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I. CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Le système immunitaire

Le système immunitaire assure la protection de l'organisme par discrimination entre le soi et le non-soi (Bensa, 2003). La pénétration de microorganismes (antigènes) induit une réponse immunitaire adaptative, qui met en jeu deux types de réponses: des réactions non spécifiques et des réactions spécifiques.

D'action rapide, l'immunité innée constitue la première ligne de défense contre les infections, capable de limiter la prolifération des agents pathogènes dans l'organisme. Cette réponse non spécifique s'exerce de la même façon quel que soit l'antigène. Une réaction inflammatoire située au niveau du foyer infectieux, attire sur place les cellules phagocytaires (Köllner *et al.*, 2002). Les macrophages, cellules présentatrices d'antigènes, phagocytent l'agent pathogène et dégradent ses macromolécules antigéniques en peptides par digestion dans le phagolysosome. C'est cette forme modifiée de l'antigène qui, associée aux molécules du complexe majeur d'histocompatibilité (CMH), génère les ligands spécifiques des récepteurs antigéniques des lymphocytes T, capables de déclencher une réaction immunitaire spécifique (Janeway, 2001). En effet, si l'immunité innée n'est pas suffisante, les macrophages interagissent avec les lymphocytes afin d'activer l'immunité acquise, qui est spécifique et donc plus efficace. Elle se manifeste sous deux formes : l'immunité à médiation humorale qui fait intervenir les lymphocytes B, responsable de la

production d'anticorps et l'immunité à médiation cellulaire qui dépend de la reconnaissance spécifique de l'antigène par les lymphocytes T (Nakanishi *et al.*, 1999).

1.2 Le complexe majeur d'histocompatibilité

Les molécules du complexe majeur d'histocompatibilité (CMH) sont impliquées dans de nombreux aspects de la reconnaissance immunitaire (Bernatchez et Landry, 2003). Contrairement aux cellules B qui lient directement l'antigène, les cellules T ne reconnaissent l'antigène qu'en association avec les molécules du CMH de classes I et II. Ces deux classes de glycoprotéines membranaires de structure très similaire, mais de fonction distincte, ont toutes deux un rôle crucial à jouer dans l'apprêtement des peptides antigéniques aux cellules T (Grimholt *et al.*, 2000).

Les molécules de classe I, exprimées à la surface de pratiquement toutes les cellules nucléées, présentent des antigènes peptidiques endogènes (intracellulaires) aux cellules T_C (cytotoxiques). S'il s'agit d'un peptide étranger, un lymphocyte T cytotoxique (CD8+) se fixera au complexe CMH-peptide et finira par tuer la cellule infectée. Dans le cas des molécules de classe II, exprimées essentiellement sur les cellules présentatrices d'antigènes (macrophages, cellules dendritiques et cellules B), les peptides antigéniques exogènes (extracellulaires) sont présentés aux cellules T_H (auxiliaires) (Trevor, 1996). Les lymphocytes T auxiliaires (CD4+) ne tuent pas directement leurs cellules cibles, ils se divisent dans un premier temps, augmentant ainsi leur nombre, puis sécrètent des cytokines.

Celles-ci ont un rôle de régulation des phénomènes immunitaires; soit elles inhibent l'antigène, soit recrutent et stimulent d'autres cellules qui se joignent à la réponse immunitaire. Les molécules de classe II sont nécessaires pour que les cellules T communiquent avec les macrophages et les cellules B (Bernatchez et Landry, 2003).

Les molécules du CMH de classe II sont composées de domaines externes, d'un segment transmembranaire et d'un segment d'ancrage cytoplasmique. Ces glycoprotéines membranaires forment de façon non covalente un hétérodimère de chaînes α et β, respectivement de masse moléculaire 34 et 28 kDA . Chaque chaîne est composée de deux domaines extracellulaires ($\alpha 1$ et $\alpha 2$; $\beta 1$ et $\beta 2$). Les domaines $\alpha 2$ et $\beta 2$ proches de la membrane (proximaux), présentent une homologie de séquence avec la structure du repliement immunoglobulinique; c'est pour cette raison que les molécules de classe II du CMH sont classées dans la superfamille des immunoglobulines (Sültmann *et al.*, 1994). Le polymorphisme de ces molécules est principalement situé dans les domaines $\alpha 1$ et $\beta 1$, éloignés de la membrane (distaux), qui forment la cavité de liaison au peptide. Cette variation structurale représente la base fondamentale permettant la spécificité antigénique (Bouillon et Mourad, 2003).

Les gènes du CMH font partie des gènes les plus polymorphiques connus jusqu'ici, avec de multiple loci et un nombre considérable d'allèles pour chaque locus donné (Grimholt *et al.*, 2003). Cette diversité augmente le nombre de peptides antigéniques pouvant être reconnus, ce qui s'avère avantageux pour l'espèce. Différentes études ont

montré que les gènes de classes I et II sont situés sur des groupes de liaison génétique différents et que le saumon Atlantique possède un seul locus codant pour le CMH de classe I et un seul codant pour le CMH de classe II (Grimholt *et al.*, 2002; Stet *et al.*, 2002). Les gènes de classe II sont organisés en une série d'exons et d'introns qui reproduisent la structure en domaines des chaînes α et β. Notre étude porte sur le domaine β1, correspondant à la région la plus polymorphique, codée par l'exon 2 (Ono *et al.*, 1993).

1.3 Les maladies bactériennes en aquaculture

De nombreuses maladies bactériennes sont liées à des facteurs qui stressent ou affaiblissent les poissons, les rendant ainsi moins résistants aux infections. Elles peuvent se classer en deux catégories, celles qui affectent des zones spécifiques du corps (infections localisées) et celles qui affectent le corps en entier (infections systémiques). De nombreuses pertes causées par les infections bactériennes systémiques ont été répertoriées dans les élevages de salmonidés à travers le monde (Avendaño-Herrera *et al.*, 2005). La furonculose (agent pathogène *Aeromonas salmonicida*), la maladie de la bouche rouge (agent pathogène *Yersinia ruckeri*), et la maladie bactérienne du rein (agent pathogène *Renibacterium salmoninarum*) sont identifiées à la fois en eau douce et marine. La vibriose (agent pathogène *Vibrio anguillarum*), et la vibriose en eau froide (*Vibrio salomonicida*) touchent uniquement les élevages en eau de mer contrairement à l'aéromonose (agent pathogène *Aeromonas hydrofila*) qui n'apparaît qu'en eau douce (Trevor, 1996). De nombreux agents pathogènes (*Cytophaga* sp, *Flavobacterium* sp et *Flexibacter* sp) appartenant anciennement

au genre *Flavobacterium-Cytophaga* sont regroupés dans le phylum *Cytophaga-Flavobacterium-Bacteroides* (Bernadet, 1997) et causent également des infections systémiques à la fois chez des poissons cultivés en eau douce ou chez ceux produits en eau marine. L'espèce *Flexibacter maritimus* a été récemment reclassée dans le genre *Tenacibaculum*, appartenant à la famille des *Flavobacteriaceae* (Suzuki *et al.*, 2001).

1.4 Le stress et la résistance aux maladies

Les poissons d'élevage sont soumis à des situations de stress répétés qui peuvent avoir des conséquences négatives plus ou moins importantes sur la croissance, la reproduction ou la résistance aux agents pathogènes (revue de Pickering, 1992). Le stress et la résistance aux maladies sont liés, mais les mécanismes de cette relation sont extrêmement complexes (Ellis, 1981) et jusqu'à récemment peu connus. Des paramètres de tolérance aux stress ont été trouvés et associés avec la résistance des poissons aux maladies (Fevolden *et al.*, 1991; 1993; 1994; 1999). On parlera de résistance pour la réaction à une modification brusque et intense, et de tolérance pour l'adaptation à des modifications durables des caractéristiques du milieu (Vandeputte et Prunet, 2002). Le cortisol est considéré comme étant un bon indicateur de stress chez le poisson (Wendelaar Bonga, 1997). Sa libération est à court terme favorable pour le poisson (mobilisation de l'énergie, rétablissement de l'homéostasie...) mais a par contre des effets négatifs sur différentes fonctions physiologiques (reproduction, croissance, immunité...) lorsqu'elle se maintient dans le temps (stress chroniques ou répétés) (Pottinger et Pickering, 1997).

1.5 Problématique et objectifs

L'émergence de nouvelles filières concernant le développement d'une production d'ombles chevalier (*Salvelinus alpinus*) et d'ombles de fontaine (*Salvelinus fontinalis*) en eau salée, se heurte à un problème de santé. De nombreux travaux effectués sur les ombles anadromes à la station aquicole de Pointe-au-Père ont montré que différents stress pouvaient provoquer des lésions de types opportunistes (localisées sur les branchies, les flancs et la queue) chez les animaux élevés en eau de mer (Audet, communication personnelle). Les analyses bactériologiques effectuées par le MAPAQ indiquaient des infections causées par des *myxobactéries*. Toutefois, une analyse plus poussée s'avère nécessaire puisqu'à l'été 2004, les résultats d'identification des pathogènes étaient différents de ceux obtenus les années précédentes pour des symptômes similaires. De plus, ces lésions n'affectent pas les différentes familles de la même façon, certaines sont peu ou pas touchées alors que d'autres peuvent être décimées (Bastien, communication personnelle). Il semblerait donc qu'il existe une relation entre les caractéristiques génétiques et la résistance.

Une étude portant sur le saumon Atlantique a permis d'établir pour la première fois une corrélation entre la variation allélique du complexe majeur d'histocompatibilité de classe II et la résistance à un agent pathogène bactérien *Aeromonas salmonicida* (Langefors *et al.*, 2001). L'association entre la résistance aux maladies infectieuses, de nature virale et bactérienne, et le polymorphisme du CMH classes I et II chez le saumon Atlantique a été confirmée (Grimholt *et al.*, 2003). D'Autray Tarte (2004) a étudié si certains allèles du

CMH classe II, domaine β1 chez l'omble de fontaine (*Salvelinus fontinalis*) (souche Rupert) étaient liés à une résistance accrue face à une infection de furonculose causée par l'agent pathogène *Aeromonas salmonicidae*. Cinq allèles (A, B, D, E et F) provenant de 62 géniteurs ont été répertoriés et ont permis d'effectuer des croisements générant 24 familles de génotype différents. Cependant, aucune famille n'avait pu être constituée à partir des allèles E et F car les géniteurs porteurs de ces allèles n'étaient pas prêts pendant la période de frai (d'Autray Tarte, 2004). Il semblerait qu'une corrélation soit observable entre le nombre de copies de l'allèle A et le temps accru de survie post infection. Cette corrélation se retrouverait également avec l'allèle B mais la résistance serait moindre que celle procurée par l'allèle A (d'Autray Tarte, 2004). Au cours de son projet, d'Autray Tarte avait également répertorié quatre allèles (P, H, I et L) du CMH classe II, domaine β1 provenant de 68 géniteurs d'ombles de fontaine (*Salvelinus fontinalis*) de la souche Laval. L'étude de Nassif (2006) visait le même objectif mais pour les allèles du CMH classe I, domaines α1 et α2. À partir des 24 familles d'ombles de fontaine (souche Rupert) générées par d'Autray Tarte, deux allèles (A et B) ont été identifiés concernant le domaine α1 et cinq allèles (A, B, C, D et E) concernant le domaine α2. En se basant sur ces études, l'hypothèse selon laquelle certains allèles du CMH pourraient prédisposer certains individus à une résistance accrue aux infections améliorant leur survie en milieu marin sera vérifiée pour les ombles (*Salvelinus fontinalis*) et (*Salvelinus alpinus*).

Des croisements inter et intraspécifiques de familles en demi-frère, permettant d'observer l'effet maternel, ont été générés à partir d'ombles chevalier (*Salvelinus alpinus*) anadromes (souche Fraser) et d'ombles de fontaine (*Salvelinus fontinalis*) anadromes et

résidentes (souche Laval). Des biopsies non-létales ont été effectuées sur les géniteurs afin de constituer une banque de données génétiques. Les différentes familles ainsi produites ont été soumises à des essais de résistance en milieu marin dans le but de mesurer les différences inter-croisements et ainsi d'identifier les combinaisons alléliques du CMH menant à une meilleure réponse immunitaire. Au cours de cette étude, trois objectifs ont été poursuivis: 1°) l'identification moléculaire des agents pathogènes opportunistes qui affectent les élevages d'ombles en milieu marin; 2°) l'identification du génotype des parents des différents croisements effectués pour les allèles β1 du complexe CMH; 3°) la mesure des différences inter-croisements de résistance aux infections de type opportuniste par des mesures d'occurrence et de suivis de mortalités. L'hypothèse nulle, « HO : il n'y a pas de différences inter-croisements pour la résistance aux maladies », a alors été vérifiée.

Afin de vérifier si une résistance accrue peut être associée à la présence ou à l'absence de certains allèles, et d'examiner si les différences de résistance sont liées à des différences alléliques au niveau de la région β1, ce projet devra être suivi d'une étude de corrélation entre les résultats obtenus pour les objectifs 2 et 3, permettant de cibler les progénitures résistantes versus non-résistantes et du génotypage des progénitures.

II. CHAPITRE II

RESISTANCE OF CHARR (*Salvelinus fontinalis*) AND (*Salvelinus alpinus*) TO OPPORTUNISTIC INFECTIONS IN SEAWATER

2.1 Introduction

Fish protect themselves against pathogenic microorganisms by an immune system comparable to that of humans and other vertebrates (Anderson, 1990). The first line of defence is based on non specific immune mechanisms. If a pathogen penetrates into the organism, the resulting inflammation attracts phagocytic cells that destroy the invader. This natural resistance is normally effective enough to protect fish from infectious diseases until specific immune responses are being induced (Köllner *et al.*, 2002). If the non specific response is unsuccessful, the major histocompatibility complex (MHC) cannot activate the specific immune response by the presentation of antigenic peptides to T cells. To be recognized, a foreign antigen must be degraded into small antigenic peptides that form complexes with class I or class II MHC molecules. These molecules are structurally and functionally distinct cell surface glycoproteins involved in antigen presentation to T-cells. Class I molecules, which are expressed on the surface of all nucleated cells are involved in the presentation of endogenously derived peptides to CD8-positive (cytotoxic) T lymphocytes. On the other hand, class II molecules, expressed mainly in the antigen-presenting cells (B lymphocytes, macrophages, and activated T cells) present exogenously derived peptides to CD4-positive (helper) T lymphocytes (Iwama and Nakanishi, 1996).

The MHC genes represent some of the most polymorphic genes, with multiple loci and a considerable number of alleles at each given locus (Grimholt *et al.*, 2002). They play an important role in disease resistance to pathogens by their efficacy to present a wide

variety of antigenic peptides derived from pathogens (Van Muiswinkel *et al.*, 1999). It was recently found that the teleostean class I and class II genes reside on different linkage groups. Furthermore, several studies in salmonids have shown that each class contains one major and dominantly expressed locus (Grimholt *et al.*, 2003).

Class II MHC proteins are heterodimers composed of two non-covalently linked glycoproteins, the α (34 kDA) and β (28 kDA) chains, which are encoded by separate class II genes organized into a series of exons and introns. Each chain consists of two external domains (α1 and α2 or β1 and β2), a connecting peptide, a transmembrane segment, and a cytoplasmic tail. The α2 and β2 domains are charracterized by an immunoglobulin fold, which places the MHC molecules in the family of immunoglobulin-like molecules. The membrane-distal domain composed by α1 and β1 domains is involved in the interaction with peptides and constitutes the peptide binding region (PBR) (Sültmann *et al.*, 1994). The ability of both class I and II genes to face various pathogens is believed to be mainly related to sequence variation among MHC alleles in the peptide binding region PBR (Bernatchez and Landry, 2003). Variation in exon 2, which codes for β1 domain, is responsible for most of the polymorphism observed in the class II genes; most of it is concentrated in the codons specifying the PBR (Sültmann *et al.*, 1994).

