Université du Québec
à Rimouski

Utilisation de la chimie des otolithes pour évaluer la croissance et la consommation de nourriture de l'omble de fontaine anadrome (*Salvelinus fontinalis*)

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RÉSUMÉ

L’omble de fontaine (*Salvelinus fontinalis*) est l’espèce la plus prisée par les amateurs de pêche sportive depuis de nombreuses années au Québec, en raison de son omniprésence et sa combativité, laquelle engendre de fortes retombées économiques dans le secteur. Cependant sa surexploitation dans les territoires fauniques structurés mena à un déclin de certaines populations, suggérant ainsi la nécessité de mettre en place une gestion efficace de l’espèce par le biais de mesures de réglementation et la réalisation d’ensemencements. La gestion des stocks d’ombles de fontaine requiert des connaissances approfondies de la physiologie (croissance, alimentation et cycle de vie) de ces poissons. Cependant les informations sur certains écotypes de l’omble de fontaine tel que l’écotype anadrome sont trop peu nombreuses. Il devient donc primordial d’améliorer nos connaissances sur cet écotype en vue d’adapter au mieux les mesures relatives à sa conservation. Dans cette étude nous avions pour objectif de corréler la croissance ainsi que la consommation de nourriture chez les ombles de fontaine anadromes de pisciculture et les signatures d’éléments traces retrouvées dans leurs otolithes. Cette corrélation permettrait de mieux comprendre ces signatures chez les poissons sauvages. Parmi ces éléments, le magnésium, le phosphore, le zinc et le cuivre ont été considérés car ils ont déjà montré, dans de précédentes études, des variations semblant pouvoir se rapporter à la croissance et à l’alimentation des poissons. Nous avons pour cela mené une expérience de croissance de six semaines pendant lesquelles des ombles de deux lignées (dont une lignée sélectionnée pour la croissance et la maturité sexuelle tardive) ont été nourris à l’aide de deux traitements de rations alimentaires différents. Nos résultats indiquent que les poissons sélectionnés ont connu une croissance moindre et ont moins consommé de nourriture que les poissons non sélectionnés et que ces deux variables ne semblent pas affecter les concentrations des éléments étudiés. Nous avons observé un effet partiel de la lignée sur les concentrations en zinc suggérant des effets de la maturation sur son incorporation dans l’otolithe. Notre travail ouvrira des perspectives sur l'utilisation des otolithes en tant qu'outil pour comprendre la croissance et l'alimentation des poissons sauvages.

Mots clés : Omble de fontaine, otolithes, sélection, croissance, consommation de nourriture, maturation

ABSTRACT

Brook Trout (*Salvelinus fontinalis*) have been the most popular species among sport fishing enthusiasts in the province of Quebec, Canada, due to their ubiquity and combativeness, which generates significant economic benefits in the sector. However, their overexploitation has led to a decline in some populations, suggesting the need for effective species management through regulatory measures and stocking efforts. Managing Brook Trout stocks requires in-depth knowledge of the physiology (growth, feeding and life cycle) of these fish. Nevertheless, information on the anadromous ecotype remains scarce. Therefore, it is crucial to enhance our understanding of this ecotype to better tailor conservation measures. In this study, our goal was to correlate the growth and food consumption of aquaculture-reared anadromous Brook Trout with the signatures of trace elements found in their otoliths. This correlation would provide a better understanding of these signatures in wild fish. Among these elements, magnesium, phosphorus, zinc, and copper were considered, as earlier studies have shown variations that appear to be related to growth and feeding related metrics of fish. To achieve this, we conducted a six-week growth experiment in which Brook Trout from two lineages (including one lineage selected for growth and late sexual maturity) were fed with two different food ration treatments. Our results indicated that the selected fish exhibited slower growth and reduced food consumption compared to the non-selected fish, while these two variables did not appear to affect trace element concentrations. We observed a partial lineage effect on zinc concentrations, suggesting sexual maturation effects on its incorporation into the otolith. Our work will open perspectives on the use of otoliths as a tool to understand the growth and feeding of wild fish.

Keywords: Brook Trout, otoliths, selection, growth, food consumption, maturation

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ISMER Institut des Sciences de la Mer de Rimouski

MDDEFP Ministère du Développement Durable, de L'Environnement, de la Faune et des Parcs

MDIFW Maine Department of Inland Fisheries and Wildlife

MELCCFP Ministère de l'Environnement, de la Lutte contre les Changements Climatiques, de la Faune et des Parcs

MFFP Ministère des Forêts, de la Faune et des Parcs

MPO Pêches et Océans Canada

TFS Territoires Fauniques Structurés

UQAC Université du Québec à Chicoutimi

UQAR Université du Québec à Rimouski

ZEC Zone d'Exploitation Contrôlée

INTRODUCTION GÉNÉRALE

1. EXPLOITATION DE L'OMBLE DE FONTAINE AU QUEBEC

La pêche sportive a toujours suscité un grand intérêt au Canada avec plus de 3,2 millions de pêcheurs dont plus de 650 000 au Québec de 2005 à 2015 (MPO 2019). Cet engouement pour la pêche se traduit par de fortes dépenses, avec plus de 2,5 milliards de dollars dépensés au Canada en 2015 contre plus de 398 millions de dollars au Québec (MPO 2019) contribuant ainsi à l'économie locale, régionale et nationale (Arlinghaus et al. 2002; Plourde-Lavoie 2014). L'omble de fontaine (*Salvelinus fontinalis*) fait partie des espèces les plus prisées au Québec pour la pêche sportive due à sa combativité et sa disponibilité (Gagné 2023; Magnan 1990). Chaque année, 16,4 millions d'ombles sont capturés en moyenne correspondant à 40% des poissons capturés par tous les pêcheurs québécois (Gagné 2023; MPO 2019). Les zones les plus exploitées pour la pêche sportive de l'omble de fontaine sont surtout les territoires fauniques structurés ou TFS (comme les ZEC, les réserves fauniques et les pourvoiries), où se concentre près de la moitié de l'effort de pêche sportive dans les régions de la Capitale-Nationale, du Saguenay-Lac-Saint-Jean, de la Mauricie, du Bas Saint-Laurent, de Lanaudière et des Laurentides (Gagné 2023). Ainsi, l'exploitation de l'omble de fontaine au Québec génère 340 millions de dollars de dépenses annuelles et plus de 3000 emplois (Gagné 2023; ÉcoRessources 2014). Chez l'omble de fontaine il existe un écotype, c'est-à-dire une sous-population de l'espèce adaptée à des conditions écologiques spécifiques (habitat, climat, régime alimentaire), appelé omble de fontaine anadrome qui a pour particularité d'effectuer des migrations entre des milieux d'eau douce et d'eau salée. Cet écotype est très apprécié pour la pêche sportive mais aussi pour la pêche commerciale en Basse-Côte-Nord (13 à 33 tonnes de débarquements annuels) et pour la pêche à visée alimentaire, rituelle et sociale menée par les communautés autochtones dans les régions de

Gaspésie-Îles-de-la-Madeleine, du Bas-Saint-Laurent, de la Côte-Nord et du Nord-du-Québec (MFFP 2020). La forte demande associée à la pêche sportive pour l'omble de fontaine au Québec nécessite cependant un suivi et un contrôle des populations dans une optique de préservation de l'espèce.

2. ÉTAT ET GESTION DES STOCKS D'OMBLE DE FONTAINE

Une analyse des données d'exploitation recueillies par le MFFP de 1980 à 2009 dans les TFS a montré que le succès de pêche de l'omble de fontaine a diminué durant cette période malgré la diminution de la pression de pêche (Figure 1; Plourde-Lavoie 2014). En effet, une diminution de la pression de pêche dans ces zones devrait signifier une augmentation de l'abondance des poissons et donc un succès de pêche plus important. Néanmoins, ce n'est pas ce qui a été observé lors de l'analyse de ces données (Gagné 2023). Ce déclin est d'ailleurs plus marqué dans les territoires où la pression de pêche a augmenté et bien qu'aussi influencé par la réduction du temps de pêche, il semble indiquer une baisse de l'abondance des populations d'ombles de fontaine au Québec démontrant une surexploitation possible de certaines populations de l'espèce (Plourde-Lavoie 2014).

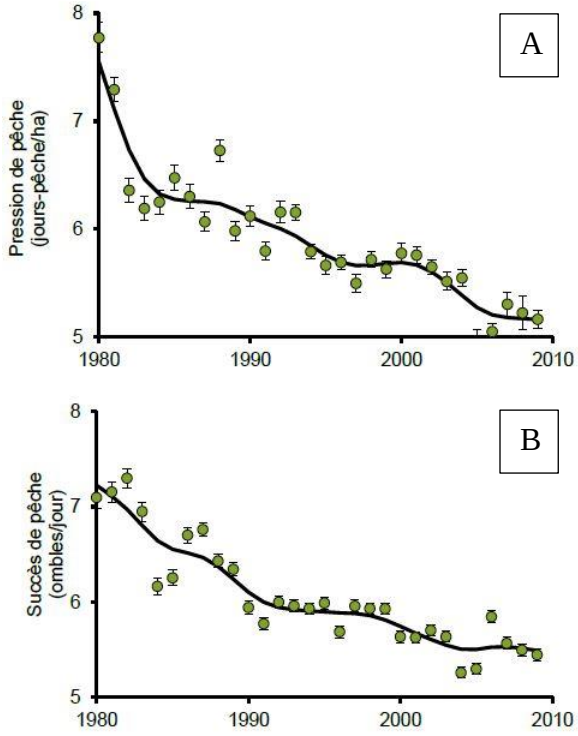

Figure 1. A. Pression et B. succès de pêche de l'omble de fontaine dans les TFS au cours du temps (tirée de Plourde-Lavoie 2014)

