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Blandine Thommerel

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Morphological variation within juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, around the Loch Lomond, Scotland

Variation morphologique au sein des juvéniles saumon atlantique, *Salmo salar*, et truite brune, *Salmo trutta*, autour du Loch Lomond, en Ecosse

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2023



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Résumé

Les saumons Atlantiques et les truites brunes sont des espèces de salmonidés qui partagent de nombreux points communs, dont la possibilité de se reproduire ensemble et créer des hybrides. Dans le but d'évaluer plus précisément le taux d'hybridation qui augmente en Ecosse et de comprendre comment ces individus se caractérisent, une étape de cela est d'étudier la variation morphologique au sein des populations de saumons et truites. Ces deux espèces sont connues pour posséder des différences génétiques et phénotypiques intraspécifiques. Dans ce contexte, une quantification de ces différences permettrait par la suite une reconnaissance plus facile des hybrides. C'est ce que l'étude, ici présentée, a permis de réaliser. Au sein du bassin versant du Loch Lomond, les populations de truites et de saumons sont variées. Dans cette étude, les objectifs étaient de quantifier les variations morphologiques au sein des juvéniles saumons et truites, entre différentes populations, entre les sexes et également voir l'effet des paramètres environnementaux sur les morphologies. Pour cela, huit sites différents ont été étudiés. Sur ces sites, sept populations de truites brunes (133 individus) et trois de saumons Atlantiques (59 individus) ont été collectés par pêche électrique. À l'aide de photos de chaque individu, une analyse géométrique morphométrique puis statistique ont été réalisées, avec un prélèvement de nageoire adipeuse une détermination du sexe a pu être faite, et un relevé de paramètres environnementaux basiques a permis de voir l'effet de l'habitat sur les morphologies. Les résultats de cette étude conduisent à une variation morphologique des populations à petite échelle ; sept des dix groupes de populations de saumons et truites sont significativement différents. Ces groupes possèdent des morphologies variées qui sont traduites par les scores des variables canoniques de l'analyse géométrique morphométrique. Il semblerait que pour un site contenant saumons et truites, l'environnement agit sur les deux espèces de la même manière. Il n'y a pas d'effet différent de l'habitat sur les espèces. Les variations entre les femelles et les mâles ne montrent pas de significativité. Enfin, l'analyse statistique des paramètres environnementaux montre que seulement la température a un effet significatif sur les morphologies des individus.

Mots clés : *Saumon Atlantique – Truite brune – Analyse géométrique morphométrique – Hybridation – Loch Lomond*

Abstract

Atlantic salmon and brown trout are salmonid species that share many common features, including the ability to reproduce together and create hybrids. In order to assess more precisely the rate of hybridisation that is increasing in Scotland and to understand how these individuals are characterised, one step is to study the morphological variation within salmon and trout populations. These two species are known to possess intraspecific genetic and phenotypic differences. In this context, quantifying these differences would make it easier to recognise hybrids. This is what the present study has achieved. Within the Loch Lomond catchment, trout and salmon populations are varied. In this study, the objectives were to quantify the morphological variations within juvenile salmon and trout, between different populations and between the sexes, and also to see the effect of environmental parameters on morphologies. Eight different sites were studied. At these sites, seven populations of brown trout (133 individuals) and three of Atlantic salmon (59 individuals) were collected by electrofishing. Using photos of each individual, a geometric, morphometric and then statistical analysis was carried out, with adipose fin sampling to determine sex, and a survey of basic environmental parameters to see the effect of habitat on morphology. The results of this study conclude that there is morphological variation in populations on a small scale; seven of the ten groups of salmon and trout populations are significantly different. These groups have varied morphologies that are reflected in the scores of the canonical variables of the geometric morphometric analysis. It would appear that for a site containing salmon and trout, the environment affects both species in a similar way. There was no significantly different effect of habitat on the species. Variations between females and males were not significant. Finally, statistical analysis of the environmental parameters showed that only temperature had a significant effect on the morphologies of the individuals.

Keywords: *Atlantic salmon - Brown trout - Geometric morphometric analysis - Hybridisation - Loch Lomond*

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Abbreviation summary

ASSI: Area of Special Scientific Interest

CV: Canonical variate

CVA: Canonical variate analysis

CSS: Composite Shape Score

Cu: Cooper

DFA: Discriminant function analysis

Fe: Iron

JNCC: Joint Nature Conservation Committee

LLAIA: Loch Lomond Angling Improvement Association

LLFT: Loch Lomond Fisheries Trust

NASCO: North Atlantic Salmon Conservation Organization

NH₄: Ammonium

NO₂: Nitrogen dioxide

NO₃: Nitrate

O₂: Dioxygen

PCA: Principal Component Analysis

PC: Principal Component

PO₄: Phosphate

PVE: Percentage of variance of eigenvalues

SAC: Special Area of Conservation

SCENE: Scottish Centre for Ecology and the Natural Environment

SD: Standard Deviation

SiO₂: Silicon dioxide

SSSI: Site of Special Scientific Interest

UK: United Kingdom

Summary in French

Introduction

Le saumon atlantique, *Salmo salar*, et la truite brune, *Salmo trutta*, sont deux espèces de salmonidés que l'on trouve en Écosse et qui ont une valeur non seulement écologique, mais aussi économique, culturelle et sociale. Leur cycle de vie particulier fait de ces deux poissons de bons exemples pour les études scientifiques. Ils naissent en eau douce, où ils se nourrissent et grandissent pendant plusieurs années avant d'éventuellement migrer en mer. Lorsque les poissons atteignent la fin de leur stade de parr, ils peuvent soit commencer leur migration en la mer, auquel cas on parle d'espèce anadrome, soit rester en eau douce et migrer, ou non, entre différentes rivières et/ou différents lacs, auquel cas on parle de résidents d'eau douce (Klemetsen et al., 2003 ; NASCO, 2019). Les saumons sont généralement anadromes, et c'est l'une des raisons de leur déclin. Le processus de migration est une phase critique dans la vie des salmonidés. C'est un processus qui coûte beaucoup d'énergie et qui est très risqué pour la survie des individus (Ferguson et al., 2019 ; Lilly et al., 2022 ; Thorstad et al., 2012). Pour entamer sa migration, le poisson doit subir d'importants changements physiologiques, morphologiques et comportementaux. Ensuite, qu'il migre ou non, il doit faire face aux nombreuses menaces présentes dans son milieu : pollution de l'eau, dégradation de l'habitat, prédateurs, espèces invasives, surpêche, aquaculture intensive, etc. (NASCO, 2019). Les dangers en milieu aquatique sont nombreux et variés, et comme beaucoup d'espèces sauvages, les saumons et les truites sont en danger (JNCC, s.d.). Les étudier permet de comprendre leur mode de vie et leur écologie, de comprendre les enjeux pour leurs espèces et d'identifier les facteurs clés à prendre en compte pour les protéger. C'est notamment l'un des objectifs du Scottish Centre for Ecology and the Natural Environment (SCENE). En Écosse, l'eau douce est une ressource naturelle importante. Avec plus de 30 000 lacs d'eau douce et 125 000 km de rivières, le saumon et la truite sont des espèces clés dans le pays (Scotland's Freshwater | Scotland's Environment Web, n.d.). D'un point de vue écologique et économique, il est alors important de les conserver.

Cependant, depuis quelques décennies, un nouveau phénomène affecte ces deux espèces. Le taux d'hybridation entre truites et saumons augmente, notamment dans le Loch Lomond (Adams, et al., 2014). Ce phénomène fait donc l'objet de plus en plus d'études afin de comprendre les processus et les enjeux qui y sont liés. Une première étape de ces études consiste à déterminer les caractéristiques morphologiques des individus hybrides. Pour ce faire, il est important de s'intéresser aux espèces pures de saumon et de truite. Il est connu qu'au sein de ces deux espèces, il existe des variations génétiques et phénotypiques entre les adultes, et que l'habitat des individus joue un rôle sur la morphologie notamment (Alexander & Adams, 2000 ; Bush & Adams, 2007 ; Ferguson, 1989). Mais qu'en est-il des juvéniles ?

C'est dans ce contexte que l'étude suivante a été réalisée, avec les questions suivantes : **"Autour du Loch Lomond, comment la variation morphologique diffère-t-elle entre les saumons atlantiques juvéniles, *Salmo salar*, et les truites brunes, *Salmo trutta*, également au sein des espèces, entre différents habitats et entre les sexes ?"** et **"Les caractéristiques environnementales des différents habitats ont-elles un effet sur la morphologie des populations ?"** Les objectifs sont les suivants (1) quantifier les variations morphologiques entre les saumons Atlantiques et les truites brunes juvéniles autour du Loch Lomond, (2) quantifier ces variations également au sein de l'espèce, entre les différentes populations, (3) voir s'il existe également des différences entre les sexes, et enfin (4) examiner les effets des paramètres environnementaux sur la morphologie des populations.

Pour répondre à ces questions, une analyse morphométrique géométrique a été réalisée. Dans un premier temps, le rapport suivant présente ces deux espèces de salmonidés et les différentes problématiques qui y sont associées, afin de comprendre l'objectif final du projet. Après une description de la méthodologie utilisée et des différentes techniques de collecte et d'analyse, les résultats sont présentés. Enfin, les résultats sont discutés dans la perspective de la littérature existante et des recommandations pour la poursuite de l'étude sont proposées.

Les résultats attendus de cette étude seraient, premièrement, de caractériser quantitativement la forme du corps du saumon et de la truite. Comme il s'agit déjà de deux espèces différentes, bien qu'appartenant à la même famille, il est inévitable que leur morphologie soit différente. Deuxièmement, une comparaison entre les différentes populations pourrait mettre en évidence les variations intraspécifiques. Dans certaines des rivières étudiées, des différences de taille des individus ont été constatées. Dans les ruisseaux Balmenoch et Walton, les truites étaient généralement plus grandes et plus larges que les autres. A l'inverse, dans les rivières Cashel et Leven, les saumons étaient globalement plus petits. Dans les plus grandes rivières, telles que Endrick et Blane, les individus collectés présentaient une plus grande variation individuelle. Enfin, un effet de l'habitat sur la morphologie est attendu, comme de nombreuses études l'ont déjà montré. Cependant, les sites d'étude étant assez proches les uns des autres, il ne serait pas surprenant que les variations ne soient pas très importantes. Ce qui pourrait influencer l'effet, c'est l'environnement des rivières étudiées et non l'habitat lui-même. En ce qui concerne le sexe des individus, étant donné que les femelles consomment plus d'énergie en général, notamment pour la reproduction, ce qui implique qu'elles doivent constituer plus de réserves, etc. On suppose que les femelles sont plus grandes et/ou plus profondes que les mâles. Mais comme il ne s'agit que de juvénile, il ne serait pas surprenant qu'il n'y ait pas de différence significative entre eux.

Collecte des données

Pour cette étude, les données nécessaires étaient des photos des saumons et des truites pour l'étude de géométrie morphométrique, ainsi que des échantillons de nageoire des individus pour déterminer le sexe génétiquement. Enfin des paramètres environnementaux et un échantillon de l'eau de la rivière ont aussi été collectés.

La méthode de pêche électrique a été utilisée pour collecter les photos et les échantillons de nageoires. Cette méthode est généralement utilisée pour évaluer les populations de poissons dans les cours d'eau et est réalisée par des experts. Dans cette étude, la pêche électrique a été effectuée par des équipes du Loch Lomond Fisheries Trust (LLFT), dans le cadre de leur surveillance dans les rivières et les ruisseaux des différents affluents du Loch. La photographie des saumons et des truites juvéniles et le prélèvement d'échantillons de nageoires ont été ajoutés au processus initial. La figure 12 montre l'environnement de travail nécessaire pour ce travail de terrain. Lorsque les poissons étaient pêchés, ils étaient placés dans un seau (seau jaune figure 12) contenant de l'eau et de l'anesthésiant. Quand ils perdaient leur équilibre de nage, c'est-à-dire leur réflexe et leur position naturelle de nage, ils étaient alors placés sur une feuille graduée et pris en photo. La photo du poisson doit répondre à certain critère afin de pouvoir être analysé par la suite (Figure 13) : individus aussi droits que possible, bouche fermée, nageoires et points de marquages pour l'analyse visibles. Un tube étiqueté et rempli d'éthanol était également placé dans le champ de la photo. Le numéro correspondait à l'identification du poisson et le tube servait ensuite à conserver l'échantillon de nageoire prélevé directement après la photo. Après ce processus, qui durait moins d'une minute, le poisson était placé dans un second container (seau noir figure 12), où il pouvait récupérer. C'est seulement à la fin de la pêche, et après s'être assuré que tous les poissons avaient récupéré leur équilibre de nage, qu'ils étaient relâchés dans leur cours d'eau.

La pêche électrique étant menée par le LLFT, le choix des sites d'études s'est fait selon leur planning de travail. Au final 8 cours d'eau ont été réalisés, certains avec plusieurs points de collecte mais pour le reste de l'analyse ils étaient regroupés en un unique site (Table 1 ; Figure 15). Sur l'ensemble, 173 saumons Atlantiques ont été collectés et 372 truites brunes. Cette faible abondance de saumon était un point attendu. En Ecosse, entre 1971 et 2016, un déclin de 72% a été remarqué (Marine Scotland), et les derniers rapports de densité des populations de saumons dans le bassin versant du Loch Lomond montrent également une forte baisse (Table 3) (Loch Lomond Fisheries Trust, 2022). Enfin les données environnementales collectées ont été les suivantes : profondeur et largeur du cours d'eau, température, conductivité, pH et taux d'oxygène dissous. Des données sur la végétation alentours ont

aussi été relevés : Type de substrat de la rivière, végétation dominante de la rive directe et des alentours, ainsi que la structure générale (voir feuille de terrain annexe 9).

Après le travail de terrain, un travail en laboratoire a été mené afin de déterminer le sexe des individus. A l'aide de la nageoire adipeuse collecté sur les poissons, une extraction d'ADN était réalisée selon un protocole optimisé et simplifié par Arne Jacobs et Peter Koene, chercheurs de SCENE. Après l'extraction, un test PCR permettait d'amplifier deux gènes ; *sdY* présent uniquement chez les mâles et *I8S* présent chez les deux sexes (Yano et al., 2013). Ensuite, grâce à une électrophorèse, les résultats pouvaient être visualisé (Figure 16). Pour cette détermination, uniquement 74 échantillons ont pu être réalisés, par manque de temps et de moyens. Mais étant donné qu'il n'était pas attendu de différence significative entre les sexes, ce nombre était suffisant pour les analyses.