In aquaculture, stress factors increase fish susceptibility to opportunistic infections by compromising the functioning of the immune system (Köllner *et al.*, 2002). Different studies realised on anadromous brook charr in our laboratory showed that stress factors

may affect fish raised in salt water by opportunistic lesions (Audet, personnal communication). However, observations from a genetic study indicated that these lesions are not present in all families; some are affected whereas others are not (Bastien, personal communication). A study realised on Atlantic salmon by Langefors *et al.* (2001) showed a correlation between MHC class II allelic variation and resistance to the bacterial pathogen *Aeromonas salmonicida*. This association between resistance towards infectious diseases and MHC class I and II polymorphism was confirmed by Grimholt *et al.* (2003). In *Salvelinus fontinalis*, Rupert strain, d'Autray Tarte (2004) and Nassif (2006) respectively studied MHC class II (domain β1) and MHC class I (domains α1 and α2). Five alleles (A, B, D, E, F) were found for the MHC class II β1, two alleles (A, B) for the MHC class I α1, and five alleles (A, B, C, D, E) for the MHC class I α2. In addition, d'Autray Tarte also identified four alleles (P, H, I, L) for the MHC class II (domain β1) in the Laval strain.

Based on these different studies, the hypothesis that some MHC alleles should predispose some charr to a better resistance to a bacterial pathogen was verified. Different cross-breeding between Arctic charr (*Salvelinus alpinus*) and brook charr (*Salvelinus fontinalis*) were planned and samples were collected to identify alleles present in broodstock. Stress resistance of the different families was challenged in order to measure differences in sensitivity between cross-breeding and identify allelic combination leading to a better immune response. The aims of the present study were: 1) to identify opportunist pathogens that affect charr in salt water; 2) to identify the parental genotype of MHC class

IIβ alleles for resistance to an infectious disease by focusing on exon 2; and 3) to compare familial resistance to this pathogen.

2.2 Material and methods

2.2.1 Fish

Intra and interspecifics cross-breeding between anadromous Arctic charr, *Salvelinus alpinus*, (strain issued from the Fraser River) and both, anadromous and resident brook charr, *Salvelinus fontinalis*, (strains issued from the Laval River), were realised in fall 2003 at the Station aquicole de Pointe-au-Père, Québec (48°31'N; 68°29'W). Five half-sib families for each following crosses were produced: 1) *S. fontinalis* anadromous*anadromous, anadromous*resident, resident*resident; 2) *S. alpinus* anadromous*anadromous; 3) anadromous hybrid *S. alpinus**S. *fontinalis* (female is indicated first) (Table 1). The twenty-five families had their own marking (left, right or both pelvic fins cut). In May 2005, families were reduced in number to 150 individuals per family (at the exception of one family CF7: only 90 fish). Fish were sampled randomly. They were raised into ten different 500 L tanks (fish from three families per tank) supplied with a constant flow (10 L min^{-1}) of recirculating water. Fish were fed with commercial pellets (HiPro Fretin Corey Fry Fish Feed, Canada) at a rate of 2.5% (from July 2004 to September 2004) or 1% (from September 2004 to September 2005) body weight per day, and raised under natural photoperiod, temperature, and salinity conditions. Weight and

forklength measurements were realised on 50 individuals per family according to the following schedule: July, and September 2004; February, April, July, and September 2005. Before measurements, fish were anaesthetized with 3-aminobenzoic acid ethyl ester (1.2 μmol L^{-1} of MS222) (Sigma-Aldrich, Germany), and their markings checked.

Cross		Family	Dam	Sire
AA	anadromous *S. fontinalis*	A1	J062	BC041*
	X	A2	J075	BC045*
	anadromous *S. fontinalis*	A3	J151	BC043
		A4	J152	BC044
		A6	B014	BC046*
AR	anadromous *S. fontinalis*	AR1	J157	V002*
	X	AR2	J153	V000*
	resident *S. fontinalis*	AR3	J056	V001
		AR4	J159	V060*
		AR5	J155	V005*
RR	resident *S. fontinalis*	R2	R005	V005*
	X	R3	R000	V002*
	resident *S. fontinalis*	R6	R053	V062
		R7	R049	V060*
		R8	R058	V000*
CF	anadromous *S. alpinus*	CF1	BC054	BC046*
	X	CF2	BC053	BC041*
	anadromous *S. fontinalis*	CF5	BC040	BC057
		CF6	BC039	BC047
		CF7	BC070	BC045*
CC	anadromous *S. alpinus*	C2	BC061	BC060
	X	C4	BC066	BC065
	anadromous *S. alpinus*	C5	BC055	BC062
		C6	BC056	BC068
		C9	BC069	BC079

Table 1. *Salvelinus fontinalis* and *Salvelinus alpinus* - *Salvelinus fontinalis* cross-breeding. Female is indicated first. Asterisks indicate that the same sire was used for two crosses in half-sib families.

2.2.2 Parental genotypes of MHC class II (region β1) alleles

DNA extraction

Adipose fins from the parental Arctic and brook charr were collected for genetic analysis. DNA was extracted from these 43 ethanol-preserved adipose fins using the Montage BAC_{96} Miniprep Kit (Millipore, USA). Each sample was disposed on 96-well culture block and received 500µL of lysis buffer (1% sodium dodecyl sulfate (SDS), 50mM Tris-HCl, pH 8.0, 0.1M EDTA, pH 8.0, and 0.1mg mL^{-1} protease K) before being incubated overnight at 37°C with constant agitation. DNA was isolated from protein residues by following the Millipore protocol. Then, purified DNA resuspended in 35µL of 10mM Tris-HCl, pH 8.0, was stocked at -20°C.

Polymerase chain reaction amplifications

To amplify the MHC class II (β1 region) sequences from brook and Arctic charr, a forward and reverse primers located on the beginning and the end of exon 2 were used. A sense primer TVS 4501 (5'-CCTGTATTTATGTTCTCCTTTC-3') was selected from the study of Langefors *et al.* (2001), whereas the corresponding antisense primer SP4502 (5'-TAAGTGTTGCTACGGAGCC-3') designed by d'Autray Tarte (2004) was used. Nevertheless, for seven samples, the antisense primer AL1002 (5'-CACCTGTCTTGTCCAGTATG-3') used by Langefors *et al.* (2001) was preferred due to better results obtained with SP4502. The amplification was carried out in the DNA Thermal Cycler Biometra T Personnal under the following conditions: denaturation for 3 min at 94°C, 34 cycles of denaturation for 30 sec at

94°C, annealing for 30 sec at 47°C, extension for 1 min at 72°C, followed by a final extension for 10 min at 72°C. The 50µL polymerase chain reaction (PCR) mixture contained: 3µl of DNA, 1µl of each primer (5pmol), 0.5µl of Taq polymerase Expand High Fidelity PCR system (Roche, Germany), 5µl of Expand Taq reaction buffer (10X), 1µl of Expand Taq $MgCl_2$ (25mM), 1µl of dNTP mix (10mM). When run on a 1% agarose gel, the PCR products showed one distinct band corresponding to 315 bp with SP4502 or 296 pb with AL1002.

Analysis of Single-Stranded Conformation Polymorphism (SSCP)

In order to detect whether or not DNA fragments from the 43 samples were identical in sequence, we used the SSCP technique. It allowed us to detect DNA polymorphisms, which result in change in mobility of the single strands on gel electrophoresis. At the end of the PCR cycling, 10µl of each PCR products was mixed with 10µl of formamide loading (0.25% xylene cyanol and 0.25% bromophenol blue). Samples were denatured for 3-4 min at 95°C in a water bath and were placed directly on ice. From this preparation, 15µl was loaded onto 10% non-denaturing polyacrylamide gels. Gels were composed of 2.5ml of acrylamide (49:1) (40%), 1.25ml of glycerol (40%), 1 ml of TBE (10X), 5.25ml of distilled water, 100µl of ammonium persulfate (10%), and 20µl Temed. The TBE (1X) running buffer was prepared the night before and was refrigerated at 4°C. The electrophoresis tank as well as the TBE were maintained at 4°C during the migration. The conditions for electrophoresis (100V for 10 min, then 200V for 2 hours at 4°C) were standardized for

optimal resolution of bands. At the end of the migration, gels were silver stained according to the protocol of Budowle *et al.* (1991).

Cloning

SSCP analyses allowed to group individuals with similar band patterns. DNA of individuals from each group was cloned and sequenced. After purification by chloroform extraction, PCR products were ligated to the pT7Blue-2 Vector and cloned into *Escherichia coli* DH10B competent cells according to the Perfectly Blunt Cloning Kits Manual (Novagen, New England). We used the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) (20mg/ml in dimethylformamide) to visualize recombinant colonies by blue/white screening; bacteria carrying recombinant plasmids form white colonies. To confirm the presence of the appropriate insert, ten white colonies, picked with a sterile tip conserved in 2YT preparation at 4°C, were firstly amplified by PCR and depending on the PCR product size, were brought under cultivation overnight. Then, plasmid DNA was extracted by minipreparation SDS/NaOH following the protocol described by Birnboim and Doly (1979).

Digestion

The orientation of the insert was determined by the analysis of the digestion profile, realised with the restriction enzyme Alu I (New England Biolab). Digestion was realised with 10µl of DNA extracted by minipreparation, 2µl of buffer #2 (1X) (New England

Biolab), and 0.5µl of Alu I. Samples were incubated one hour at 37°C, before being loaded onto 20% polyacrylamide gels. Gels were composed of 6.67ml of acrylamide (29:1) (30%), 1ml of TBE (10X), 2.33ml of distilled water, 100µl of ammonium persulfate (10%) and 20µl of Temed. The migration conditions were the same as described with SSCP. At the end of the migration, gels were immerged 30 min in 100ml of distilled water with 5µl of ethidium bromide. Bands were visualised with Chemi Imager 5500 (Alpha Innotech Corporation). Two inserts with different orientations were chosen for each sample and were purified with Sephaglas™ BandPrep Kit following the manufacturer's instructions (Amersham Pharmacia Biotech, UK).

Sequencing and identification of alleles

Our selected samples were sent to the sequencing services provided by the immunogenetic department at the Notre-Dame campus of the Centre hospitalier de l'Université de Montréal (CHUM), which used an ABI 3100 DNA sequencer with 16 capillaries. All sequence alignments were carried out using ClustalW (European Bioinformatics Institute) and amino acids sequences were obtained using MEGA version 3.1 (Kumar *et al.*, 2004). From the different sequences found, our different alleles were determined. Another PCR amplification from parental DNA was realized. Purified PCR products were sent to the sequencing services in order to determine genotypes and to confirm the different alleles. By analysing the different chromatograms using Sequencher (Gene Codes Corporation), heterozygotes versus homozygotes individuals were determined.

2.2.3 Identification of pathogens

Media preparation

Different selective culture medium were prepared and stocked at 4°C in order to facilitate the isolation and identification of the pathogens responsible of opportunists lesions affecting charr in salt water. Marine agar (MA) was used to detect total bacteria from water samples, TCBS for the detection of *Vibrio spp*, Anacker and Ordal agar (AOA) prepared with seawater (contained in gl^{-1}: 5g tryptone, 0.5g yeast extract, O.2g beef extract, 0.2g sodium acetate, 15g Agar, 1L of salt water, pH : 7.2-7.4) for the detection of *Flavobacterium spp* (Anacker and Ordal, 1959) and *Flexibacter maritimus* Medium (FMM) also prepared with seawater (contained in gl^{-1}: 5g peptone, 0.5g yeast extract, 0.01g sodium acetate, 15g agar, 1L of salt water, pH : 7.2-7.4) for the detection of *Flexibacter maritimus* (Pazos *et al.*, 1996).

Sampling

Two series of samplings were done: one in June at the beginning of the experiment and another in August at the end, to ensure that it was the same pathogen in both cases. Five sick fish were removed before feeding in the different tanks. These fish were transported alive to the laboratory and analysed directly upon arrival to avoid degeneration of tissue that could cause an uncertain diagnostic. Fish were sacrificed by a blow on the head, adipose and caudal fins were sectioned and stored in 95% ethanol for future analysis whereas weight and forklength were measured. External lesions were the most prominent at

gills, tale or flank. These lesions were cleaned with 70% ethanol, then incised with a sterile scalpel. Sowing by scratches in aseptic conditions were made with an inoculation loop on the different culture medium. Triplicate plates labelled for each charr sampled were maintained in the dark at room temperature and frequently examined. Three isolations were realised to obtain a single type of bacteria. We picked representative isolates of each colony type observed for characterization and identification. A drop of the suspended culture to be examined was transferred on a microscope slide with an inoculation loop and was stained using the 77730 Gram Staining Kit following the manufacturer's indications (Fluka, Neu-Ulm, Germany). Representative isolates were removed, placed in aliquot of 1 ml containing 150µl of autoclaved water and stocked at –20°C. In order to confirm our preliminary results, a nested polymerase chain reaction system developed by Cepeda *et al.* (2003) was applied to detect *Flexibacter maritimus* from fish tissue. Clinical isolate of *Flexibacter maritimus* (ATCC 43398) provided by Uhland (Faculté de médecine vétérinaire, Université de Montréal, Canada) was used to test the sensitivity of the nested PCR protocol as reference strain. The stain was stored in vials at –80°C in Marine agar broth (Difco Laboratories, USA) with 20% glycerol added. DNA from the representative isolates was purified by Phenol: Chloroform: Isoamyl (Alcohol 25: 24: 1 saturated with 10mM Tris, pH 8.0, 1mM EDTA) (Sigma-Aldrich, Germany). The reference strain was used as positive control whereas an *Escherichia coli* DNA and a tube without any DNA constituted our negative controls.

Primers

According to the protocol described by Cepeda *et al.* (2003), two universal primers, 20F (5'-AGAGTTTGATCATGGCTCAG-3') and 1500R (5'-GGTTACCTTGTTACGACTT-3'), complementary to conserved regions of most eubacterial 16S rRNA, were used for the first PCR step. For the second PCR, two specific primers Mar1 (5'-TGTAGCTTGCTACAGATGA-3') and Mar2 (5'-AAATACCTACTCGTAGGTACG-3') expected to give a product of 400 bp were used. Nucleotides were synthesized by Biocorp Inc. (Montréal, Canada). The cycling conditions for PCR1 were 30 cycles of denaturation (95°C for 30 seconds), annealing (57°C for 30 seconds), and extension (72°C for 60 seconds). A preheating step at 95°C for 5 minutes and a final extension step of 5 minutes at 72°C were done. The cycling conditions for PCR 2 consisted of a preheating step of 94°C for 2 min, followed by 40 cycles at 94°C for 1 second, 54°C for two seconds and 5 seconds at 72°C, followed by a final extension step of 72°C for 4 minutes. The PCR products were analysed on a 1% agarose gel.

2.2.4 Stress exposure

The experiments were conducted from the beginning of June to mid-September 2005. At the beginning of June, both temperatures of fresh and seawater reached 8°C and, charr aged of 1+ were gradually transferred to salt water ($2^{\circ}/_{\circ\circ}$ per day over 10 days) until salinity reached $20^{\circ}/_{\circ\circ}$. Ten days later, an antibiotic Onycin 1000 (Vetoquinol, Canada) treatment was applied to follow regular rearing procedure (tetracycline HCl powder: 75 mg kg^{-1} body weight day^{-1} for 10 days). Twice a week, salinity was adjusted with a refractometer. Charr

were monitored throughout the day, sick fish were removed, and mortalities were recorded in the morning before the feeding and late afternoon.

Samples of DNA were obtained from either adipose or caudal fin from sick fish and preserved in 95% ethanol to constitute a genetic bank. Clinical signs, forklength and weight were also noted for dead fish. At the beginning of August (1st and 8th August), two acute stress were applied in sequence. The water in each tank was drained to three quarters. Fish stayed in low water depth for a period of 10 minutes before tank was refilled. In mid-August, salinity was increased to 30°/∞. The experiment stopped in mid-September, when charr were transferred back to freshwater.