La pêche sportive étant aussi sélective, les individus de plus grande taille et ceux âgés de deux à trois ans sont plus ciblés et très peu de captures de poissons de plus de quatre ans ont lieu (Curry et al. 2003; Gagné 2023; Lewin et al. 2006), induisant ainsi des réponses adaptatives et populationnelles chez l'omble de fontaine. En effet, l'étude de populations de 17 lacs du bouclier canadien a montré que l'intensité de pêche de l'espèce était négativement corrélée à l'âge à maturité et positivement corrélée à l'indice gonado-somatique des femelles (Magnan et al. 2005). Les longueurs à la fourche moyennes des ombles semblent aussi inversement corrélées à l'activité de pêche (Heggelin 2008). Ainsi le retrait d'ombles adultes aurait pour conséquence d'accentuer les maturations sexuelles précoces générant ainsi un accroissement de l'effort reproducteur, mais avec des géniteurs de plus petite taille, caractéristique des stratégies de survie des populations sujettes à une exploitation plus intense

(Magnan et al. 2005). Les observations de Plourde-Lavoie (2014) semblent attester de ces stratégies de survie. En effet lors de son étude, une augmentation de la masse moyenne des ombles de fontaine dans les TFS entre 1980 et 2010 a été observée tandis que l'effort de pêche était réduit. Cette même tendance a été observée en rivière et en estuaire chez certaines populations d'ombles de fontaine anadromes au cours des 20 dernières années (MFFP 2020). La diminution des populations d'ombles de fontaine au Québec découle également d'autres facteurs anthropiques tels que l'introduction d'espèces compétitrices comme le meunier noir (*Catostomus commersonii*), le mulet à cornes (*Semotilus atromaculatus*) ou encore la perchaude (*Perca flavescens*) et d'espèces exotiques comme la truite arc-en-ciel (*Oncorhynchus mykiss*) et la truite brune (*Salmo trutta*). Ces différentes espèces entrent en compétition pour les ressources alimentaires et pour l'occupation des habitats favorables, ce qui résulte en une baisse des taux de croissance (Fausch et White 1981; Magnan et al. 1998; Marschall et Crowder 1996). La dégradation et la fragmentation des habitats dues à l'aménagement de structures telles que les ponceaux et les barrages, tout comme l'augmentation des températures due aux changements climatiques font aussi partie de ces facteurs anthropiques pouvant réduire l'abondance des ombles de fontaine au Québec (Hudy et al. 2008; Jourdain Bonneau 2022; Lachance et al. 2008).

Les principales mesures prises pour la conservation de l'espèce au Québec comprennent la réglementation de la pêche, passant par l'abaissement des limites de prises quotidiennes, la définition des périodes de pêche optimales et la gestion des quotas dans les TFS (Gagné 2023). Les ensemencements jouent un rôle très important dans la gestion des stocks de l'espèce. En effet ils ont pour but de conserver et de restaurer les populations en déclin mais aussi de mettre en valeur la pêcherie en permettant aux pêcheurs sportifs d'avoir une meilleure qualité de pêche (Gagné 2023). L'omble de fontaine est ainsi l'espèce la plus vendue pour l'ensemencement au Québec avec 94 % de la production annuelle en pisciculture destinées à ces fins (MDDEFP 2013). La mise en œuvre efficace de ces mesures nécessite une connaissance approfondie de l'écologie de l'omble de fontaine. Pour parvenir à une meilleure compréhension de l'écologie des poissons, il est impératif de maîtriser les paramètres physiologiques qui y sont associés.

3. CONNAÎTRE LA PHYSIOLOGIE DES POISSONS POUR CONTRIBUER À LA GESTION DES PÊCHES

Les adaptations physiologiques à l'environnement et aux changements environnementaux (variations de température, de salinité et d'oxygène dissous) chez les poissons sont des facteurs déterminants pour leur survie dans des milieux variés. Connaître ces adaptations faciliterait une meilleure compréhension des réponses des poissons à différents habitats et des conséquences des variations environnementales sur la distribution des populations de poissons. Par exemple, plusieurs espèces de chabot d'une même famille (Cottidae) acquièrent une meilleure tolérance à l'hypoxie dans les zones intertidales les plus variables en oxygène. L'adaptation à ces conditions particulières est marquée par une baisse de la tension critique en oxygène (P_{crit}) chez les espèces tolérantes, qui est déterminée par la variation de divers paramètres physiologiques tels que la consommation d'oxygène de routine, la surface branchiale spécifique à la masse ainsi que l'affinité de liaison à l'hémoglobine pour l'oxygène (Mandic et al. 2009; Richards 2011). De plus, la connaissance de paramètres physiologiques tels que la croissance, la maturation sexuelle et la reproduction fournit des informations essentielles sur le cycle de vie des poissons et la dynamique des populations auxquelles ils appartiennent. Par exemple chez les salmonidés, pour de nombreuses espèces (l'omble de fontaine, la truite brune et la truite arc-en-ciel) certains poissons effectuent des migrations lors de la maturation sexuelle tandis que d'autres ne le font pas. Les poissons en migration passent une partie de leur vie en eau salée afin de se nourrir, de grandir et de devenir matures, puis reviennent en eau douce pour se reproduire (anadromie), tandis que d'autres poissons restent en eau douce durant la maturation sexuelle (résidence) (Klemetsen et al. 2003). Connaître l'évolution des paramètres liés à la maturation sexuelle tels que les taux métaboliques standard, les taux de croissance et les teneurs en lipides chez les poissons est essentiel pour déterminer leur stratégie de cycle de vie et le moment et la nature de leur migration. Par exemple, les individus ayant des taux métaboliques plus élevés ou des ressources énergétiques plus faibles auront tendance à migrer alors que les individus ayant de faibles taux métaboliques ou des ressources

énergétiques plus élevées adopteront la stratégie de résidence (Birnie-Gauvin et al. 2021). Afin de soutenir la gestion des populations de poissons, il est aussi crucial de déterminer les réponses au stress d'origine anthropique ou environnementale afin d'ajuster au mieux les mesures prises pour la conservation et la survie des poissons. En effet, le stress a des effets sur la physiologie des poissons qui présentent des réponses inadaptées telles que la réduction de l'immunocompétence en cas de stress prolongé (Yada et Tort 2016), de la capacité de reproduction (Barton et Iwama 1991; Pankhurst 2016) et de la quantité d'énergie allouée à la croissance (Sadoul et Vijayan 2016). On peut citer la pollution des milieux aquatiques (effluents industriels, débordements et dérivations d'eaux usées) comme source de stress chez les poissons (Stoliar et Lushchak 2012). Les individus soumis à ces conditions connaissent un état de stress oxydatif lors duquel une augmentation excessive de radicaux réactifs à l'oxygène (ROS) peut causer des dommages et des modifications cellulaires au niveau des lipides, de l'ADN et des protéines. La mesure de modifications telles que la peroxydation des lipides permet de déterminer l'état de stress oxydatif chez un poisson (Winston et Di Giulio 1991). De plus, la manipulation des poissons occasionnée dans la pêche sportive est aussi une source de stress chez ces animaux. Plus celle-ci est prolongée et plus le poisson est stressé. Par exemple, on observe une augmentation significative du niveau de cortisol et de lactate plasmatique (mesurés pour quantifier le stress chez les poissons) de la truite arc-en-ciel lorsque la durée de manipulation (le temps de capture et le temps de retrait de l'hameçon) dépassait deux minutes (Meka et McCormick 2005).

Les principales mesures de paramètres physiologiques des poissons sur le terrain comprennent l'échantillonnage sanguin pour estimer les niveaux d'hormones tels que le cortisol (Meka et McCormick 2005), les biopsies musculaires ou tissulaires pour estimer le contenu énergétique des poissons par exemple à l'aide de la calorimétrie (Schreck et Moyle 1990), la respirométrie pour estimer les taux métaboliques (Rummer et al. 2016) ainsi que l'analyse des otolithes de poissons dont les récentes avancées technologiques (notamment en chimie des otolithes) constituent une approche innovante (Morissette et al. 2021).

4. LA CHIMIE DES OTOLITHES ET SES POSSIBILITÉS

Les otolithes sont des paires de concrétions calcifiées dans l'oreille interne des poissons présentes chez tous les téléostéens et qui leur permet d'entendre et de se tenir en équilibre (Campana 1999; Popper et al. 2005). Il existe trois paires d'otolithes: les sagittae, les lapilli et les asterisci. Les sagittae étant les plus grands otolithes chez les poissons non-ostariophysiens, ils sont généralement les plus utilisés dans la détermination de l'âge et de la croissance, ainsi que pour l'analyse de la composition chimique des otolithes (Campana et Neilson 1985). Ces structures croissent progressivement de manière concentrique au cours de la vie du poisson, laissant apparaître des anneaux de croissance journaliers (Pannella 1971). Des bandes de croissance annuelles, formées par ces anneaux, sont distinguables et leur décompte permet d'estimer la taille à l'âge d'un poisson. Le taux de croissance de l'otolithe, calculé à l'aide de la distance entre les anneaux de croissance et l'âge du poisson, est proportionnel à la croissance somatique d'un poisson. Il est donc possible de déterminer les taux de croissance des poissons au cours de leur vie (Campana et Neilson 1985).