Ensuite, grâce au logiciel TPSdig 2 (Rohlf, 2017) des points de marquages prédéfinis ont été placés sur les photos (Figure 17). Photos qui ont été triées et sélectionnées en amont, selon leur qualité. C'est donc finalement 59 saumons, de trois sites différents, et 133 truites, répartis sur 7 sites, qui ont été analysés par géométrie morphométrique (Table 4). Puis c'est avec le logiciel MorphoJ que les analyses statistiques ont été faites (Klingenberg, 2011). Avec les points de marquages, des coordonnées étaient obtenues et ont permis de comparer les individus entre eux, mais aussi les populations, et selon les sexes. Une analyse en composante principale (ACP) a, dans un premier temps, permis d'obtenir une description générale des morphologies des poissons. Puis une analyse des variables canoniques (AVC) et une analyse des fonctions discriminantes (AFD) ont identifié et quantifié les variations entre les différentes populations. En effet, les poissons ont été classés selon leur espèce et leur site. Alors il était possible de comparer les morphologies moyennes de chacun des groupes. Ces deux dernières analyses (AVC et AFD) déterminaient également la valeur de la distance Procruste entre chaque paire de groupes comparés, ce qui permettait de voir la significativité ou non des résultats. La distance Procruste est une mesure de la différence entre les deux groupes comparés, basée sur une analyse de Procruste. Enfin, un score de composition de forme (CSS) basé sur les scores des composantes principales (PC) (formule suivante) ainsi que l'écart type ont été calculés avec Excel, afin de quantifier la variation des morphologies au sein des populations : $CSS = (PC1 * PVE1) + (PC2 * PVE2) + (PC3 * PVE3) + (PC4 * PVE4)$ – Avec PCx pour le score PC ; $PVEx$ pour le pourcentage de variation expliqué par le PCx ; x nombre de PC utilisés

Pour finir, les échantillons d'eau prélevés pour chaque site ont été analysés grâce à un kit d'analyse de la chimie de l'eau (JBL GmbH & Co. KG, n.d.). Les éléments testés sont l'ammonium (NH_4), le nitrite (NO_2), le cuivre (Cu), l'oxygène (O_2), le nitrate (NO_3), le phosphate (PO_4), les silicates (SiO_2) et le fer (Fe). Les résultats obtenus ont ensuite été placés dans un jeu de données contenant les 192 individus et les variables suivantes : leur valeur de score de composition de forme, leurs scores des variables canoniques une et deux ainsi que l'ensemble des données environnementales collectées et testées (annexe 16). Puis sur R Studio, un modèle linéaire et une ANOVA ont été réalisés entre chaque paramètre et les trois valeurs représentant la morphologie (CSS, VC1 et VC2) (Script en annexe 17). Le modèle linéaire a permis de connaître l'effet de chaque variable sur les individus. L'ANOVA a ensuite été réalisée sur ce modèle pour justifier la significativité des différences entre les différents groupes.

Résultats

Deux ACP ont été faites en amont sur l'ensemble des données et également selon les groupes classés par espèce et site. Elles permettent d'obtenir des descriptions de morphologies moyennes selon les différentes composantes principales (Table 5). Mais les principaux résultats de cette étude sont montrés grâce à l'analyse des variables canoniques (AVC). 79% des variations sont expliquées par les trois premières variables canoniques (VC) (Table 7). D'après le scatter plot de la VC2 et VC1, les truites et les saumons sont clairement distincts (Figures 21 et 22) et les truites possèdent un score plus élevé de VC1. Alors, le graphique montrant la forme du poisson s'il a un score élevé (bleu foncé ; annexe 22) semble montrer une morphologie approximative de truite brune.

Ensuite, le VC2 montre une légère distinction du site Boquhan, tant pour la truite (bleu turquoise) que pour le saumon (rouge). Sur ce site, les poissons ont une tête plus petite et un corps légèrement plus mince. Les truites de Cashel Burn semblent également correspondre à cette description. Les truites de Balmenoch et Walton ont un score VC2 élevé. À l'inverse, elles ont un corps large et épais et une tête plus grosse. En termes de description de la VC3, les groupes de truites de Blane, Cashel et Kirkhouse semblent avoir des têtes et des yeux plus petits, contrairement au groupe de truites Boquhan. Pour un résumé clair des descriptions approximatives de chaque groupe sur la base des scores VC, voir l'annexe 23. Aussi le site ne semble pas avoir d'impact sur la morphologie des poissons. Pour les sites abritant à la fois des saumons et des truites (Boquhan, Blane et Endrick), les VC2 et VC3 ne distinguent pas les groupes. L'environnement semble alors influencer les deux espèces de la même manière.

Les distances de Procruste, et leurs valeurs p correspondantes, fournies par la AVC montrent comment les groupes sont différents les uns par rapport aux autres (Figure 23). À l'exception d'une paire de groupes de saumons (Endrick et Boquhan) et d'une paire de population de truites (Balmenoch et Walton), toutes les comparaisons entre les différentes populations montrent des résultats significatifs (valeurs $p < 0,05$). Les populations sont morphologiquement différentes. Enfin, avec les écarts types (SD) calculés sur les valeurs des composantes, les saumons montrent une plus grande variation morphologique que leurs congénères ($SD\ salmon = 0.00548$; $SD\ trout = 0.00389$). Toutefois, ce résultat doit tenir compte du fait que les saumons représentent un échantillon beaucoup plus petit que la truite, 59 individus saumon pour 133 truites.

Enfin les résultats de la comparaison des femelles et des males ne montrent aucun significativité (Figure 24). Comme attendu au sein des espèces, les juvéniles ne sont pas morphologiquement distinct selon leur sexe. Les analyses statistiques des effets des paramètres environnementaux amènent à la conclusion que la température semble être le seul paramètre à avoir un impact significatif sur les morphologies des individus.

Discussion, limites et perspectives

L'objectif principal de cette étude était de comparer les populations entre les sites. Les résultats montrent des différences significatives entre huit des dix groupes de la classe espèce/site. Ceci est cohérent avec les études montrant des morphologies divergentes entre les populations allopatriques et sympatriques (Fraser et al., 2011 ; Koene et al., 2020 ; Piggott et al., 2018). L'analyse génétique n'a pas été réalisée dans cette étude, mais des travaux antérieurs sur la truite brune et le saumon atlantique dans les affluents du Loch Lomond ont conclu qu'il existe des divergences génétiques significatives entre les populations dans différents cours d'eau (Coulson et al., 2013 ; Rodger, comm. pers., 2023). Il est donc intéressant de noter que la variation entre les populations dans le bassin versant du Loch Lomond n'est pas seulement génétique, mais aussi phénotypique. À une petite échelle, il existe des différences morphologiques intraspécifiques, entre les populations de saumons et truites. Elles ont été notamment quantifiées ici, mais une augmentation de l'échantillon des saumons seraient pertinent pour améliorer la qualité des résultats.

Les groupes qui ne sont pas significatifs peuvent s'expliquer par la très faible distance entre les sites, comme le Balmenoch Burn et le Walton Burn qui ne sont séparés que de 250 mètres. Peut-être auraient-ils pu être regroupés au début des analyses. Ou encore par le fait que la plupart des cours d'eau de l'étude sont des affluents de la River Endrick (Figure 15). Mais, dans cette étude, il a été supposé que tous les individus provenaient de l'endroit où ils ont été capturés. Il n'est donc pas surprenant que le saumon de Boquhan ne présente pas de différences significatives par rapport au saumon d'Endrick. Inversement, il peut être surprenant que les autres affluents de cette rivière soient significatifs (Walton et Balmenoch ou Blane). Cependant, malgré l'importance des distances de Procruste, ce lien entre les rivières se traduit par le fait que la River Endrick présente les valeurs de distance les plus faibles avec tous les autres groupes (Figure 23). Ce constat est également à nuancer car Endrick est l'un des cours d'eau présentant le plus grand échantillon d'individus, saumons et truites

confondus. Afin d'identifier si les cours d'eau Balmenoch et Walton, qui semblent être très souvent à l'écart des autres groupes de truites, une AVC a été faite sans les individus de ces deux sites. Cependant les résultats obtenus sont similaires à l'analyse initiale. Leur présence dans l'analyse ne change donc pas grand-chose.

La température est le seul paramètre à avoir montré des effets significatifs sur la morphologie. Toutefois, dans cette étude, les données relatives à la température ne sont pas représentatives de la température au cours des saisons ou de la durée de vie des poissons, car elles ont été recueillies à un moment précis. En outre, la différence entre les sites ne peut être considérée comme constante tout au long de l'année. Mais de nombreuses études ont démontré l'impact significatif de ce paramètre sur le cycle de vie des salmonidés ; Leur croissance, leurs chances de survie pendant la migration, leur succès reproductif, etc. La température est largement connue pour être un facteur déterminant dans la plupart des moments clés de la vie des poissons (Enders & Boisclair, 2016 ; Ferguson et al., 2019 ; Mobley et al., 2021 ; Réalis-Doyelle et al., 2016 ; Sloat & Reeves, 2014 ; Thorstad et al., 2012).

L'obtention des valeurs des variables canoniques (VC) donne une moyenne pour le saumon atlantique et une autre pour la truite brune. Une sorte d'échelle de score de forme pourrait être obtenue. Il serait alors possible de positionner les individus hybrides selon cette échelle de valeurs. En fonction de leurs analyses morphométriques, ils pourraient être identifiés comme ressemblant ou non à l'une des espèces pures. Les résultats de l'étude distinguent clairement le saumon de la truite. Il devrait donc être possible de créer ce type d'échelle. Cependant, un échantillon plus important de saumons atlantiques serait plus favorable afin de garantir la significativité et la généralisation des résultats. Par ailleurs, au vu des scores obtenus, il semblerait que l'hypothèse de l'absence d'hybrides dès le début de l'étude soit correcte. En effet, dans les résultats obtenus, aucun groupe d'individus ne semble se situer morphologiquement entre les deux espèces, du moins pas distinctement (Figure 21). La question de l'hybridation s'était posée pour les individus de la rivière Endrick. Ceux-ci ont finalement été classés comme saumons. Finalement, ce groupe (S/En) a montré des résultats clairs et distincts, ce qui semble valider l'appartenance des individus en question à l'espèce choisie.

Si l'étude morphométrique des juvéniles doit être poursuivie ou répétée, il peut être nécessaire de revoir certains aspects de la méthode, comme le choix des sites. Il serait plus intéressant d'avoir des sites contenant à la fois des saumons et des truites. Il faudrait donc soit étudier la densité des populations des années précédentes et choisir en fonction de ces statistiques, soit disposer de plus de temps et faire plus de sites de collecte. L'identification du sexe pourrait être une analyse à laisser de côté, mais la collecte de la nageoire adipeuse reste un point clé. Elle permettra tout de même d'obtenir l'ADN de l'individu et d'identifier génétiquement les hybrides, en utilisant une technique différente d'identification des différences connues dans les microsatellites, si nécessaire. Il serait également intéressant d'approfondir les paramètres environnementaux. Tout d'abord, il faudrait revoir la précision de la collecte des données. Dans cette étude, il n'est malheureusement pas possible d'affirmer que tous les sites ont été collectés de la même manière. Au vu des résultats, très peu significatif, de l'analyse chimique des échantillons d'eau, il n'est pas nécessaire de la répéter. D'autant plus que les tests présentaient également certaines limites de précision. Les tests étaient basés sur la capacité de la personne à différencier à l'œil nu les nuances de couleurs des nuanciers. Bien que les résultats restent des approximations, l'erreur de lecture pouvait être facile et différente si la lumière de la pièce changeait, par exemple. Les résultats liés à la chimie de l'eau sont intéressants, mais l'influence sur la morphologie n'est pas claire. Il pourrait être utile d'aller plus loin dans l'utilité de chaque élément de l'eau sur la vie du poisson pour comprendre leur effet.

Cette analyse serait également pertinente pour examiner la qualité de l'eau des cours d'eau écossais et leur impact sur la morphologie des poissons. Les milieux aquatiques tels que les rivières sont très changeants. Il a également été démontré que l'impact du changement climatique sur ces habitats est significatif et représente un problème majeur. Les saumons et les truites sont déjà soumis à de nombreuses pressions tout au long de leur vie, notamment lors de la migration (Figure 5). Elles doivent déjà s'adapter à de nombreux changements. La qualité de leur milieu de vie est donc probablement un facteur clé de leur survie. Il serait intéressant d'examiner de plus près comment les

polluants présents dans l'eau, les résidus agricoles ou les déchets humains influencent la morphologie des poissons et pourraient fournir des informations sur les adaptations possibles des espèces aux changements environnementaux. Dans un contexte où les ressources en eau sont de plus en plus menacées, il est pertinent d'examiner de plus près la manière dont les poissons s'adaptent sur le plan comportemental. D'autant plus pour le saumon et la truite, qui sont des espèces économiques clés en Écosse.

Conclusion

En conclusion, il existe une différence significative de morphologie entre le saumon atlantique et la truite brune juvénile. Plus important encore, entre 8 cours d'eau du sud et du sud-est du bassin versant du Loch Lomond, les individus d'une même espèce ont des physiques différents. Cette étude morphométrique rapide et efficace montre les variations qui existent entre les espèces dans différentes rivières. Elle permet de tirer des conclusions sur la forme moyenne des populations et de faire des comparaisons entre elles. Chez la truite, cette variation, bien que faible, reste importante. À l'exception de deux cours d'eau très similaires, les populations de truites sont morphologiquement différentes selon leur cours d'eau. Il en va de même pour le saumon atlantique. Cependant, l'échantillon de saumons atlantiques étant très faible, la conclusion ne peut être tirée que de la comparaison de trois cours d'eau. Il est néanmoins possible de conclure qu'il existe, à petite échelle, des variations caractéristiques au sein des populations de ces deux espèces. La morphologie des poissons est potentiellement liée aux effets de l'environnement, notamment la température, mais une étude plus détaillée de ces effets serait intéressante pour conclure avec certitude aux causes de ces phénotypes et éventuellement comparer ces effets sur des populations différentes dans des sites différents.

Conclusion d'un point de vue personnel

Dans le cadre de ce travail, j'ai eu la chance de vivre de nombreuses expériences. Tout d'abord, travailler dans la recherche. Il y a trois ans, j'avais dit que je ne voulais pas faire de recherche. Je ne savais pas vraiment ce que cela signifiait. Ces six mois dans ce domaine m'ont donc donné un bon aperçu de tout le travail que l'on peut faire et m'ont prouvé que j'avais tort. Chez SCENE, les projets sont divers et variés, l'environnement et l'ambiance de travail sont parfaits. Après cette expérience, je dirais que la recherche, c'est aussi le partage d'idées, l'entraide et le travail acharné pour relever les défis de demain. J'ai eu la chance de pouvoir réaliser une étude par moi-même. Avec le soutien et l'aide nécessaires, j'ai appris à monter un projet. Je peux maintenant dire qu'il est possible de continuer dans ce domaine.