Fish derived from a diverse set of crosses, failed to cluster into "resistant" and "susceptible" phenotype groups. Based on the different families responses observed, we determined four scales of disease susceptibility: 1°) unaffected (0 mortality) or very resistant (less than 10 mortalities all along the experiment); 2°) very sensitive: affected by the transfer to 20°/∞ (more than 10 mortalities); 3°) moderately sensitive: affected by acute stress (more than 10 mortalities); 4°) slightly sensitive: affected by the transfer to high salinity 30°/∞ (more than 10 mortalities).

2.2.5 Statistical analysis

Data on growth performance were gathered into databases and statistical analyses were performed using STATISTICA 6.0 (Statsoft Inc., France). In order to analyse our two

different types of hybrids (intra and interspecific), the 25 families were pooled in two different groups representing intra- and interspecific crosses. One was composed of *Salvelinus fontinalis*: anadromous*anadromous (AA), anadromous*resident (AR), resident*resident (RR) and the other one of *S. fontinalis*: anadromous*anadromous (AA), anadromous hybrids *S. fontinalis*S. alpinus* (CF), *S. alpinus*: anadromous* anadromous (CC). Two-way ANOVAs ($\alpha = 0.05$) were performed (family, time of sampling) on the following variables: body weight, fork length and condition factor ($100*(\text{weight/length})^3$). The normality and homogeneity of variances were checked by Komolgorov-Smirnov and Levene tests respectively. Weight, length and condition factor data had to be transformed as log (x) to obtain normality. When there were significant interactions, a posteriori one-way ANOVAs were realised followed by Tukey HSD post hoc tests. In absence of heteroscedasticity, pairwise comparisons were realised using Games and Howell's test (Sokal and Rohlf, 1995).

2.3 Results

2.3.1 Parental genotypes of MHC class II (region β1) alleles

A total of 21 MHC class II (region β1) exon 2 alleles were identified from broodstock and assigned by an alphabetic letter from a to u (Figure 1). Among the 271 nucleotides and 91 amino acid positions, 79 (29%) nucleotide positions and 41 (45%) amino acids residues were variable (Figures 1 and 2).

```
  1           11          21          31          41          51          61          71          81
a GATGGATATT  TTTGAACAGG  TTGTGAGACA  GTGCCGATAC  TCCTCAAAGG  ACCTGCATGG  TATAGAGTTT  ATAGACTCTT  ATGTTTTCAA
b ..........  ..-......A  GG........  ........T.  ..........  ..........  ..........  ..........  ..........
c ..........  ..-T.T....  ..........  ...T......  ..........  ..........  .GC.......  ..........  ..........
d ..........  ..AAT-..TA  GGT..GT...  ..........  ..........  ..........  .........G  ..........  ..........
e ..........  ..AAT-..TA  GGT..GT...  ..........  ..........  .......C..  .........G  ..........  ..........
f ..........  ..AAT-..TA  GGT..GT...  ..........  ..........  ..........  .........G  ..........  ..........
g ..........  ...CTT...A  CG....C...  ..........  ..........  ..........  .........G  ..........  ..........
h ..........  .........A  GG........  .........T  ..........  ..........  ..........  ..........  ..........
i ..........  .........A  GG........  .........T  ..........  ..........  ..........  ..........  ..........
j ..........  ...-.T...A  GG...G....  .........T  ..........  ..........  ..........  ..........  ..........
k ..........  ...AT-..TA  GGT..GT...  ..........  ..........  ..........  ..........  ..........  ..........
l ..........  ...AT-..TA  GGT..GT...  ..........  ..........  ..........  ..........  ..........  ..........
m ..........  ...AT-...A  GG...G....  ..........  ..........  ..........  ........A.  ..........  ..........
n ..........  ...AT-...A  GG...G....  ..........  ..........  ..........  ........A.  ..........  ..........
o ..........  ...AT-...A  GG...G....  ..........  ..........  ..........  ........A.  ..........  ..........
p ..........  ...AT-..TA  GGT..GC...  ..........  ..........  ..........  ..........  ..........  ..........
q ..........  ...AT-..TA  GGT..GC...  .C........  ..........  ..........  ..........  ..........  ..........
r ..........  .........TA  GG..TG.T..  ..........  ..........  ..........  ..........  ..........  .CTAC.....
s ..........  .........TA  GGT..GT...  ..........  ..........  ..........  ..........  ..........  .CTAC.....
t ..........  ...-....TA  AC...GATA.  A.........  ..........  ..........  .GC.......  ..........  .CTAC.....
u ..........  ...GT-..TA  GG...G....  ..........  ..........  ..........  .GC.......  ..........  .CTAC.....

  91          101         111         121         131         141         151         161         171
a TCAGATTGAA  CACATCAGAT  TCAACAGCAC  TGTGGGGAAG  TATGTTGGAT  ACACTGAGCA  TGGTGTGAAG  AATGCAGAAA  CATGGAACAA
b .A..G.....  G.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
c .A..GC....  T.TG......  ..........  ..........  ..........  ..........  .......T.C  ......A...  ..........
d ....G.....  A.........  ..........  ..........  .T........  ........GT  G...T...G.  .........G  ..........
e ....G.....  A.........  ..........  ..........  .T........  ........GT  G...T.....  .........G  ..........
f ....G.....  ..A.......  ..........  ..........  .T........  ........GT  G...T.....  .........G  ..........
g ....G.....  A.........  ..........  ..........  ..........  ........GT  G...T.....  ..........  ..........
h .A..G.....  G.........  ..........  ..........  ..........  .........T  G...T.....  .C........  ..........
i .A..G.....  G.........  ..........  ..........  ..........  .........T  G...T.....  .C........  ..........
j .A..G.....  G.........  ..........  ..........  ..........  .........T  G...T.....  .C........  ..........
k ....GC....  T.TG......  ..........  ..........  ..........  ........T.  ..........  .........G  .........G
l ....G.....  T.TG......  ..........  ..........  ..........  ........T.  ..........  .........G  .........G
m .A..GC....  T.TG......  ..........  ..........  ..........  ........T.  ..........  .........G  T........G
n .A..GC....  T.TG......  ..........  ..........  ..........  ........T.  ..........  .........G  T........G
o .A..GC....  T.TG.T....  ..........  ..........  ..........  ........T.  ..........  .........G  ..........
p ....G.....  G.T.......  ..........  ..........  .T........  ..........  .......T.C  ..........  ..........
q ....G.....  G.T.......  ..........  ..........  .T........  ..........  .......T.C  ..........  ..........
r ....G.....  G.T.......  ..........  ..........  ..........  ..........  ..........  .........G  ..........
s ....G.....  G.T.......  ..........  ..........  ..........  ..........  ..........  .........GG  ..........
t ....G.....  G.T.......  ..........  ..........  .T........  ..........  .......T.C  ..........  ..........
u ....G.....  G.T.......  ..........  ..........  ..........  ..........  ..........  ..........  ..........
```

```
    181         191         201         211         221         231         241         251         261
a   AGGTTCTGTC  ---CTGGCTC  GAGAGCTAGG  GGAGCTGGAG  CGTTTCTGTA  AGCATAACGC  TGATCTCTAC  TACAGCACCGT  ACTGGATAAG
b   ..........  ---.......  ..........  ..........  ..........  ..........  ....A..C..  ......G..A.  ..........
c   ........AG  ---....T..  ..........  ..........  ..........  ..........  ....A..C..  ......G..A.  ..........
d   ........A.  ---.......  ..........  ..........  ..........  ..........  ..C.AA.C..  ......G.---  ----------
e   ........A.  ---.......  ..........  ..........  ..........  ..........  ..C.AA.C..  ......G....  ..........
f   ........A.  ---.......  ..........  ..........  ..........  ..........  ..C.AA.C..  ......G.---  ----------
g   ........AG  ---.......  A.........  ..........  ..........  ..........  ....A..C..  ......G.---  ----------
h   ..A.....GG  ATT.......  A.........  ..........  ........C.  ..........  .......C..  ......G..A.  ......C...
i   ..A.....GG  ATT.......  A.........  ..........  ........C.  ..........  .......C..  ......G..A.  ..........
j   ..A.....GG  ATT.......  A.........  ..........  ........C.  ..........  .......C..  ......G..A.  ..........
k   ..A..---A.  ---...-...  A.........  ..........  ....A...C.  ..T.......  ..C.A.....  .......A...  ..........
l   ..A..---A.  ---.......  A.........  ..........  ...GA...C.  ..........  ..C....C..  .......A...  ..........
m   ..A..---A.  ---.......  A.........  ..........  ........C.  ..T.......  .......C..  ...G..G..A.  ......C...
n   ..A..---A.  ---.......  A.........  ..........  ........C.  ..T.......  .......C..  ...G..G....  ..........
o   ........AG  ---.......  A.........  ..........  ........C.  ..........  ..C.AA.C..  ......G..A.  ..........
p   ........AG  ---.......  A.........  ..........  ....A...C.  ...C.C....  ....A.....  ......G....  ..........
q   ........AG  ---.......  A.........  ..........  ........C.  ...C......  ..C.AA.C..  ......G.---  ----------
r   ..AG....A.  ---.......  A.....AG.C  .C........  A...A...C.  ..T.......  ..C.A.....  .......A...  ..........
s   ..AG....A.  ---.......  A.....AG.C  .C........  A...A...C.  ..T.......  ..C.A.....  .......A...  ..........
t   ...G....AG  ---.......  A.........  ..........  ...G....C.  ..T...C...  ..C.A..G.A  ......G....  ..........
u   ........AG  ---.......  ..........  ..........  ...G....C.  ...C......  .......C..  ...G..G....  ..........

    271
a   ACAGGTG
b   .......
c   .......
d   -------
e   .......
f   -------
g   -------
h   .......
i   .......
j   .......
k   .......
l   .......
m   .......
n   .......
o   .......
p   .......
q   -------
r   .......
s   ....---
t   .......
u   .......
```

Figure 1. Exon 2 sequences of the MHC class IIβ1 alleles in *Salvelinus alpinus* and *Salvelinus fontinalis*. Dots indicate identity with the top sequence.

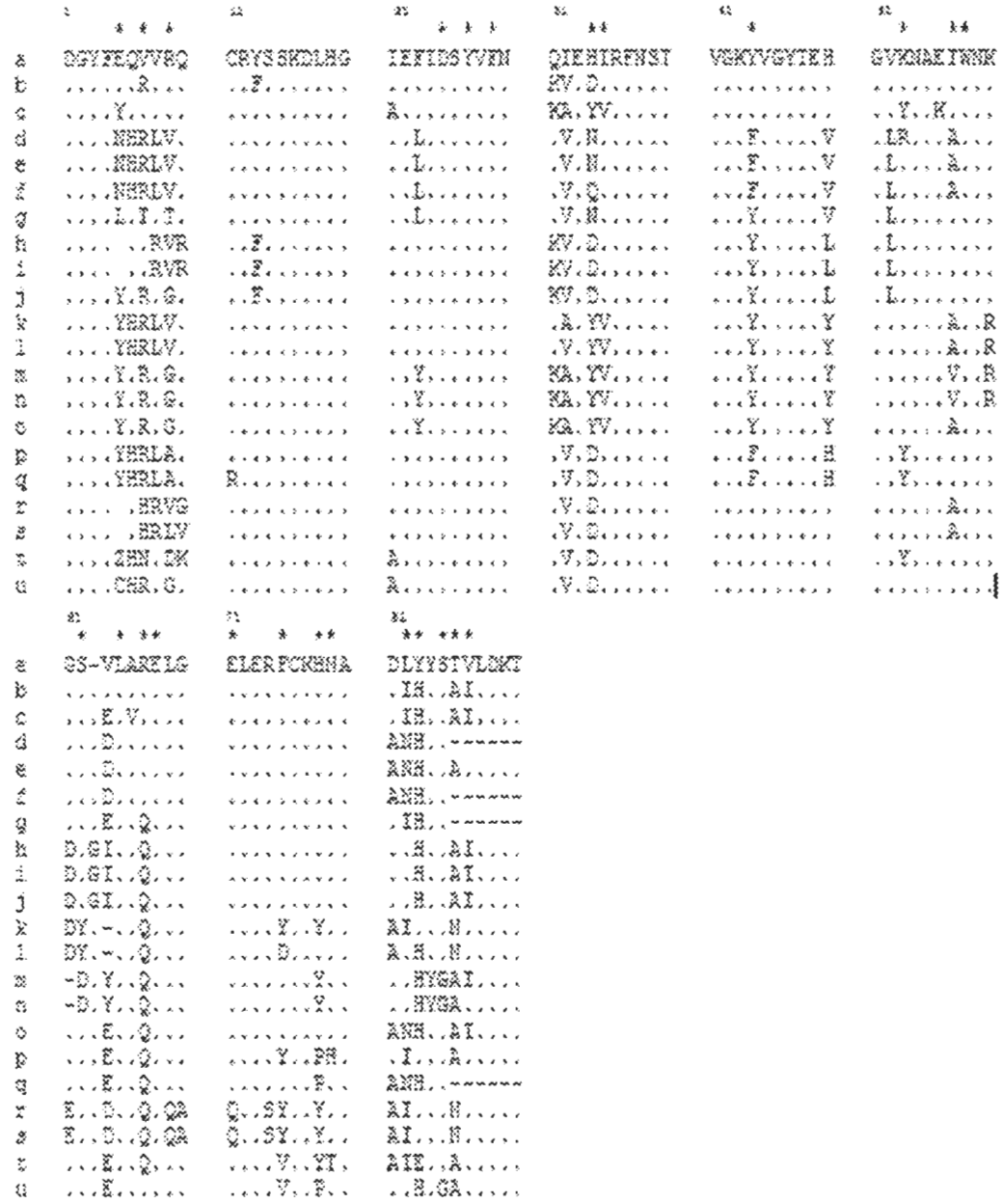


Figure 2. Amino-acid sequences for MHC class IIβ1 alleles in *Salvelinus alpinus* and *Salvelinus fontinalis*. Codons involved in antigen binding in human (Langefors *et al.*, 2001) are denoted by asterisks.

From the 25 positions of the antigen-binding sites (noted by asterisks), 19 (76%) were variable (Figure 2). All alleles encoded distinct amino-acid sequences, at the exception of alleles i and j, which differed from one nucleotide. Five specific alleles (a, c, g, q and t) were found only in anadromous *Salvelinus alpinus*. It also appeared that six alleles (e, f, k, l, o and u) and four alleles (h, j, p and r) were respectively specific to anadromous and resident *Salvelinus fontinalis*. Six others (b, d, i, m, n, and s) were shared between anadromous Arctic and anadromous or resident brook charr. Three (P, I, L) of the four alleles (P, H, I, L) identified by d'Autray Tarte (2004) in *Salvelinus fontinalis* (Laval strain), were identical to ours, and corresponded respectively to alleles h, d and m. SSCP analysis revealed that some parents present the same sequence as their DNA strands assume the same shape, and consequently similar gel mobility (Sunnucks *et al.*, 2000) (Figure 3). Looking at the allele frequencies, it appeared that seven alleles (i, c, d, s, p, n, g) occured more frequently than others (over 5) and that six alleles (e, f, k, o, r and u) were only found in one individual (Table 2). Only three dams and three sires were homozygotes, the 37 others were heterozygotes (Table 3). From the parental genotypes, an estimation of possible allelic combinations that should be found in progeny was made (Table 3). There was no similarity in allelic combinations among the different families.