Les otolithes sont principalement composés de carbonate de calcium ($CaCO_3$), d'une matrice de protéines et également d'une variété d'éléments à des concentrations très faibles (de l'ordre de 10 ppm à 1000 ppm); les éléments traces (Campana 1999). On compte parmi ceux-ci le strontium (Sr) et le baryum (Ba) qui sont incorporés dans l'otolithe selon les conditions environnementales telles que les concentrations ambiantes de l'eau dans laquelle réside le poisson, la température et la salinité (Bath et al. 2000; Elsdon et Gillanders 2004; Miller 2011), ou encore des éléments incorporés selon la croissance, l'alimentation et la maturation sexuelle comme le magnésium (Mg), le phosphore (P), le zinc (Zn) et le cuivre (Cu) (Friedrich et Halden 2010; Halden et Friedrich 2008; Halden et al. 2000; Hamer et Jenkins 2007; Ranaldi et Gagnon 2008; Stanley et al. 2015; Sturrock et al. 2015; Sturrock et al. 2014; Willis et Sunda 1984). Par exemple, Elsdon et Gillanders (2004) ont mené une expérience sur des *Acanthopagrus butcheri* soumis à des traitements de température (17 °C et 26 °C), de salinité (5‰ et 32‰) et à des concentrations de Sr et Ba huit fois supérieures aux concentrations ambiantes. Ces paramètres et leurs interactions ont eu un effet significatif

sur l'incorporation de Sr et Ba dans l'otolithe du poisson. Hamer et Jenkins (2007) eux ont trouvé que le Mg était plus élevé dans les zones d'accrétion plus rapides des otolithes de *Pagrus auratus* et de *Platycephalus bassensis* sauvages capturés. Enfin, Ranaldi et Gagnon (2008) ont montré que des *Pagrus auratus* nourris avec une diète enrichie en Zn avaient des concentrations plus élevées en Zn dans l'otolithe. Le processus d'incorporation des éléments traces se divise en plusieurs étapes et fait intervenir plusieurs barrières entre l'environnement et l'otolithe. D'abord ceux-ci sont absorbés par le poisson lors de sa respiration par ses branchies, lors de son alimentation (paroi intestinale) et à travers sa peau (Hüssy et al. 2021). Les éléments sont assimilés dans le sang, puis transportés à travers le corps lors de sa circulation. Ensuite, ils passent dans l'endolymphe, liquide dans lequel baigne l'otolithe (Campana 1999; Hüssy et al. 2021). Les éléments ainsi incorporés le sont de façon permanente car l'otolithe est inerte, ce qui apporte une absence de résorption suite à la formation de celui-ci (Campana et Neilson 1985). La chimie des otolithes est souvent utilisée afin de caractériser l'environnement d'un poisson, de déterminer son origine natale pour mieux comprendre la structure des stocks, de retracer les migrations effectuées par le poisson et d'évaluer sa croissance et son régime alimentaire (Buckel et al. 2004; DiMaria et al. 2010; Kalish 1989; Morissette et al. 2016; Ranaldi et Gagnon 2008).

5. LA BIOLOGIE DE L'OMBLE DE FONTAINE

L'omble de fontaine (Figure 2) fait partie de la famille des salmonidés. Il est caractérisé par une silhouette fuselée et se distingue des autres poissons de cette famille par la présence de points rouges entourés d'un halo bleu nettement défini, de marbrures sur le dos et de nageoires pectorales et pelviennes délimitées par une bande blanche puis d'une bande noire (Gagné 2023).

Figure 2. Omble de fontaine (MELCCFP 2023)

Originaire du nord-est de l'Amérique du Nord, l'espèce est distribuée dans l'est du Canada et le nord-est des États-Unis couvrant ainsi le Québec, les Maritimes, l'ouest de la baie d'Hudson allant du Manitoba aux Grands Lacs et le long des Appalaches (MacCrimmon et Campbell 1969; Raleigh 1982). L'omble de fontaine a ensuite été introduit dans le sud-ouest du Canada et l'ouest des États-Unis. Il est retrouvé quasiment partout au Québec dans divers plans d'eau de toutes tailles (Figure 3). En milieu côtier, l'espèce est constituée de deux écotypes distincts qui adoptent des stratégies de vie différentes, l'omble de fontaine résident qui va passer l'entièreté de son cycle de vie en eau douce et l'omble anadrome qui va migrer vers des zones estuariennes pour se nourrir afin d'améliorer sa croissance avant de revenir en eau douce pour se reproduire (MFFP 2020). La forme résidente vit dans les eaux oxygénées, fraîches et de bonne qualité dans les lacs, les rivières ou les ruisseaux tandis que la forme anadrome favorise les eaux côtières et estuariennes comme l'estuaire et le golfe du Saint-Laurent ou le fjord du Saguenay (Bastien et al. 2011; Gagné 2023; MFFP 2020; Raleigh 1982).

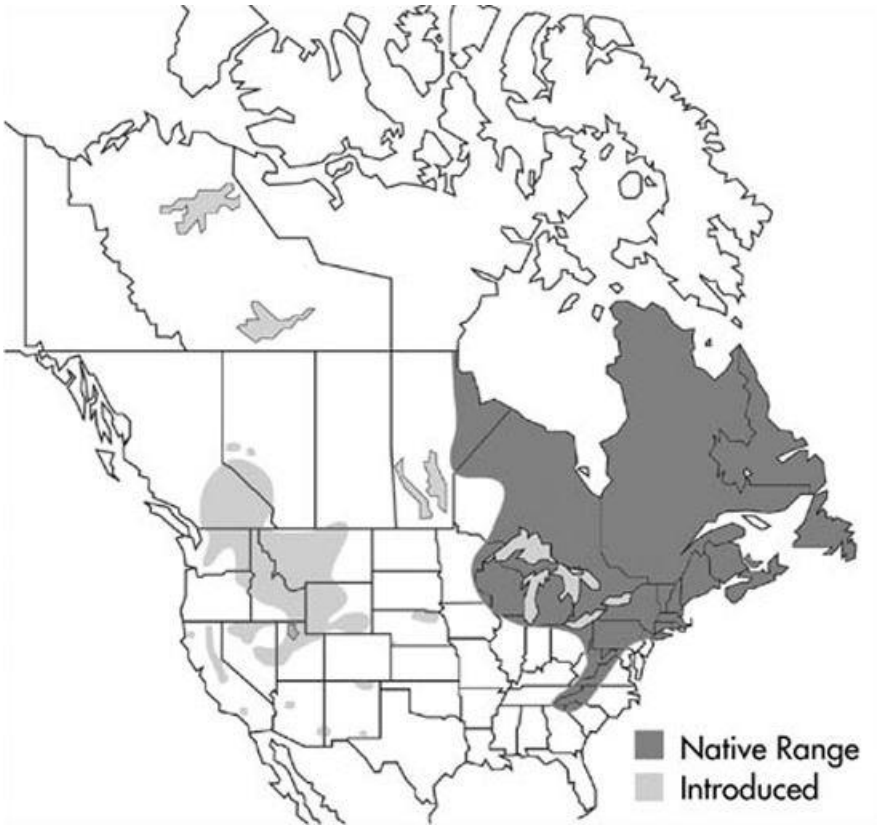

Figure 3. Distribution de l'omble de fontaine en Amérique du Nord (MDIFW 2024)

Les ombles de fontaine anadromes atteignent leur maturité sexuelle au bout de deux à trois ans et vont se reproduire en fin d'été ou en automne (Naiman et al. 1987). Avant d'être aptes à la reproduction, les ombles anadromes vont se rendre vers le mois de mai en eau plus salée (près de leur rivière natale) pour s'alimenter pendant deux à trois mois en proies plus grosses et plus disponibles telles que les amphipodes et les mysidacés pour les migrateurs de première année, puis les poissons marins et les crustacés pour les migrateurs de seconde année et plus (Morinville et Rasmussen 2006). On observe ensuite, à partir de juillet, le retour en eau douce pour la reproduction des ombles matures. Généralement, les ombles anadromes sont de plus grandes tailles, ont une plus grande longévité et sont plus vieux à la maturité que leurs congénères résidents (Castonguay et al. 1982; Naiman et al. 1987). Les poissons anadromes montrent des taux de consommation de nourriture plus élevés que les résidents tout en ayant des efficacités de croissance plus faibles que ces derniers. Cela signifie

possiblement des coûts métaboliques plus importants engendrés par les migrations observées chez cet écotype (Morinville et Rasmussen 2003). Les taux de croissance et l'âge de ces poissons pourraient être déterminés à l'aide des otolithes afin de mieux déterminer leurs différentes caractéristiques.

6. PROBLÉMATIQUE ET OBJECTIFS

Les conséquences de la surexploitation de certaines populations d'omble de fontaine soulèvent la nécessité d'intégrer une gestion plus efficace de ces dernières. Cependant, les populations anadromes restent assez peu documentées (MFFP 2020) et il importe d'accroître nos connaissances sur celles-ci et sur les paramètres physiologiques déterminant leur écologie. La chimie des otolithes pourrait représenter un outil utile pour la compréhension des effets qu'ont une consommation de nourriture, une croissance plus importante ainsi qu'une maturité plus tardive sur ces individus. L'analyse de l'incorporation d'éléments traces selon ces paramètres a montré que des éléments comme le Mg, le P, le Zn et le Cu semblaient être positivement corrélés aux variations de croissance et à l'alimentation (Friedrich et Halden 2010; Halden et Friedrich 2008; Halden et al. 2000; Hamer et Jenkins 2007; MFFP 2020; Ranaldi et Gagnon 2008; Stanley et al. 2015; Sturrock et al. 2015; Willis et Sunda 1984) tandis que Zn et Cu semblent négativement corrélés à la maturité (Sturrock et al. 2014). Ces analyses permettraient donc de déterminer les variations de ces paramètres et de les dater sur l'otolithe.

L'objectif principal de notre recherche était de vérifier comment les concentrations des éléments traces dans l'otolithe des ombles de fontaine anadromes sont affectées par les variations de paramètres physiologiques du poisson. Nous nous intéressons plus particulièrement à l'effet que pourraient avoir une croissance et une consommation de nourriture accrues sur ces concentrations afin d'approfondir nos connaissances sur cet écotype. Pour ce faire, nous avons évalué le lien entre la croissance, la consommation de nourriture, la lignée (une lignée contrôle et une lignée sélectionnée) et les rations alimentaires

chez des ombles de fontaine anadromes d'élevage en milieu contrôlé lors d'une expérience de six semaines. Nous avons ensuite étudié les concentrations de Mg, P, Zn et Cu contenus dans les otolithes de ces poissons afin de corréler les variations observées avec les différents traitements de l'expérience. Nous avons aussi voulu vérifier si les ombles sélectionnés avaient bien des taux de croissance et une consommation de nourriture plus importants et une maturité sexuelle amoindrie par rapport aux ombles de la lignée contrôle. Nous supposons ici que les concentrations de Mg, P, Zn et Cu retrouvées dans les otolithes des poissons de la lignée sélectionnée seront plus élevées que dans la lignée contrôle.