Ensuite, j'ai développé et amélioré de nombreuses compétences et connaissances professionnelles et personnelles. J'ai entrepris un projet pour lequel je n'avais aucune expérience. Je ne connaissais rien au saumon et à la truite, ni à l'Écosse. Je savais que l'adaptation ne serait pas un problème, car j'avais déjà passé sept mois à l'étranger l'année dernière. Ce qui m'a probablement demandé le plus d'efforts au cours des premières semaines, c'est d'apprendre le vocabulaire de base de la région afin de pouvoir communiquer avec tout le monde. Ou encore de me réhabituer à communiquer en anglais tous les jours, et de m'habituer à ma nouvelle vie quotidienne dans la campagne écossaise, sans voiture pour me déplacer. Mais grâce à ma capacité d'adaptation et à ma force de caractère, je pense avoir réussi ce défi. D'autant que vivre dans la campagne écossaise, entre Loch Lomond et montagnes, n'est pas le pire des quotidiens !

Enfin, mon apprentissage et mes compétences d'ingénieur m'aideront à comprendre les enjeux de la préservation de l'environnement que je souhaite défendre. Ce stage de fin d'études a largement confirmé mon envie de travailler à l'international et dans la conservation des espèces. De plus, grâce à ce stage, j'ai eu l'occasion de découvrir de nombreux projets scientifiques pour la préservation de l'environnement, dont Darwin200, auquel j'ai la chance de participer prochainement. Ce stage de six mois a été une très belle conclusion, tant sur le plan professionnel que personnel.

Introduction

Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, are two species of salmonid found in Scotland that have not only ecological but also economic, cultural, and social value. They have a particular life cycle that makes these two fishes good examples for scientific study. They are born in freshwater, where they feed and grow for several years before potentially migrating to the sea. When the fish reach the end of their parr stage, they can either begin their migration to the sea, in which case they are referred to as an anadromous species, or they can also remain in freshwater and migrate, or not, between different rivers and/or lakes, in which case they are referred to as freshwater residents (Klemetsen et al., 2003; NASCO, 2019). Salmon are usually anadromous, and this is one of the reasons for their decline. The migration process is a critical phase in the lives of salmonids. It is a process that costs a lot of energy and is very risky for the survival of the individuals (Ferguson et al., 2019; Lilly et al., 2022; Thorstad et al., 2012). To begin its migration, the fish must undergo significant physiological, morphological, and behavioural changes. Then, whether they migrate or not, they must deal with the many threats that affect them: water pollution, habitat degradation, predators, invasive species, overfishing, intensive aquaculture, etc. (NASCO, 2019). The dangers in the aquatic environment are many and varied, and like many wild species, salmon and trout are at risk (JNCC, s.d.). Studying them helps us to understand their way of life and their ecology, so we can understand what is at stake for their species and identify the key factors that need to be considered to protect them. This is one of the aims of the Scottish Centre for Ecology and the Natural Environment. In Scotland, freshwater is a significant natural resource. With over 30,000 freshwater lakes and 125,000 km of rivers, salmon and trout are key species in the country (*Scotland's Freshwater | Scotland's Environment Web*, n.d.). From both an ecological and economic point of view, it is important to conserve them.

However, over the last few decades, a new phenomenon has affected these two species. The rate of hybridisation between trout and salmon is increasing, particularly in Loch Lomond (Adams, et al., 2014). This phenomenon is therefore the subject of more and more studies in order to understand the related processes and issues. A first step in these studies is to determine the morphological characteristics of hybrid individuals. To do this, it is important to look at pure species of salmon and trout. It is known that within these two species, there are genetic and phenotypic variations between adults, and that the habitat of the individuals plays a role in morphology in particular (Alexander & Adams, 2000; Bush & Adams, 2007; Ferguson, 1989). But what about juveniles?

It was in this context that the following study was carried out, with the following questions: “**Around Loch Lomond, how does the morphological variation differ between juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, also within the species, between different habitats and between the sexes?**” and “**Do the environmental characteristics of the different habitats have an effect on the morphology of the population?**” The objectives are: (1) to quantify the morphological variations between juvenile Atlantic salmon and brown trout around Loch Lomond, (2) to quantify these variations also within the species, between different populations, (3) to see if there are also differences between the sexes, and finally (4) to look at the effects of environmental parameters on the morphology of the populations.

To answer these questions, a geometric morphometric analysis was carried out. Firstly, the following report presents these two salmonid species and the various issues associated with them, in order to understand the ultimate aim of the project. Following a description of the methodology used and the various collection and analysis techniques, the results are presented. Finally, the results are discussed from the perspective of the existing literature, and recommendations for the continuation of the study are proposed.

1. Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta* – species with a high value

1.1. Freshwater in Scotland – a natural and significant resource

When one thinks of Scotland, one thinks of its lochs, its islands, its castles and mountains, its special nature, its typical landscapes. One might also think of its history, its myths, and legends, which are rooted in every corner of the country. Scotland has over 5.5 million inhabitants and covers an area of 79,000 km² (*Scotland Population 2023 | Population UK*, 2023). It also has over 125,000 km of rivers and streams and more than 30,000 freshwater lochs, including Loch Ness, a natural freshwater reservoir containing more water (7,452 million m³) than all the English and Welsh lakes combined (*Scotland's Freshwater | Scotland's Environment Web*, n.d.). Both groundwater and surface water are valuable resources in Scotland and are regularly monitored. It is important for the supply of drinking water, for agriculture and industry, and also in times of drought for wetlands and watercourses. Scotland has a significant natural resource.

Water resources around the world are widely recognised as essential to modern society. Water, and freshwater in particular, has always been used by humans for hydration, food, and navigation (Maitland, 1990). Towns have long been built around watercourses, and there are many recreational, educational, and professional activities based around this resource (Raven et al., 1998). The quantity of water required to meet society's needs is increasing daily. Whether for domestic, industrial, agricultural, or commercial use, water is an integral part of people's daily lives, even though it is unevenly distributed throughout the world and between societies. Water is also home to an unimaginable diversity of animal and plant species. Throughout the world, due to climate, geomorphology, topography, etc., aquatic environments are all different (Maitland, 1990).

More than 70% of the earth's surface is covered by water, 97% of which represents the oceans. The rest is found in glaciers, soils, rivers, lakes, and the atmosphere (Dobson & Frid, 1998). Rivers, lakes, reservoirs, wetlands, etc., freshwater environments are numerous and diverse. Yet freshwater, and water in general, is having a major impact on environmental change and the ecological crisis, which is intensifying year by year. Water resources have been affected many times: drought, flooding and acidification of waters, pollution, degradation of aquatic habitats, etc. (Loudière & Gourbesville, 2020; Raven et al., 1998).

Scotland contains 69% of Great Britain's lakes and reservoirs (Appendix 1) (Smith & Lyle, 1979). In order to maintain this freshwater resource and its beneficial contributions from an ecological as well as an economic and social point of view, the country can count on scientists, fishermen, environmental associations and organisations, who are committed to preserving it. The Scottish Centre for Ecology and the Natural Environment (SCENE) is one of the organisations working to preserve Scotland's heritage. A field research centre of the University of Glasgow, SCENE was created and built in 1946 on the bank of Loch Lomond, and now lies within the Loch Lomond and the Trossachs National Park (Figure 1) (*University of Glasgow - Scottish Centre for Ecology and the Natural Environment*, n.d.).

The Loch Lomond and the Trossachs National Park covers more than 1.865 km². It encompasses a diversity of landscapes typical of Scotland: from rolling plains in the south to high mountains in the north, intersected by numerous lakes and rivers or forests and woodlands (Loch Lomond & The Trossachs National Park, n.d.). Loch Lomond is often regarded as the most impressive lake in the country. It has the largest surface area in Scotland, covering more than 70 km², and the second largest volume (after Loch Ness) with 2.652 million m³. The morphology of the lake, as well as its flora and fauna, are strongly influenced by the Highland Boundary Fault, which runs through the southern basin (Tippett R., 1994). It also contains a significant diversity of plant and animal communities, living in a wide variety of aquatic habitats (Maitland, 1990). The Loch Lomond's two main tributaries are Falloch Water to the north and Endrick Water to the south-east. Finally, the lake flows into the river Leven to the south, which joins the sea via the Firth of Clyde near Glasgow (Tippett R., 1994).

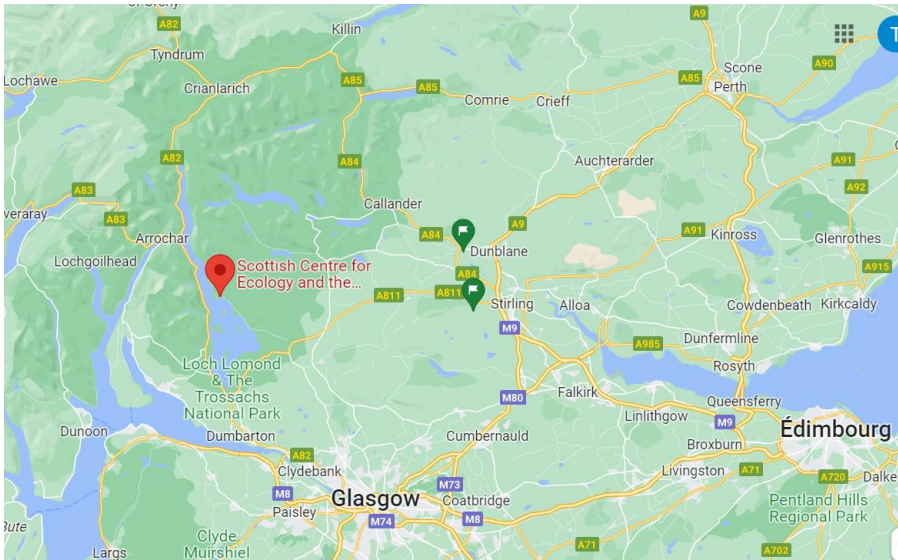


Figure 1: Location of the Scottish Centre for Ecology and the Natural Environment by the Loch Lomond, on the Trossachs National Park, Scotland (source: google map)

Figure 1 : Emplacement du Scottish Centre for Ecology and the Natural Environment au sein du parc national du Loch Lomond et des Trossachs, en Ecosse

With this favourable environment, SCENE hosts many teaching courses in its buildings, but its main activity is research. The centre contributes to many fundamental questions about the functioning of biological processes among species and their habitats, as well as about environmental change and species adaptation. While freshwater fish are a major focus of research projects, birds, mammals, and insects are also studied. Promoting knowledge and skills to reconcile environmental conservation and human use of natural resources is one of its main guidelines. At a time of major environmental change, the centre is working, in particular, on the impact on species, their habitats and their ways of life. These various studies highlight how species adapt or fail to adapt to change and provide crucial information on protecting biodiversity for the future (*University of Glasgow - Scottish Centre for Ecology and the Natural Environment*, n.d.). Aware of the importance of Scotland's natural freshwater resources, the research centre is carrying out numerous studies on the subject, particularly on the species of freshwater fish present in the country. SCENE and its partners, such as the Loch Lomond Fisheries Trust (LLFT), the Loch Lomond Angling Improvement Association (LLAIA) and Loch Lomond & The Trossachs National Park, are making it possible to carry out long-term studies on aquatic species, set up restoration projects and increase knowledge of the state of populations and their habitats, with the aim of proposing improvements to the management of this resource (Loch Lomond Fisheries Trust, 2022).

At the heart of these studies and projects are often two species: Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. Scotland is one of Europe's leading salmon producers, supplying the UK with 70% of its salmon (Capital.fr, 2014). There are many scientific articles and studies on Atlantic salmon and brown trout. The countries of northern Europe generally feature prominently in these articles. In number of publications, Scotland is the 9th country to publish on brown trout (Appendix 2) and 4th behind Norway, Canada, and the United States for Atlantic salmon (Appendix 2) (according to the Web of Science platform). In fact, as Appendix 3 shows, these two species are very important in the country's fishing industry. Although there was a significant decline in the 2000s, the numbers are still significant (Marine Scotland, 2023). In addition, the country's lakes and rivers are considered to be in relatively good condition and favourable to migratory fish species such as salmon and trout (*Scotland's Freshwater | Scotland's Environment Web*, n.d.). This is why the conservation of these two species in Scottish waters is so important, both ecologically and economically.

1.2. Atlantic salmon and brown trout – a unique life cycle

The Atlantic salmon, *Salmo salar*, and the brown trout, *Salmo trutta*, are two closely related species of fish in the Salmonidae family. This family includes no fewer than hundreds of species of fish, known and often studied for their particular and varied life cycles. The life cycles of salmon and trout are broadly similar, they are both migratory species, their strategies and tactics of survival are common. But each have distinct individual idiosyncrasies, such as their ecology and population dynamics. That is making these two fishes a good example of the great diversity of this family (Klemetsen et al., 2003).

1.2.1. Atlantic salmon, *Salmo salar* – anadromous species

The Atlantic salmon, *Salmo salar*, is an anadromous species. As its name says, Atlantic salmon is spread across the North Atlantic Ocean. From the Connecticut River (United States) to Ungava Bay (Canada), in the northwest Atlantic or from Portugal to the Barents and White Sea areas (Finland, Northwest of Russia) in Europe. Atlantic salmon occupies a wide array of various environments (Klemetsen et al., 2003). As anadromous, it is born in freshwater areas and migrates to the ocean at sea-age maturity, before returning in freshwater to spawn (Figure 2).

