L.*) (Borrell *et al.*, 2004) and in arctic charr (*Salvelinus alpinus*) (Ditlecadet *et al.*, Ms. Accepted).

The first part of this study aims to compare growth performances between and within two commercial strains of arctic charr available in Quebec (Fraser and Buteux). The objectives were: 1) to determine growth performance of each strain and 2) to determine if there is genetic variation for growth performances among families.

The second part of this study will examine physiological indicators of growth performance. Food costs represent a high percentage of fishery industries. Optimization of food conversion efficiency is important to consider in a selective breeding program for growth

enhancement. Growth is a complex and energy requiring process. Organisms must ensure digestive and metabolic enzyme syntheses (Blier *et al.*, 1997) that in turns allow food digestion and protein synthesis respectively (Houlihan *et al.*, 1995). Determination of the physiological pathways involved in growth is the subject of numerous studies in fish. A wide diversity of experiments has been used to obtain different growth rates: temperature manipulation (Pelletier *et al.*, 1993a; Pelletier *et al.*, 1993b; Pelletier *et al.*, 1994; Pelletier *et al.*, 1995; Overnell and Batty, 2000), growth hormone gene insertion (Stevens *et al.*, 1999; Blier *et al.*, 2002; Stevens and Devlin, 2005), food content (Le François *et al.*, 2000) or ration (Bélanger *et al.*, 2002). All of these treatments can be confounding since they can act directly or indirectly on the expression of digestive and metabolic enzymes. This may explain the unclear link currently observed between digestive and metabolic capacities of fish and growth performance. Growth performance shows strong variation between and within arctic charr strains, and even among fish from a same cohort making this species an interesting model for growth studies. Lemieux *et al.* (2003) used two strains of arctic charr known to have different growth performances to explore the relationship between digestive and metabolic capacities and growth performance in early fish development. Their results showed that the Yukon Gold strain with the highest growth rate exhibited lower metabolic capacities, suggesting that higher growth performance were linked with lower metabolic capacities in the early stages of arctic charr. Since Lemieux *et al.* (2003) worked on whole larvae, their study is of primary relevance for digestive and metabolic capacities development but the relationship between enzymes activities and growth performance should be examined in older life stages when tissues are sufficiently developed to be

considered independently. In the second part of this study, we examined the relationship between key enzymes of oxidative (citrate synthase), glycolitic (pyruvate kinase and lactate dehydrogenase), and amino acid (Aspartate aminotransferase and glutamate dehydrogenase) metabolism and digestion (trypsin) and growth performances of Arctic charr of two strains (Buteux and Fraser). Pyloric caeca size adjustments have been shown in Atlantic cod in response to food restriction (Bélanger *et al.*, 2002) and in GH-transgenic coho salmon (Stevens and Devlin, 2005) suggesting the potential for the pyloric caeca to limit growth. Other studies have suggested trypsin activity as a potential limiting factor of growth rate (Lemieux *et al.*, 1999). Since trypsin digestion takes place mostly in the pyloric caeca, this tissue seems to be the scene of important processes involved in growth performance. We hypothesized that variation in growth performance is linked to variation in pyloric caeca size and/or trypsin activity. Liver is known to have a high metabolism and protein turnover. High metabolic rate has already been linked to lower growth rate in Arctic charr (Lemieux *et al.*, 2003). High protein turnover is also linked to lower growth performances. As shown by Dobly *et al.* (2004), the activity of a proteolytic enzyme (20S proteasome) has been negatively correlated to growth efficiency in rainbow trout liver. Considering these results we hypothesize that liver metabolism is higher in fish with low growth performance. Finally white muscle is the most important tissue in fish representing more than 40% of the total weight. It is thus one of the most important tissue and it is hypothesized that muscle metabolism will be tightly linked to growth performances.

To resume, the growth performances of two arctic charr strains will be estimated. We will also examine the effect of breeder relatedness on family growth performance as well as the

relationship between metabolic and digestive enzyme activities and growth performance in pyloric caeca, liver and white muscle.

Material and methods

Experimental design

In September 2003, twelve and fourteen full-sib families were obtained from the Pisciculture des Alléghanys Inc., Ste Émélie-de-l'Énergie, Qc, Canada, for the Fraser and the Buteux strains respectively. Adipose fins of all breeders were sampled and placed in 100% ethanol for genotyping. The incubation took place in the Centre Aquacole Marin de Grande-Rivière, Quebec (CAMGR), until the eyed egg stage. At eyed egg stage, a portion of eggs of each family were transferred to the second experimental place, Marinard Aquaculture Ltée in Rivière-au-Renard, Quebec (Marinard). Eggs from each family were incubated separately at 6°C in drawer incubators in both locations. A classification of the families was done depending on their hatching days to avoid high size variability between larvae at the beginning of the experiments. The earliest hatched families were classified as Week1 families and the latest one as Week-2 families. Since a high correlation was observed between families hatching dates in CAMGR and in Marinard, the same families were classified on the same way as Week1 (F1...F6 and B1...B7 for the Fraser and the Buteux strains respectively) or Week2-families (F7...F12 and B8...B14 for the Fraser and the Buteux strains respectively). At first feeding in March 2003, larvae were transferred in tanks as shown in figure 3. Transfer of Week1 fish in their respective tanks was done five days before the Week2 fish transfer. In CAMGR, four groups were created by pooling fifty

larvae per family depending on the strain and on the hatching day group (Week1-Fraser, Week1-Buteux, Week2-Fraser and Week2-Buteux). In Marinard, two groups were created by pooling one hundred larvae per family depending on the hatching day group (Week1 and Week2). Each group of both places was represented by two tanks to evaluate tank effect (A and B). All experimental conditions (light, temperature, densities, food …) were as similar as possible between both stations and were chosen according to Johnston (2002).

Growth monitoring and sampling

Fifty individuals were weighted monthly in each tank in both research and commercial stations until December 2003. Since the growth model used applies to periods of undisturbed growth, the first individual weightings were done three weeks after fish transfer in tanks for first feeding. This delay allowed fish to acclimate to this new environment. In January 2004, the adipose fins of 50 and 20 fish for each of the upper and lower size tails were sampled in each tank in Marinard and CAMGR respectively and placed in 100% ethanol for genotyping analysis. In CAMGR, 5 of the upper tail fish were sacrified in each tank and their liver, pyloric caeca and white muscle sampled and conserved at -80°C until enzymatic analyses. Fish were starved 48 hours before sampling. Five of the lower tail fish were pit-tagged, replaced in their respective tank and let grown until April 2004 when their mean weight had attained that of the upper tail fish of January 2004. All tagged fish where sacrified and the same sampling as January 2004 occurred for enzymatic analysis.