CHAPITRE 1

USE OF OTOLITH CHEMISTRY TO ASSESS GROWTH AND FOOD CONSUMPTION OF ANADROMOUS BROOK TROUT (*SALVELINUS FONTINALIS*)

1.1 ABSTRACT

Otoliths, calcified structures found in the inner ear of fish, serve as remarkable recorders of their life history, providing essential insights into physiological parameters. Understanding the complexity of fish physiology is primordial for effective species management and conservation measures. Despite the importance of otoliths in understanding the life history of various fish species, critical information on specific ecotypes, such as the anadromous variant of Brook Trout (*Salvelinus fontinalis*) in Quebec, Canada, remains notably scarce. Therefore, it is crucial to enhance our understanding of this ecotype to better tailor conservation measures. In this study, our goal was to correlate the growth and food consumption of aquaculture-reared anadromous Brook Trout with the signatures of trace elements found in their otoliths. This correlation would provide a better understanding of these signatures in wild fish. Among these elements, magnesium, phosphorus, zinc, and copper were considered, as earlier studies have shown variations that appear to be related to growth and feeding related metrics of fish. To achieve this, we conducted a six-week growth experiment in which Brook Trout from two lineages (including one lineage selected for growth and late sexual maturity) were fed with two different food ration treatments. Our results indicated that the selected fish exhibited slower growth and reduced food consumption compared to the non-selected fish, while these two variables did not appear to affect trace element concentrations. We observed a partial lineage effect on zinc concentrations, suggesting sexual maturation effects on its incorporation into the otolith. Our work will open perspectives on the use of otoliths as a tool to understand the growth and feeding of wild fish.

Keywords: Brook Trout, otoliths, selection, growth, food consumption, maturation

1.2 INTRODUCTION

Brook Trout (*Salvelinus fontinalis*) is a freshwater fish species originating from the northeast region of North America and which is found throughout the province of Quebec, Canada, in various water bodies (Gagné 2023). Since the end of the 19th century, this species was introduced in western Canada and United States (MacCrimmon and Campbell 1969). In Quebec, Brook Trout are the most sought after species when it comes to sport fisheries with a total of 16 million captures per year and 340 M$ of annual expenditures generated by this fishery, in average (Gagné 2023).

This intense fishing activity led to an overexploitation causing declines in some population in the province. A decrease of the fishing success from 1980 to 2010 in multiple lakes in TFS was observed (from approximately 7.2 trout·angler-day^{-1} in 1980 to 5.5 trout·angler-day^{-1} in 2010) even though fishing pressure also decreased (from 7.5 to 5.2 angler-day·ha^{-1} during the same period), which reflects the impact of these activities on Brook Trout populations (Plourde-Lavoie 2014). Indeed, a reduction in fishing pressure in these areas should result in an increase in fish abundance and, consequently, a greater fishing success. These populations would no longer be able to sustain similar long-term fishing pressure, especially under climate change, which could affect growth and food consumption of the species (Ries and Perry 1995). Also subjected to overfishing, a decrease in abundance and size in some populations of anadromous Brook Trout has been observed since 2000. In the commercial fisheries of the Basse-Côte-Nord region, landings of the species have been found to fluctuate from 13 to 33 tons per year since 1998 (MFFP 2020).

Brook Trout reach sexual maturity around two to three years and spawning occurs during late summer and fall (Naiman et al. 1987). Approaching the spawning season, the species can adopt two strategies that mark its life cycle. Indeed, Brook Trout can be freshwater residents that feed and reproduce in streams, rivers and lakes during their whole life, or anadromous with trophic migration to brackish water in the spring. Anadromous Brook Trout remain in brackish water two to three months and up to a year before they return

to freshwater in late summer for reproduction (Castonguay et al. 1982; White 1940; Wilder 1952). Anadromous Brook Trout find larger prey in estuaries (amphipods, mysids for first year migrants and marine fish for second year migrants) than in freshwater (freshwater aquatic invertebrates and insects) that are in a wider range of sizes meaning better opportunities to grow (Morinville and Rasmussen 2006). This food availability observed in brackish water allows sea-run Brook Trout to grow faster than the freshwater resident ecotype (Rikardsen et al. 2000). In Crespel et al. (2017), the critical swimming speed (U_{crit}) of two strains of Brook Trout (anadromous and resident) were measured at different salinities (fresh and salt water). The two strains reached the same U_{crit} in both fresh and salt water but with different underlying factors linked to their metabolism: anadromous fish benefit from their streamlined body shape and osmoregulatory capacity and resident fish relied more on anaerobic components (higher lactate dehydrogenase capacity and higher glycogen storage) and their higher energy reserves. The difference between these two strategies thus highlights the importance of knowing the specificities related to physiological parameters like growth and food consumption in Brook Trout to better assess their ecology and the status of fish populations in a given environment. Indeed, the effective implementation of management measures requires an in-depth understanding of the ecology of Brook Trout and therefore, to comprehend the associated physiological parameters.

Fish exhibit crucial physiological adaptations to environmental changes, such as variations in temperature, salinity, and dissolved oxygen, influencing their survival across diverse habitats. Understanding these adaptations is essential for comprehending how fish respond to different environments and the implications of environmental fluctuations on fish population distribution. The main field methods for assessing fish physiological parameters include blood sampling to estimate hormone levels such as cortisol (Meka and McCormick 2005), muscle or tissue biopsies for estimating fish energy content, for example, using calorimetry techniques (Schreck and Moyle 1990), respirometry to estimate metabolic rates (Rummer et al. 2016), and the analysis of fish otoliths. Recent technological advancements, particularly in otolith chemistry, represent an innovative approach to studying fish ecology (Morissette et al. 2021).

In this study, anadromous Brook Trout otolith chemistry was used to enhance our understanding of intrinsic and extrinsic factors of fish growth and feeding. Otoliths are mineral concretions found in the inner ear of all bony fish, and which serve for maintaining their balance (Popper et al. 2005). They are formed primarily of calcium carbonate (CaCO3), constituting approximately 96% of the otolith weight, enveloped in an organic matrix constituting approximately 3% of the otolith and with the remainder constituted of trace elements (Campana 1999). These structures show opaque and translucent bands called annuli that represent annual growth (Grønkjær 2016; Stevenson and Campana 1992) and daily growth increments (Pannella 1971) that can inform on fish age and growth rates. Otoliths incorporate trace elements present in the waters in which fish develop reflecting their environmental concentrations. This suggests that these elements can be used as environmental tracers to describe the conditions in which fish lived (e.g., salinity, temperature and environmental concentrations), and infer stock identification, migration history and pollution exposure using otolith microchemistry (Campana 1999). Trace elements analysis assumes that the elements accumulated on the otolith surface are metabolically inert and so cannot be resorbed, leaving a permanent fingerprint on the otolith (Campana and Neilson 1985). Such elements as strontium (Sr) and barium (Ba) have been used as tracers of salinity and environmental concentration (Hüssy et al. 2021). For example, it has been shown that otolith Sr/Ca and Ba/Ca are incorporated in proportion to their respective ratios in ambient waters for Spot Croaker (*Leiostomus xanthurus*) and that there was a significant effect of the interaction between salinity and ambient concentrations for Black Bream (*Acanthopagrus butcheri*; Bath et al. 2000; Elsdon and Gillanders 2004). However, other trace elements appear to be under physiological control and thus could also be used as tracers of processes like growth, food consumption, maturation, and reproduction (Campana 1999). Hüssy et al. (2021) have focused on phosphorus (P), zinc (Zn) and copper (Cu) to represent this category of tracers, with magnesium (Mg) being also a potential candidate. Some studies found that these elements are positively correlated mostly with growth (Friedrich and Halden 2010; Halden and Friedrich 2008; Halden et al. 2000; Hamer and Jenkins 2007; Stanley et al. 2015; Sturrock et al. 2015), positively with food consumption

for Zn (Ranaldi and Gagnon 2008; Willis and Sunda 1984) and negatively with sexual maturation for Cu and Zn (Sturrock et al. 2014). These element concentration estimates can be related to the time during which they were deposited using the growth rings.

The lack of information for anadromous populations and the difficulty encountered in their monitoring because of their migratory behaviour justified the establishment of an action plan by the MFFP for 2019-2023. The main points of this plan are to distinguish habitat distribution and use, to develop monitoring of abundance and demography methodologies and to set up standardized monitoring of fishing pressure (MFFP 2020). So, it becomes increasingly necessary to improve our understanding of Brook Trout physiology to optimise their population distribution and abundance. Indeed, deepening our knowledge on wild fish food availability and consumption, as well as on sexual maturation and reproductive cycle, would help to better understand their adaptation to environments and the variability on their reproductive success. Additionally, the study of growth mechanisms linked to dietary needs for juveniles could help better anticipate the survival of young fish and their contribution to the adult population.

The main objective of this study was to relate otolith element concentrations to the underlying physiology, which encompasses the internal mechanisms governing growth and the utilization of food resources in anadromous Brook Trout. This correlation was determined based on the known growth and food consumption of the fish over a given period and involved conducting an experiment within a controlled environment. We aimed at observing the variations in elemental concentrations induced by a faster growth and a higher food consumption considering late maturity by using selected fish. We hypothesized that selected fish and fish with higher food intake would demonstrate higher trace element concentrations than unselected fish for Mg, P, Cu and Zn. Indeed, the purpose of this study was to better understand trace element signature response to further comprehend production dynamics of this species in the wild.