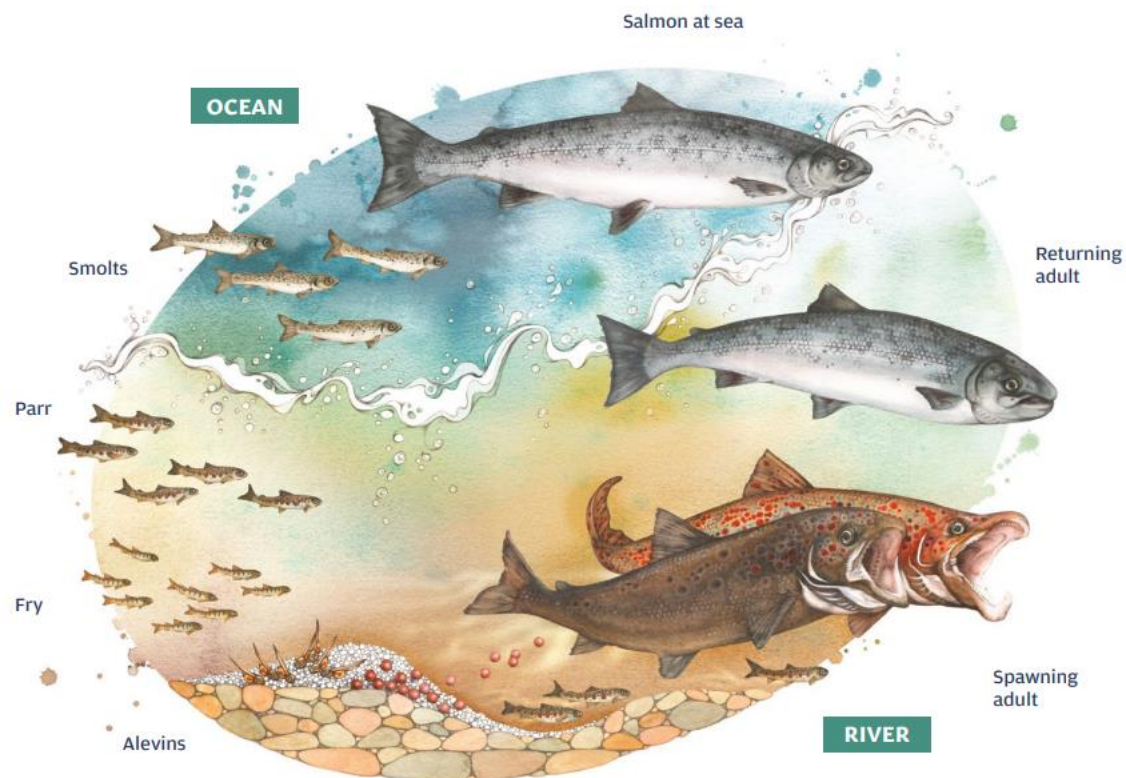


Figure 2: Life cycle of the Atlantic salmon, *Salmo salar*. Example of an anadromous salmon (source: NASCO, 2019)

Figure 2 : Cycle de vie du saumon Atlantique, *Salmo salar*. Exemple d'un saumon anadrome

After incubating in gravel for one or several months, salmon hatch during early springtime, and spend around eight weeks hiding in the gravel as *alevins*. Very small and fragile, fish must remain under the gravel to protect themselves from predators or other risks. They grow up and feed themselves thanks to a large yolk sac on their belly, full of nutrients. When this yolk sac is all absorbed, the salmon develop to the *fry* stage. Smaller than five centimetres, they can emerge from the gravel to find food on their own in the stream. But as they are easy prey for bigger fish and birds, they stay under good streamside cover and in shallow pools near the edge of the stream. The third stage, as *parr*, salmon are the size of a human finger and start to change physiologically in order to become anadromous or

a freshwater resident. This parr stage may last one or more years (NASCO, 2019). These first three stages of the Atlantic salmon's life may precede an important stage which is the *smolt* stage of anadromous salmon. These initiate their migration downstream toward the ocean, when all their characteristics are ready. Indeed, the migration is hazardous and very risky. Salmon face many predators, man-made infrastructures, and pass through different habitats. They will need to be in good condition before migrating. That is why they start physiological, morphological, and behavioural changes, known as the process of smolting (NASCO, 2019). Unfortunately, these changes between freshwater and ocean areas also means a high mortality rate (Lilly et al., 2021; Lothian et al., 2018; Thorstad et al., 2012). For those which succeeded in reaching the ocean, a new life may start. Habituated to the salt water, Atlantic salmon grow rapidly thanks to the ocean, which is a huge food resource for them. They spend two or more years in the ocean, until they are mature for reproduction and strong enough for their upstream return migration. Salmon have a homing instinct; they return to spawn in their rivers of origin. Finally, after spawning, either they die or sometimes turn over a new cycle from salmon at sea stage.

1.2.2. Brown trout, *Salmo trutta* – several life-history strategies

The brown trout, *Salmo trutta*, is native to Europe, north-west Africa, and western Asia. It has a wide geographical distribution, largely due to its ecological variability and ability to adapt to a wide range of habitats. Like its cousin the Atlantic salmon, the trout may or may not be anadromous. Yet, this species is more commonly freshwater resident (Klemetsen et al., 2003). Its life cycle includes the same major stages as its cousin. However, the brown trout has a greater variety of potential life-history strategies (Figure 3).

Like salmon, trout spawn in autumn and winter. The eggs are laid between stones and gravel in streams. The female leaves her nest (or *redd*) at the end of the spawning period, while the male may stay for several days potentially to fertilise other eggs. After incubating for one or more months, the eggs hatch, usually in spring. The fish remain hidden near the gravel and feed with their yolk sac for several weeks. Once the yolk sac has been completely absorbed, the fish emerge from the gravel and start feeding in the watercourse. This is the fry stage. Young trout can be very territorial and aggressive in defending themselves and their territory. A hierarchy and intensive competition for resources is established. Brown trout are also opportunistic carnivores. The fry stage can be a critical stage for the fish. If it is to survive, it will need to feed and grow quickly (Klemetsen et al., 2003). For the rest of its cycle, the trout must make an initial 'decision' (Figure 4). Either it becomes a resident, or it decides to migrate. At this stage, the difference is not visible morphologically, but this 'choice' is generally made according to the genetics of the fish and the environmental conditions (Ferguson et al., 2019). When the first choice is made, the parr can become river-resident, where it will remain in its river of origin (Figure 3), or become migratory, where it will continue to grow before leaving its river to feed more extensively. Depending on its growth rate, size, and environmental parameters, the parr stage may last one or more years ('decision' 2 in Figure 4). When the parr trout are ready to migrate, they usually have all the right physiological characteristics, because this process may demand a lot of energetic costs. They can then migrate to different areas: to a lake where they can become lake-resident or migrate between different adjacent rivers (fluvial-adfluvial strategy; Figure 3), or between adjacent rivers and lakes (lacustrine-adfluvial) or not (allacustrine), or finally, they become anadromous or semi-anadromous, and they enter in the smolt stage. These last two life-history strategies lead the smolt to a different environment: estuary and sea. This sea migration generally takes place in spring. Like their salmon congeners, trout can feed more quickly and to a greater extent in this new habitat, thanks to the environment's greater resources. However, they also have to go through the smolting process and all the associated physiological, morphological and behavioural changes. In the end, no matter what life-history 'choice' the fish makes, once it has reached the adult stage, it will return to its river of origin to spawn again.

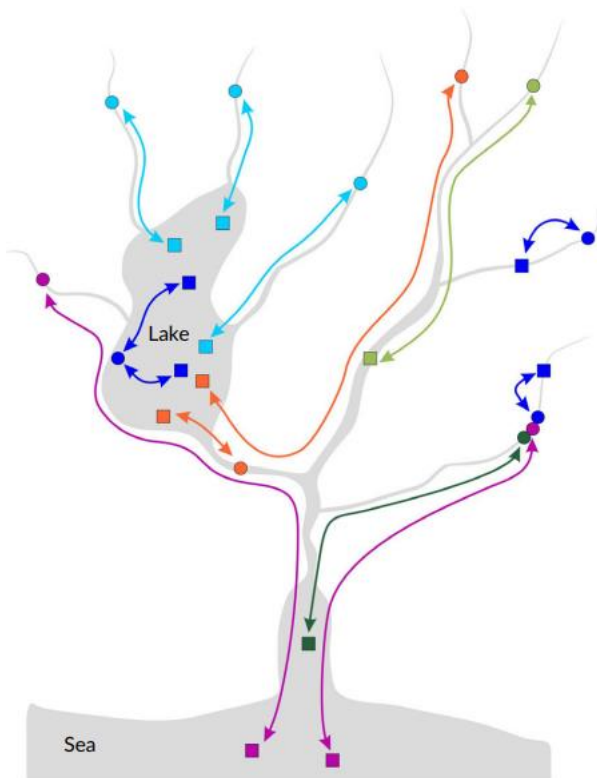


Figure 3: Potential life-history diversity of *Salmo trutta* in a typical catchment with a lake. Dot: Spawning location; Square: Adult feeding sites; Dark blue: Lake or river-resident; Light green: Fluvial-adfluvial; Light blue: Lacustrine-adfluvial; Orange: Allacustrine; Dark green: Semi-anadromous; Pink: Anadromous (Source: Ferguson et al. 2019)

Figure 3 : Diversité des potentielle stratégie de vie des *Salmo trutta* dans un bassin versant typique d'un lac. Point : Emplacement de ponte ; Carré : Site d'alimentation des adultes ; Bleu foncé : Résident de lac ou de rivière ; Vert clair : Fluvial-adfluvial ; Bleu clair : Lacustre-adfluvial ; Orange : Allacustrine ; Vert foncé : Semi-anadrome ; Rose : Anadrome

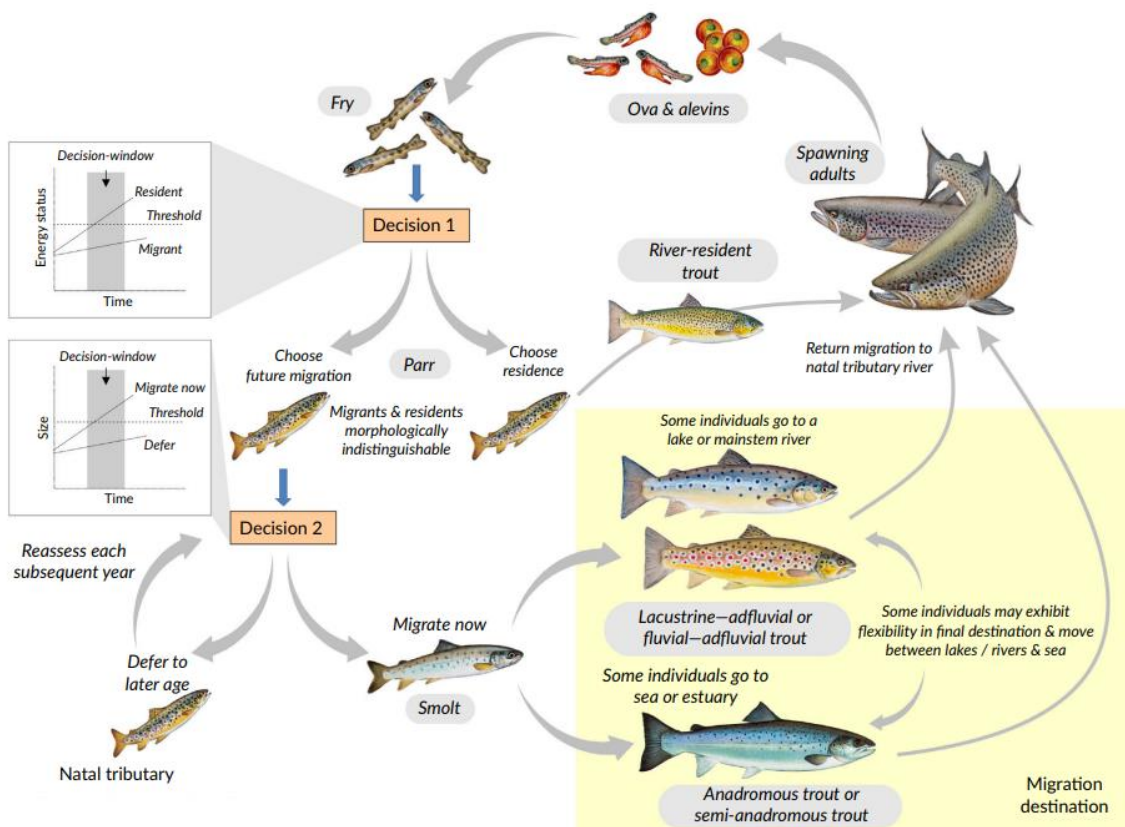


Figure 4: Lifecycle of the Brown trout, *Salmo trutta*, with the various potential strategies. Note that lake-resident and allacustrine strategies are not included (Source: Ferguson et al. 2019)

Figure 4 : Cycle de vie de la truite brune, *Salmo trutta*, avec les différentes stratégies potentielles. Les stratégies 'résident de lac' et 'allacustrine' ne sont pas représentées

1.2.3. Migration – a complex, risky, and beneficial process

Migration is a process that takes animals to a new environment and during which they move through different habitats in order to reproduce and feed (Hendry, A. P., et al., 2004). In the ocean, salmonids spend several years feeding and growing rapidly due to the large food supply (Klemetsen et al., 2003). The quality of the food source has a major influence on the animal's growth rate. The size of the salmonid is important and has been shown to influence reproductive success. Indeed, smolts that migrate early will spend more time at sea and thus grow larger and return to spawn with greater reproductive fitness (Mobley et al., 2021). It has also been shown that growth and size can vary considerably between slow-growing brown trout populations in rivers and streams and fast-growing anadromous populations. Thus, for both salmon and trout, migrating to the sea represents an opportunity and an important strategic 'choice' for their survival, but it also represents a risk (Klemetsen et al., 2003).

To migrate, salmonids must undergo physiological, morphological, and behavioural changes (Lilly et al., 2021; Thorstad et al., 2012). There is a very significant energy cost that the fish must pay in order to habituate itself to a future salty environment. But this 'choice' of life-history strategy is a compromise: a compromise between a greater fecundity that comes with greater size and the survival of the individual because of higher risk. Indeed, migrating to the sea means rapid and significant growth, i.e. better reproductive success and, ultimately, survival for the species. But it also means a high risk of mortality for the individual during this process (Lilly et al., 2021; Lothian et al., 2018; Thorstad et al., 2012).

There are many factors driving the migration and the process of smolting. Before, during and after the migration, the fish undergo many changes. First, they have physiological, morphological, and behavioural transformations, which are critical for the processes of smolting and for the migration. When salmonids begin their migration, they need to be physically ready. It has been shown that smolting is a function of the individual growth rate of the fish, fry and parr size, its energetic state during the season preceding the process and genetic and ecological factors. Various studies have shown that smolt size is correlated with freshwater age (Mobley et al., 2021; Thorpe et al., 1998). That has shown the influence of the parr's state before the migration (Figure 4, parr to smolt for the trout depending on the size, graph 'decision' 2). Morphologically, the salmon change colour (Figure 2, from smolt to salmon at sea), grow longer, and fatter. Then, physiologically, the increase of certain hormones, such as prolactin or growth hormone, during smolting is an important change too. Linked with other initiating factors, hormones will enable the activation of molecular processes that lead to the maturation of the salmon. In both salmonids and vertebrates, the Brain-Pituitary-Gonadal (BPG) axis is the control centre for maturation. Activation of this axis is induced by internal and external stimuli including sex and growth hormones (Mobley et al., 2021; Taranger et al., 2010). As well as hormones, the fish's lipid storage and ion regulation change as well to adapt to salt water. In the gills the activity of Na^+/K^+ -ATPase increases, which will allow the fish to overcome the changes of osmotic pressure in marine water. Also, studies have shown that depending on the level and type of lipid stored, maturation is delayed or not (Mobley et al., 2021; Sheridan & Harmon, 1994). The sooner salmon and trout begin to store lipids and feed as fry, the sooner they may migrate and become mature quickly (Rowe et al., 1991). Finally, the behaviour of the fish changes as well. They will naturally change their swimming direction in order to migrate to the sea: downstream migration (Mobley et al., 2021). Or adjusting their swimming movement when crossing different habitats (such as lakes) (Lilly et al., 2021), or their speed for example (Lothian et al., 2018). The processes within the organism therefore allow the salmon to grow. The sooner it may reach the smolt stage with the right conditions (sufficient size, high metabolic energy, etc.), the sooner it may migrate to the sea and finish its sexual maturation. Furthermore, during the sea phase, the physical conditions of the fish are very important in order to avoid larger predators (Thorstad et al., 2012). That is why the physiological conditions of the salmon are key for the timing of migration. Not having good characteristics at the beginning may significantly reduce survival rate.