2.3.2 Identification of pathogens

Samples obtained from lesions on ten moribund fish were first characterized by efficient selective media. After incubation in dark at room temperature for 2 to 3 days,

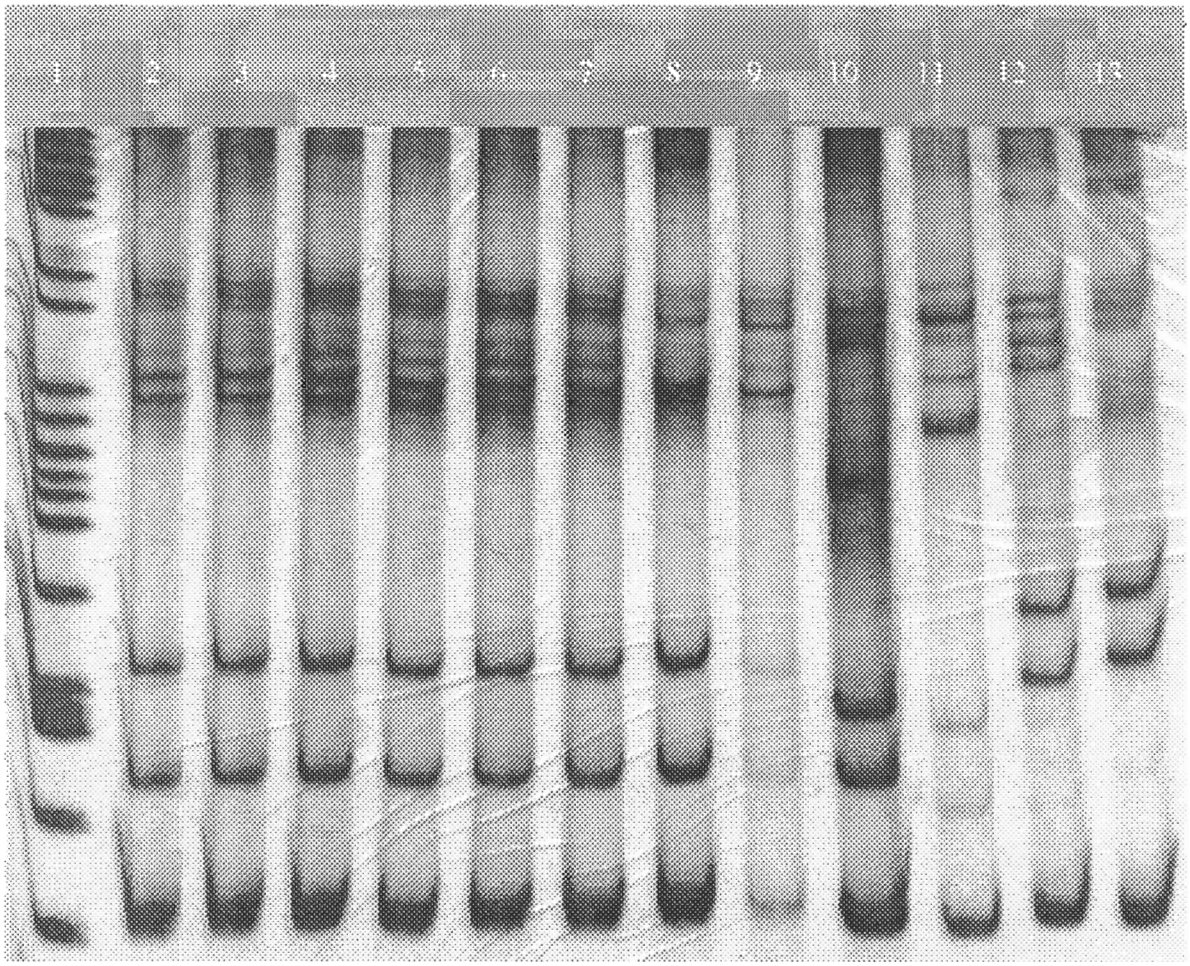


Figure 3 : Silver-stained SSCP gel of the MHC class II β1 from *Salvelinus fontinalis* and *Salvelinus alpinus*. Lanes: 1, molecular weight marker; 2, anadromous *Salvelinus fontinalis* sire BC046; 3, anadromous *Salvelinus fontinalis* sire BC045; 4, anadromous *Salvelinus fontinalis* dam J151; 5, anadromous *Salvelinus fontinalis* dam J152; 6, anadromous *Salvelinus fontinalis* sire BC044; 7, anadromous *Salvelinus fontinalis* dam J150 not used for the experiment; 8, anadromous *Salvelinus fontinalis* dam J056; 9, anadromous *Salvelinus fontinalis* dam J157; 10, resident *Salvelinus fontinalis* sire V002; 11, anadromous *Salvelinus alpinus* sire BC079; 12, resident *Salvelinus fontinalis* dam R000 res; 13, resident *Salvelinus fontinalis* dam R006 not used for the experiment (refer Table 1).

Allele	Frequence	Dams	Sires
a	3	**BC070**	**BC068, BC060**
b	2	**J159**	V002
c	8	**BC061, BC066, BC069, BC054, BC053, BC070**	**BC060, BC062**
d	8	R000, R049, R058, **BC040, BC039**	V060, V000, V001
e	1		V001
f	1	**R053**	
g	5	**BC055, BC056, BC069, BC053**	**BC065**
h	2	**J153, J155**	
i	10	*J157, J056, J151*	V005, *BC045, BC043, J152, BC047, BC046, BC044*
j	2	*J062*	**BC057**
k	1	R005	
l	2	R005, R000	
m	3	*J159,* **BC040**	V060
n	6	R058, *J062, J075, B014,* **BC039**	V002
o	1		V062
p	7	**J153, J155, J075, B014**	**BC043, BC057, BC047**
q	3	**BC061, BC056**	**BC079**
r	1	**J157**	
s	8	*J056, J152*	V005, *BC041, BC045, J151, BC046, BC044*
t	4	**BC055**	**BC065, BC062, BC068**
u	1	R049	

Table 2 : Allele frequencies from broodstock. Bold character indicates anadromous *Salvelinus alpinus* individuals, italic indicates anadromous *Salvelinus fontinalis* individuals and normal character indicates resident *Salvelinus fontinalis* individuals. Five alleles (a, c, g, q and t) are specific to anadromous *Salvelinus alpinus*, six alleles (e, f, k, l, o, u) are specific to resident *Salvelinus fontinalis*, and four alleles (h, j, p, r) are specific to anadromous *Salvelinus fontinalis*.

Cross	Family	Dam	**Sire**	Dam genotype	Sire genotype	Expected progeny genotypes
AA	A1	J062	BC041	nj	sh	ns, nh, js, jh
	A2	J075	BC045	np	si	ns, ni, ps, ip
	A3	J151	BC043	si	ip	si, sp, ii, ip
	A4	J152	BC044	si	si	ss, si, ii
	A6	B014	BC046	np	si	ns, ni, ps, ip
AR	AR1	J157	V002	ri	nb	rn, rb, ni, bi
	AR2	J153	V000	hp	dd	hd, dp
	AR3	J056	V001	si	de	sd, se, di, ie
	AR4	J159	V060	mb	md	mm, md, mb, bd
	AR5	J155	V005	hp	si	hi, sh, ip, sp
RR	R2	R005	V005	kl	si	li, ls, ks, ki
	R3	R000	V002	dl	nb	nd, nl, bd, bl
	R6	R053	V062	ff	oo	fo
	R7	R049	V060	ud	md	um, dd, ud, md
	R8	R058	V000	nd	dd	nd, dd
CF	CF1	BC054	BC046	cc	si	cs, ci
	CF2	BC053	BC041	cg	sh	cs, ch, gs, gh
	CF5	BC040	BC057	md	jp	mj, mp, dj, dp
	CF6	BC039	BC047	nd	ip	ni, np, di, dp
	CF7	BC070	BC045	ca	si	cs, ci, as, ai
CC	C2	BC061	BC060	cq	ca	cc, ca, cq, qa
	C4	BC066	BC065	cc	gt	cg, ct
	C5	BC055	BC062	gt	ct	cg, gt, ct, tt
	C6	BC056	BC068	gq	at	ga, gt, qa, qt
	C9	BC069	BC079	cg	qq	cq, gq

Table 3. Parental and expected progeny genotypes for *Salvelinus fontinalis* and *Salvelinus alpinus*.

white-yellow colonies, appeared on AOA and FMM, whereas nothing was observed on TCBS at the exception of the triplicate plates from one fish. Nevertheless, colonies did not exhibit the typical *Vibrio* sp morphology characteristics, which are flat and large yellow colonies (Difco and BBL Manual, 2003).

Further analysis showed the presence of long and thin gram-negative rods. The presence of *Flexibacter maritimus* was presumed by the appearance of colonies morphology, pale-yellow, flat with uneven edges, as described by Toranzo *et al.* (2005). This preliminary diagnosis of marine flexibacteriosis was supported by the use of specific molecular DNA-based methods. The nested PCR amplification generated a 400 bp product for the reference strain *Flexibacter maritimus* (ATCC 43398), whereas amplification products were not obtained from our two negative controls. Each batch of samples was amplified with positive and negative controls and for both series of samplings, one single band at 400 bp length was observed (Figure 4). *Flexibacter maritimus* was isolated and identified as the causative agent of opportunist lesions in all cases.

2.3.3 Stress exposure

Fish condition during the growing season

All families were transferred to salt water at the beginning of June. Weight and length were measured in April, July and September to follow their growth performance during summer. From these data, condition factors, which indicate fish condition, were

calculated. Familial differences were found for each sampling period, families did not keep the same profile in time. This led us to pool and analyse them according to two groups: intraspecific (AA, AR, RR), and interspecific (AA, CF, CC) crosses. Significant interactions among intraspecific families and sampling time were present. The analysis of variance results are summarized in (Table 4).

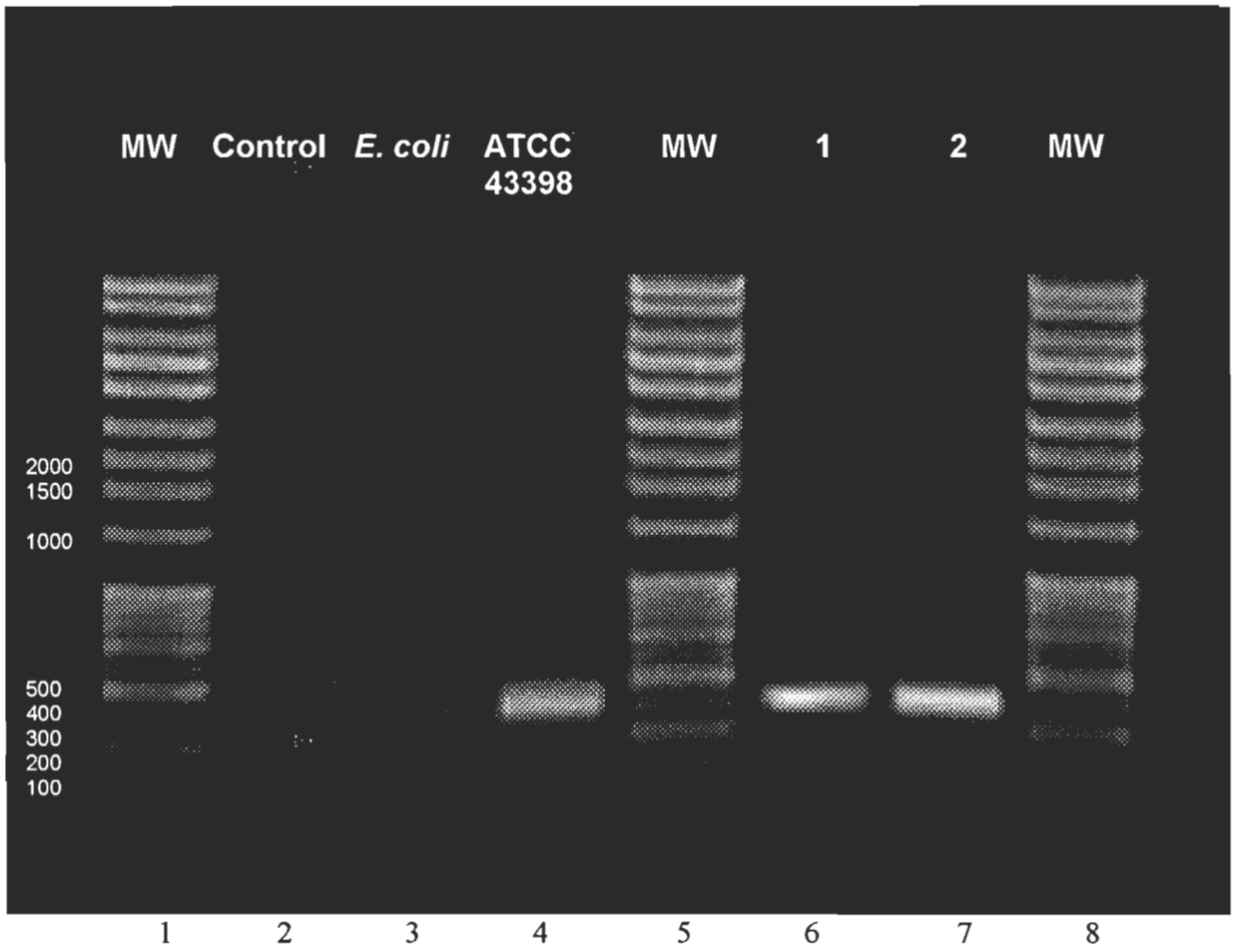


Figure 4. Nested PCR products obtained from sick fish. Lanes: 1, molecular weight marker; 2-3, negative controls: one without DNA and one with *Escherichia coli* DNA; 4, positive control: reference strain *Flexibacter maritimus*, clinical isolate provided by Uhland (ATCC 43398); 5, molecular weight marker; 6-7, two examples from each batch of samples to show the presence of a distinct single band at 400 bp but it was also the case for all others samples; 8, molecular weight marker.

Group	Variables	Effect	df	F	p-level
Intraspecific	Weigth	Family	14	62.4	<0.001
		Sampling	2	1768.1	<0.001
		Family*Sampling	28	2.4	<0.001
	Length	Family	14	44.4	<0.001
		Sampling	2	990.0	<0.001
		Family*Sampling	28	1.3	<0.001
	Condition factor	Family	14	63.8	<0.001
		Sampling	2	582.0	<0.001
		Family*Sampling	28	5.6	<0.001
Interspecific	Weigth	Family	14	43.4	<0.001
		Sampling	2	1307.3	<0.001
		Family*Sampling	28	1.9	<0.001
	Length	Family	14	67.0	<0.001
		Sampling	2	1517.4	<0.001
		Family*Sampling	28	1.8	**0.125**
	Condition factor	Family	14	45.1	<0.001
		Sampling	2	632.3	<0.001
		Family*Sampling	28	8.8	<0.001

Table 4. Two-way ANOVA results. Comparisons among intraspecific *Salvelinus fontinalis* (AA, AR, RR) and among interspecific *Salvelinus alpinus* - *Salvelinus fontinalis* progenies (AA, CF, CC) and among the three sampling periods (April, before the transfer in salt water; July, during the transfer; and September, just after the transfer) (refer to Table 1).

In spite of these differences, we could notice that during the whole summer, the family of smallest size was A2 and the largest one, R6 (Figures 5-6). However, these families did not have the lowest and the highest condition factors (Table 4). Condition factors were particularly variable in April, ranging from 0.749 (CF2) to 1.076 (R8). *Salvelinus fontinalis* resident families (principally families R8 and R2) tended to have the highest condition factor all along the summer. In April, anadromous families had the lowest condition factors and were among the smallest and the largest families. All families grew well during summer time, but the differences observed in April were generally maintained throughout the season. Conditions factors were generally higher in September, at the exception of family AR2, which had a higher one in July, period corresponding to a remarkable growth performance.