1.3 MATERIALS AND METHODS

1.3.1 Fish husbandry

The fish used for this study originated from a wild strain of anadromous Brook Trout captured in the Laval River in the province of Quebec (Crespel et al. 2011). Two fish lineages were reared at the Pointe-au-Père aquaculture station of UQAR-ISMER (Université du Québec à Rimouski, Institut des Sciences de la Mer de Rimouski): The "selection" (S) lineage included fish selected over multiple generations to favour growth and delayed sexual maturity (Audet and Bernatchez 2004; Bastien et al. 2011) while the "control" (C) lineage included fish that did not go through the selection process. 1+ year old Brook Trout were distributed in five tanks of 250 L that were permanently supplied with dechlorinated tap water at ambient temperature. The photoperiod was set weekly according to local sunrise and sunset. Before the beginning of the experiment, fish were fed with a ration (2.0 mm size COREY Aquafeeds Optimum™ feed) equivalent to 1.5% of the tank biomass five times a week.

1.3.2 Growth experiment

A six-week growth experiment with 1+ year old Brook Trout was performed during the summer of 2021 at the aquaculture station of UQAR-ISMER. For this experiment, a total of 240 fish was distributed equally in 12 tanks of 125 L, with a total of 20 fish per tank, and the two fish lineages were split into six tanks each, among the 12. For each lineage, we used four different families (for a total of eight families), with five individuals per family placed in each experimental tank. Fork length (cm ± 0.1 cm) and mass (g ± 0.01 g) were measured for all fish. To conduct the experiment, we chose the fish families with mean fork length most similar to the population mean, avoiding fish families with outlier fork length. The water supplied in the experimental tanks was firstly put in a head tank, to degas it using a degassing column, before being pumped in the experimental tanks. Fish were not fed the day

before the beginning of the experiment. During the experiment, fish were fed daily with two different rations (2.0 mm size COREY Aquafeeds Optimum™ feed), one at 1% of total biomass for each tank and the other at 1.5% of total biomass. The experimental design thus used triplicate tanks for the four treatments (two lineages [C and S] and two ration levels [1.0% and 1.5%] leading to the following treatments: C 1.0 %, C 1.5 %, S 1.0% and S 1.5%). The repartition of fish lineages and feed ration treatments were randomly assigned among the 12 experimental tanks.

On day one of the experiment, Brook Trout were sampled from their rearing tanks and were anesthetized in tricaine methanesulfonate (MS-222 at 0.080 $g \cdot L^{-1}$) before being measured, weighed, tagged with a 12 mm Passive Integrated Transponders (PIT) tags (Power Tracker II, Avid Identification Systems, Inc. California, USA) and placed in their experimental tanks for recovery. Once all fish had been handled, they were exposed to brackish water (14 ‰) for 48 hours, by adding salt water from St. Lawrence Estuary to the fresh water in the head tank of the experimental system, to attempt marking the beginning of the experiment by creating a peak in strontium concentration (^{88}Sr) in their otoliths. Once the 48 hours of exposure had elapsed, the saltwater supply was cut off and the water flow was readjusted. Following this exposure and for the duration of the experiment, tank temperature was ambient, the water flow was continuous and maintained at 2.0 $L \cdot min^{-1}$ in each tank using flowmeters. The photoperiod was set in the same way as in the rearing rooms at the time of the experiment.

Each day during the experiment, the water flow was checked, and the tanks were cleared of fish feces using a siphon. Tank temperature, pH, salinity, and dissolved oxygen were also measured daily with a multiparameter probe (YSI Pro Series Quatro Field Cable, YSI Incorporated, Yellow Springs, Ohio USA). Fish were fed around noon each day according to their feed ration treatment with possible leftovers weighed to later estimate food consumption. Feeding efficiency was variable among the tanks, forcing the use of an acclimation period to feeding at the beginning of the experiment. The acclimation followed this regimen; only half the ration was given in all tanks for the first week. During the second

week, Brook Trout were fed twice a day, with half the ration each time for most tanks. However, some tanks received a single total ration (good feeders) or twice with a quarter of the ration (poor feeders). From the third week to the end of the experiment, all tanks were fully fed, and fish consumed the complete ration in a single event.

At the end of the experiment, the fish were removed from their experimental tanks, anesthetized in tricaine methanesulfonate (MS-222 at 0.080 $g \cdot L^{-1}$), and identified by scanning their PIT tag number. They were then measured and weighed, before being euthanized. Gonads and liver were dissected and weighed, and the sagittal otoliths were removed with fine point high precision forceps made of stainless steel. Otoliths were then rinsed with deionized water and dried using Kimwipes before being left to air dry for 24 hours in opened Eppendorf microtubes (0.6 mL). All manipulations as part of this experiment were realized according to UQAR's animal care committee certificate CPA-85-21-233.

1.3.3 Otolith preparation

The otolith chemical analysis took place at the Université du Québec à Chicoutimi (UQAC) in the Laboratoire des Matériaux Terrestres (LabMaTer) in January 2022. Otoliths of 48 fish out of the 240 used for the growth experiment were randomly picked ensuring one fish per family within each experimental tank. The right sagitta was used when possible, otherwise the left sagitta was selected when inclusions of vaterite were observed (it occurred on 10 otoliths) to avoid elemental differences linked to crystal composition and elemental affinity. The otoliths (length < 3.0 mm and width < 2.0 mm) were fixed on standard microscope slides (76 mm × 26 mm) using thermoplastic glue (Crystalbond™509; Aremco™ products, NY, USA) and then sanded using successively sandpaper (1200 grit Wetordry™, 3M™) and two categories of aluminium oxide lapping films (5 µm and 1 µm lapping film, 3M™) to progressively reveal the core of the otoliths. Six otoliths were sanded down too much, and we had to use another otolith (either the left sagitta of the same fish or the right sagitta from another fish of the same family and experimental tank). Afterwards, otoliths

were transferred on petrographic slides (46 mm × 26 mm) with 12 otoliths per slide, sonicated twice in ultrapure water for two minutes each time, rinsed in ultrapure water and dried under a laminar flow fume hood for 24 h before storage in slide boxes waiting for laser ablation.

1.3.4 Otolith analysis

Otoliths were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The LA-ICP-MS was composed of a 193 nm Excimer RESOlution laser ablation system (Australian Scientific Instruments) equipped with a S-155 dual volume sample cell (Laurin Technic) and combined with an Agilent Technologies 7900 ICP-MS. The ablation was on a straight transect made from the ventral to the dorsal margins, passing through the core of the otolith. The laser ablation was realized using a 19 µm diameter ArF laser beam, at a frequency of 20 Hz, a moving speed set to 5 $\mu m \cdot s^{-1}$, a fluence of 5 $J \cdot cm^{-2}$ and a dwell time of 0.266 s. The ablated otolith sample was carried into the mass spectrometer by an Ar gas (0.8-1 $L \cdot min^{-1}$) to determine the concentrations of detected elements. The elemental concentrations of 40 elements were quantified (^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{27}Al, ^{29}Si, ^{31}P, ^{34}S, ^{39}K, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{61}Ni, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn, ^{69}Ga, ^{75}As, ^{79}Br, ^{85}Rb, ^{86}Sr, ^{87}Sr, ^{88}Sr, ^{111}Cd, ^{114}Cd, ^{118}Sn, ^{119}Sn, ^{120}Sn, ^{136}Ba, ^{137}Ba, ^{138}Ba, ^{202}Hg and ^{208}Pb). Three reference materials were ablated: a glass standard reference material (NIST-610) measured for calibration of the ablation system, and USGS MACS-3 and GP-4 assessed for quality control. Ablation of standard material was made every hour (after the laser ablation of approximately five otoliths) during 60 s. Each laser ablation (for the references and otoliths) was preceded by a gas blank measure set for 30 s which was then subtracted from the observed signal values.

The element concentrations were estimated (data integration) using LA-ICP-MS raw data with the software Iolite (Paton et al. 2011), a non-commercial package in Igor Pro software (Wavemetrics Inc., Portland, Oregon, United States). For each otolith analysis, ^{44}Ca concentration is assumed to be constant at 40% of otolith calcium content (Campana 1999)

and comparison of measured values and reference material were used to correct possible instrumental drifts. Element-specific limits of detection (LOD) were calculated as three times the standard deviation of the gas blank (SDblank) divided by the sensitivity of the signal (Lazartigues et al. 2014). Element concentrations that did not exceed the LOD were excluded from statistical analysis (^{7}Li, ^{11}B, ^{29}Si, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{69}Ga, ^{75}As, ^{79}Br, ^{85}Rb, ^{111}Cd, ^{114}Cd, ^{118}Sn, ^{119}Sn, ^{120}Sn, ^{202}Hg, ^{208}Pb). Data points corresponding to low ^{43}Ca concentrations (which indicate cracks on the otolith) were removed from the statistical analysis.

After the ablation, pictures of otoliths were taken using a Leica MC170 HD camera coupled to a Leica M60 microscope with 40X magnification (0.866 pixel·µm^{-1}). The pictures were analyzed using ImageJ software, the distance between the core and the edge of each otolith, which correspond to the lifetime of the fish, was measured (µm) and the age of fish (year) was determined by counting the annuli directly on the image with the software, thus checking they were one year old.

1.3.5 Data analysis

1.3.5.1 Growth and food consumption analysis

Growth rates during the experiment were calculated as the difference between fish fork length (or mass) at the end (L_f and W_f) and the beginning (L_i and W_i) of the experiment, divided by the duration (in days) of the experiment (n = 42 days).

Hepatosomatic index, gonadosomatic index and the Fulton's condition factor variation were also calculated. The hepatosomatic and gonadosomatic indices (HSI and GSI) were calculated at the end of the experiment by dividing the mass of the liver (W_L) and gonad (W_G), respectively, by the total mass of the fish and multiplying by 100.