Growth rate estimation

The growth model developed by Iwama and Tautz (1981) and Iwama (1996) for salmonids in intensive aquaculture conditions was applied to each tank to determine their growth coefficient. The basic form of the model is: $Wf^{1/3} = Wi^{1/3} + Gs \times Time$

where $Wf^{1/3}$ is the cubic root of the final mean weight in grams; $Wi^{1/3}$ is the cubic root of the initial mean weight in grams; Gs is the growth slope (calculated by dividing the mean water temperature in Celsius (T) by 1000) and Time is the number of days between weightings. The basic model is applicable to all salmonids but has to be adjusted for each species. The correction factor, called the growth coefficient (Gc), is calculated as a ratio between the actual growth slope (Gs) and the theoretical growth slope (Gs'), where Gs' is the water temperature in Celsius divided by 1000. Corrected formula of the model can be rewritten as: $Wf^{1/3} = Wi^{1/3} + (T / 1000 \times Gc) \times Time$

SYSTAT 10.2© (SYSTAT software inc.) was used for statistical analyses. Nonlinear Model was used to determine the mean Gc values of each tank. Differences between tanks were then estimated by comparison of the the confidence interval (95%).

Genetics and growth

Relatedness estimation of the breeder pairs

Breeders of each family were genotyped at 7 microsatellites loci according to Ditlecadet *et al.* (Ms. Accepted). The relatedness coefficients (R_C) of each mating pairs were estimated using KINSHIP 1.3.1 (Queller and Goodnight, 1989). The calculation is based on frequency of the alleles in the population sample, in the current individual and in the current individual's partners. This measure of relatedness range from -1 to +1, where a

positive value indicates that two individuals share more alleles that are identical by descent than expected by chance.

Parentage assignment

Initially the seven microsatellites used to genotype breeders were assessed for offspring assignment. Only four of them, Sfo-23, Sfo-8, Sco-19 and Mst-85 were retained for the parentage assignment since the three others were not informative. Variability among the overall breeder population was 4, 6, 6 and 10 alleles for Mst-85, Sfo-8, Sfo-23 and sco-19 respectively. Eleven of the 26 alleles detected had frequencies lower than 0.02 (Table 2). Parentage assignment was performed using the Probmax Parentage assignment program 1.2 (Danzmann, 1997).

Family effects

Families were classified as Fast or Slow growers depending on the number of fish in each tail (lower or upper). Only families with significant differences between upper and lower tails were retained. Significance was measured with a Chi-Square (χ^2) test for each family comparing number of fish observed in the upper and lower tails with the theoretical numbers expected if there was no family effect. Tank effect was then estimated for each family retained. Families not classified in the same way in both duplicate tanks from a group were eliminated. The same process was followed in each experimental location.

Relatedness and growth performance

Tests of relatedness effect on growth performance were estimated on families classified by the Chi-squared method and on all individuals assigned to only one family at both experimental stations. SYSTAT 10.2© (SYSTAT software inc.) was used for statistical

analyses. Covariance analyses (ANCOVA) were used to evaluate the effects of relatedness coefficients (R_C) on growth performance. Independence of the estimates and of the residuals and normality of the residuals were estimated for each test.

Enzymatic assays

Enzyme activities measured in each tissue are presented in Table 3. Tissues were thawed on ice and homogenised in 9 volume of Tris-HCl buffer (pH 7.5). Pyloric caeca homogenate (200µl) were centrifuged at 13 000g for 15 minutes for trypsin analysis. Except for trypsin, all homogenates were centrifuged 30 seconds at 3000g to eliminate tissue residuals. All enzyme activities were measured in duplicates in a Lambda 11 UV/VIS spectrophotometer (Perkin Helmer) at 15°C. Enzymes analyses were performed following parameters presented in Table 4. All activities were expressed in Unit per gram of tissue (U/g_{tissue}).

Enzymatic analyses

Fish from the upper tail of size range were classified as fast growing fish (Fast) whereas fish from the lower tail were classified as slow growing fish (Slow). Covariance analyses (ANCOVA) were used to test for enzyme activity differences between Fast and Slow individuals using SYSTAT 10.2© (SYSTAT software inc.). Because of the complexity of the experimental design, analyses were performed independently on each hatching group. Tank duplicates (A and B) were nested to take into account potential tank effect. Independence of the estimates and residuals and normality of the residuals were estimated for each test performed. Log transformations were performed when the assumptions were not respected. Log transformations were used for mass and for trypsin activity.

Results

Growth rate estimation

Growth information's summary for each tank and experimental station is given in Table 5.

CAMGR (Research station)

Coefficient of determination of growth was high for all tanks with a minimum value of 0.996. No tank effect was detected for growth rate (Gc) for any of the four groups established in this station. Gc was neither dependant of the strain nor of the hatching group with mean values of 1.4032, 1.4000, 1.3897, and 1.4049 for the Week1-Fraser, Week1-Buteux, Week2-Fraser and Week2-Buteux groups respectively. A tank effect was detected for $Wi^{1/3}$ in the Week1-Fraser group, Week1-FraserA being bigger than Week1-FraserB during all the measurements. Excepting this group, $Wi^{1/3}$ was higher in the Week2 than in the Week1 groups.

Marinard (Commercial station)

Coefficient of determination of growth was also high for all tanks with a minimum value of 0.988. A tank effect was detected for growth rate (Gc) in the Week2 group, Week2-A growing faster than Week2-B. Week2-B displayed a lower Gc than all other three tanks with mean values of 1.5534, 1.5163, 1.5414 and 1.4119 for Week1-A, Week1-B, Week2-A and Week2-B respectively. No tank effect was detected for $Wi^{1/3}$ for any of the hatching groups. $Wi^{1/3}$ was higher in the Week2 than in the Week1 group.

Growth rates exhibited in Marinard were higher than in CAMGR for all groups but no difference was detected for $Wi^{1/3}$ between the two stations.

Growth performance and genetics

Mean weight displayed by the upper and the lower tails of growth in January 2004 are presented in Table 6 for each tank of each experimental station. No tank effect was detected in any of the groups. In CAMGR, mean weight of the upper or of the lower tail was not different between strains or hatching groups (F-ratio= 1.145; df= 1; p=0.4325). In Marinard, mean weight of the upper or of the lower tail was not significantly different between hatching groups but a strong tendency was observed (F-ratio= 18.02; df= 1; p= 0.0513). Mean weight among the upper or among the lower tails displayed in Marinard were higher than in CAMGR (F-ratio= 136.4; df= 1; p= 0.0000). In CAMGR, mean weights varied from 105.7 to 119.5g and from 41.2 to 45.0 g in the upper and lower tails respectively. In marinard, mean weights were 187.2 and 209.4g for the upper tails and 69.7 and 72.2g for the lower tails.