Considering the interspecific comparisons, families differed significantly in weight and condition factor but not in length between sampling time (Table 4). As with intraspecific families, in April, anadromous families were among the smallest and the largest size families, but in this case, their condition factors were among the highest. During the whole summer, the smallest families were CF5 and A2, whereas the largest ones, A4 and CF6 (Figure 7-8). In April, conditions factors were low, majority was inferior to 0.9, however in September, all families had condition factors over 1.0 (Table 5). All families grew well during summer time, but C9, C5, CF7, and C6 grew considerably from July to September (Figure 8 and Table 5). In April, brook charr families had the highest

condition factors, but the situation changed after seawater transfer. In September, Arctic charr had higher condition factors than brook charr.

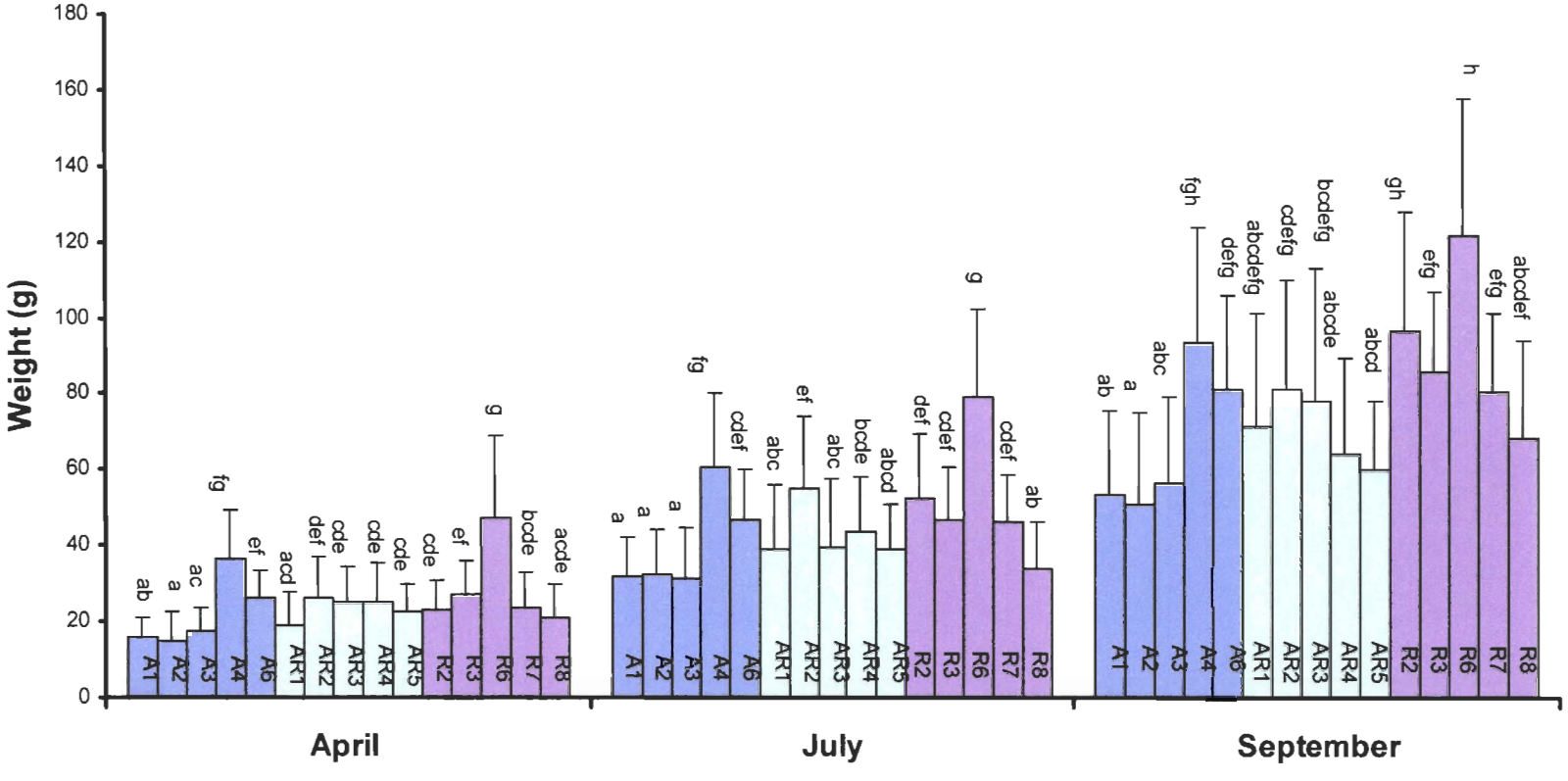


Figure 5. Fish weigth of the intraspecific *Salvelinus fontinalis* families (AA, AR, RR) during summer time. Mean ± standard deviation. Significant interactions were found for each time of sampling, results of a posteriori test are represented by a letter: abcdefg.

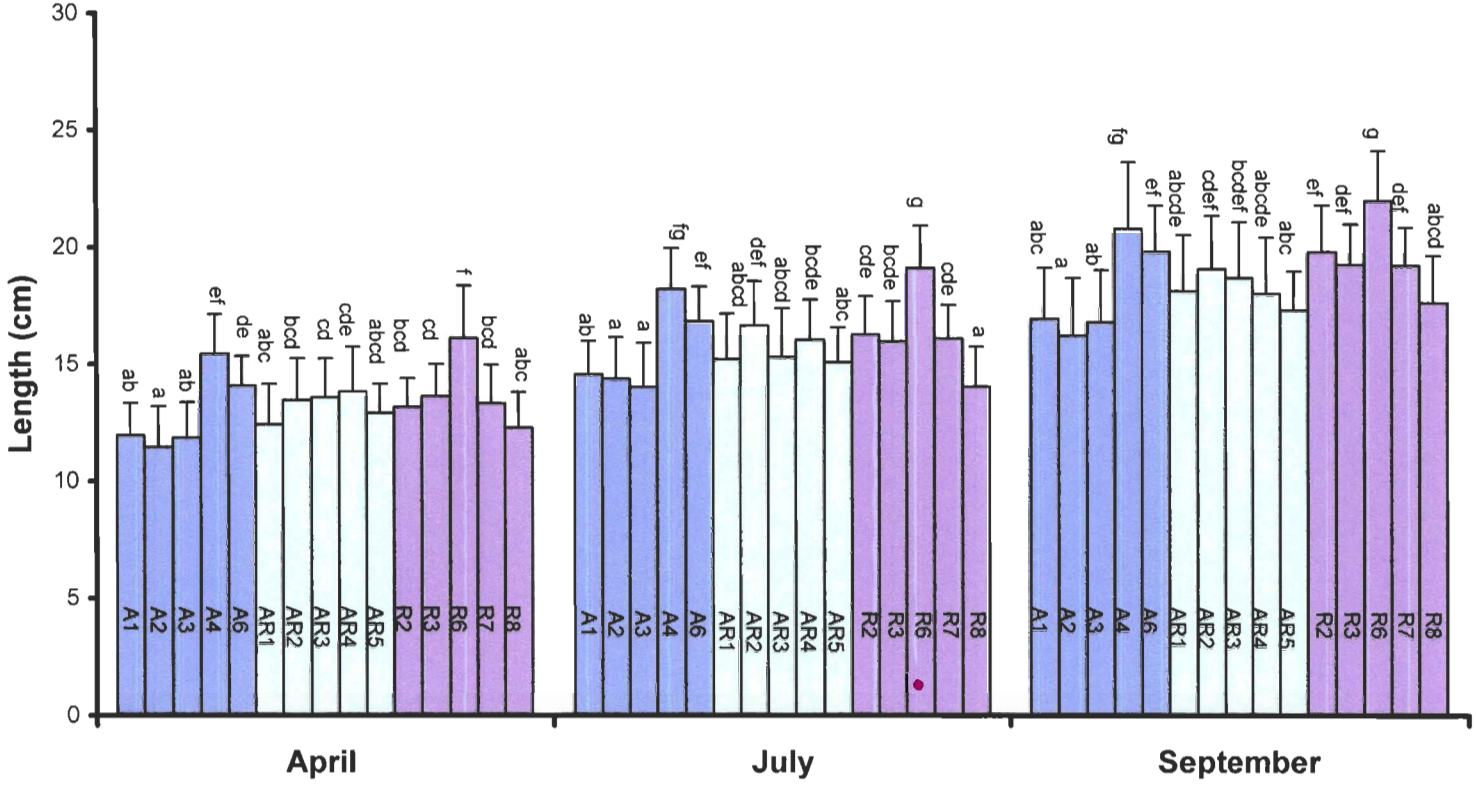


Figure 6. Fish length of the intraspecific *Salvelinus fontinalis* families (AA, AR, RR) during summer time. Mean ± standard deviation. Significant interactions were found for each time of sampling, results of a posteriori test are represented by a letter: abcdefg.

Group	Family	Condition factor		
		April	July	September
Intraspecific	A1	0.897 ±0.064 (50) [a]	1.003 ±0.053 (30) [abc]	1.035 ±0.062 (50) [ac]
	A2	0.926 ±0.047 (50) [ab]	1.044 ±0.049 (30) [bcde]	1.118 ±0.162 (50) [bcdf]
	A3	0.981 ±0.069 (50) [bcd]	1.084 ±0.043 (30) [ef]	1.129 ±0.078 (50) [bdef]
	A4	0.956 ±0.049 (50) [b]	0.970 ±0.055 (30) [a]	1.029 ±0.197 (50) [ab]
	A6	0.908 ±0.041 (50) [a]	0.960 ±0.083 (30) [b]	1.006 ±0.056 (50) [a]
	AR1	0.923 ±0.073 (50) [ab]	1.051 ±0.046 (30) [cde]	1.122 ±0.074 (50) [bde]
	AR2	1.027 ±0.063 (50) [de]	1.138 ±0.069 (30) [fg]	1.115 ±0.076 (50) [bde]
	AR3	0.961 ±0.057 (50) [bc]	1.037 ±0.055 (30) [abcde]	1.126 ±0.073 (50) [abcdef]
	AR4	0.897 ±0.049 (50) [a]	1.028 ±0.038 (30) [abcd]	1.039 ±0.042 (50) [ac]
	AR5	1.011 ±0.060 (50) [cde]	1.102 ±0.063 (30) [efg]	1.123 ±0.074 (50) [bde]
	R2	0.987 ±0.115 (50) [abcde]	1.175 ±0.055 (30) [g]	1.200 ±0.069 (50) [f]
	R3	1.044 ±0.082 (50) [de]	1.109 ±0.074 (30) [efg]	1.172 ±0.053 (50) [ef]
	R6	1.056 ±0.084 (50) [de]	1.100 ±0.046 (30) [ef]	1.130 ±0.142 (50) [bde]
	R7	0.950 ±0.075 (50) [abc]	1.079 ±0.055 (30) [def]	1.108 ±0.071 (50) [bd]
	R8	1.076 ±0.083 (50) [e]	1.172 ±0.059 (30) [g]	1.194 ±0.058 (50) [f]
Interspecific	A1	0.897 ±0.064 (50) [a]	1.003 ±0.053 (30) [abc]	1.035 ±0.062 (50) [ac]
	A2	0.926 ±0.047 (50) [ab]	1.044 ±0.049 (30) [bcde]	1.118 ±0.162 (50) [bcdf]
	A3	0.981 ±0.069 (50) [bcd]	1.084 ±0.043 (30) [ef]	1.129 ±0.078 (50) [bdef]
	A4	0.956 ±0.049 (50) [b]	0.970 ±0.055 (30) [a]	1.029 ±0.197 (50) [ab]
	A6	0.908 ±0.041 (50) [a]	0.960 ±0.083 (30) [b]	1.006 ±0.056 (50) [a]
	CF1	0.938 ±0.088 (50) [de]	1.063 ±0.134 (30) [bcde]	1.088 ±0.089 (50) [bcd]
	CF2	0.749 ±0.058 (50) [a]	0.818 ±0.084 (30) [a]	1.003 ±0.059 (50) [a]
	CF5	0.790 ±0.092 (50) [ab]	0.907 ±0.107 (30) [ab]	1.104 ±0.090 (50) [bcd]
	CF6	0.988 ±0.103 (50) [e]	1.129 ±0.138 (30) [e]	1.147 ±0.150 (50) [bcd]
	CF7	0.892 ±0.071 (50) [cd]	1.002 ±0.124 (30) [bcde]	1.045 ±0.161 (50) [ab]
	C2	0.928 ±0.115 (50) [de]	0.991 ±0.074 (30) [bcd]	1.090 ±0.080 (50) [bcd]
	C4	0.811 ±0.122 (50) [abc]	0.937 ±0.082 (30) [bd]	1.078 ±0.068 (50) [bc]
	C5	0.963 ±0.104 (50) [de]	1.005 ±0.077 (30) [bcde]	1.163 ±0.076 (50) [d]
	C6	0.878 ±0.151 (50) [bce]	0.898 ±0.144 (30) [abcd]	1.141 ±0.097 (50) [cd]
	C9	0.963 ±0.125 (50) [de]	1.051 ±0.096 (30) [cde]	1.175 ±0.096 (50) [d]

Table 5. Condition factor of intraspecific *Salvelinus fontinalis* (AA, AR, RR) and interspecific *Salvelinus alpinus* - *Salvelinus fontinalis* (AA, CF, CC) families during summer time (refer to Table 1). Mean ± standard deviation. Significant interactions were found for each time, results of a posteriori test are represented by a letter: abcdef.

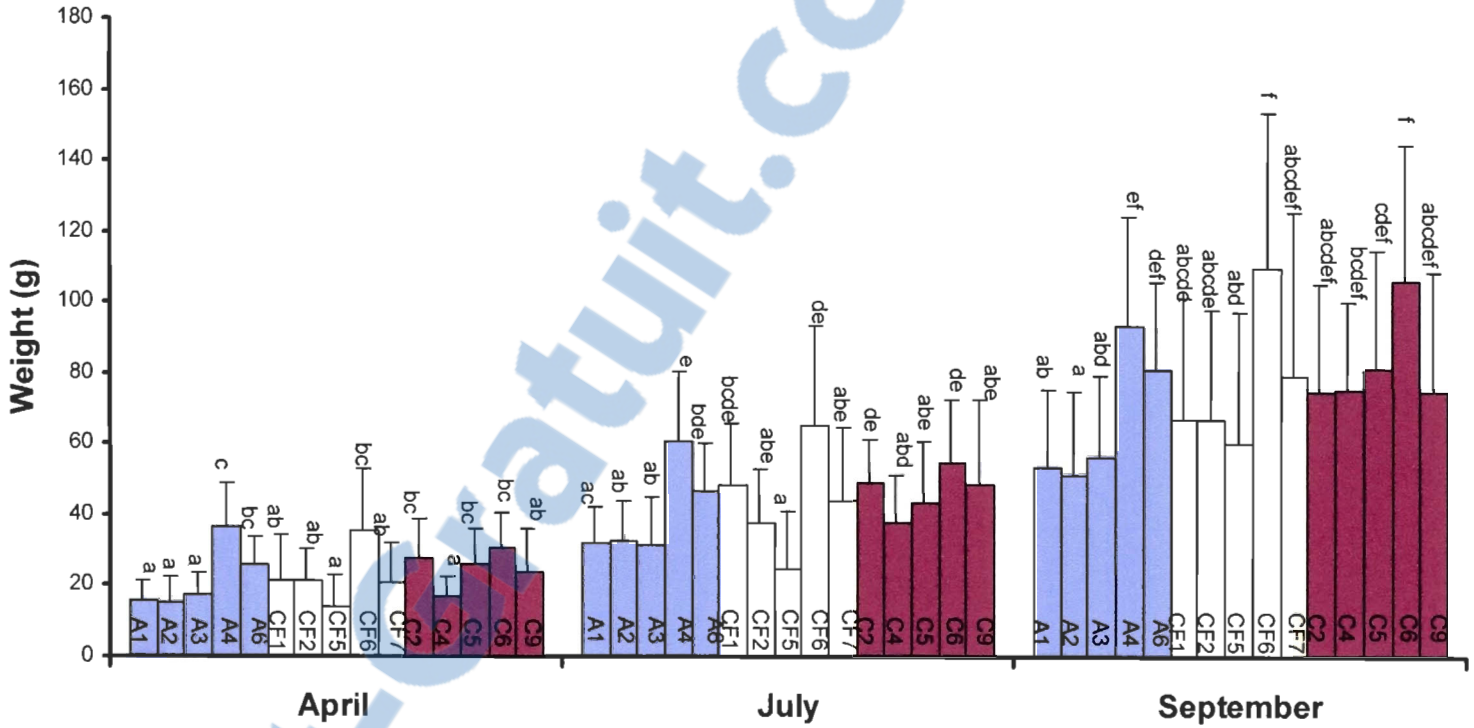


Figure 7. Fish weigth of the interspecific *Salvelinus alpinus* Salvelinus fontinalis* families (AA, CF, CC) during summer time. Mean ± standard deviation. Significant interactions were found for each time of sampling, results of a posteriori test are represented by a letter: abcdef.