The Fulton's condition factor variation (ΔK; Ricker 1975) was expressed as the difference between final and initial Fulton's condition factor (K_f and K_i), with K measured as:

(1) $$K = \frac{W}{L^3} \times 100$$

(2) $$\Delta K = K_f - K_i$$

For 15 fish, the final length was less than the initial length likely due to precision error (the difference being a maximum 0.5 cm). In these cases, the final length was set to equal the initial length. The data were analyzed using random effect linear mixed models with growth rates, growth indices (HSI, GSI and Fulton's condition factor) and food consumption as response variables, ration and lineage as fixed effects, and family and experimental tank as random effects. For GSI, sex was also used as a fixed effect, as the maturation schedule and gonads size can vary greatly between salmonid sexes (Klemetsen et al. 2003) influencing the GSI values (Rizzo and Bazzoli 2020). For food consumption, the experimental day was also used as a fixed effect because of the change of appetite throughout the duration of the experiment while only the experimental tank was used as a random effect because the food consumption variable of each family could not be isolated. The data for growth rates, HSI, GSI, Fulton's condition factor and food consumption follow a normal distribution and no transformation was used.

1.3.5.2 Trace element analysis

For trace elements data analysis, it was first intended to determine the initiation of the experiment using ^{88}Sr peak corresponding to the 48 hours of brackish water exposure (see methods), but this approach was inconclusive. Indeed, most of the time no obvious peaks were observed along the end of the ^{88}Sr concentration profile (i.e., corresponding to the start

of the experiment). Some rapid increases were observed, but the reached concentrations did not stand out from the rest of the profile. Instead, a daily otolith growth rate was determined by dividing the distance between the annuli (first year) and the margin of the otolith by the duration between post-winter growth recovery (estimated at early April when fish feeding frequency was increased from twice a week to five times a week) and fish sacrifice at the end of the experimental period (late September), representing 141 days. Then, these growth rates were multiplied by the duration of the experiment (42 days) to obtain the length of otolith growth (in µm) during the experiment for every fish. The difference between the distance to the margin of the otolith and the calculated otolith growth approximates the position of the point on the transect corresponding to the beginning of the experiment.

Average concentrations of magnesium ^{24}Mg, phosphorus ^{31}P, zinc ^{64}Zn and copper ^{65}Cu were calculated for all the otolith transects representing the experimental period. Then, the summed concentrations were also calculated for these elements using the last 12 data points, corresponding to the average number of data points for all fish used in the analysis, because the distance calculation did not lead to the same amount of data points depending on the fish and its growth rate (e.g., the experimental period on the otolith corresponded to five data points for one fish but 24 for another). Thus, the sum of trace elements concentrations was determined using the same amount of data points for all otoliths.

The average concentrations and the summed concentrations for each retained trace elements were analyzed using linear mixed models with the trace element concentrations as the response variables. Ration and lineage were used as fixed effects and the family and experimental tank as random effects. For ^{64}Zn, GSI was also used as a fixed effect. The data for trace elements concentrations follow a normal distribution and a log transformation was used only for the summed concentrations of ^{65}Cu. The linear mixed models used in this study were performed using R studio version 4.1.0 (R Core Team 2021) with the lme4 package (Bates et al. 2014). For each response variable, multiple models were used: one model including all fixed effects and their interactions, and others with at least one of the fixed effects removed. Models were ranked by Akaike information criterion, or AIC, to determine

which model explained more of the variability for each response variables. The selected best model was the one with the smallest AIC among the others. Indeed, the smallest AIC among all the models gives the best trade-off between the goodness of fit and the complexity of the model.

For each model chosen, the assumption of normality, homoscedasticity and independence of the residuals were verified respectively with Q-Q plots and histograms, plots of residuals versus predicted values and plots of residuals versus each fixed effect and each random effect in the model. Then, analysis of variance (ANOVA) of the chosen model was done to determine the significance of each fixed effect. This applies for the growth, food consumption and trace elements analyses and the significance threshold used in each statistical analysis was 0.05.

1.4 RESULTS

1.4.1 Mortality

On day two of the experiment, seven fish were found dead in four different experimental tanks and were replaced by other fish from the same families. Another fish was found dead the day before the end of the experiment and was measured and weighed at that time, but the otoliths of this fish were not used for the analysis. At the end of the experiment, it was noticed that some fish had been misplaced at the beginning of the experiment with one fish from tank B3 found in tank B7 and another one from tank B11 found in B12. However, the fish found in B12 had the same treatment as those of B11 (treatment C 1.5%) and the otoliths of this fish were not used for trace elements analysis. Regarding the fish of B3 found in B7, whose otoliths were used, the treatments were not the same (C 1.5 % in B3 and C 1% in B7) but no statistical differences were observed when including this fish or not in the analysis.

1.4.2 Food consumption

From the third week of the experiment (daily food ration completely eaten), the mean daily food consumptions (mean ± SD) recorded were 6.68 ± 0.396 g, 9.86 ±0.365 g, 6.84 ± 0.743 g and 10.2 ± 0.552 g for the treatments C 1 %, C 1.5 %, S 1 % and S 1.5 %, respectively. Fish from the control lineage began to eat all their food ration after the first week of the experiment whereas fish from the selection lineage ate all their food ration after nine days for the S 1 % treatment and after 24 days for the S 1.5 % treatment. Overall, similar food consumption was observed between the two lineages for the 1.0 % ration treatment, but it was higher in the control lineage than in the selection lineage for the 1.5 % ration treatment (Table 1). However, these observations were not statistically significant.

Table 1.Mean (± SD) values of initial mass and fork length, growth rates, ΔK (Fulton's condition factor variation), HSI (Hepatosomatic Index), GSI (Gonadosomatic Index), food consumption and ratio of male (M), female (F) and undetermined (UND) for each treatment of the growth experiment

Variables/treatments	C 1.0 %	C 1.5 %	S 1.0 %	S 1.5 %
Initial mass (g)	33.4±11.3	32.8±12.8	34.2±11.5	34.1±12.7
Initial fork length (cm)	14.5±1.51	14.4±1.77	15.0±1.63	15.0±1.81
Mass growth rate ($g \cdot day^{-1}$)	0.34 ± 0.15^{a}	0.54 ± 0.23^{b}	0.31 ± 0.22^{a}	0.42 ± 0.38^{b}
Length growth rate ($cm \cdot day^{-1}$)	0.033 ± 0.013^{a}	0.050 ± 0.014^{b}	0.030 ± 0.019^{a}	0.038 ± 0.031^{b}
ΔK ($g \cdot cm^{-3}$) $\times 10^{-2}$	9.56 ± 6.26^{a}	14.1 ± 12.2^{b}	7.59 ± 7.54^{c}	8.78 ± 9.57^{d}
HSI (%)	1.35 ± 0.427^{a}	1.58 ± 0.362^{b}	1.15 ± 0.309^{c}	1.32 ± 0.419^{d}
GSI (%)	3.06 ± 2.42^{a}	3.02 ± 2.30^{a}	0.298 ± 0.778^{b}	0.252 ± 0.558^{b}
Daily mean food consumption ($g \cdot day^{-1}$)	5.87 ± 2.03^{a}	8.70 ± 2.78^{b}	5.89 ± 2.28^{a}	7.57 ± 3.78^{b}
Sex ratio M/F/UND	29/28/3	29/30/1	30/26/4	25/26/9

Superscript letters (^{a,b,c,d}) represent significant differences with $p < 0.05$

The effects of ration, lineage and the day on the food consumption (mass of food eaten) were statistically tested and the ration, the day and the interactions between the ration and the day, the lineage and the day, and the ration, the day and the lineage had a significant effect on the food consumption (Table 2).

Table 2.Significant effects observed for the ANOVA of each best model chosen for food consumption, growth rates, ΔK (Fulton's condition factor variation), HSI (Hepatosomatic Index), GSI (Gonadosomatic Index) and ^{64}Zn mean concentrations

Models	Fixed effects*	DF	Sum square	Mean square	F value	P (>F)
Food consumption (g)	R	1	19.4	19.4	54.9	< 0.001
	D	41	3.14×10^{3}	76.6	2.17×10^{2}	< 0.001
	R × D	41	1.97×10^{2}	4.82	13.6	< 0.001
	L × D	41	1.56×10^{2}	3.80	10.8	< 0.001
	R × L × D	41	1.07×10^{2}	2.61	7.39	< 0.001
Mass growth rate ($g \cdot day^{-1}$)	R	1	1.44	1.44	23.8	< 0.001
Length growth rate ($cm \cdot day^{-1}$)	R	1	9.85×10^{-3}	9.85×10^{-3}	25.5	< 0.001
	R × L	1	1.49×10^{-3}	1.49×10^{-3}	3.86	5.06×10^{-2}
ΔK ($g \cdot cm^{-3}$)	R	1	4.85×10^{-2}	4.85×10^{-2}	5.91	1.58×10^{-2}
	L	1	7.22×10^{-2}	7.22×10^{-2}	8.79	1.80×10^{-2}
HSI	R	1	2.40	2.40	17.4	< 0.001
	L	1	1.37	1.37	9.93	1.36×10^{-2}
GSI	L	1	34.4	34.4	13.9	1.99×10^{-3}
	S	2	38.1	19.0	7.69	< 0.001
	L × S	2	36.3	18.2	7.34	< 0.001
Mean ^{64}Zn (ppm)	GSI	1	5.49×10^{2}	5.49×10^{2}	5.18	2.81×10^{-2}
	L × GSI	1	5.29×10^{2}	5.29×10^{2}	4.99	3.10×10^{-2}

Fixed effects*: Ration (R), lineage (L), day (D) and sex (S)

1.4.3 Growth

At the beginning of the experiment, Brook Trout initial mass was slightly higher on average for the upcoming 1% ration treatment than for the upcoming 1.5% ration treatment for the control lineage. For the selection lineage, the initial masses were approximately equal on average between the two upcoming ration treatments. Moreover, the initial fork lengths were approximately equal between the ration treatments for both lineages. Finally, the initial mass and fork length was slightly higher on average for the selection lineage than for the control lineage for both ration treatments. At the end of the experiment, Brook Trout growth rates (in mass and in length) were higher, on average, for the control lineage than for fish from the selection lineage, but both lineages had higher growth rates with the 1.5 % ration than with the 1 % ration (Table 1). Regarding ΔK, the highest ΔK was observed for the C 1.5 % treatment but on average the values of Fulton's condition factor variation were close (~0.10 $g \cdot cm^{-3}$) between the different treatments (Table 1). As for HSI, the highest HSI were found in the control lineage, but the values are quite similar between the treatments. The same applies to the GSI but the values showed a clear difference between the two lineages, with the male to female ratio being similar among the different treatments with a minority of fish of undetermined sex (Table 1). Note that these results are not significant.