Relatedness estimation of breeder pairs

Relatedness coefficients (R_C) of each pair of breeders are given in Table 7. Values varied from -0.607 to 0.364 and from -0.415 to 0.389 for the Fraser and the Buteux strains respectively. Half of the families from each strain had negative R_C. Most of these non-related families were from the first Hatching group for the Buteux strain whereas they were from the second one (Week2) for the Fraser strain.

Microsatellites and parentage assignment

Upon all genotyped offspring, the four microsatellites used allowed 70.7% matching to one family, 20.9% to two families, and 7.2% to more than 3 families. 1.3% could not be

assigned to any possible families. These 1.3% may represent fish that jump from other incubators.

In CAMGR, The Week1-Buteux group displayed a higher unique assignment percentage than the Week2-Buteux group (75 vs. 66.2%) while the Week1-Fraser group displayed a lower unique assignment percentage than the Week2-Fraser group (68.7 vs. 75%)

Growth performance and family effect

In Marinard only 8 of the 26 families (14 Buteux and 12 Fraser) were retained and classified as Fast or Slow growers. At this station, all families classified as Fast growers were from the Buteux strain. Slow grower families from the week1 group were all from the Fraser strain whereas those from the week2 group were all from the Buteux strain (Table 8). In CAMGR, only 7 upon the 26 families were retained (Table 8). Four of these families (B12, F1, F2 and B14) have also been detected in Marinard and classified on the same way. Surprisingly, family B7 was classified as Slow grower in CAMGR while it was classified as Fast grower in Marinard. Two families from the Fraser strain were assigned as Fast grower in CAMGR whereas no families of this strain were assigned as Fast growers in Marinard (Table 8).

Growth performance and relatedness coefficient (R_C)

Using only families classified as Fast or Slow growers, a significant effect of the relatedness coefficient of the parents on growth performance was detected in both experimental stations (F-ratio= 18.63; df= 1; p= 0.0012). Families classified as Slow growers had higher R_C (Figure 4). Using all genotyped individuals without consideration of the family performance classification, a highly significant effect of the relatedness

coefficient of the parents on growth performance of the offspring was still observed in both experimental stations (F-ratio= 86.97; df= 1; p= 0.0000) with a small effect of the location. Fish from the upper tail exhibited lower relatedness coefficients than those from the lower tail (Figure 5). The effect was not as strong in CAMGR but was still highly significant (F-ratio= 28.14; df= 1; p= 0.0000).

Digestive and metabolic capacities and growth performance

Mean weight, hepato-somatic index (HSI) and pyloric caeca somatic index (PCI) for Fast and Slow growers of each group are presented in Table 9. No tank effect was detected for any group and no difference was detected between the Fast and Slow growers for mean weight, HSI and PCI.

Enzymatic activity in pyloric caeca

Difference in enzyme activities between Fast and Slow growers was only detected for CS and trypsin (Table 10). CS activities were higher in Slow fish of both strains of the Week1 hatching group (F-ratio= 6.699; df= 1; p= 0.0149). No difference was detected for this enzyme in any of the Week2 groups. In contrast, trypsin activities were higher in Fast fish of both strains of the Week2 hatching group (F-ratio= 32.566; df= 1; p= 0.00002) but no differences were detected in the Week1 groups.

Enzymatic activity in liver

In liver, CS activity was significantly higher in the Slow group of both strains of the Week2 group (F-ratio=7.73; df= 1; p=0.0087) (Table 10). The same tendency was observed in the Week1 group (F-ratio=3.962; df= 1; p= 0.0546). GDH activity was higher in the Slow

group of both strains of the Week2 group (F-ratio= 13.71; df= 1, p= 0.0007). No tendency was observed for AAT, PK or LDH in any of the groups.

Enzymatic activity in White muscle

In white muscle, differences in activities were detected in all enzymes tested (Table 10). CS, AAT and GDH all exhibited higher activities in Fast growing individuals in both strains of the Week1 group (F-ratio= 15.038; df= 1; p= 0.0005, F-ratio= 45.61; df= 1; p= 0.0000 and F-ratio= 29.94; df= 1; p= 0.0000 for CS, AAT and GDH respectively). The same results were observed in the Buteux strain of the Week2 group for CS and AAT and in both strains of the Week2 group for GDH. PK and LDH exhibited higher activities in Fast fish of both strains of the Week2 group (F-ratio= 6.680; df= 1; p= 0.0142 and F-ratio= 23.75; df= 1; p= 0.0000 for PK and LDH respectively).

Discussion

Growth rate

Linearity of the weight ($Weight^{1/3}$) in function of the time was high with coefficient of determination higher than 0.99. This suggests high accuracy of the growth model developed by Iwama and Tautz (1981) and allows accurate predictions concerning size gain, temperature and time needed to obtain a specific mean weight in arctic charr. The only variable to be estimated depending on the conditions is the growth coefficient Gc. Significant differences in growth coefficients were observed between CAMGR (mean Gc= 1.3994) and Marinard (Gc= 1.5058) suggesting an effect of experimental conditions on growth rate. Even when treatments were maintained as similar as possible between the

stations, some factors such as water characteristics or technician practices are difficult to control and could explain the growth rate variability observed. Growth coefficient (Gc) values varied from 1.3859 to 1.4205 for the research station (CAMGR) and from 1.4086 to 1.5534 for the commercial one (Marinard). These results agree with those estimated by Iwama (1996) where a mean Gc of 1.27 was estimated for arctic charr from 32 datasets with the highest Gc value (superior to 2.0) exhibited by a Harmmerfest strain ant the lowest (around 1.1) by a Nauyuk strain. Family composition of tanks did not appear to affect growth rate since Gc was similar among all tanks of a same place, Week2-B tank in Marinard being the only exception. Low Gc estimated in the Week2-B tank seems to be dictated by a tank effect what is a common phenomenon of unknown nature (Herbinger et al., 1999). In both stations, the Week2 groups displayed higher initial weights (Wi) at the beginning of weight measurements witch is quite surprising since fish of the Week2 groups hatched after fish from the Week1 groups. Fish from the Week2 groups must then have displayed a higher growth rate from hatching to the first weight measurement in April. Since incubation and tank rearing conditions were the same for both hatching groups, higher initial growth rate of the Week2 groups could be related to difference in egg quality or egg size. However, no differences in survival rates were detected at eyed stage or at hatching (results not shown). Egg maturation duration before fertilization should be investigated to highlight differences observed between these hatching groups.