Secondly, ecological factors, such as water temperature, photoperiod, or water flow, may play a determining role (Ferguson et al., 2019; Lothian et al., 2018; Mobley et al., 2021; Thorstad et al., 2012). Various studies on salmonid migration show that these three factors and several others, by initiating physiological changes in fish, also initiate the migration process (Hanssen et al., 2022; Lothian et al., 2018; McCormick et al., 1998; Mobley et al., 2021; Thorstad et al., 2012). There is thus ample evidence that the smolting and migration processes are dependent on the physiological state of salmon and trout and the environmental conditions that occur. For example, temperature appears to be the crucial factor in the life of salmonids. Influencing their development from the embryonic stage, it also plays a role in water quality or oxygen saturation, food resource availability, or in fish metabolisms and lipid stocks, and in direct migration movements (Enders & Boisclair, 2016; McLennan et al., 2018; Réalis-Doyelle et al., 2016; Sogard et al., 2012). The latter parameters are notably physiological factors for migration initiation. There are numerous studies showing the effects of temperature on lipid storage in salmonids, depending on the life stage and sex of the fish (Appendix 4), or on their growth rates (Appendix 5) (Brannon et al., 2004; Sloat & Reeves, 2014). For example, salmonids are known to prefer low temperatures, generally below 20-25°C. Furthermore, in Scotland, migration has been shown to occur most often at night, during periods when water temperatures are 8°C (Lilly et al., 2022). This dependence on temperature has also been shown on a European scale with northern European populations lagging those in the south who migrate earlier (Klemetsen et al., 2003). Linked to the season, temperature is a key element for the migration of salmon, depending on the timing of their migration they will have a greater or lesser chance to survive and to return for spawning. Like temperature, photoperiod is related to the season and indicates to the fish the beginning of their smolting. In particular, it may affect hormonal levels (Mobley et al., 2021). A study on the effect of the photoperiod on migration movements showed that the earlier the days are long, the earlier the migration will take place (Zydlewski et al., 2014).

The sea-migration of salmonids is a complex process involving many physiological and environmental aspects. The fish undergo many physical, morphological, and behavioural changes which, linked to environmental factors, lead them to begin their migration to the ocean. This journey will be very costly and risky for them, but it is necessary for the survival of their species. The understanding of the various transformations that take place, what are the optimal environmental conditions, and the dangers around migration, are widely studied across Europe and North America. This understanding is essential in order to manage and preserve this species. Because like numerous species in the world, salmon and trout are in ever-greater danger, too.

1.3. Salmon and trout – endangered species

Salmonid populations have been in decline for several decades, in part due to their diversified lifestyles and sometimes risky life-history strategy choices. Although the threats causing the decline in salmonid populations are not always proven to be the real causes of their decline, they have been observed for years and are therefore strongly considered as such. Many of these are attributable to human activities (Figure 5). For example, power stations, dams or other river regulation usually lead to changes in water flow, temperature, and water quality (the same factors influencing migration). Also, polluted or degraded water becomes increasingly the main cause of their loss (Belletti et al., 2020; NASCO, 2019). Acid rain caused by atmospheric pollution, the complete drying up of certain rivers caused by repeated droughts, and water contamination by agricultural and other industries, all alter the fish's habitat and put significant pressure on the species. The construction of dams blocks fish migration, preventing them from accessing habitats suitable for spawning or survival. Commercial or recreational overfishing represents a major threat to populations; regulations are not always respected. Since adult salmon returning to the river to spawn are fished out, this has a considerable impact on the species' numbers. Moreover, intensive aquaculture creates numerous risks of parasites and diseases affecting wild salmon and trout populations. These various threats do not act alone in reducing numbers. But rather, all at the same time, and added to climate change, fish mortality is largely impacted by these threats. The increase of temperature is a major concern for

salmonids because they need low temperature to live and because cold water holds more dissolved oxygen than warm water, and this oxygen level is very important for them. This could explain the significant decline in numbers over the years. (Almodóvar et al., 2012; NASCO, 2019; Parrish et al., 1998; Zimmerli et al., 2007).

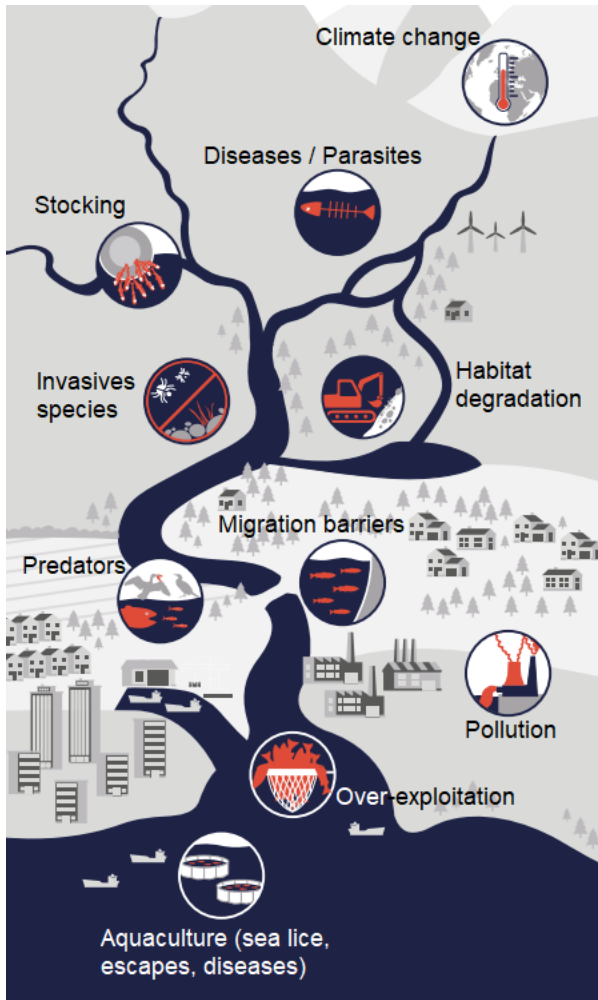


Figure 5: Pressure point that threatens salmonid populations throughout their lives (Source: modified from NASCO, 2019)

Figure 5 : Points de pression qui menacent les populations de salmonidés au cours de leur vie

Despite these numerous threats, trout are not considered to be endangered in the UK, but Atlantic salmon are on the list of species with priority status. In fact, in over thirty years Atlantic salmon abundance has been reduced by more than 60% (Figure 6). Their presence in the UK represents a significant proportion of the total European stock, particularly in Scotland. In addition, their abundance in certain rivers and their special status have influenced the selection of Special Areas of Conservation (SAC). These are created for the conservation of the species found there (Appendix 6) (JNCC, s.d.).

Salmon and trout are key species in many countries. Not only for the environment or the aquatic ecosystem, but also from an economic, social, and cultural point of view. Fishing is a predominant economic and social activity throughout the world. Whether commercial, recreational, or even aquaculture, the fish industry generates millions of dollars for society. Salmon is a species of socio-economic importance (Mobley et al., 2021). It contributes to the diet of society, to the quality of life of many people, but they also represent a cultural and social value for some. In eastern Canada, it is the object of unique local fishing methods. In Norway, it is possible to swim with salmon as a tourist activity. It has a spiritual aspect in some religions or, more locally, it is on the current Glasgow City coat of arms, coming from the myth of St Mungo, the salmon, and the ring. Its contribution to society is wide and diverse but endangered. It is not just a fish species, “It is more than a fish” (NASCO, 2019). Understanding this species and its cousin, the brown trout, is of paramount importance to

society, both economically and environmentally. Even more so in these times of environmental change when all species must adapt and evolve if they are to survive.

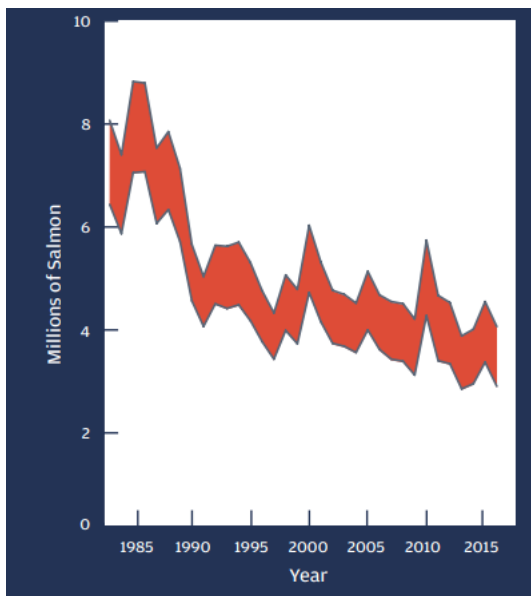


Figure 6: Salmon abundance between 1983 and 2016 across the North Atlantic Ocean (Source: NASCO, 2019)

Figure 6 : Abondance de saumon entre 1983 et 2016 dans la partie nord de l'océan Atlantique

1.4. Understanding the evolution of species to understand their future

Evolution is a long and natural process during which species have changed through the action of several forces and mechanisms. It may be defined by the change over time in allele frequency via descent with modification, or as the change in form and behaviour of organism between generations. The main mechanism of this process is natural selection, random event also plays a role through genetic drift (Ridley, 2004). Natural selection acts on the phenotype of an individual, through the fact that a particular trait is passed on from generation to generation during reproduction; this trait is considered to be favourable to the survival of the individual (Geo.fr, 2022). Natural selection is based on the principles of variation (all individuals are different), adaptation (the most adaptable individuals survive longer) and heredity (characteristics favourable to survival are passed on to descendants). It is influenced by a number of factors, such as environmental changes, various pressures (predation, competition for resources, etc.), genetic mutations and the migration of certain species. While this mechanism acts for all species at each new reproduction, over the generations the accumulation of these very traits, linked to changes and factors, can lead to the appearance of new species. This appears by the appearance of reproductive isolation between the new species and the old one, which then reduces gene flow between populations (Ridley, 2004). The definition of a species is complex and often differs. However, we can simply define it as a group of living organisms with common characteristics (physical, genetic, behavioural, etc.) that can reproduce together, giving rise to individuals that are themselves fertile (Larousse, n.d.).

1.4.1. *Salmo salar* and *Salmo trutta* – naturally diverse species

Evolution therefore naturally leads to a diversity of individuals and a diversity of species. This diversity can be manifested genetically and phenotypically. The Salmonidae family is particularly well known and studied for its polymorphism, the ability of a species to exist in different forms or morphologies (Alexander & Adams, 2000; Bush & Adams, 2007; Ferguson, 1989). The Atlantic salmon, *Salmo salar*, and the brown trout, *Salmo trutta*, therefore display a wide variety of morphology, ecology and behaviour, due partly to their variety of potential life-history strategies, but also due to their genetic and phenotypic adaptation to their environment (Drinan et al., 2012; Ferguson, 1989). For example, depending on their stage of development, or their sex, fish may not be the same colour (Figure 2, adult male more colourful than female), or depending on the population,

their physical characteristics may be different. While it has been widely demonstrated that salmonids show significant phenotypic plasticity, as well as considerable genetic diversity, the source of these variations is still debated (Alexander & Adams, 2000; King et al., 2001; Landry & Bernatchez, 2002; Taggart et al., 1981).

Genetic diversity, defined as the measure of allelic variation within a population, is a fundamental source of biodiversity (Hughes et al., 2008). There are many factors acting on genetic diversity, as well as the consequences of its diversity (Figure 7). The effects of genetic variation are important within an ecosystem and for the survival of populations (Booy et al., 2000). A key element in the evolution of species and linked to natural selection, genetic diversity may enable species to cope with change. Only the most resistant allele will survive and be passed on to their descendants. Within salmon and trout, as with species in general, genetic diversity is high (Alexander & Adams, 2000; Allendorf et al., 1976; Ferguson, 1989; Landry & Bernatchez, 2002). Generally, the greater the geographical distance between populations, the greater the genetic diversity. King et al (2001) showed that there is significant variation in genes in Atlantic salmon, whether on a large or small scale, between two different continents, within a continent or within the same country. Thus, although they are the same species, individuals may possess different alleles within the same environment, such as a lake (Allendorf et al., 1976). The result of natural selection, this genetic variation also may allow the species to change habitat more or less easily. Levis & Pfennig (2016) call that “Genetic accommodation” defined as “a mechanism of evolution wherein a phenotype, generated by either a mutation or environmental change, is refined into an adaptive phenotype through selection driving quantitative genetic changes”. Indeed, the direct consequence of genome is the expression of genes, in other words, the phenotype.