Statistical analyses showed a significant effect of ration on growth rates, both for mass and fork length, and a significant effect of the interaction between the ration and the lineage for the growth rate in length. For ΔK, there was a significant effect of both food ration and lineage on ΔK. For HSI, there was a significant effect of both ration and lineage. For GSI, there was a significant effect of lineage, sex and the interaction between lineage and sex (Table 2).

The linear mixed effects models used for the statistical analysis of growth rates, ΔK, HSI, GSI and food consumption were listed and ranked by AIC (Table 3).

Table 3. Linear mixed models used during the statistical analysis of growth rate, ΔK (Fulton's condition factor variation), HSI (Hepatosomatic Index), GSI (Gonadosomatic Index) and food consumption ranked by AIC

Response variables	Model rank	Models*	AIC
Growth rate (Mass (g))	1	Ration	31.154
	2	Ration × Lineage	31.744
	3	Lineage	43.797
Growth rate (Length (cm))	1	Ration × Lineage	-1181.8
	2	Ration	-1179.8
	3	Lineage	-1166.2
ΔK ($g \cdot cm^{-3}$)	1	Ration × Lineage	-456.94
	2	Lineage	-454.26
	3	Ration	-453.50
HSI	1	Ration × Lineage	225.97
	2	Ration	228.88
	3	Lineage	234.72
GSI	1	Lineage × Sex	928.28
	2	Ration × Lineage × Sex	939.35
	3	Lineage	942.01
	4	Ration × Lineage	945.96
	5	Sex	951.04
	6	Ration × Sex	955.52
	7	Ration	957.02

Food consumption	1	Ration × Lineage × Day	1287.0
(Mass of food eaten (g))	2	Ration × Day	1578.0
	3	Lineage × Day	1638.7
	4	Day	1694.1
	5	Lineage × Ration	2474.2
	6	Ration	2475.7
	7	Lineage	2491.5

Models*: Ration, lineage, day (for food consumption) and sex (for GSI) were used as fixed effects and family and experimental tanks as random effects for all models (except for food consumption where only experimental tanks were used as random effects)

1.4.4 Trace element analysis

^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu concentrations varied respectively between 10 ppm and 55 ppm, 38 ppm and 536 ppm, 13 ppm and 101 ppm and 1 ppm and 15 ppm on average with a mean of 18±2.5 ppm, 101±36.7 ppm, 56±10 ppm and 6±5 ppm.

Trace elements mean concentrations (Table 4) and sum of the concentrations (Table 5) were also calculated for each treatment. The mean concentration models of ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu (Table 6) and the sum of these concentrations (Table 7) were also listed and ranked by AIC. The first ranked model was chosen to determine the significant effects for each response variables.

Table 4. Mean concentrations of ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu ± SD for each treatment of the growth experiment

Element mean concentrations (ppm)/treatments	C 1.0 %	C 1.5 %	S 1.0 %	S 1.5 %
^{24}Mg	17.00±1.164	18.54±3.751	18.13±2.968	17.64±1.011
^{31}P	91.39±16.13	117.2±50.44	88.30±30.32	107.0±37.97
^{64}Zn	57.67±9.262	50.12±8.213	61.12±6.274	54.67±14.18
^{65}Cu	5.492±5.030	7.324±5.476	6.073±4.285	5.890±4.619

Table 5. Sum of the concentrations of ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu ± SD for each treatment of the growth experiment

Element sum concentrations (ppm) /treatments	C 1.0 %	C 1.5 %	S 1.0 %	S 1.5 %
$^{24}Mg \times 10^2$	25.17± 2.614	28.78±5.481	27.79±5.220	26.97±2.266
$^{31}P \times 10^3$	11.06±3.499	15.65±7.854	11.72±4.268	13.56±6.658
$^{64}Zn \times 10^3$	8.491±1.209	7.770±1.139	9.343±1.010	8.374±2.305
^{65}Cu	54.48±71.59	90.19±88.90	89.87±122.4	72.38±83.82

Table 6. Linear mixed models used during the statistical analysis of magnesium ^{24}Mg, phosphorus ^{31}P, zinc ^{64}Zn and copper ^{65}Cu mean concentrations ranked by AIC

Response variables	Model rank	Models*	AIC
^{24}Mg	1	Lineage	366.10
	2	Ration	366.28
	3	Ration × Lineage	368.26
^{31}P	1	Ration	636.84
	2	Lineage	636.93
	3	Ration × Lineage	640.27
^{64}Zn	1	Lineage × GSI	375.88
	2	Lineage	376.06
	3	Ration	376.52
	4	GSI	376.78
	5	Ration × Lineage	379.18
	6	Ration × GSI	379.64
	7	Ration × Lineage × GSI	382.05
^{65}Cu	1	Lineage	318.39
	2	Ration	318.54
	3	Ration × Lineage	321.84

Models*: Ration and lineage were used as fixed effects and family and experimental tanks as random effects for all models. For ^{64}Zn, GSI were also used as a fixed effect of the model.

Table 7. Linear mixed models used during the statistical analysis for the sum of magnesium ^{24}Mg, phosphorus ^{31}P, zinc ^{64}Zn and copper ^{65}Cu concentrations ranked by AIC

Response variables	Model rank	Models*	AIC
^{24}Mg	1	Ration	606.28
	2	Lineage	606.54
	3	Ration × Lineage	608.76
^{31}P	1	Ration	875.81
	2	Lineage	878.67
	3	Ration × Lineage	878.99
^{64}Zn	1	Ration	611.46
	2	Lineage	611.80
	3	GSI	611.86
	4	Lineage × GSI	613.20
	5	Ration × GSI	614.10
	6	Ration × Lineage	614.27
	7	Ration × Lineage × GSI	617.72
^{65}Cu	1	Ration	159.73
	2	Lineage	160.10
	3	Ration × Lineage	162.77

Models*: Ration and lineage were used as fixed effects and family and experimental tanks as random effects for all models. For ^{64}Zn, GSI were also used as fixed effect of the model.

For the distance calculated corresponding to the growth experiment period, there was no effect for ^{24}Mg, ^{31}P and ^{65}Cu mean concentrations but for ^{64}Zn, significant effects of the GSI and the interaction between lineage and GSI was observed (Table 2) and a p-value that was close to 0.05 for the effect of lineage ($F_{[1,18]} = 4.13$, $p = 5.69 \times 10^{-2}$). For the normalized distance, there was no significant effect either for the ration or for the lineage for the sum of ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu concentrations (although the p-value was close to 0.05 for the effect of ration on the sum of ^{31}P with $F_{[1,11]} = 4.03$ and $p = 6.98 \times 10^{-2}$). There was also no effect of GSI on the sum of ^{64}Zn concentration.

1.5 DISCUSSION

Our study aimed at correlating Brook Trout otolith elemental signature to their growth and food consumption to help in the understanding of trace element incorporation in wild fish. Rearing of two lineages of farmed Brook Trout in a controlled environment allowed for the analysis of otolith trace element concentrations for a 6-weeks period. Results showed that fish from the control (C) lineage ate and grew more than fish of the selected (S) lineage, with ^{31}P and ^{64}Zn patterns that seemed to vary positively and negatively with factors linked to higher growth rates without any significant statistical effects observed. The difference between the growth rates observed between the two lineages is contradictory to the expectations we had for the selected lineage. The element concentration patterns noticed confirm (P) but also refute (Zn) or do not offer any insights (Mg and Cu) on the observed relationships between trace elements concentrations and growth, feeding or maturation. These results suggest that other phenomenon, which may have slowed the growth of selected fish, and which may have lowered Zn concentrations (e.g. stress and maturation), could provide some answers to the contradictions between our hypothesis and our results.

One striking aspect of our dataset is that we observed unexpected differences between the growth rates of control and selection lineages. Such difference could be explained by the consumption of the entire ration observed in each lineage. Brook Trout from the selection

lineage, because of their selection history, were expected to have better growth rates early in life (Bastien et al. 2011). We observed, however, the opposite with the main driver for growth being the ration consumption rather than lineage. For the 1% ration treatment, no difference in growth rate was observed between the two lineages, but the distinction between growth rates for the 1.5% ration treatment and the better condition observed for the control lineage, which is contrary to what was observed in a similar study (Jourdain Bonneau 2022), highlights the possible influence of food ration consumption on growth, where the control lineage needed a shorter acclimation period to fully consume their entire ration. Martinez-Silva et al. (2023) did not find differences in the expression of genes related to appetite regulation between the same two lineages of Brook Trout used in this study but did find different expressions of genes controlling appetite within fish families. Indeed, in slow-growing juveniles, the upregulation of leptin receptor results in decreased food consumption.