Growth performance and genetics

70.7% of all genotyped fish were assigned to one family. This percentage was not enhanced by addition of other chosen microsatellites and is likely related to the low genetic

variability of both strains. Only eight and seven among the 26 possible families were categorized as Fast or Slow growers in Marinard and CAMGR respectively. All Fast families detected in Marinard were from the Buteux strain. In CAMGR, where both strains were grown separately, one Fraser strain family was categorized as Fast growers in both of the Week1 and Week2 group suggesting the possibility of aggressive behaviour of the Buteux strain against the Fraser strain when both strains were mixed (Marinard). Brown *et al.*, (1996) showed that growth rate was higher and that size variation was less important in full-sib arctic charr groups compared to mixed groups and proposed reduction in aggressive behaviour as a potential explanation. Effects of kinship on aggression were also shown in juvenile arctic charr (Olsén and Järvi, 1997). If family recognition can induce aggressive behaviour it is more likely to occur among mixed families of different strains. However, aggressive behaviour of the Buteux strain should result in a decrease of growth performance of individuals from the Fraser strain. This was the case in the Week1 group in Marinard where both families categorized as Slow growers were from the Fraser strain but not in the Week2 group where both families categorized as Slow growers were from the Buteux strain. Furthermore, aggression often results in a decrease of growth rate (Brown *et al.*, 1996; Olsén and Järvi, 1997) but it is not the case in Marinard where growth rate was higher than in CAMGR suggesting that better growth performance exhibited by the Buteux strain when both strains were mixed were more hazardous than dictated by an aggressive behaviour. Only one family (B7) was placed in opposite categories in the two stations (Fast growing in Marinard and Slow growing in CAMGR). A poor correlation between family growth performances in single tank and in mixed tank environment had already been

observed for Atlantic Salmon (Herbinger *et al.*, 1999). Authors suggested that growth performance differences may reflect environment differences among tanks rather than genetic differences among families. Difference in growth performance of B7 between Marinard and CAMGR could be explained similarly. However, four of the 8 and 7 families retained in Marinard and CAMGR respectively obtained similar ranking suggesting that their growth potential was dictated by genetic differences. All individuals from the B12 family were designated as Fast growers and families F1, F2 and B14 were classified as Slow growers in both places. Using all families retained as Fast or Slow growers, a strong effect of the relatedness coefficient (R_C) was observed in Marinard and in CAMGR ($F= 18.63$; $p= 0.0012$). Except for family B7 which was not placed in the same category in both stations, all Fast families had negative R_C suggesting low relatedness among the parents whereas all Slow families had R_C higher than 0.14 suggesting high relatedness among parents. Furthermore consistency of this result was shown using all uniquely assigned offspring. A significant effect of the relatedness coefficient of the parents on growth potential of the offspring was again shown (F-ratio= 28.14; $p= 0.0000$) suggesting the high usefulness of R_C in estimation of growth potential of families in these strains. This is in accordance with results of Herbinger *et al.* (1995) where an indication of depressed performances due to inbred crosses was found. These results are of primary relevance for commercial growers since it could allow them to enhance their production using only breeder's information. It could allow family selection without the need of numerous genotyping. Furthermore, by mating less related individuals, producers will reduce risks of inbreeding and will maintain genetic variability.

Growth performance and digestive and metabolic capacities

No significant differences were detected for pyloric caeca somatic index (PCI) or hepatosomatic index (HSI) between the Fast and Slow groups after the latter were allowed to catch up in growth. Difference in enzyme activities between Fast and Slow groups was detected for CS and trypsin in pyloric caeca, CS and GDH in liver and CS, AAT, GDH, PK and LDH in white muscle. Trypsin and all enzymes investigated in white muscle were higher for the Fast growers whereas CS in pyloric caeca and CS and GDH assayed in liver were higher for the Slow growers. Statistical significant differences between Fast and Slow groups seems to be affected by either hatching period or strain but trends observed in both (hatching period and strain) were always in the same direction. Stevens *et al.* (1999) have shown an important increase in pyloric caeca size in GH-transgenic coho salmon (*Salmo salar* L.) displaying a high growth rate compared to non transgenic fish. Plasticity of pyloric caeca was also found in Atlantic cod (*Gadus morhua*) in response to food restriction (Bélanger *et al.*, 2002). Without any treatment, no difference in relative pyloric caeca mass was detected in any of the groups, suggesting that difference observed in both studies was induced by the treatment rather than by changes in growth by itself. In our study, growth performance seemed to be more dependent on the digestive capacities through trypsin activities. Trypsin is a major enzyme involved in protein digestion in fish (Üeberschar, 1988) since it acts directly and indirectly through chymotrypsin activation in protein digestion (Horton *et al.*, 1994). In this study, trypsin activities were higher in Fast growers suggesting difference in protein digestion capacities as previously observed in cod

Gadus morhua by Lemieux *et al.* (1999) who showed a correlation of trypsin activities with growth rate but also with food conversion efficiency.

CS activities in the pyloric caeca and in the liver as well as GDH in the liver were higher in Slow growers. This suggests that slow growers have higher metabolic rates, as found by Lemieux *et al.* (2003) in juvenile stages of arctic charr. By contrast, all enzymes investigated in white muscle had higher activities in Fast growers. Comparison with results obtained by Lemieux *et al.* (2003) is difficult since these authors assessed enzyme activities in whole larvae whereas we measured tissue specific activities. Liver is known to have a high metabolism and protein turnover. In our study, fish with the lowest growth performance displayed higher GDH and CS activities than those with the highest performance. The glutamate dehydrogenase enzyme (GDH) can be associated to anabolism or catabolism: it catalyzes both the reversible conversion of ammonium nitrogen into organic nitrogen (glutamate production) and the oxidative deamination of glutamate, resulting in 2-oxoglutarate (Timmerman *et al.*, 2002). Dobly *et al.* (2004) have shown that rainbow trout (*Onchorynchus mykiss*) that displayed poor growth efficiency also had higher rates of protein turnover. In parallel they showed that proteolytic enzyme activities were lower in fish with high specific growth rates. Our results agree with this previous study if we consider the catabolic function of GDH in the liver. Fish may compensate the lower nutrients supply trough digestion by increasing protein degradation in the liver. In white muscle, higher GDH and AAT activities in Fast growing fish may be explained by their higher capacity to supply amino acids for growth trough protein deposition. Both protein synthesis and protein degradation are energetically demanding processes and have to be

sustained by energetic metabolism (Houlihan *et al.*, 1995). This may partly explain the parallel higher CS activity in both tissues.