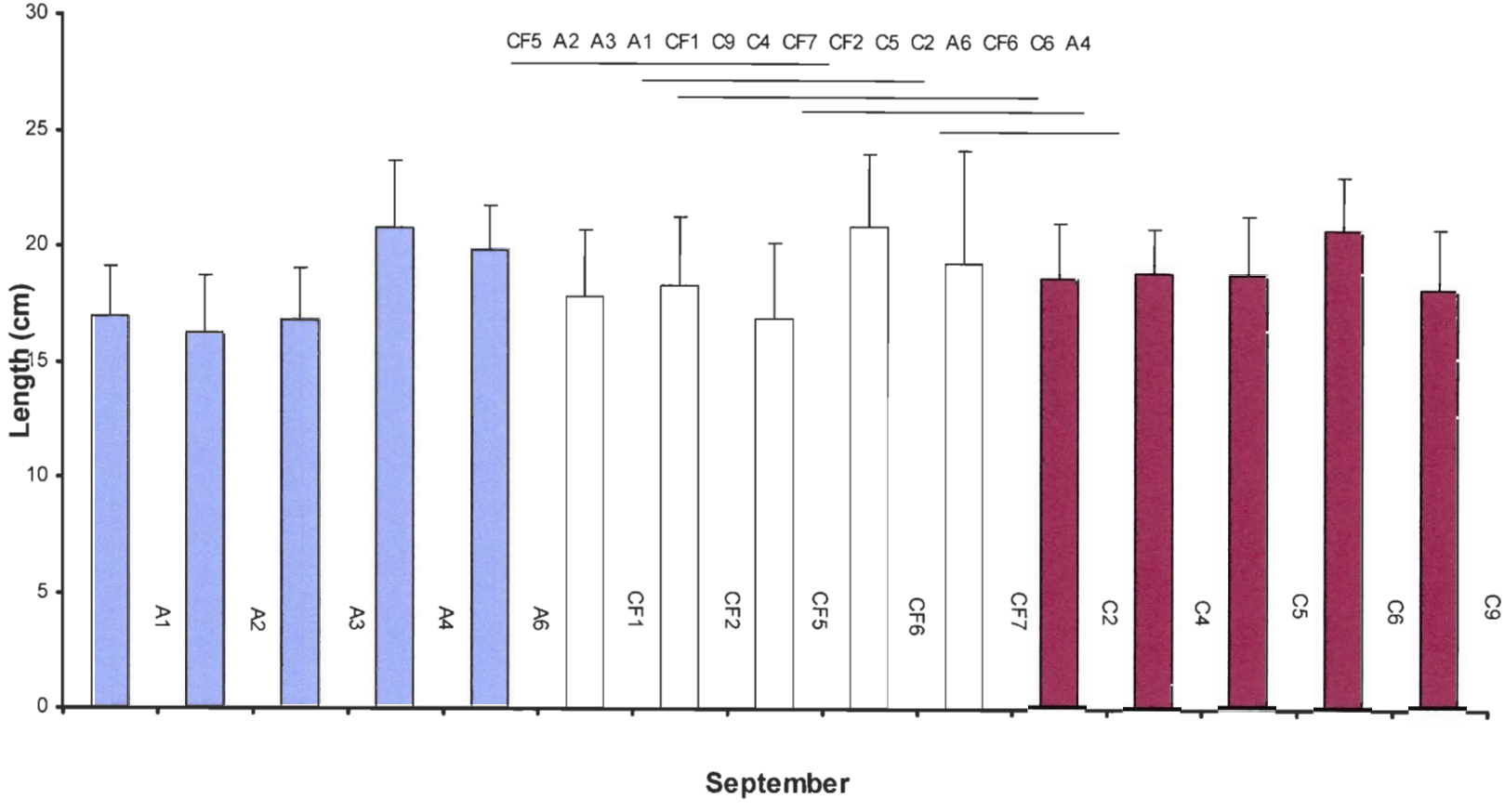


Figure 8. Fish length of the interspecific *Salvelinus alpinus* Salvelinus fontinalis* families (AA, CF, CC) in September. Mean ± standard deviation. There was no significant difference between time of sampling. Interfamilial differences for September are represented here but the same profile has been found in April and July. Results of pairwise comparisons using Games and Howell's test are presented here for fish length of the interspecific families.

Disease susceptibility

Disease susceptibility varied tremendously among families. The most resistant ones, in which no mortality occurred during summer time were *S. fontinalis* anadromous (A4, A6), resident (R7) and interspecific hybrids *S. fontinalis** *S. alpinus* (CF6, CF7) (Figures 9, 10, 12). In some families, apparition of lesions and loss of appetite occurred two weeks after transfer to 20°/∞. Lesions were superficial when mortalities occurred early, but developed into eroded ulcers when mortalities took place later during the experiment. The most prominent clinical signs in both species were erosive lesions on the head, dorsal and lateral sides of fish. Many cases of fin or tail rot, jaw erosion or different combinations of these were reported. It was not rare to find dead fish without visible lesions.

These families in which mortalities occurred early in summer were considered as the less resistant to opportunistic infections. These families were *S. fontinalis* anadromous (A1, A2, A3) and resident (R8, R2, R3) (Figures 9-10). Following exposure to acute stress, few mortalities were recorded (less than 3) in *S. fontinalis* resident (R3, R8), intraspeficic hybrids (AR1, AR5), interspecific hybrids (CF2, CF5), and *S. alpinus* (C4, C6) (Figures 10-13). On the other hand, important mortalities occurred upon transfer on individuals to high salinity conditions. Susceptible families after transfer to 30°/∞ were *S. fontinalis* resident (R8), intaspecific hybrids (AR5, AR3, AR1, AR2), and *S. alpinus* (C4, C2, C9) (Figures 10; 11; 13). Some families, *S. fontinalis* resident (R6), intraspecific hybrid (AR4), interspecific hybrids (CF1, CF2, CF5), and *S. alpinus* (C5, C6), were considered very resistant due to few mortalities recorded during the entire summer (less than 10) (Figures 10-13).

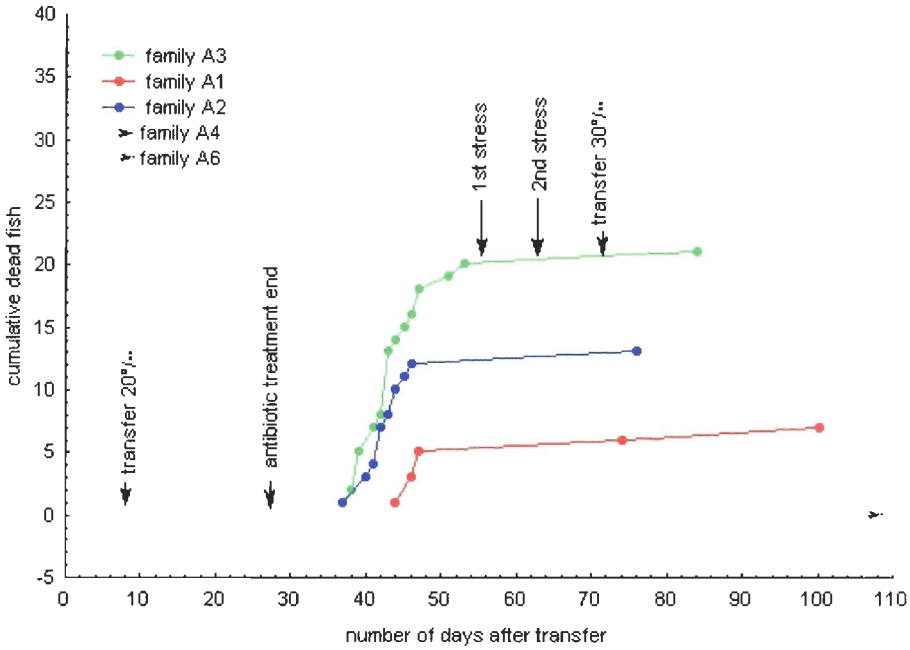


Figure 9. Cumulative mortalities in anadromous *Salvelinus fontinalis* families (AA) during summer time. Dotted arrows indicate that no mortality occurred in families A4 and A6.

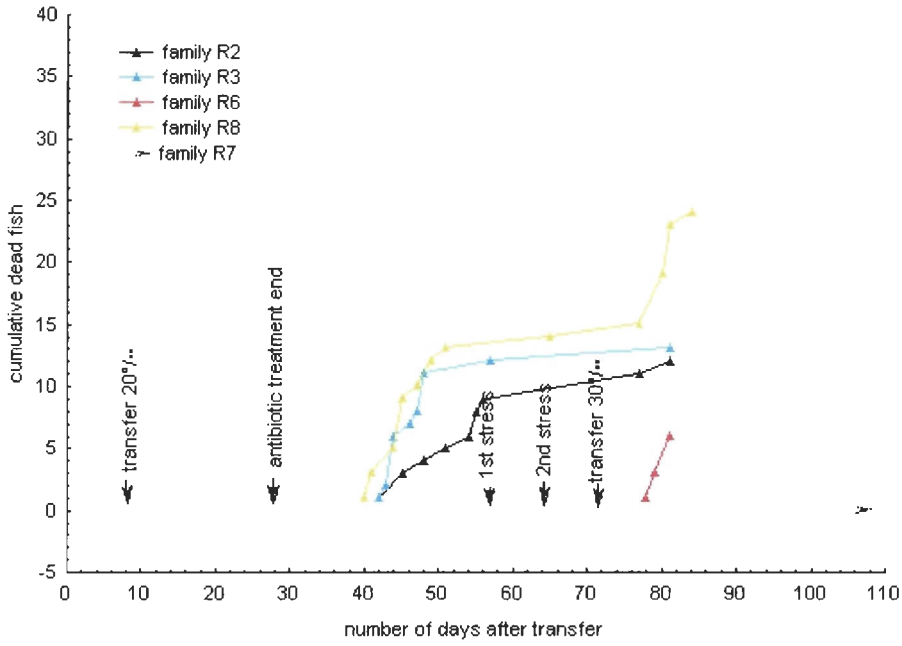


Figure 10. Cumulative mortalities in resident *Salvelinus fontinalis* families (RR) during summer time. Dotted arrows indicate that no mortality occurred in family R7.

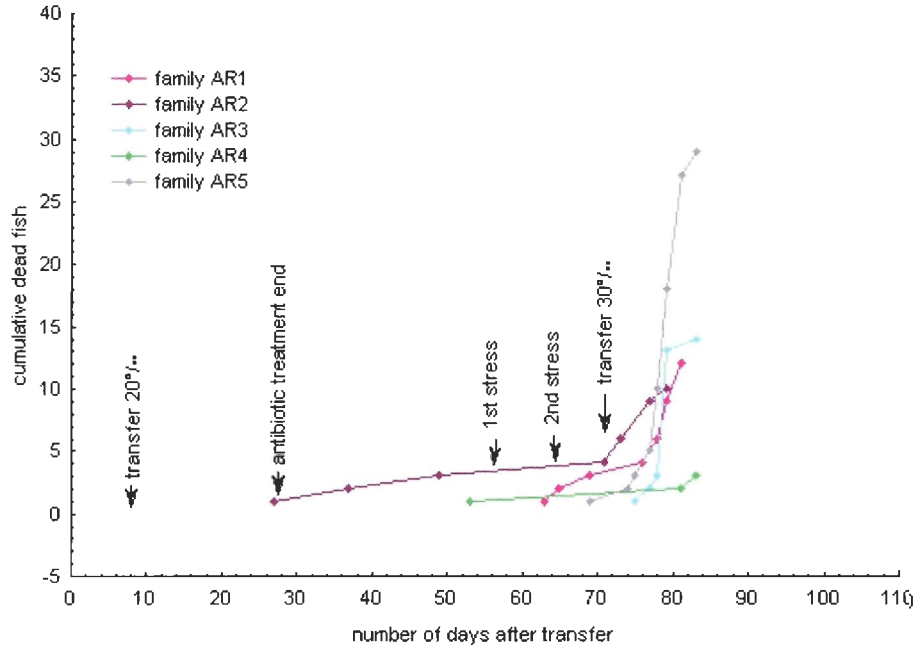


Figure 11. Cumulative mortalities in anadomous-resident *Salvelinus fontinalis* families (AR) during summer time

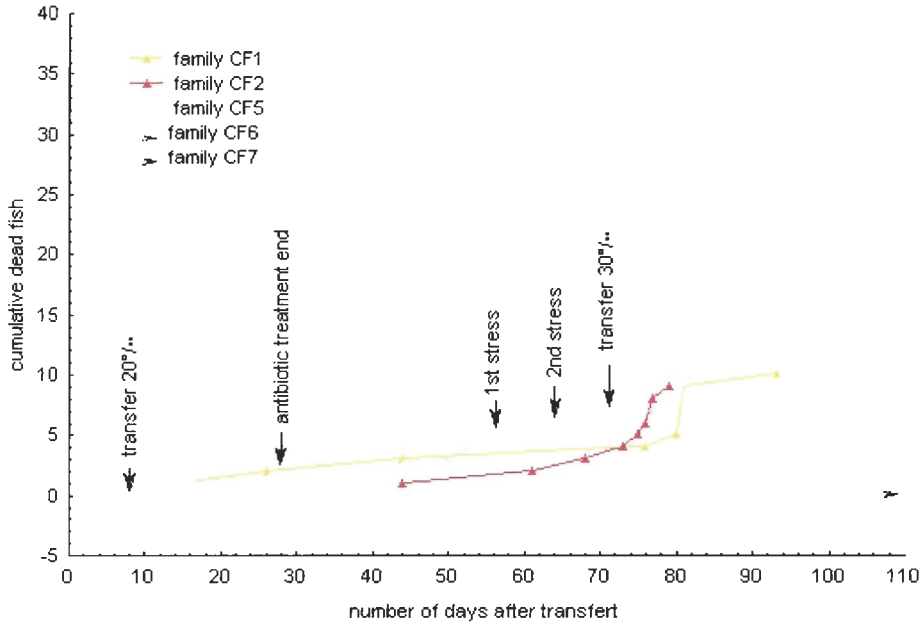


Figure 12. Cumulative mortalities in anadromous *Salvelinus alpinus* * Salvelinus *fontinalis* families (CF) during summer time. Dotted arrows indicate that no mortality occurred in families CF6 and CF7.