Barton and Iwama (1991) have classified changes in growth as a tertiary response to stress that could be used to assess it (Goede and Barton 1990). Indeed, stress exposition could cause growth rate reduction with one of the known stressors being the handling of fish (Barton and Iwama 1991; Peters and Schwarzer 1985; Pickering 1990). In this study, it is possible that fish from the S treatments were more stressed following the initiation of the experiment compared to fish from the C treatments, which is reflected by the differences observed in their behavior, growth, and overall food consumption. Barton and Iwama (1991) pointed out that plasma cortisol, an indicator for stress response in fish, in Brook Trout increased significantly after 30 s of handling, meaning an increased stress that could have occurred in this case as well. However, in the present study, the reduced growth was only visible on selected fish while all fish were handled in a similar fashion, suggesting that fish could have tolerated stress differently depending on their lineage. In their study, Bastien et al. (2011) also used fish from the Laval strain and stated that there were different stress responses between the two lineages, with different plasma cortisol variation patterns among the selected families. The lower HSI observed in this study in the selection lineage could also indicate that these fish spent more, or stored less, energy than those of the control lineage, thus reflecting that they are not adapted to cope with acute stressful events. On the other

hand, the GSI was clearly lower in the selection lineage, showing that energy was not mobilized for maturation but for growth as expected.

Our study of variations of elemental composition of otolith margin after the experiment allowed for the analysis of the effects of our experimental conditions, which are linked to various physiological metrics on elemental concentrations. The observed concentrations for ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu in this study were in the same order of magnitude than those found in the literature (Campana 1999) for marine fish such as bluefish (*Pomatomus saltatrix*) (Buckel et al. 2004), snapper (*Pagrus auratus*) (Hamer and Jenkins 2007; Ranaldi and Gagnon 2008), sand flathead (*Platycephalus bassensis*) (Hamer and Jenkins 2007), Atlantic croaker (*Micropogonias undulatus*) (Hanson and Zdanowicz 1999), red drum (*Sciaenops ocellata*) (Hoff and Fuiman 1995), cod (*Gadus morhua*) (Miller et al. 2006), for freshwater fish such as Lake Trout (*Salvelinus namaycush*) and burbot (*Lota lota*) (Melancon et al. 2009), and for diadromous such as chinook salmon (*Oncorhynchus tshawytscha*) (Gauldie 1986), Arctic char (*Salvelinus alpinus*) (Halden et al. 2000) and barramundi (*Lates calcarifer*) (Milton et al. 2000).

Notably, we sought to observe effects of ration consumption and gonadosomatic index (i.e., maturation) on Mg, P, Zn and Cu, all elements expected to show some variations with those metrics (Hoff and Fuiman 1995; Marohn et al. 2009; Milton and Chenery 2001; Ranaldi and Gagnon 2008; Sturrock et al. 2015; Sturrock et al. 2014; Willis and Sunda 1984). While our results were not significantly different, we observed higher concentrations of ^{31}P with factors linked to higher growth rate (control lineage and higher ration). Conversely, we observed higher ^{64}Zn with all factors linked to slower growth rate (selection lineage and lower ration). For ^{24}Mg and ^{65}Cu, no such patterns could be identified whereas otolith magnesium concentrations seemed to vary positively with fish growth in other studies (Hamer and Jenkins 2007; Stanley et al. 2015; Sturrock et al. 2015). For copper, the absence of patterns for the concentrations coincides with the incorporation of the element not being affected significantly by food (Friedrich and Halden 2010; Marohn et al. 2009; Milton and Chenery 2001). The influence of growth metric (lineage and ration) on otolith ^{31}P and ^{64}Zn

is an interesting observation, but is not entirely linked to what is expected in the literature. Hüssy et al. (2021) suggested that phosphorus and zinc elemental patterns correspond with otolith growth patterns, with higher concentrations detected during growth periods and for faster growing fish. The observed concentrations in this study support that this positive correlation was only with ^{31}P and not for ^{64}Zn although several studies have mentioned that dietary Zn is likely a major source of Zn in fish otoliths and that higher Zn concentrations were noticed during faster growth periods (Friedrich and Halden 2010; Halden and Friedrich 2008; Halden et al. 2000; Ranaldi and Gagnon 2008; Willis and Sunda 1984). Studies found that Zn concentrations tend to decrease in mature females of European Plaice (*Pleuronectes platessa*; Sturrock et al. 2015; Sturrock et al. 2014) thus exposing a negative relationship with GSI. This depletion of Zn concentrations agrees with the hypothesis of the rerouting of Zn in the blood plasma to the ovaries in mature female fish suggested in a few studies (Fletcher and King 1978; Fletcher and Fletcher 1980; Thompson et al. 2002). In our study, Brook Trout from the C lineage were mostly sexually mature and showed a higher GSI than the selection lineage. Combining this with the significant effects of the GSI and the interaction between lineage and GSI on mean ^{64}Zn concentrations suggests that a higher transport of Zn to the gonads could have occurred causing a decrease of blood plasma Zn that could be transported to the otoliths.

The absence of significant effect of ration and lineage alone on the means and sums of the observed concentrations of ^{24}Mg, ^{31}P, ^{64}Zn and ^{65}Cu could suggest too small a sample size. Yet for the sum analysis, even with the last 100 data points, which correspond approximately to the Brook Trout's first year of life, the results were the same with no effects of the lineage on trace elements concentrations (the lineage being the only known parameter for this period). Even so, this interval includes data that do not result from the growth experiment, so this leads to the question of the duration of the latter. In some studies, experiments on larval and juvenile fish that lasted between 30 and 60 days by varying temperature, salinity and diet showed no evidence of growth rate or diet effects on otolith concentrations of trace elements such as Mg, Zn and Cu (Buckel et al. 2004; DiMaria et al. 2010; Marohn et al. 2009; Woodcock et al. 2012); while another study that lasted longer (124

days) also failed to show any effects of diet on otoliths elemental composition such as Mg (Hoff and Fuiman 1995). The significant effects however observed for the GSI and the interaction between lineage and GSI on ^{64}Zn concentrations supports that the element is influenced partially with lineage and that coupled with GSI, indicates that ^{64}Zn is more likely to be influenced by the maturation factor alone as mentioned in the literature (Sturrock et al. 2014).

This study was conducted to seek evidence of correlations between trace element incorporation in otoliths, growth rate and food consumption with environmental parameters such as temperature and salinity kept constant. It suggested that element concentrations known as physiological tracers may not be influenced directly by growth and food consumption under short experimental periods but could still be affected by maturation. Further studies based on this one and with similar experimental designs might help to gather more refined information on the use of otolith trace elements to improve our understanding of anadromous Brook Trout physiology to aid in the management of the species.

CONCLUSION GÉNÉRALE

Ce projet a permis de mettre de l'avant la possibilité d'utiliser les otolithes comme outil pour estimer les paramètres physiologiques chez l'omble de fontaine anadrome en élevage en vue d'une application sur les poissons sauvages. Il nous a permis d'évaluer les liens existants entre les concentrations d'éléments traces incorporés dans les otolithes et la croissance de l'omble de fontaine anadrome. Les taux de croissance observés ont montré une contradiction avec les résultats attendus chez les ombles de fontaine anadromes sélectionnés pour la croissance et la maturité sexuelle tardive comme mentionné dans la littérature (Bastien et al. 2011). En effet, les poissons issus de la lignée sélection ont montré des taux de croissance et une consommation de nourriture plus faibles que les poissons de la lignée contrôle. Ce constat, couplé au refus de ces poissons de s'alimenter durant plusieurs semaines, pourrait suggérer l'existence d'un stress dû à la manipulation, aux interactions sociales et aux conditions d'expérience (Barton and Iwama 1991; Pickering 1993). L'étude des concentrations de Mg, P, Zn et Cu n'a pas permis de confirmer notre hypothèse sur la relation entre leur incorporation dans l'otolithe, la croissance et la consommation de nourriture, étant donné les plus faibles taux de croissance relevés chez les ombles sélectionnés. Par contre, l'étude nous a tout de même permis d'établir un lien entre la sélection, donc implicitement la maturation, et les taux de Zn dans l'otolithe tel qu'observé dans la littérature (Sturrock et al. 2014). Lors de ce projet nous n'avons malheureusement pas pu déterminer précisément le début de l'expérience de croissance sur l'otolithe ce qui aurait pu apporter plus de précisions à nos analyses. L'utilisation de marqueurs fluorescents visibles sur l'otolithe tel que l'oxytétracycline pourrait remédier à ce problème. Nous avions voulu en utiliser dans nos bassins expérimentaux cependant les quantités rejetées dans les eaux usées auraient été trop importantes selon la vétérinaire du comité de protection des animaux de l'UQAR. Une approche comme celle de Ranaldi et Gagnon (2008), qui eux en ont injecté directement dans les poissons, pourrait être utilisée. De plus, notre design

expérimental n'a pas rendu possible l'acclimatation de nos poissons à nos conditions expérimentales ayant donc une possible incidence sur nos résultats. Ajouter une période d'acclimatation de deux à trois semaines (en se basant sur nos observations) dans notre protocole, où l'on s'assure que les poissons mangent tous avant le début de l'expérience, pourrait permettre d'éviter un stress potentiel après leur échantillonnage et un stress dû au mélange des familles d'une lignée dans un bassin. Ensuite, il pourrait être judicieux de faire durer l'expérience un peu plus longtemps afin de comparer les résultats obtenus avec ceux de cette étude. L'incorporation des éléments dans l'otolithe pourrait ne pas avoir été visible sur la courte période où tous les poissons de notre expérience ont commencé à manger l'entièreté de la ration. Enfin, l'utilisation du facteur maturation (i.e. l'indice gonado-somatique) plutôt que la lignée pourrait également renforcer nos analyses. Ainsi l'étude des variations de concentrations de Mg, P, Zn et Cu dans nos conditions tenant compte de ces quatre points pourrait mener à des résultats différents et favoriser l'approfondissement de nos connaissances.

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