Glycolytic enzyme (PK and LDH) activities in white muscle were higher in Fast growing fish than in Slow growing fish. Such results have been shown in various studies on Atlantic cod (*Gadus morhua*) (Pelletier *et al.*, 1993a,b; Pelletier *et al.*, 1994; Pelletier *et al.*, 1995; Couture *et al.*, 1998). LDH is involved in anaerobic glycolysis and has been used as an index of fish capacity for burst swimming (Somero and Childress, 1980). The increase of muscle glycolytic enzyme activities with growth rate were suggested to reflect an autocatalytic process since the greater burst swimming capacity should enhance locomotion capacity of fish (Pelletier *et al.*, 1994). Burst swimming capacities can affect fitness of predatory fish like salmonids. Low burst swimming capacities could limit predator avoidance or prey capture capacities and would have negative consequences on life time and/or growth. High glycolytic enzyme activities in white muscle could have a positive adaptive value in wild environment when prey abundances are low or where fish predators occur but also in captivity where access to food is limited and where aggressive behaviours are observed.

Conclusion

The use of microsatellite markers allowed us to highlight the usefulness of relatedness coefficients for predicting familial growth performance in two strains of arctic charr. Using this information, producers will be able to enhance growth performance and also to avoid inbreeding since families with the highest performance were the most unrelated ones. The physiological approach allowed us to establish relationships between digestive and

metabolic capacities and growth performance. Our results are relevant in a growth selection context since selection of Fast growing fish will favor the selection of individuals with the highest digestive and metabolic capacities.

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Table 2. Allele frequencies at the four loci used for assignment for the overall breeder population, and for each strain depending on hatching date.

Locus	***Alleles (bp)***	***Overall frequency***	***Week1-Fraser***	***Week1-Buteux***	***Week2-Fraser***	***Week2-Buteux***
Sco-19	*179*	0.010	0.000	0.036	0.000	0.000
	191	0.077	0.125	0.143	0.042	0.000
	193	0.173	0.083	0.250	0.167	0.179
	209	0.010	0.000	0.000	0.000	0.036
	215	0.240	0.292	0.321	0.167	0.179
	223	0.010	0.000	0.000	0.000	0.036
	231	0.010	0.000	0.000	0.042	0.000
	235	0.279	0.417	0.214	0.417	0.464
	239	0.077	0.083	0.000	0.125	0.107
	245	0.019	0.000	0.036	0.042	0.000
Sfo-23	*175*	0.240	0.208	0.250	0.208	0.286
	181	0.019	0.000	0.036	0.000	0.036
	183	0.317	0.458	0.286	0.375	0.179
	201	0.260	0.292	0.214	0.333	0.214
	205	0.010	0.000	0.214	0.083	0.036
	209	0.154	0.042	0.000	0.000	0.250
Sfo-8	*255*	0.548	0.000	0.000	0.000	0.036
	277	0.279	0.000	0.000	0.000	0.036
	281	0.010	0.000	0.000	0.042	0.036
	285	0.163	0.208	0.214	0.333	0.179
	289	0.010	0.750	0.679	0.583	0.714
	297	0.010	0.042	0.107	0.042	0.000
Mst-85	*205*	0.019	0.583	0.571	0.542	0.500
	207	0.231	0.292	0.357	0.167	0.286
	209	0.683	0.000	0.000	0.000	0.036
	211	0.048	0.125	0.071	0.292	0.179

Table 3. Enzymes investigated in each tissue. CS= citrate synthase; AAT= Aspartate aminotransferase, GDH= Glutamate dehydrogenase; PK= Pyruvate kinase and LDH= Lactate dehydrogenase.

Enzymes	Pyloric caeca	Liver	White muscle
Metabolic enzymes	CS	CS	CS
	AAT	AAT	AAT
	GDH	GDH	GDH
	PK	PK	PK
	LDH	LDH	LDH
Digestive enzyme	Trypsin		

Table 4- Substrates and pH used for enzyme analysis

Enzyme	Substrate	pH	Reference
CS	Oxaloacetate (0.15 mM)	8.0	Thibault *et al.*, 1997
AAT	Aspartate (22 mM)	7.4	Pelletier *et al.*, 1994
GDH	A-ketoglutaric acid (14mM)	8.5	Pelletier *et al.*, 1994
PK	Phosphoenolpyruvate (5 mM)	7.4	Pelletier *et al.*, 1994
LDH	Pyruvate (0.4 mM)	7.0	Thibault *et al.*, 1997
Trypsin	Benzoyl-L-arginine-p-nitroanilide	8.4	Prieser et al., 1975; Torrissen *et al.*, 1994

Table 5- Growth information's summary showing growth coefficients (Gc), cube root of initial weight ($Wi^{1/3}$) and coefficient of determination of growth (r^2) for each tank in each experimental location. A.S.E corresponds to Asymptotic Standard Error.

Experimental location	**Tank**	**$Wi^{1/3}$ (A.S.E)**	**Gc (A.S.E)**	**r^2**
Research station	Week1-FraserA	0.4093 (0.0174)	1.4205 (0.0116)	0.996
	Week1-FraserB	0.4846 (0.0170)	1.3859 (0.0113)	0.996
	Week1-Fraser	**0.4469 (0.0122)**	**1.4032 (0.0082)**	**0.996**
	Week1-ButeuxA	0.4330 (0.0170)	1.4058 (0.0113)	0.996
	Week1-ButeuxB	0.4482 (0.0161)	1.3935 (0.0107)	0.996
	Week1-Buteux	**0.4406 (0.0117)**	**1.4000 (0.0078)**	**0.996**
	Week2-FraserA	0.5200 (0.0130)	1.3922 (0.0090)	0.997
	Week2-FraserB	0.5054 (0.0140)	1.3872 (0.0096)	0.997
	Week2-Fraser	**0.5126 (0.0096)**	**1.3897 (0.0066)**	**0.997**
	Week2-ButeuxA	0.5586 (0.0169)	1.4076 (0.0116)	0.996
	Week2-ButeuxB	0.5051 (0.0166)	1.4021 (0.0114)	0.996
	Week2-Buteux	**0.5318 (0.0120)**	**1.4049 (0.0083)**	**0.996**
Commercial station	Week1-A	0.4331 (0.0216)	1.5534 (0.0155)	0.991
	Week1-B	0.4481 (0.0236)	1.5163 (0.0169)	0.989
	Week1	**0.4406 (0.0160)**	**1.5349 (0.0115)**	**0.990**
	Week2-A	0.6051 (0.0221)	1.5414 (0.0163)	0.990
	Week2-B	0.5442 (0.0232)	1.4119 (0.0172)	0.988
	Week2	**0.5743 (0.0175)**	**1.4768 (0.0129)**	**0.987**

* Bolded data correspond to results obtained by pooling duplicate tank values.