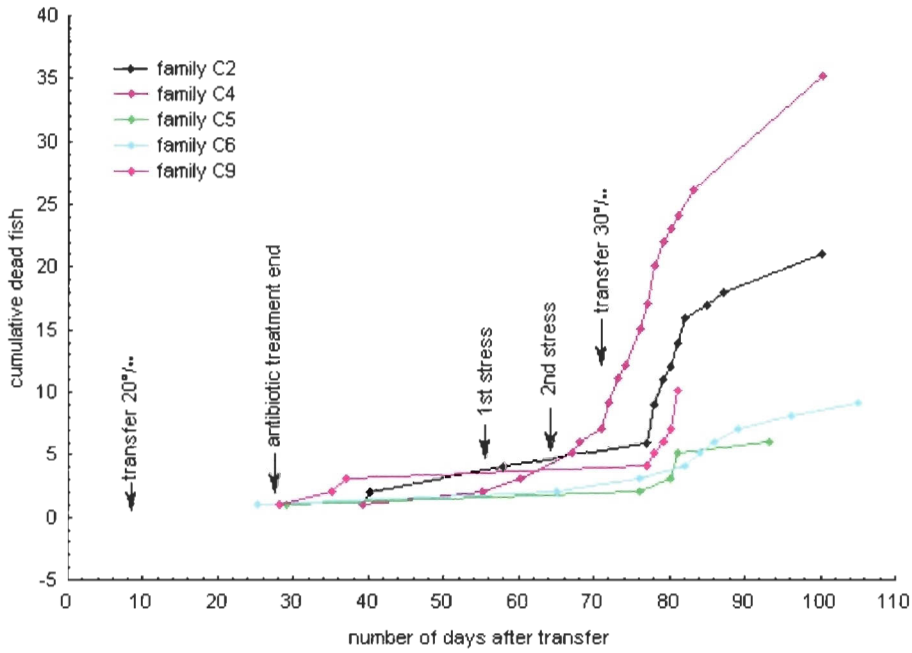


Figure 13. Cumulative mortalities in anadromous *Salvelinus alpinus* families (CC) during summer time.

2.4 Discussion

Based on an efficient specific medium selection, we were able to make an initial diagnosis of the pathogen affecting fish. FMM was chosen due to its ability to allow better growth of *Tenacibaculum maritimum* in comparison to the growth of heterotrophic halophilic bacteria, such as *Vibrio*, *Pseudomonas*, and *Aeromanas* species, which usually overgrow the plates (Avendaño-Herrera *et al.*, 2005). Results were then confirmed through the nested PCR protocol of Cepeda *et al.* (2003) considered as the most adequate for an accurate detection of *Tenacibaculum maritimum* in diagnostic pathology as well as in epidemiological studies of gliding bacterial disease in marine fish (Avendaño-Herrera *et al.*, 2004).

One of the major problems that may be encountered is to distinguish *Tenacibaculum* from other phylogenetically and phenotypically similar species, particularly those of the genera *Flavobacterium* and *Cytophaga* (Toranzo *et al.*, 2005). Formerly, many water bacteria belonging to *Cytophaga* and *Cytophaga*-like bacteria were classified by mistake as myxobacteria. In fact, until recently, those organisms belonged to the *Cytophaga-Flavobacterium-Bacteroides* phylum (Reichenbach, 1999). The heterogeneity of the genera *Cytophaga*, *Flexibacter*, and *Flavobacterium* has caused considerable confusion in differentiating these bacteria (Toranzo *et al.*, 2005). Suzuki *et al.* (2001) reclassified *Flexibacter maritimus* into the genus *Tenacibaculum* belonging to *Flavobacteriaceae*

family. The application of PCR was a great benefit for the characterization of specific pathogens.

The filamentous organism *Tenacibaculum maritimum* (formerly, *Cytophaga marina*, *Flexibacter marinus* and *Flexibacter maritimus*) is the causative agent of marine flexibacteriosis, an important disease widely distributed in cultured marine fishes (Toranzo *et al.*, 2005). This etiological agent has the potential to cause severe mortalities in several fish species reared in seawater around the world (Avendaño-Herrera *et al.*, 2005). It was first identified in Japan in 1979 (Bernadet, 1997) and was reported as a salt-water requiring organism producing infections characterized by eroded mouth and fins and skin lesions (Wakabayashi *et al.*, 1986). Young fish suffer a more severe form of the disease than juveniles (Margariños *et al.*, 1995). The disease is influenced by a multiplicity of environmental (stress) and host-related factors (skin surface condition) and could be more severe if temperatures are above 15°C (Margariños *et al.*, 1995). Until recently, no vaccines were available to prevent the disease (Bernadet, 1997), except one for turbot. However, it is not effective in other marine fish (Santos *et al.*, 1999).

One of our main objective was to verify if differential familial genetic resistance to this opportunist pathogen in seawater exist. Our results supported this hypothesis as disease susceptibility varied tremendously among families. Even though, it appeared that the interspecific hybrids *S. alpinus**S. fontinalis* (CF) were the most resistant all along the experiment. On the other hand, anadromous and resident *S. fontinalis* were the less resistant

to opportunistic infections. However, these families were affected early in summer but there was no mortality following exposure to acute stress neither to high salinity conditions. Furthermore, some of the anadromous and resident *Salvelinus fontinalis* families were highly or very resistant, showing no or very few mortalities during the whole experiment. This observation suggested that fish with susceptible phenotype were eliminated from the beginning of the experiment, and that only the most resistant ones remained.

In general, growth performance was important in all families in seawater, and especially in *S. alpinus* families. Furthermore, in both groups intraspecific (AA, AR, RR) and interspecific (AA, CF, CC), condition factors were higher and differences reduced in September, which indicates that at the end of the experiment, all families were in good condition. Our results showed that in September, resident *S. fontinalis* for intraspecific families and *S. Alpinus - S. fontinalis* for interspecific families were in better condition than others. The fact that anadromous *S. fontinalis* had lower condition factors than resident throughout the whole summer could be explained by their physiology. Anadromous *S. fontinalis* are found to be more streamlined (narrower and shallower bodies) than resident brook trout (Morinville and Rasmussen, 2003), our data seemed to confirm it.

Families that performed better (A4, A6, CF6, and R6) were highly resistant, and the smallest families (A1, A2 and A3) were the less resistant ones. However, these observations should be interpreted with caution because the presence of correlations between growth performance and disease resistance was not tested. Furthermore, highly

resistant families (CF7 and R7), were not among those with largest individuals but were rather intermediate in size. Afterwards, it will be interesting to calculate dead fish condition factors in order to compare them with condition factors of surviving fish. If significant genetic variation in disease resistance is found as it has been the case in many studies, this will suggest the possibility of selection to enhance resistance to opportunistic infections (Chevassus and Dorson, 1990; Fjalestad *et al.*, 1993; Wiegertjes *et al.*, 1996; Cipriano *et al.*, 2002; Henryon *et al.*, 2002; Roed *et al.*, 2002). According to these studies, in both cases, inter-specific and intra-specific differences in susceptibility of fish to diseases were obtained (Cipriano *et al.*, 2002). However, an important problem in studying susceptibility in fish populations is to identify and use measurable traits correlating with disease resistance (Sahoo *et al.*, 2004). The present study could help in developing strategies for the selection of marine flexibacteriosis-resistant strains of fish in genetic selection programs.

We isolated and characterized the parental class II β1 alleles in brook and Arctic broodstocks. Alignment of the 21 alleles' sequences revealed that most of the variability (76%) resides in the peptide-binding sites. Much of the variation found in the charr sequences corresponds to polymorphic sites of the β1 domain in higher vertebrates. As Langefors *et al.* (2001) noted, this indicates that the polymorphic sites in the fish alleles differed only slightly from those in human alleles. The high variability of exon 2 was in accordance with observations made on mammalian class IIβ genes (Ono *et al.*, 1993). It is interesting to note that some alleles only differed by a single (d and f; n and m; h and i) or two nucleotides (d and e; i and j). The fact that most of them encoded for different amino-

acid sequences (at the exception of alleles h and i) confirmed the high variability of exon 2. In their study, Langefors *et al.*, (2001) also found two different alleles that differed by a single nucleotide. They concluded that one of these two alleles could be 'an example of a new allele that has been arisen and been maintained through frequency-dependent selection due to its association with resistance to furonculosis' (Langefors *et al.*, 2001).

Specific alleles to anadromous Arctic charr, anadromous and resident brook charr were found, as well as alleles being shared between both species. Miller and Withler (1996) indicated that comparison of MHC exons within and between closely related species often reveals the existence of shared allelic lineages, which indicates that the formation of alleles predated speciation.

The estimation of allelic combinations was realized from the parental genotype. The progeny inherits two genes, one from each parent, so the progeny proportion is ¼ for each allele. Nevertheless, future analysis for genotype identification of offspring will be planned and will confirm the allelic combinations. Based on the high level of heterozygosity from the parental generation, an important range of allelic combinations is expected to occur in the progeny. These data will allow to evaluate if progeny survival to infection was associated with specific alleles. In addition, paternal half-sib crosses will allow us to study the maternal effect and to determine if genetic differences exist between resistant and susceptible individuals.

DISCUSSION GÉNÉRALE

Le cadre général de cette étude dépasse un projet de maîtrise car au départ, il avait été conçu comme un projet de doctorat. La suite du projet sera par conséquent finalisée par d'autres que moi. Dans le cadre de ma maîtrise, j'ai établi le répertoire des allèles du CMH classe IIβ1 chez les géniteurs, identifié l'agent pathogène *Flexibacter maritimus* responsable d'infections opportunistes chez les ombles en milieu marin, noté des différences interfamiliales de résistance lors du transfert en eau de mer et suggéré des pistes d'analyses pour la poursuite du projet.

Les croisements intra et interspécifiques ont été élaborés pour réunir différentes combinaisons d'allèles du CMH classe IIβ1. Les différences interfamiliales de résistance/susceptibilité, la présence de 21 allèles différents, et le fait que la majorité des parents soient hétérozygotes assurent qu'une importante gamme de combinaisons alléliques sera retrouvée. Une estimation des combinaisons alléliques possibles sachant qu'un géniteur ne transmet que la moitié de son génome à la descendance (un gène parmi les deux qu'il possède) a été réalisée. Les analyses à venir sont tout d'abord de déterminer le génotype de la progéniture permettant ainsi de confirmer l'estimation allélique établie. En effet, à partir des prélèvements effectués à la fois chez tous les poissons infectés et chez cinquante individus pour les familles résistantes, il sera possible de vérifier s'il existe une disproportion par rapport à l'estimation attendue. Ces résultats couplés avec ceux obtenus chez les géniteurs, permettront d'établir si les différences de résistance sont corrélées à des

différences alléliques au niveau de β1. Il sera alors possible de déterminer si certaines combinaisons alléliques ou si un allèle spécifique mènent à une plus grande résistance. Si une variation génétique significative est trouvée, l'identification des allèles conférant une résistance accrue aux infections permettra d'optimiser la production et de mettre en place un programme de sélection. Les reproducteurs ayant les meilleures valeurs phénotypiques pour le caractère recherché, meilleure résistance, pourront être sélectionnés.

Bien que le domaine β1 soit la région la plus polymorphique, une étude similaire concernant le domaine α1 pourrait être envisagée car cette région est également associée à la présentation de l'antigène. Chez les poissons, le CMH de classe IIα attire moins l'attention que le classe IIβ du fait que chez les mammifères, il est beaucoup moins polymorphique (Marsh *et al.*, 2000). Cependant, Stet *et al.*, (2002) ont observé un nombre comparable d'allèles du CMH de classe IIα que du CMH de classe IIα chez le saumon atlantique, démontrant ainsi un niveau de polymorphisme de la chaîne α comparable à la chaîne β.

Les méthodes habituelles de génétique quantitative, pour lesquelles on applique les lois de Mendel aux caractères présentant une variation discontinue, ne s'appliquent pas dans notre cas (Falconer and Mackay, 1996). En effet, pour la susceptibilité aux maladies, on retrouve deux classes phénotypiques : affectés ou non-affectés, mais il n'y a pas d'intermédiaires. L'infection n'a pas été déclenchée volontairement contrairement à la plupart des études qui utilisent ainsi le temps de survie comme variable principale. D'après

la littérature, la seule analyse envisageable serait d'utiliser un modèle pour lequel les individus sont affectés s'ils dépassent un certain seuil de susceptibilité (Yang *et al.*, 1998). Ce modèle : « the liability/threshold model » a été développé par Falconer (1965) pour traiter les données de « caractères seuils » présentant des distributions discontinues dans le cas de susceptibilité aux maladies. Selon lui, ces caractères peuvent être analysés de la même façon que des caractères continus s'ils sont considérés comme exhibant des valeurs seuils entre lesquelles on observe une discontinuité. Il suggère l'utilisation d'une variable appelée « liability » qui ne peut être mesurée mais dont la valeur moyenne au sein d'un groupe peut être déterminée à partir de l'incidence d'apparition d'un caractère (ex : l'apparition d'une maladie). Le point sur l'échelle de « liability » où tous les individus sont affectés et au-dessous duquel tout le monde est « normal » est appelé un seuil.

Un exemple a été réalisé pour la famille R8 (Annexe 1). Quatre classes phénotypiques ont été définies : non-affectés, affectés par le transfert à 20°/₀₀, affectés par les stress aigus et affectés par le transfert à 30°/₀₀. Ensuite, trois seuils séparant ces quatre classes ont été désignés: T1 correspondant au transfert à 20°/₀₀ (du jour 0 au jour 55); T2 correspondant aux stress aigus (du jour 56 à 70), et T3 correspondant au transfert à 30°/₀₀ (du jour 71 à 105). A partir de ce modèle, on peut remarquer que sous le seuil T1, 84% des poissons n'ont pas été affectés par les infections opportunistes, alors qu'au-delà de ce seuil, les infections apparaissent. Pour la famille R8, la majorité des poissons infectés est due principalement au transfert à 20°/₀₀ (ce qui représente 8.66%), ainsi qu'au transfert à 30°/₀₀ (6.66%), alors que seulement (0.66%) ont été affectés par les stress aigus. L'annexe 2

regroupe les données pour l'ensemble des familles. Il serait sans doute opportun de considérer l'application d'un tel modèle pour des comparaisons statistiques ultérieures.

Afin de comparer les différences interfamiliales de résistance, l'approche du « threshold/liability model» a été envisagée. Rappelons cependant que ce modèle ne doit pas être vu comme une preuve scientifique, mais plus comme une hypothèse (Turnpenny et Ellard, 2004). Dans les analyses à venir et ce avant la publication, l'héritabilité sera calculée. On pourrait envisager de donner un indice pour chaque individu en fonction du seuil, lequel indice pourrait être utilisé comme valeur individuelle dans des calculs d'héritabilité.

De plus, les croisements en demi-frère devraient être pris en compte. Il faudra également regarder les différences de résistance en fonction du type d'hybrides. Les données actuelles des facteurs de conditions indiquent que la majorité des familles sont en bonne santé. Cependant, ces facteurs ont été calculés chez les poissons vivants. Il serait donc intéressant de les comparer avec ceux des poissons morts (des mesures ayant été prises pour chaque poisson mort) afin de vérifier si ce sont les plus petits qui étaient les plus sensibles. Par contre les mortalités étant survenues à divers moments au cours de l'été, il faudrait sans doute trouver le moyen de réajuster l'évolution des facteurs de condition en fonction des degrés jours si on voulait procéder à des comparaisons.

CONCLUSION GÉNÉRALE

Ce travail a permis d'une part de dresser le répertoire des allèles du CMH de classe IIβ1 des géniteurs. A partir du génotype parental, l'estimation des combinaisons alléliques pouvant possiblement être retrouvées chez la progéniture a pu être établie. D'autre part, la détermination des bases génétiques de la susceptibilité/résistance naturelle des ombles et l'identification de l'agent pathogène opportuniste *Flexibacter maritimus* permettront de cibler des individus naturellement plus résistants. Ces résultats serviront à la suite du projet qui vise à déterminer si les différences de résistance sont corrélées à des différences alléliques au niveau de β1. La détermination du génotype de la progéniture confirmera l'estimation allélique établie et permettra de mettre en évidence la présence ou l'absence d'un allèle conférant une résistance accrue.

Ce travail s'insère dans un projet qui vise à mettre à la disposition de l'industrie les outils génétiques de sélection de lignées performantes pour l'élevage d'ombles en milieu côtier. On cherche ainsi à optimiser la production et améliorer l'adaptabilité à l'eau de mer des populations d'élevage ciblées.