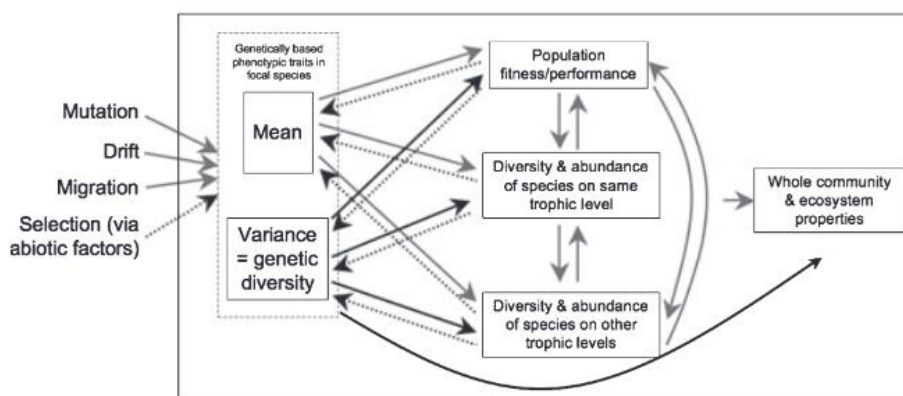


Figure 7: Processes underlying potential direct and indirect effects of genetic diversity on the ecological properties of populations, communities, and ecosystems. Solid black lines indicate direct ecological consequences of genetic diversity per se; dotted black line indicate effects of natural selection, which depend on genetic diversity; grey line represent causal effect not directly related to genetic diversity per se. (Source: Hughes et al. 2008)

Figure 7 : Processus sous-jacents des effets directs et indirects potentiels de la diversité génétique sur les propriétés écologiques des populations, des communautés et des écosystèmes. Les lignes noires pleines indiquent les conséquences écologiques directes de la diversité génétique en tant que telle ; les lignes noires pointillées indiquent les effets de la sélection naturelle, qui dépendent de la diversité génétique ; les lignes grises représentent un effet causal qui n'est pas directement lié à la diversité génétique en tant que telle

Phenotypic plasticity is defined as the capacity of an organism to modify its morphology and/or physiology, as well as its behaviour, in response to environmental changes, or as the capacity of a given genotype to express alternative phenotypes in response to environmental stimuli (Levis & Pfennig, 2016). The salmon and trout possess this plasticity and may express it many times during their live. Depending on their habitat, lake or river or sea, the individual's physique will not be the same. Anadromous fish will have a larger body mass than the others (Hutchings & Jones, 1998; Mobley et al., 2020), and even between two distinct lakes or a natural lake and a reservoir, the shape and size of the individual's head may differ significantly (Koene et al., 2020). The effect of environmental conditions on fish morphology has been studied and highlighted several times. Not only between two different rivers, but also within the same river. Vahanen and Huusko (2011) also

showed the effect of living conditions at birth on trout development. A population of trout reared in a hatchery showed different and delayed morphological development compared to trout reared in the wild (Vehanen & Huusko, 2011). Other studies have also assessed the impact of environmental characteristics, such as the availability of food resources, lake size, depth, etc., on the shape and behaviour of fish (Koene et al., 2020; Piggott et al., 2018). Finally, this phenotypic plasticity, which may be expressed according to the sex of the individual, also leads to a different use of energy reserves during the period at sea. So, the effect on offspring is also important. While female Atlantic salmon are usually bigger than male, they may spend more energy in the reproduction process, such as 20-25% on egg production, males may spend only 3-9% into gonad (Mobley et al., 2021; Rowe et al., 1991). The parental effect is an important factor in the physical attributes and survival of offspring. Depending on time at sea or choice of residence, juveniles may not have the same characteristics (Heinimaa & Heinimaa, n.d.; Van Leeuwen et al., 2016). Also, as with adults, the environment in which juveniles live may have an effect on their morphology. Drinan et al. (2012) showed local adaptation of salmon and trout parr to different and changing habitats. Plasticity is therefore also expressed in the early stages of life. All these physiological, morphological, and behavioural differences are evidence within salmon and trout, and show their abilities to adapt to their living conditions.

1.5. Hybridization – an expanding process that increase the diversity

Why take an interest in the morphology of salmon and trout? It is now certain that Atlantic salmon populations are declining in Scotland and all in the north Atlantic Ocean in general (NASCO, 2019). There are many reasons for this, as we saw earlier. One of them, aquaculture, i.e., salmon and trout farms, is causing a risk that is increasing every day. Incidence of salmon trout hybrids have been increased in rivers situated near intensive salmon farming in Norway and Scotland (Matthews et al., 2000). The increasing rate of hybridisation in aquaculture also increases the risk of decline in wild populations (Adams, et al., 2014). Indeed, when a fish, hybrid or even non-hybrid, escapes from the farm, it presents a risk of introducing pathogens or parasites into the natural environment (Hindar & Balstad, 1994). Youngson et al. (1993) showed that a female Atlantic salmon escaped from a farm was eight times more likely to reproduce with a male brown trout, in particular because she would be differently adapted to local reproductive conditions and would present fewer constraints than a wild female. When it is a hybrid that escapes, if it is fertile, it could be able to display either the better or the less favourable traits from both species, and it may lead to a predominance of these traits that will gradually make those of the pure species disappear, in the end. However, rearing hybrids under the same conditions as the parent species shows intermediate or similar growth rates to the best of the parents. Their survival rate is also often similar, and sometimes even higher, than that of the parents (Chevassus, 1979). Despite everything, it is interesting to study the hybridisation of salmon and trout in greater depth, with a view to conserving the traits of these declining species or for economic purposes.

In the natural environment, hybridisation also occurs. Atlantic salmon and brown trout are two species that share a same lineage which diverged over 3.3 million years ago (Shedko et al., 2012). It is therefore naturally, in an environment that they share with no reproductive barriers between them, that they create hybrid offspring. Hybridisation rates vary. In Sweden, for example, a high rate of 13% has been found in the Grönan river (Jansson et al., 1991), while in Spain only 2.3% of hybrids have been recorded (Garcia De Leaniz & Verspoor, 1989), and only 0.9% in North America (Verspoor, 1988) and 0.4% in Northern Ireland (Crozier, 1984). The rates are generally very low, but over the years an increase has been widely observed. Recently, a hybridisation rate of 10.4% was recorded in the Loch Lomond catchment in Scotland. In a country where Atlantic salmon are under threat, an increase in hybrids is not always good news. Although hybrids do not represent a direct risk to salmon and trout species, they do have an indirect impact on interactions and have negative consequences for the life cycle of wild species. Hybridisation in Scotland is therefore not a threat to be ignored (Adams,

et al., 2014). Moreover, some literature written about the rate of hybridisation increasing as salmon populations decline (Perrier et al., 2011). Because there are few salmon, the individual does not have a lot of choice for reproduction and then sometime, must mate with a trout (Koene, pers. comm. 2023).

Recognising a hybrid from a pure salmon or pure trout is difficult. It can sometimes be done by eye, according to the morphology of the fish, but the traits of a hybrid are often intermediate, and sometimes closer to one of the two parents. The hybrid is then often confused with a pure species. Jones (1948), for example, identified the specific characteristics of hybrids from a female salmon and a male trout. Depending on the age of the offspring, the physical resemblance to the parents varied, but overall, the hybrid fish had more traits in common with trout than with salmon. Conversely, Solomon and Child (1978) found that hybrid smolts had more characteristics of salmon, while parr resembled trout more closely. It is important to be cautious about identifying hybrids simply based on their morphology. These criteria are not reliable. The use of genetic techniques and electrophoretic methods in particular should be taken into account (Perrier et al., 2011; Wilkins et al., 1994).

Although morphology is not a 100% reliable criterion, it is nevertheless necessary to consider the physical differences between species, and between individuals with different living environments. Adult individuals have been extensively studied and are known to be phenotypically different, particularly in relation to their habitat (Allendorf et al., 1976; King et al., 2001; Koene et al., 2020; Piggott et al., 2018). The juvenile, fry and parr, life stages before potential migration to the sea or remain in freshwater, are key moments in the life of salmonids. These two stages determine the survival capacity of the adult individual. Growing up as quickly as possible is likely a key to the survival of juvenile fish. There are very few studies on the morphological variation of juveniles. They are generally studied for their link and their importance in the smolting and migration processes. However, recognising clear and diagnostic characteristics of juvenile individuals could also be a determining factor in the recognition of hybrids.

In the Loch Lomond catchment, salmon × trout hybrids are present (Adams, et al., 2014). It has also been shown that habitat characteristics influence the shape of trout (Koene et al., 2020). Increasing our knowledge of the morphology of juvenile salmon and trout around the lake would make it possible to identify typical morphologies of pure salmon or pure trout. Quantifying their morphology more precisely would enable hybrids to be classified in relation to pure species. This would give a more accurate idea of the hybridisation rate in the lake's tributaries. Although a genetic study would then be necessary. In addition, quantifying the impact of environmental characteristics on fish morphologies would be a plus. In Indonesia, Adro'i et al (2018) showed a significant effect of water quality and abiotic factors on the size and colour of guppy fish (*Poecilia reticulatae*), so it would be interesting to know whether the same effect would be found with salmonids in Scotland.

2. Problematic study and hypothesis

It is in this context that the following study is being carried out. With the future aim of identifying salmon x trout hybrids, the study attempts to answer the following questions: **“Around Loch Lomond, how does the morphological variation differ between juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*?, also within the species, between different habitats and between the sexes?”** and **“Do the environmental characteristics of the different habitats have an effect on the morphology of the population?”**

To do this, we will first carry out a geometric morphometric analysis of the physical characteristics of the two species. This analysis will reveal a typical morphology of the individuals. The results will then be compared between the two species and between the different populations, using multivariate statistical analyses to quantify the morphological variation in juvenile salmon and trout around Loch Lomond. A quantified difference will contribute to obtaining a shape scale and will make it possible to classify hybrids that are more or less similar to pure salmon or trout. Finally, a comparison of habitats has also been added in order to identify a potential effect of the environment on morphological differences. To do this, a map of land use will be drawn up and related to the differences in morphology, and then potential effects can be tested. Finally, a genetic analysis will be carried out to determine the sex of the individuals studied, with the aim of observing potential variation between the sexes.

The expected results of this study would be, firstly, to characterise salmon and trout body shape quantitatively. As they are already two different species, albeit from the same genus, it is inevitable that their morphology will be different. Secondly, a comparison between the different populations could highlight variations within each species. In some of the streams studied, differences in the size of individuals were noted. In the Balmenoch and Walton streams, trout were generally larger and deeper. Conversely, in the Cashel and Leven rivers, the fish were smaller overall. In the larger streams, such as Endrick and Blane, the individuals collected showed more individual variation. Finally, an effect of habitat on morphology is expected, as numerous studies have already shown. However, as the study sites are fairly close to each other, it would not be surprising if the variations were not very significant. What could influence the effect is the environment surrounding the rivers studied, not the habitat itself. As far as the sex of the individuals is concerned, given that females use more energy in general, particularly in reproduction, which implies that they need to build up more reserves, etc., it is assumed that the latter will be less affected. It is assumed that females will be larger and/or deeper than males. But, as they are only juvenile, it would not be surprising that there are no significant differences either.

In order to address these issues and verify these hypotheses, the following report presents all the methods for data collection, and the various analysis. Then, the results are presented and discussed in the light of the literature. Finally, a few recommendations are made before concluding the study.

3. Material and method

3.1. Data collecting – simple and efficient methods

The first stage of this study is data collection. Photos of juvenile salmon and trout and fin samples are required. The photos will be used for morphometric analysis and the samples for sex determination. The electrofishing method is used to obtain these data.

3.1.1. Electrofishing – a scientific fishing method

This technique is commonly used for scientific purposes, for example to assess fish populations in streams. Electrofishing was a major development in the fishing industry in the 1950s and 1960s. It is a fast and efficient method of collecting fish and is safe if used correctly. Several factors come into play when electrofishing: external factors such as the type of current, biological factors such as the physiology of the target species, and physical factors such as the conductivity, temperature and quality of the water (Bohlin et al., 1989). Electrofishing can be carried out in two ways: on foot, in shallow water, or from a boat in deeper water. In both cases there are three main components (Figure 8):

- The energy source (battery or generator): which produces an alternating current and supplies energy according to the effect of the unit, which determines the maximum voltage of the water.
- A transformer: which converts the original current into direct current of different voltage and determines the characteristics of the pulse (its shape, length, frequency, etc.).
- The electrodes, anode, and cathode: depending on their shape, they determine the strength of the pulse.

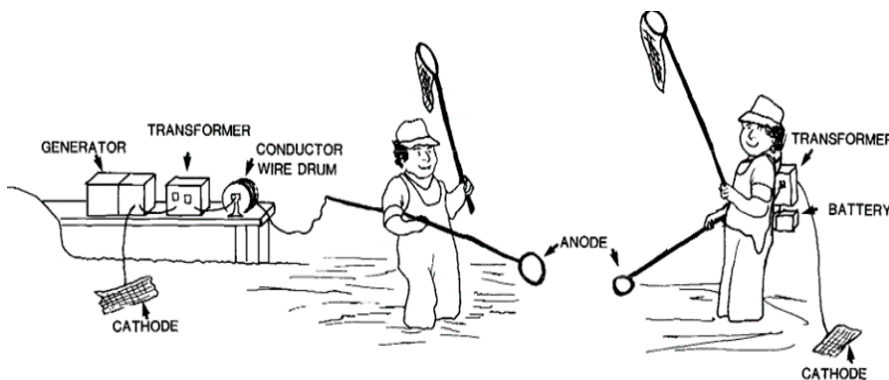


Figure 8: Scheme of the equipment used for electrofishing with stationary (left part) and mobile (right part) systems (Source Bohlin et al. 1989)

Figure 8 : Schéma des équipements utilisés pour la pêche électrique sur place (partie gauche) et mobile (partie droite)

During this process, the question of animal welfare is a legitimate one. Despite the recommendations and rules to be followed, injuries to fish do occur, as do cases of mortality (Reynolds & Dean, 2020; Snyder, 2003). Studies have shown, for example, that the sensitivity of fish to electrofishing varies according to species (Figure 9) and frequency (Figure 10). Authors have noted that salmonids are more sensitive to electric field exposure (Pottier G et al., 2022). The influencing factors are numerous and sometimes complex, which is why this method is used by professionals and certified personnel. In addition, the choice of current and voltage is very important in reducing the impact on fish. It is often advisable to use direct current (DC). This type of current is known for its very low negative impact on fish. With this type of current, the electrons move constantly and in the same direction. The fish's muscles are not stimulated repeatedly, but only once, when it enters the anode's electric field zone. This is when the fishermen will be able to retrieve the fish. Once out of the field, the fish will recover normal movements (Smith-Root, 2019). In all cases, the conductivity of the watercourse must be considered when choosing the right voltage to apply (Pottier G et al., 2022). The streams in the Loch Lomond catchment, generally, have poor conductivity, which lowers even further after rain. The voltage we tend to use ranges between 200-400 Hz (Koene, pers. comm., 2023)