Table 6 - Mean weight in grams of the upper (W_{Fast}) and lower (W_{Slow}) tails for each tank in each experimental location. SD corresponds to standard deviation.

Experimental location	Tank	W_{FAST} ±SD	W_{SLOW} ±SD
Research station	Week1-FraserA	109.7 ±12.5	42.9 ±13.4
	Week1-FraserB	113.8 ±13.3	43.7 ±12.5
	Week1-Fraser	**111.7 ±12.7**	**43.3 ±12.6**
	Week1-ButeuxA	135.8 ±51.6	35.5 ±13.7
	Week1-ButeuxB	104 ±14.7	47.6 ±12.4
	Week1-Buteux	**119.9 ±40.4**	**41.6 ±14.2**
	Week2-FraserA	111.8 ±15.5	49.4 ±16.6
	Week2-FraserB	99.4 ±17.7	40.5 ±15.9
	Week2-Fraser	**105.6 ±17.4**	**45.0 ±16.4**
	Week2-ButeuxA	111.6 ±19.8	42.9 ±11.4
	Week2-ButeuxB	125.9 ±28.4	39.4 ±9.6
	Week2-Buteux	**118.7 ±24.9**	**41.1 ±10.4**
Commercial station	Week1-A	203.7 ±26.2	74.7 ±18.3
	Week1-B	215 ±30.9	69.6 ±19.5
	Week1	**209.4 ±29.0**	**72.1 ±19.0**
	Week2-A	189.1 ±35.2	71.1 ±20.1
	Week2-B	185.1 ±32.4	68.2 ±20.3
	Week2	**187.1 ±33.7**	**69.7 ±20.2**

Table 7- Relatedness coefficient (R_C) estimated for each pair of breeders used to produce families of the Fraser and Buteux strains.

	Fraser		***Buteux***	
Hatching group	***Family***	***RC***	***Family***	***RC***
Week1	F1	0.295	B1	-0.274
	F2	0.144	B2	-0.040
	F3	0.066	B3	0.169
	F4	-0.531	B4	-0.324
	F5	-0.607	B5	-0.070
	F6	0.123	B6	0.389
Week2	F7	0.364	B7	-0.186
	F8	-0.387	B8	0.360
	F9	0.197	B9	-0.174
	F10	-0.285	B10	0.050
	F11	-0.514	B11	0.272
	F12	-0.116	B12	-0.415
			B13	0.370
			B14	0.286

Table 8- Families classified as Fast or Slow growers for each group in each experimental location.

Experimental location	Group	Fast growers	Slow growers
Research station	Week1-Fraser	F4	F1 F2
	Week1-Buteux	none	B7
	Week2-Fraser	F8	none
	Week2-Buteux	B12	B14
Commercial station	Week-1	B1 B2 B7	F1 F2
	Week-2	B12	B11 B14

Table 9: Sample size (N), Weight, Lenght, Pyloric caeca somatic (PCI) and Hepatosomatic (HSI) indexes (mean ± standard deviation) of Fast and Slow growers for each group.

Group	Week1-Fraser		Week1-Buteux		Week2-Fraser		Week2-Buteux	
Sampling date	January	April	January	April	January	April	January	April
Growers' category	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
N	10	10	10	9	10	10	10	9
Weight (in grams)	121.37 ±10.60	125.8 ±8.26	121.81 ±14.52	128.1 ±21.50	119.02 ±8.58	126.04 ±9.50	135.85 ±24.47	133.2 ±12.26
Length (in cm)	22.57 ±0.81	23.43 ±1.43	22.37 ±0.84	28.43 ±1.42	22.60 ±0.55	22.77 ±1.31	23.77 ±1.22	24.13 ±0.89
PCI	0.0314 ±0.0034	0.0315 ±0.0065	0.0287 ±0.0030	0.0327 ±0.0038	0.0275 ±0.0019	0.0245 ±0.0068	0.0269 ±0.0044	0.0299 ±0.0062
HSI	0.0179 ±0.0017	0.0183 ±0.0024	0.0172 ±0.0014	0.0196 ±0.0026	0.0184 ±0.0014	0.0167 ±0.0035	0.0155 ±0.0053	0.0172 ±0.0032

Table 10- Enzyme activities in Units per gram of tissue (mean ± SD) for each hatching group and each strain

		Week1-Fraser		Week1-Buteux		Week2-Fraser		Week2-Buteux	
Tissue	Enzyme	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
Pyloric caeca	CS	**4.081 ±1.356**	**4.977 ±1.071**	**4.493 ±0.649**	**5.434 ±1.18**	5.411 ±1.002	4.591 ±1.572	5.205 ±1.158	5.249 ±1.543
	AAT	7.079 ±0.756	7.013 ±1.501	7.334 ±0.403	7.951 ±0.837	8.084 ±1.532	7.988 ±1.444	7.417 ±0.921	8.225 ±0.567
	Trypsin	4.254 ±0.986	4.658 ±1.761	4.908 ±0.827	3.901 ±1.032	**7.929 ±1.899**	**4.901 ±1.161**	**6.276 ±1.642**	**3.998 ±0.753**
Liver	CS	4.815 ±0.893	5.251 ±1.082	4.347 ±0.778	5.016 ±0.717	**4.644 ±0.61**	**5.504 ±0.97**	**4.532 ±0.806**	**5.023 ±0.777**
	AAT	56.01 ±7.95	55.38 ±4.33	58.35 ±11.75	53.08 ±7.38	54.38 ±6.28	55.50 ±6.76	53.02 ±8.42	56.29 ±4.84
	GDH	76.79 ±11.92	82.59 ±12.52	74.15 ±9.16	72.71 ±8.60	**76.64 ±9.19**	**88.09 ±11.20**	**71.75 ±14.39**	**89.47 ±13.93**
	PK	44.01 ±12.88	43.93 ±13.69	45.46 ±10.80	41.72 ±4.32	45.73 ±8.39	45.64 ±6.76	51.23 ±16.90	48.41 ±15.79
	LDH	234.0 ±25.3	227.2 ±37.3	222.7 ±35.5	218.6 ±24.0	229.9 ±26.7	251.9 ±17.2	227.5 ±19.5	224.5 ±27.7
White Muscle	CS	**8.135 ±1.949**	**5.740 ±1.577**	**7.289 ±1.44**	**5.656 ±1.401**	6.466 ±0.909	6.589 ±1.911	**7.298 ±0.736**	**5.284 ±0.593**
	AAT	**24.21 ±2.91**	**16.42 ±2.32**	**22.65 ±3.24**	**17.36 ±3.02**	21.72 ±2.37	22.99 ±7.32	**24.18 ±2.33**	**17.192 ±1.20**
	GDH	**5.106 ±0.888**	**3.668 ±0.626**	**5.217 ±0.819**	**3.61 ±1.014**	4.809 ±0.472	4.297 ±0.670	**5.024 ±0.817**	**3.525 ±0.444**
	PK	408.9 ±67.8	367.6 ±138.9	403.7 ±72.1	344.0 ±67.5	**371.5 ±133.1**	**354.6 ±130.9**	**517.2 ±66.5**	**386.4 ±72.2**
	LDH	890.1 ±96.3	853.4 ±157.2	828.3 ±126.8	724.1 ±105.8	**861.2 ±141.3**	**698.1 ±138.9**	**1002.9 ±124.0**	**794.6 ±102.3**

* Bolded data correspond to enzymes activities where significant differences were detected between the Fast and Slow group.