LISTE DES RÉFÉRENCES

Anacker, R.L., et Ordal, E.J. 1959. Studies on the myxobacterium *Chondrococcus columnaris*. I. Serological typing. Journal of Bacteriology 78: 25-32.

Anderson, D.P. 1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. American Fisheries Society Symposium 8: 38-50.

Avendaño-Herrera, R., Rodriguez, J., Magariños, B., Romalde, J.L., et Toranzo, A.E. 2004. Intraspecific diversity of the marine fish pathogen *Tenacibaculum maritimum* as determined by randomly amplified polymorphic DNA-PCR. Journal of Applied Microbiology 96: 871-877.

Avendaño-Herrera, R., Irgang, R., Núñez, S., Romalde, J.L., et Toranzo, A.E. 2005. Recommendation of an appropriate medium for in vitro drug susceptibility testing of the fish pathogen *Tenacibaculum maritimum*. Antimicrobial Agents and Chemotherapy 49: 82-87.

Bensa, J.C. 2003. Le complexe majeur d'histocompatibilité et la définition du soi. Transfusion Clinique et Biologie 10 : 209-213.

Bernadet, J.F. 1997. Immunization with bacterial antigens: *Flavobacterium* and *Flexibacter* infections. Developments in Biological Standardization 90: 179-188.

Bernatchez, L., et Landry, C. 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? Journal of Evolutionary Biology 16: 363-377.

Birnboim, H.C. et Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research 7: 1513-1519.

Bouillon, M., et Mourad, W.M. 2003. Complexe majeur d'histocompatibilité de classe II : diversité fonctionnelle. Medecine Sciences 19: 988-993.

Budowle , B. Chakraborty, R., Giusti, A.M., Eisenberg, A.J., et Allen, R.C. 1991. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. American Journal of Human Genetics 48 : 137-144.

Cepeda, C., Garcia-Márquez, S., et Santos, Y. 2003. Detection of *Flexibacter maritimus* in fish tissue using nested PCR amplification. Journal of Fish Diseases 26: 65-70.

Chevassus, B., et Dorson, M. 1990. Genetics of resistance to disease in fishes. Aquaculture 85: 83-107.

Cipriano, R.C., Marchant, D., Jones, T.E., et Schachte, J.H. 2002. Practical application of disease resistance to furunculosis. Aquaculture 206: 1-17.

d'Autray Tarte, P. 2004. Analyse du CMH de classe IIβ chez l'omble de fontaine (*Salvelinus fontinalis*) et résistance à *Aeromonas salmonicida*. Mémoire présenté à la faculté des études supérieures de l'Université de Montréal, département de Microbiologie et Immunologie.

Difco™ et BBL™ Manual. 2003. Manual of microbiological culture media. Difco laboratories, Detroit. pp. 536-537.

Ellis, A.E. 1981. Stress and the modulation of defence mechanisms in fish. In: Stress and fish (Pickering, A.D., ed.). Academic Press, London and New York. pp. 209-245.

Falconer, D.S. 1965. The inheritance of liability to certain diseases, estimated from the incidence among relatives. Annals of Human Genetics 29: 51-76.

Falconer, D.S. et Mackay, F.C. 1996. Introduction to Quantitative Genetics. 4th Edition. Addison Wesley Longman, New York. pp. 300-311.

Fevolden, S.E., Refstie, T. et Reed, K.H. 1991. Selection for high and low cortisol stress response in Atlantic salmon (*Salmo safar*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 95: 53-65.

Fevolden, S.E., Refstie, T., et Gjerde, B. 1993. Genetic and phenotypic parameters for cortisol and glucose stress response in Atlantic salmon and rainbow trout. Aquaculture 118: 205-216.

Fevolden, S.E., Reed, K.H., et Gjerde, B. 1994. Genetic components of post-stress cortisol and lysozyme activity in Atlantic salmon; correlations to disease resistance. Fish and Shellfish Immunology 4: 507-519.

Fevolden, S.E, Roed K.H., Fjalestad, K.T., et Stien, J. 1999. Post-stress levels of lysozyme and cortisol in adult rainbow trout (*Oncorhynchus mykiss*); heritabilities and genetic correlations. Journal of Fish Biology 54: 900-910.

Fjalestad, K.T., Gjedrem, T., et Gjerbe, B. 1993. Genetic improvement of disease resistance in fish: an overview. Aquaculture 111: 65-74.

Grimholt, U., Drables, F., Jorgensen, S.M., Hoyheim, B., et Stet, R.J.M. 2002. The major histocompatibility class I locus in Atlantic salmon (*Salmo salar* L.): polymorphism, linkage analysis and protein modelling. Immunogenetics 54: 570-581.

Grimholt, U., Getahun, A., Hermsen, T., et Stet, R. 2000. The major histocompatibility class II alpha chain in salmonid fishes. Developmental and Comparative Immunology 24: 751-63.

Grimholt, U., Larsen, S., Nordmo, R., Midtlyng, P., Kjoeglum, S., Storset, A., Saeb, S., et Stet, R.J.M. 2003. MHC polymorphism and disease resistance in Atlantic salmon (*Salmo salar*); facing pathogens with single expressed major histocompatibility class I and class II loci. Immunogenetics 55: 210-219.

Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., et Slierendrecht, W.J. 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209: 59-76.

Iwama, G., et Nakanishi, T. 1996. The specific immune system : cellular defenses. In: Manning, M.J., et Nakanishi, T. (ed.): The fish immune system organism, pathogen and environment. Academic Press, London. pp. 185-205.

Janeway, C.A. 2001. How the immune system works to protect the host from infection: A personal view. Proceedings of the National Academy of Sciences. 98: 7461-7468.

Köllner, B., Wasserrab, B., Kotterba, G., et Fischer, U. 2002. Evaluation of immune functions of rainbow trout (*Oncorhynchus mykiss*) – how can environmental influences be detected? Toxicology Letters 131: 83-95.

Kumar, S., Tamura, K., et Nei, M. 2004. MEGA 3 : Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5: 150-163.

Langefors, A., Lohm, J., Grahn, M., Andersen, O., et von Schantz, T. 2001. Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. Proceedings of the Royal Society of London Series B - Biological Sciences 268: 479-485.

Magariños, B., Pazos, F., Santos, Y., Romalde, J.L., et Toranzo, A.E. 1995. Response of *Pasteurella piscicida* and *Flexibacter maritimus* to the skin mucus of marine fish. Diseases of Aquatic Organisms 21: 103-108.

Marsh S.G.E., Parham, P., et Barber, L. D. 2000. The HLA factsbook. Academic Press, San Diego. In: Stet, R.J.M., de Vries, B., Mudde, K., Hermsen, T., van Heerwaarden, J., Shum, B.P., et Grimholt, U. 2002. Unique haplotypes of co-segregating major histocompatibility class II A and class II B alleles in Atlantic salmon (*Salmo salar*) give rise to diverse class II genotypes. 2002. Immunogenetics 54: 320-331.

Miller, K.M., et Withler, R.E. 1996. Sequence analysis of a polymorphic MHC class II gene in Pacific salmon. Immunogenetics 43: 337-351.

Morinville, G., et Rasmussen, J.B. 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60: 401–410.

Nakanishi, T., Aoyagi, K., Xia, C., Dijkstra, J.M., et Ototake, M. 1999. Specific cell-mediated immunity in fish. Veterinary Immunology and Immunopathology 72: 101-109.

Ono, H., O'hUigin, C., Vincek, V., et Klein, J. 1993. Exon-intron organization of fish major histocompatibility complex class II β genes. Immunogenetics 38: 223-234.

Pazos, F., Santos, Y., Macías, A.R., Núñez, S., et Toranzo, A.E. 1996. Evaluation of media for the successful culture of *Flexibacter maritimus*. Journal of Fish Diseases 19: 193-197.

Pickering, A.D. 1992. Rainbow trout husbandry: management of the stress response. Aquaculture 100: 125-139.

Pottinger, T.G., et Pickering, A.D. 1997. Genetic basis to the stress response : selective breeding for stress-tolerant fish. In: Fish stress and health in aquaculture, Iwana, G.K., Pickering, A.D., Sumpter, J.P., et Schreck, C.B. Cambridge University Press, Cambridge, UK. pp. 171-193.

Reichenbach, H. 1999. The ecology of the myxobacteria. Environmental Microbiology 1: 15-21.

Roed, K.H., Fevolden, S.E., et Fjalestad, K.T. 2002. Disease resistance and immune characteristics in rainbow trout (*Oncorhynchus mykiss*) selected for lysozyme activity. Aquaculture 209: 91-101.

Sahoo, P.K., Meher, P.K., Mahapatra, K.D., Saha, J.N., Jana, R.K., et Reddy, P. 2004. Immune response in different fullsib families of Indian major carp, *Labeo rohita*, exhibiting differential resistance to *Aeromonas hydrophila* infection. Aquaculture 238: 115-125.

Santos, Y., Pazos, F., et Barja, J.L. 1999. *Flexibacter maritimus*, causal agent of flexibacteriosis in marine fish. pp. 1-6. In: G. Oliver (ed.), ICES identification leaflets for diseases and parasites of fish and selfish, no. 55. International Council for the Exploitation of the Sea, Copenhagen, Denmark.

Sokal, R.R. et Rohlf, F.J. 1995. Biometry. The principles and practice of statistics in biological research. 3rd ed. W. H. Freeman & Co., New York. p. 887.

Stet, R.J.M., de Vries, B., Mudde, K., Hermsen, T., van Heerwaarden, J., Shum, B.P., et Grimholt, U. 2002. Unique haplotypes of co-segregating major histocompatibility class II A and class II B alleles in Atlantic salmon (*Salmo salar*) give rise to diverse class II genotypes. 2002. Immunogenetics 54: 320-331.

Sültmann, H., Mayer, W.E., Figueroa, F., O' hUigin, C., et Klein, J. 1994. Organization of MHC Class IIβ genes in the zebrafish (*Branchydanio rerio*). Genomics 23: 1-24.

Sunnucks, P., Wilson, A.C., Beheregaray, L.B., Zenger, K., French, J., et Taylor, A.C. 2000. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Molecular Ecology 9: 1699-1710.

Suzuki, M., Nakagawa, Y., Harayama, S., et Yamamoto, S., 2001. Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. International Journal of Systematic and Evolutionary Microbiology 51: 1639–1652.

Toranzo, A.E., Magariños, B., et Romalde, J.L. 2005. A review of the main bacterial fish diseases in mariculture systems. Aquaculture 246: 37-61.

Trevor, P.T.E. 1996. Infection and disease. In: Iwama, G., et T. Nakanishi. (ed.). The fish immune system: Organism, Pathogen, and Environment. Academic Press, London. pp. 339-366.

Turnpenny, P., et Ellard, S. 2004. Emery's elements of medical genetics. 12th ed. Churchill Livingstone. Chapter 9. Polygenic and multifactorial inheritance. pp. 137-146.

Vandeputte, M., et Prunet, P. 2002. Génétique et adaptation chez les poisons: domestication, résistance au stress et adaptation aux conditions de milieu. Institut National de la Recherche Agronomique, productions animales 15 : 365-371.

Van Muiswinkel, W.B., Wiegertjes, G.F. et Stet, R.J.M. 1999. The influence of environmental and genetic factors on the disease resistance of fish. Aquaculture 172: 103-110.

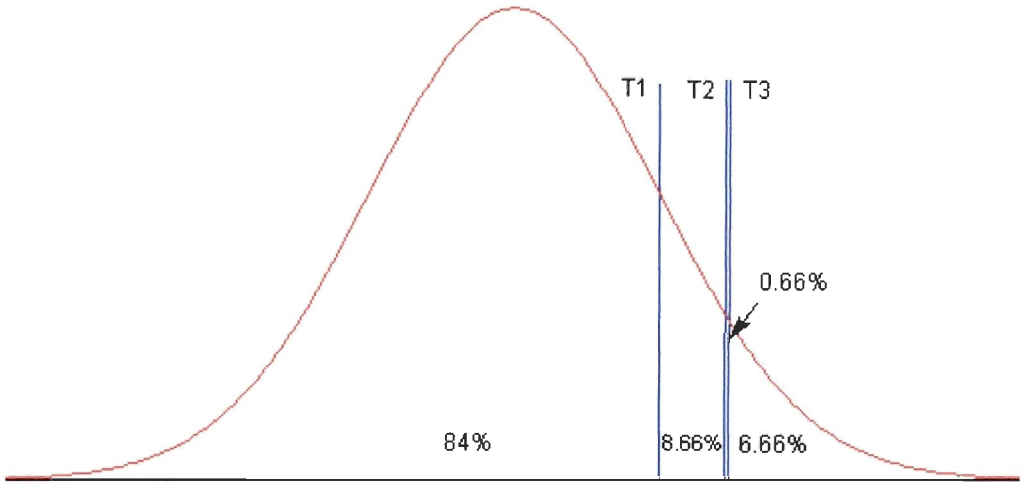
Wakabayashi, H., Hikida, et H., Masumura, K. 1986. *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. International Journal of Systematic Bacteriology 36: 396– 398.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews 77: 501-625.

Wiegertjes, G.F., Stet, R.J.M., Paramentier, H.K., et van Muiswinkel, W.B. 1996. Immunogenetics of disease resistance in fish: a comparative approach. Developmental and Comparative Immunology 20: 365-381.

Yang, R.C., Dhir, N.K. et Yeh, F.C. 1998. Intraclass correlation of polychotomous responses of lodgepole pine to infection of western gall rust: a simulation study. Silvea Genetica 47: 108-115.

ANNEXES



Annexe 1. Exemple du "liability/threshold model" pour la famille résidente *Salvelinus fontinalis* R8 (se reporter au tableau 1).

Groupe	Famille	avant T1	de T1 à T2	de T2 à T3	T3 et plus
Intraspécifique	A1	95.33%	3.33%	0%	1.33%
	A2	91.33%	8%	0%	0.66%
	A3	86.66%	13.33%	0%	0%
	A4	100%	0%	0%	0%
	A6	100%	0%	0%	0%
	AR1	92%	0%	2%	6%
	AR2	93.33%	2%	0%	4.66%
	AR3	90.66%	0%	0%	9.33%
	AR4	98%	0.66%	0%	1.33%
	AR5	80.66%	0%	0.66%	18.66%
	R2	92%	6%	0%	2%
	R3	91.33%	7.33%	0.66%	0.66%
	R6	96%	0%	0%	4%
	R7	100%	0%	0%	0%
	R8	84%	8.66%	0.66%	6.66%
Interspécifique	A1	95.33%	3.33%	0%	1.33%
	A2	91.33%	8%	0%	0.66%
	A3	86.66%	13.33%	0%	0%
	A4	100%	0%	0%	0%
	A6	100%	0%	0%	0%
	CF1	93.33%	2%	0%	4.66%
	CF2	94%	0.66%	1.33%	4%
	CF5	94%	2.66%	1.33%	2%
	CF6	99.33%	0.66%	0%	0%
	CF7	100%	0%	0%	0%
	C2	98.66%	1.33%	0%	0%
	C4	76.66%	1.33%	2%	20%
	C5	96%	0.66%	0%	3.33%
	C6	94%	0.66%	0.66%	4.66%
	C9	93.33%	2%	0%	4.66%

Annexe 2. Tableau présentant, pour les familles intraspécifiques *Salvelinus fontinalis* et interspécifiques *Salvelinus alpinus* - *Salvelinus fontinalis*, les proportions de populations pour chacune des quatre classes phénotypiques séparées par les trois seuils : avant T1 : pas de poissons infectés, de T1 à T2 : poissons susceptibles au passage en eau de mer à 20 °/₀₀, donc les plus sensibles aux infections opportunistes, de T2 à T3 : poissons un peu plus résistants mais sensibles à une surimposition de stress aigus successifs, T3 et plus : poissons encore plus résistants mais sensibles au transfert à 30 °/₀₀.