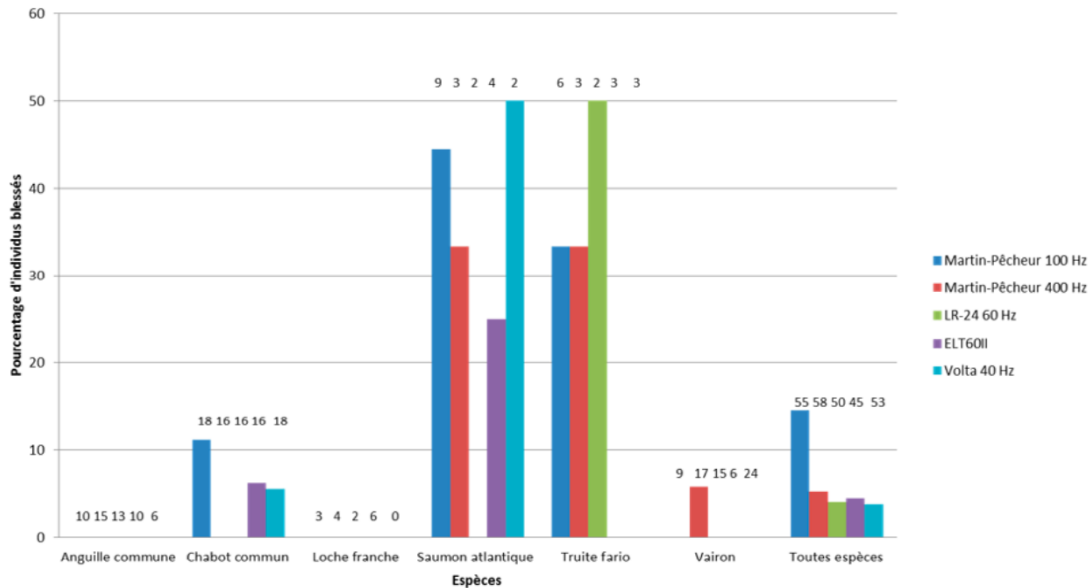


Figure 9: Percentage of individuals with a wound in the spinal column, by species or undifferentiated species. The number above the bars indicates the size of the samples analysed. Based on data from Pottier et al. 2016 (Source: Pottier et al. 2022)

Figure 9 : Pourcentage d'individus avec une blessure au niveau de la colonne vertébrale selon les espèces ou espèces indifférenciées. Les nombres au-dessus des barres indiquent la taille des échantillons analysés. Basé sur les données de Pottier et al (2016)

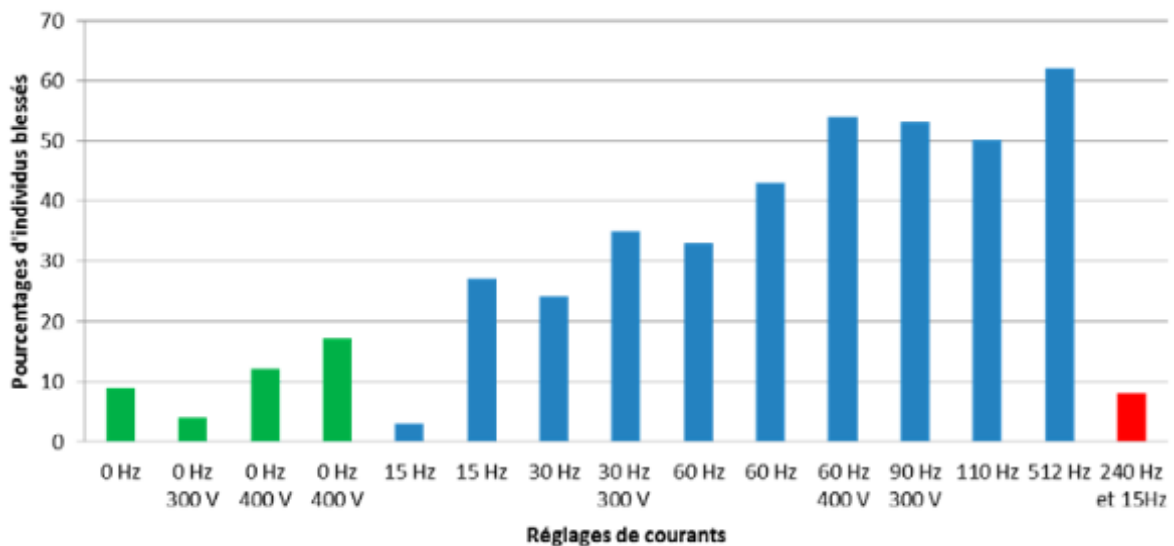


Figure 10: Percentage of individuals injured as a function of different frequencies (in Hertz) induced with a direct current (0 Hz) and pulsed crenulated current (PDC). green: DC; blue: crenulated PDC; red: CPS (burst PDC), 240 Hz is the frequency within the pattern and 15 Hz is that of the repetition of the pattern. Based on data from Dalbey et al (1996), Dolan et al (2002), McMichael (1993) and Sharber et al (1994) (Source: Pottier et al. 2022)

Figure 10 : Pourcentage d'individus blessés en fonction de différentes fréquences (en Hertz) provoquées avec un courant continu (0 Hz) et du courant pulsé crénelé (PDC). Vert : DC ; bleu : PDC crénelé ; rouge : CPS (burst PDC): 240 Hz est la fréquence à l'intérieur du motif et 15 Hz est celle de la répétition du motif. D'après les données de Dalbey et al. (1996), Dolan et al. (2002), McMichael (1993) et Sharber et al. (1994).

In this study, electrofishing was carried out by teams from the Loch Lomond Fisheries Trust, as part of their monitoring of fish populations in rivers and streams in the various tributaries of the Loch. Photographing salmon and trout and taking fin samples were added to the initial process.

3.1.2. Photographing and collecting fin clips – a simple protocol but with a few critical points for the fish

The protocol for collecting photos and fin clips is simple and rapid. Firstly, prior to the fieldwork, 2 mL tubes were filled with pure ethanol and labelled with unique numbers (Figure 11). The first letter corresponds to the code of the study site (Table 1) and the number corresponds to the number of the corresponding individual. Ethanol is used to preserve the samples over time. Then, in the field, the workplace was as shown in Figure 12. After catching the fish, the salmon and trout were placed in a bucket containing Tricaine methane sulfonate 1000 mg/g mixed with river water (yellow bucket, Figure 12). Tricaine is a powder used to sedate or anaesthetise fish. According to the recommendations in the product data sheet, a quantity of only 30-80 mg/L of water is sufficient for light anaesthesia, with a maximum immersion time of 10 to 30 minutes depending on the species (Pharmaq AS, 2022). For this work, the product was added in small quantities to the tank and increased if necessary. As the anaesthetic product, a same amount of sodium bicarbonate was also added to buffer the mixture and keep the pH of the water neutral for the fish. Taking water from the river helps to avoid creating a sudden change in temperature for the fish. When the fish lost its balance, i.e. its swimming position and reflexes, it was removed from the sedation water. This can take between one and five minutes, depending on the concentration used. It should be noted that fish that were too small, i.e. generally measuring less than five or six centimetres in length, were not taken into account. At this stage of the summer, very small fish are likely to be very young fish, born this past spring. For this study, older fish (at least one years old) were more relevant, they give a larger adipose fin for the lab work as well.



Figure 11: Box of 2-mL ethanol-filled tubes for fin samples. Numbered from 001 to 072. The letter corresponds to the code of the study site (Personal source).

Figure 11 : Boîte de tube de 2 mL, remplis d'éthanol pour les échantillons de nageoires. Numéroté de 001 à 072. La lettre correspond au code du site étudié

Figure 12: Field workplace. Bucket (yellow) with anaesthetic; Large bucket (black) with oxygen probe for fish recovery; Graduated board for photos and labelled ethanol tubes for fin samples (Personal source)

Figure 12 : Environnement de travail. Seau (jaune) pour l'anesthésie ; Grand seau (noir) avec sonde à oxygène pour la récupération des poissons : Feuille graduée pour prendre les photos et boîte de tube étiqueté.



The individual was then placed on a waterproof graduated sheet, which serves as a scale, and the fish is then photographed (Figure 12: graduated white board). A camera or even a simple mobile phone sufficed. The photo still had to be of good quality and taken in a certain way. The fish had to be well positioned, i.e., as straight as possible with its fins as visible as possible, on its right side with its head to the left, and finally a labelled tube was placed in the frame for identification. When the fish's mouth was closed, the photo was taken, trying to get the best possible light. Avoiding too much shadow in the photos is easier for the morphometric analysis afterwards but is not prohibitive. Several photos must be taken of the same individual to guarantee quality. Figure 13 as a good example, while figure 14 cannot be used. However, it should be noted that for the study, given the sometimes-insufficient number of samples for certain sites or for one of the species (mainly Atlantic salmon), a slight margin of error is accepted, i.e., even if the mouth is slightly open or the fish not completely parallel to the scale, the photo is still considered usable.



Figure 13: Correct photo of an Atlantic salmon. Mouth closed, body straight, as parallel as possible with the scales, and fins visible. Ethanol tube with identification number also placed in the frame. L for site "River Leven"; 015 for fish number 15 (Personal source)

Figure 13 : Photo correcte d'un saumon Atlantique. Bouche fermée, corps droit, aussi parallèle que possible à la graduation, et nageoires visibles. Tubes d'éthanol avec le numéro d'identification aussi placé dans le cadre. L pour le site de la rivière Leven » ; 015 pour le poisson numéro 15



Figure 14: Photo not suitable for analysis. Mouth wide open and fish too curved (Personal source)

Figure 14 : Photo non utilisable pour les analyses. Bouche très ouverte et poisson trop courbé

After the photo, the adipose fin sample was taken (Figure 13). To do this, the fish was held in one hand, and with the other, using a surgical scissors (also shown in the figure 13), a piece of the adipose fin is cut off. Fish fins are often used as markers in certain population monitoring studies or in aquaculture farms, where perforating a fin allows the individual to be recognised (Pettersson et al., 2014). Although the question of animal welfare in this situation is questionable, adipose fins are also known to be regenerative (Rosburg et al., 2022; Thompson & Blankenship, 1997). Furthermore, the survival or growth of juvenile fish with a cut adipose fin does not show a higher mortality rate than their congeners with a whole fin (Johnsen & Ugedal, 1988; Vander Haegen et al., 2005). The salmonid

adipose fin has no skeleton or musculature, which means that it is the least used for swimming compared to other fins (Reimchen & Temple, 2004; Stewart & Hale, 2013). Scales or mucous could be used instead, but they yields low amounts of DNA and require more sophisticated methods to extract the DNA (Koene, pers. comm., 2023). That is why the adipose fin is the best option, in terms of impact on the fish and for what this study requests. However, this type of fin contains several nerves and can sometimes be used as flow sensor by fish. It is therefore advisable to remove this tissue only occasionally (Koll et al., 2019). Then, thanks to anaesthesia, the operation has no painful effect on the fish. The sample was then placed in the ethanol tube corresponding to the photo.

Once these two stages have been completed (photo and fin sampling), the fish was placed in a second container filled simply with water (Figure 12: black bucket) where it recovers from the operation. An oxygen probe was also added in the bucket to ensure a regular oxygen supply. At the end of the fishing, and once all the fish have recovered their normal balance and reflexes, which can take between 5 and 15 minutes, the fish are released back into their watercourse.

3.2. Study area – the fish abundance can be unpredictable

To carry out this study, the data collection sites were chosen according to the Loch Lomond Fisheries Trust schedule. Five days of work were necessary. In one day, three or four different streams could be fished. However, electrofishing is a technique that is highly dependent on the weather. If it rains too much in the days leading up to the work, the water level in the stream will be too high, but if it does not rain enough, the water level may not be high enough. For this reason, the five days of fieldwork were carried out between mid-July and mid-August, with one or two days of work per week. The aim was to get as much data from different sites as possible, but with a time constraint: by mid-August the fieldwork had to be finished to allow time for the analysis.

Table 1: Sites with the corresponding number of Atlantic salmon and brown trout collected
Table 1 : Sites avec le nombre de saumons Atlantique et de truites brune collectées

| Site (abbreviation) | Code | Atlantic Salmon | Brown Trout |
|--------------------------|------|-----------------|-------------|
| Cashel Burn (Cas) | A | 1 | 19 |
| Kirkhouse Burn 1 (Ki1) | B | 0 | 17 |
| Kirkhouse Burn 2 (Ki2) | C | 0 | 10 |
| Blane Water 1 (B11) | D | 1 | 40 |
| Blane Water 2 (B12) | E | 0 | 66 |
| Endrick Water 1 (En1) | F | 34 | 6 |
| Endrick Water 2 (En2) | G | 27 | 43 |
| Endrick Water 3 (En3) | H | 31 | 79 |
| Walton Burn (Wal) | J | 0 | 29 |
| Balmenoch Burn (Bal) | K | 0 | 40 |
| River Leven (Lev) | L | 64 | 2 |
| Boquhan Burn (Boq) | - | 15 | 21 |
| Total per species | | 173 | 372 |

A total of eleven sites were completed for the 2023 period. One site from the 2022 period, Boquhan Burn, was also added to the data to broaden the samples. This choice was made in view of the low number of Atlantic salmon from different sites (only two sites in 2023). Adding Boquhan Burn means that this species can be compared at three different sites instead of just two. For the Kirkhouse Burn, Blane Water and Endrick Water, different collection points were set up (Figure 15). As these sites correspond to the same stream, it was decided to group them together for the analyses. In total, there are therefore 8 different sites.