Figure caption

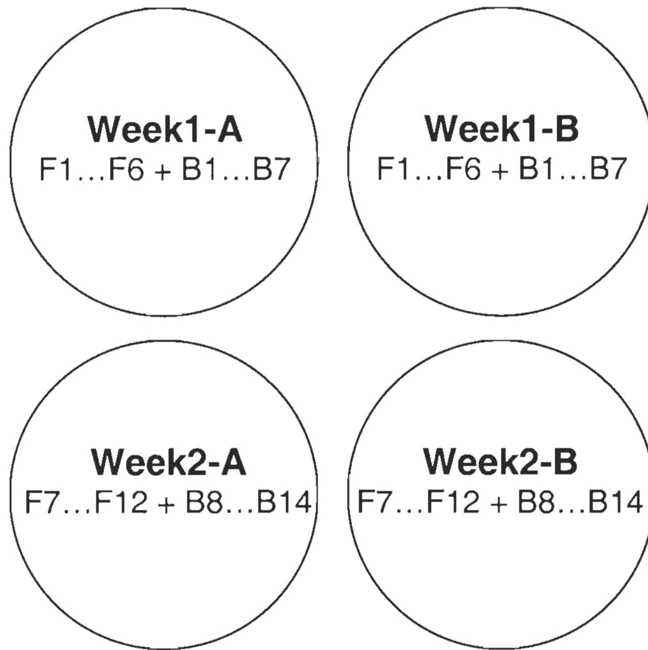
Figure 3- Experimental design of commercial and research facilities (F1...F12 and B1...B14 are Fraser and Buteux families respectively

Figure 4- Relatedness coefficients (RC) of families classified as fast or slow growers in each experimental stations.

Figure 5- Relatedness coefficients (RC) of individuals from the upper and from the lower tails in each experimental place.

Figure 3- Experimental design at commercial and research facilities (F1…F12 and B1…B14 are Fraser and Buteux families respectively

A- Commercial station



B-Research station

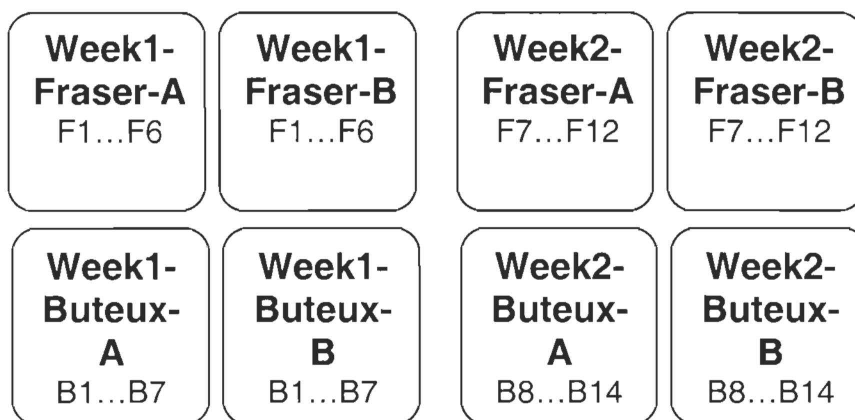


Figure 4- Relatedness coefficients (R_C) of families classified as fast or slow growers in each experimental stations.

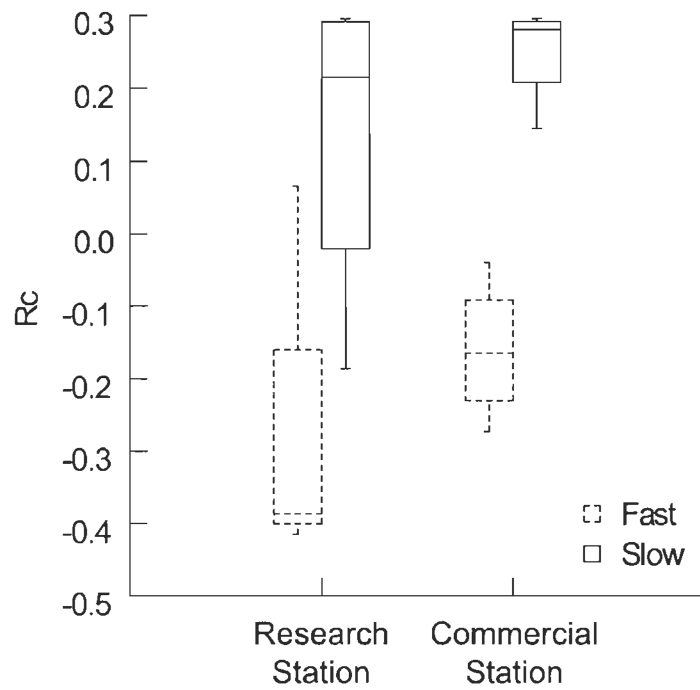
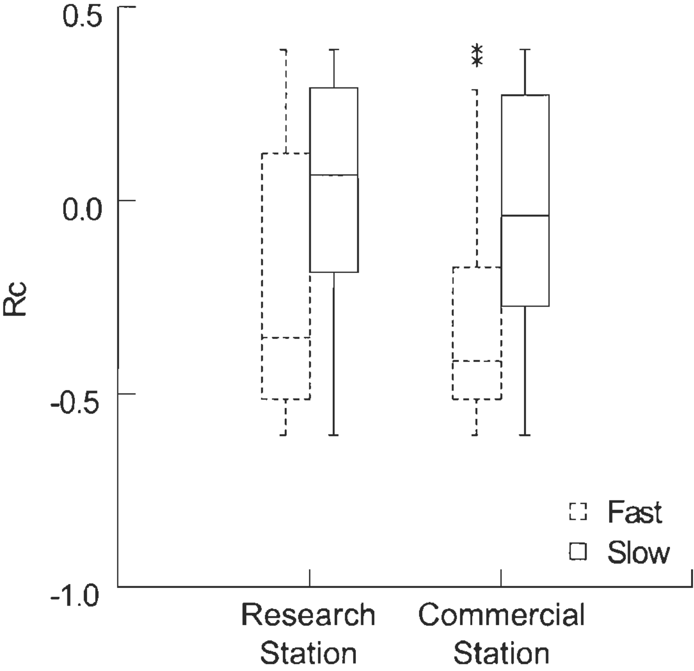


Figure 5- Relatedness coefficients (R_C) of individuals from the upper and from the lower tails in each experimental place.



CONCLUSION GÉNÉRALE

Trois hypothèses ont été proposées au début de ce projet. **La première hypothèse** était que la variabilité génétique des souches commerciales d'ombles chevalier Buteux et Fraser devait être inférieure à celle estimée pour des populations sauvages mais du même ordre que celle estimée pour d'autres populations commerciales canadiennes du fait de leur origine commune. **La deuxième hypothèse** était que le degré de parenté des géniteurs pouvait servir d'indicateur du potentiel de croissance de leur famille respective, les familles les plus performantes devant exhiber des degrés de parenté moindres. Finalement, **la troisième hypothèse** était que les performances de croissances devaient être liées à des voies physiologiques quantifiables au niveau d'activités enzymatiques.

En regard des résultats obtenus, la première hypothèse a été réfutée (Chapitre I). En effet les souches Buteux et Fraser présentent une variabilité génétique inférieure à celles de populations sauvages mais également à celles d'autres populations aquacoles. La souche Fraser présente la variabilité la plus réduite et est plus consanguine que la Buteux. Les marqueurs microsatellites s'avèrent être des outils efficaces dans l'estimation du degré de parenté. L'augmentation de la variabilité génétique et l'utilisation des microsatellites sont donc fortement recommandés dans un programme de sélection basé sur ces souches afin d'éviter l'apparition rapide d'effets néfastes dus à une consanguinité trop importante.

Le protocole expérimental de la deuxième partie de cette étude (Chapitre II) était complexe et limité par des contraintes logistiques (nombre et volume des bassins). Ces contraintes ont augmenté la difficulté d'analyse des résultats. Le potentiel de croissance de nombreuses

familles n'a pu être déterminé. Un nombre plus important de poissons par bassin aurait permis d'augmenter le nombre de poissons présentant des extrêmes de taille et le potentiel de croissance de plus de familles aurait pu être déterminé. Malgré cela, des différences significatives au niveau de la croissance ont été détectées soulignant leur importance.

La deuxième hypothèse a pu être validée pour les deux souches utilisées (Chapitre II). En effet, à l'exception d'une famille dont les performances de croissance semblent varier avec les conditions environnementales, toutes les familles à fort potentiel de croissance ont un coefficient de parenté très bas (représentatif des familles à faible degrés de parenté) alors que toutes les familles à faible potentiel de croissance présentent un coefficient de parenté très fort (représentatif des familles à degrés de parenté élevés). Ces résultats sont d'une grande pertinence dans un contexte aquacole puisque les coefficients de parenté pourraient permettre de sélectionner des familles sans avoir à suivre la croissance de leur progéniture. De plus, en croisant les individus les plus éloignés génétiquement, le producteur diminue les risques de consanguinité. Néanmoins, la pertinence de ces coefficients a été révélée au sein de ces deux souches mais devrait être validée chez d'autres populations aquacoles présentant une variabilité génétique plus forte. En effet, les souches Buteux et Fraser ont toutes les deux une variabilité génétique réduite par rapport à d'autres populations aquacoles (Chapitre I). Il se pourrait que le taux de consanguinité soit déjà suffisamment important pour avoir des effets négatifs sur les performances de croissance. Dans ce cas tout effort effectué pour croiser les individus plus éloignés génétiquement (en utilisant le coefficient de parenté par exemple) pourrait se caractériser par un effet positif sur la

croissance. Le lien observé dans cette étude entre le coefficient de parenté et les performances de croissance ne sera peut-être pas aussi marqué chez d'autres populations.

La troisième hypothèse a pu être en partie testée et attestée (Chapitre II). Le lien entre les capacités digestives et métaboliques et les performances de croissance n'est pas toujours clairement défini dépendamment de la souche ou du groupe d'éclosion mais les tendances et les différences observées vont toujours dans le même sens. Les individus les plus performants présentent des activités de trypsine supérieures au niveau des caeca pyloriques suggérant leur plus forte capacité digestive. L'activité métabolique du foie est généralement plus forte chez les individus peu performants alors que l'activité métabolique du muscle blanc est supérieure chez les individus les plus performants suggérant un rôle distinct de ces deux tissus au niveau de la croissance. Une étude a déjà démontré une activité protéolytique supérieure dans le foie de truites arc-en-ciel présentant un faible taux de croissance (Dobly *et al.*, 2004). L'activité métabolique supérieure dans le foie des poissons les moins performants de notre étude pourrait donc refléter une activité catabolique supérieure au niveau de ce tissu. En augmentant leur catabolisme, les individus à faible taux de croissance pourraient compenser le faible apport en nutriments (nécessaire à la croissance) dû à leur capacité plus faible de digérer les protéines. La mesure de l'activité de protéases telles que le protéasome 20S chez des individus à faible et à fort potentiel de croissance en parallèle à la mesure des enzymes métaboliques détectées dans cette étude dans le foie permettrait de vérifier si le métabolisme observé est bien lié à l'activité catabolique du foie.

L'activité supérieure des enzymes impliquées dans le métabolisme des acides aminés (AAT et GDH) dans le muscle blanc des individus performants suggère leur plus grande capacité de synthèse protéique ou d'utiliser les acides aminés pour la glycolyse, l'oxydation ou la néoglucogenèse. L'estimation du taux de synthèse protéique dans le muscle blanc permettrait de vérifier cette hypothèse. Une activité glycolytique (PK et LDH) supérieure dans le muscle blanc des poissons les plus performants a déjà été observée chez la morue. Une valeur adaptative a été proposée pour expliquer ces observations. Les individus ayant un métabolisme supérieur au niveau du muscle blanc sont susceptibles d'être plus rapides favorisant leur accès à la nourriture ou favorisant leur survie par une meilleure capacité d'évitement des prédateurs. C'est donc le potentiel de compétitivité des poissons qui serait mesuré à travers l'activité de ces enzymes.

La sélection des individus les plus performants s'accompagnerait de la sélection des individus présentant les meilleures capacités digestives et métaboliques ce qui présente un grand intérêt dans un contexte aquacole. En revanche il reste à vérifier si la même corrélation positive que celle observée chez la morue par Lemieux *et al.* (1999) s'observe entre les capacités digestives et l'efficacité de conversion des aliments ainsi que l'héritabilité de ces capacités.

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