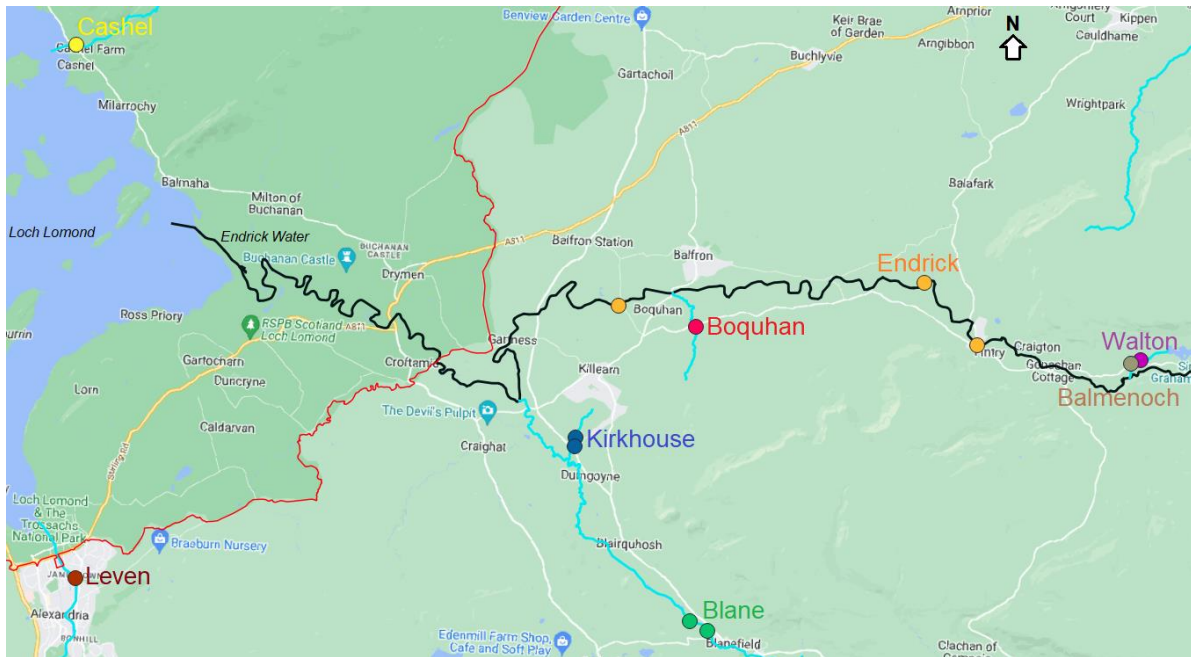


Figure 15: Map of the study sites. Coloured dot corresponded to the same-coloured name. Endrick, Kirkhouse and Blane have two or three collecting location for one site

Figure 15 : Carte des sites d'études. Les couleurs des points correspondent à celles des noms. Endrick, Kirkhouse et Blane ont deux ou trois points de collecte pour un même site

Individuals were identified based on their phenotype. Several rather clear clues help to differentiate salmon from trout (Table 2 and Appendix 7):

Table 2: Main comparison characteristics between juvenile Atlantic salmon and juvenile brown trout (Personal source)
Table 2 : Principales caractéristiques de comparaison entre les saumons juvéniles et les truites juvéniles

| Main characteristics | Atlantic Salmon, <i>Salmo salar</i> | Brown trout, <i>Salmo trutta</i> |
|----------------------|--|-----------------------------------|
| Tail | More pointed, sharper | Straighter, or slightly curved |
| Adipose fin | Brown, black or grey | Coloured: orange, yellow or red |
| Mouth and eye | Line from the end of the month crosses the middle of the eye | Line straight behind the eye |
| Pectoral fin | Longer | A bit smaller |
| Parr mark | Sometimes lower on the lateral line | In the centre of the lateral line |
| Operculum | One big spot | Several spots |
| Face | A bit more pointed | More curved |

However, several points were noted during the identification process. Firstly, the age of the fish; in fact, the study focused on juveniles, i.e., the fry and parr stages. An easy way to recognise parr is their parr mark visible along their lateral line. Fry are often less than ten centimetres long. It is therefore simple to identify these individuals. In addition, the collection period corresponds to the period when the smolt stage is no longer present in the rivers, as migration takes place in the spring. However, some of the individuals collected had non-juvenile characteristics. They were very large, over 15 cm, their parr marks were not very visible and were beginning to disappear. They were probably resident fish that had not migrated this year. Nevertheless, they were considered in the collection, and it was assumed that they were juveniles or in the early stages of maturity. The second point to note is the potential presence of hybrids, although they have only been confirmed in one study stream (Endrick Water) (Adams, et al., 2014). Some individuals displayed phenotypic traits typical of both species: adipose fin coloured (as trout) and sharpened tail (as salmon). However, for this study, no hybridisation was assumed, and suspect individuals were still classified as one of the two species according to a preponderance of the phenotypic criteria. These criteria are general but not prohibitive.

In the end, 545 samples and photos were taken for the study. Overall, 173 Atlantic salmon and 372 brown trout were identified. The low abundance of Atlantic salmon was to be expected. For several decades now, the number of salmon in Scotland and around the world has been falling sharply. Water pollution, man-made installations, overfishing and global warming (Figure 5) are the best-known causes. They affect salmon migration, a critical phase in their life cycle, as well as their health. As a result, fewer and fewer adults capable of reproduction are returning (NASCO, 2019). In Scotland, there are numerous population monitoring studies, and Marine Scotland has revealed a 72% decline in salmon between 1971 and 2016. Also, forecasts of fishing in the world, Europe and Scotland between 1960 and 2016 are quite clear and show a significant decline in salmon populations (Appendix 8) (Adams et al., 2022). The tributaries of Loch Lomond are no exception to this trend, and over the years the SCENE and LLFT teams have clearly noticed this decline. The 2022 reports produced by these teams reveal the low, even very low, density of Atlantic salmon, compared to trout, and their significant decline between years (Table 3) (Loch Lomond Fisheries Trust, 2022).

Table 3: Densities (individuals/m²) of salmonid juveniles across the catchment in 2020. Lomond tributaries refers to a number of small streams flowing directly into Loch Lomond. n/a' means that there were no individuals of that life stage found in the surveys in that specific area (Source MacDonald et al. 2022)

Table 3 : Densités (individus/m²) de juvéniles de salmonidés dans l'ensemble du bassin versant en 2020. Les affluents du Loch Lomond se réfèrent à un certain nombre de petits cours d'eau qui se jettent directement dans le Loch Lomond. "n/a" signifie qu'aucun individu de ce stade de vie n'a été trouvé lors des études dans cette zone spécifique (Source : MacDonald et al. 2022).

| Catchment (number of sites) | Salmon fry | Salmon parr | Trout fry | Trout parr |
|------------------------------------|-------------------|--------------------|------------------|-------------------|
| All sites (50) | 0.136 | 0.052 | 0.166 | 0.151 |
| Endrick (16) | 0.225 | 0.075 | 0.109 | 0.211 |
| Falloch (3) | 0.018 | n/a | 0.084 | 0.012 |
| Finlas (1) | n/a | n/a | 0.281 | 0.475 |
| Fruin (6) | 0.125 | 0.031 | 0.183 | 0.049 |
| Invergulas (2) | n/a | n/a | 0.024 | 0.081 |
| Luss (3) | 0.011 | 0.007 | 0.067 | 0.034 |
| Lomond tributaires (19) | 0.017 | 0.050 | 0.250 | 0.172 |

3.3. Environmental data collecting – habitat characterisation

Another of the aims of the study is to find out whether the environment has an effect on salmonid body morphology at the population level. To do this, environmental data were collected in the field, along with a sample of stream water. Using a riverine habitat monitoring method, physical data were collected on the stream itself (depth, width, etc.), as well as on the bank and surrounding environment (type of bank, vegetation, etc.). This data collection provides a rapid description of the study sites. The aim was then to identify potential differences between the sites and then to see the link with the morphologies of the populations. Looking at the general structure of the riparian vegetation can also give an indication of the neighbouring fauna, and possible predators for fish, such as bird for example. Finally, taking a sample of the water allows a chemical analysis to be carried out and gives an idea of its quality (Raven et al., 1998).

Environmental data collected in the field:

Watercourse:

- Depth (cm)
- Width (m)
- Temperature (°C)
- Conductivity (µS)
- pH
- Dissolved Oxygen (mg/L)

Vegetation:

- Substrate type of the watercourse
- Bank stream
- Bank stream top
- Bank stream structure

The document by Raven et al (1998) on the method for monitoring riparian habitats was used to create the lists for the different types of substrates and shoreline. Appendix 9 presents the field sheet produced upstream to facilitate data collection. To obtain averages for the watercourse data, several measurements had to be taken. However, for some parameters, such as pH, conductivity and dissolved oxygen, a single measurement was sufficient. In fact, after several tests, it was found that the values of these parameters between two points five metres apart were very similar. Water velocity (m/s) is shown on the field sheet because, at the start of the field phase, it was wanted to have an idea of this for each river, on average but also for the places where the fish were caught. Unfortunately, the equipment for this measurement was no longer working after the first site (Cashel Burn). It was therefore decided to drop this feature. With hindsight, this was probably a good decision, as velocity can be a complex measurement and depends on many parameters. It can vary greatly between two points on the same stream and at different times. As for the rest of the equipment used for the other measurements, a simple metre and ruler for width and depth, a thermometer that also measured conductivity, a pH meter, and a dissolved oxygen meter. For vegetation, simple observations were made. In addition, an observation of the land cover around the sites was also carried out, in order to get an idea of potential differences and what might influence this. To do this, a shapefile with the habitat cover was found on the NatureScot Spatial website, which provides a wealth of data on habitats in the country (NatureScot Open data, n.d.).

3.4. A wide range of analyses for maximum results

3.4.1. Lab work – determination of the sex

Following this field phase, a laboratory analysis phase was carried out, followed by statistical analysis. The laboratory work consisted of determining the sex of the fish using their adipose fin sample. SCENE scientists Arne Jacobs and Peter Koene have set up a protocol for extracting DNA and determining sex from the adipose fin of salmonids. This protocol is based on modifications and optimisation of other protocols (Meeker et al., 2007; Yano et al., 2013). Arne Jacobs' optimisation accepts a

poorer (but still sufficient) DNA quality in favour of volume, simplicity, and reduced cost. With this simplified protocol, these techniques (DNA extraction and sex determination) could be carried out in the research centre's molecular laboratory, did not require complex equipment, and the overall financial cost was lower and more profitable from an expenditure/results point of view. Finally, these optimised techniques did not require any specialist. After learning them, which only takes a day and some training, they can be performed by anyone (Koene, pers. comm. 2023).

This protocol consists of 3 main stages:

Step1 _ DNA extraction from an adipose fin (Appendix 10)

*Cut a small piece of fin clip (about 1-2 mm), with scalpel and clamp
Put it in a 1.5 mL Eppendorf tube, filled with 200 μ L of 1 mol PBS (Phosphate-Buffered Saline)
Left it there for about 10 min*

This step cleans the sample of the ethanol in which it was stored. During this step, the utensils for cutting and handling need to be cleaned with distilled water and HCl (Chloridric acid) between each sample to avoid DNA contamination between them.

*After 10 min, put the fin sample from PBS in a PCR tube filled with 60 μ L of 50 mmol NaOH (Sodium hydroxide)
Checked the tissue is completely covered
Put the tube in the PCR machine for the "Hot-Shot Program". It means heating the tissue solution to 95°C for 20 minutes. If needed to leave it in the machine longer, a hold temperature at 4°C is necessary to cool the sample.
After the program, added 6 μ L of 1 mol Tris-HCl to neutralize the solution.
Put in the centrifuge for 2-5 minutes to pellet the tissue debris
Extracted the supernatant liquid to a new tube
Store this tube, which contains the DNA extracted, in the freezer for long-term storage.*

The NaOH, pH ~ 12.0, is the buffer solution for the Hot-Shot extraction. Then add Tris-HCl, pH ~ 8.0, to stop the DNA extraction and maintain the sample in a stable solution. During the adding of Tris-HCl or for the liquid extraction, make sure to change tips for each sample.

Step 2 _ PCR test, multiplication of the sex genes

Making the Master Mix, which is going to be used as a solution for the PCR test. For one sample, in an tube added:

*Qiagen Multiplex PCR mix _ 7.5 μ L
Salmo sdY-Forward (20 mmol) _ 0.3 μ L
Salmo sdY-Reverse (20 mmol) _ 0.3 μ L
Salmo 18S-Forward (20 mmol) _ 0.075 μ L
Salmo 18S-Reverse (20 mmol) _ 0.075 μ L
H2O _ 2.25 μ L*

To this 10.5 μ L, in a PCR tube, added 3 μ L of DNA extracted sample

Mix the tube with vortex, then centrifuge for 20 seconds

Put in the PCR machine for the PCR test program:

*Initialisation: 95°C – 15 minutes – 1 cycle
PCR Amplification: 94°C – 30 seconds
63°C – 90 seconds
72°C – 90 seconds } – 35 cycles*

Final extension: 72°C – 10 minutes – 1 cycles

Salmo sdY (forward primer: CCC AGC ACT GTT TTC TTG TCT CA; reverse primer: CTT AAA ACC ACT CCA CCC TCC AT) and Salmo 18S (forward primer: GTY CGA AGA CGA TCA GAT ACC GT; reverse primer: CCG CAT AAC TAG TTA GCA TGC CG) are the primers for the two sex

genes of the salmonids. *sdY* is a sex-determining gene in most salmonids and it is present in males and not females. *I8S* is a gene present in both and it is used as a positive control (Yano et al., 2013). Amplifying these two genes will enable us to differentiate male and female for the last step. Moreover, at this step, it is important to add controls for the PCR test. One male control and one female control, they are DNA extracted samples from Brown trout's adipose fin. And a negative control with H₂O. They are all added like normal DNA extracted samples and mixed to Master Mix.

Step 3 _ Electrophoresis, visualisation of results (Appendix 11)

Making the 2% agarose gel electrophoresis: in a beaker added 4,800 mg of agarose and 240 mL of 1 mol TBE (Tris-borate-EDTA). Heat the mixture in the microwave for about 5 minutes, for a total dilution.

After heating, add as quickly as possible, 20 µL of Sybr Safe DNA Gel Stain (this will bind to the PCR product and enables us to see the DNA under certain light conditions, such as the UV light). Mix gently.

Then put the liquid in the specific mould and remove any bubbles.

Wait about 20 minutes for cooling and solidification.

When room temperature, put the gel in the electrophoresis machine, which is filled with TBE. The TBE is a conductive electrophoresis buffer and improves the migration condition of the DNA. The gel must be completely covered by the bath of TBE.

Into a reusable 96-well plate, for each well, add 3 µL of colouring and 5 µL of PCR product. Make sure both are mixed and at the bottom.

Then add each mixture in the gel well. Except for the first one where 5 µL of ladder must be add.

Close the machine and turn on at 100 Volt for 40-45 minutes

When it's done, put the gel under the UV light for the results.

During this step, it is necessary to be careful with the identification of the sample and the plate or gel well corresponding. Writing the order is important. The 100 bp ladder (ThermoFisher Scientific) acts as a positive control for the gel, to make sure the DNA migrated. Moreover, the filling of the wells might be tricky, that is why a training before is better and why it is important to be as careful and accurate as possible, to reduce the error. If the PCR product spreads in the bath, it will go into other wells and contaminate other samples.

The results are shown on figure 16. The first line represents the *I8S* gene and the second the *sdY*. So, one line is for a female and two for a male. The pictures also show the bad quality of some samples, the ones which cannot conclude because there are not any lines, or it looks just like a run-out. But the cause in the case of the first photo is a problem with the machine. For the second picture, some samples do not seem to have any DNA because there is nothing shown.

For this genetic analysis, although the method is not the most reliable, it works most of the time. It is a simple, quick, and inexpensive method. So, in the case of this study, where sex determination was only a small part of the analysis and it was also expected that there would be no morphological differences between males and females, it was more than sufficient.

A total of 164 samples were tested using these extraction and sex determination protocol. Unfortunately, only 45% (74 samples) were successfully determined. There may be many reasons for this: accuracy of the techniques, handling errors on the part of the operator, equipment problems, lack of time, etc. Of the 74 samples, only 32 could be analysed statistically due to the quality of the photos. However, that was enough in the first instance. In fact, if a significant difference in morphology was found in these 32 samples, the analysis would be taken further, and more samples would be considered. If the difference is not significant, then there would be no need to go any further.

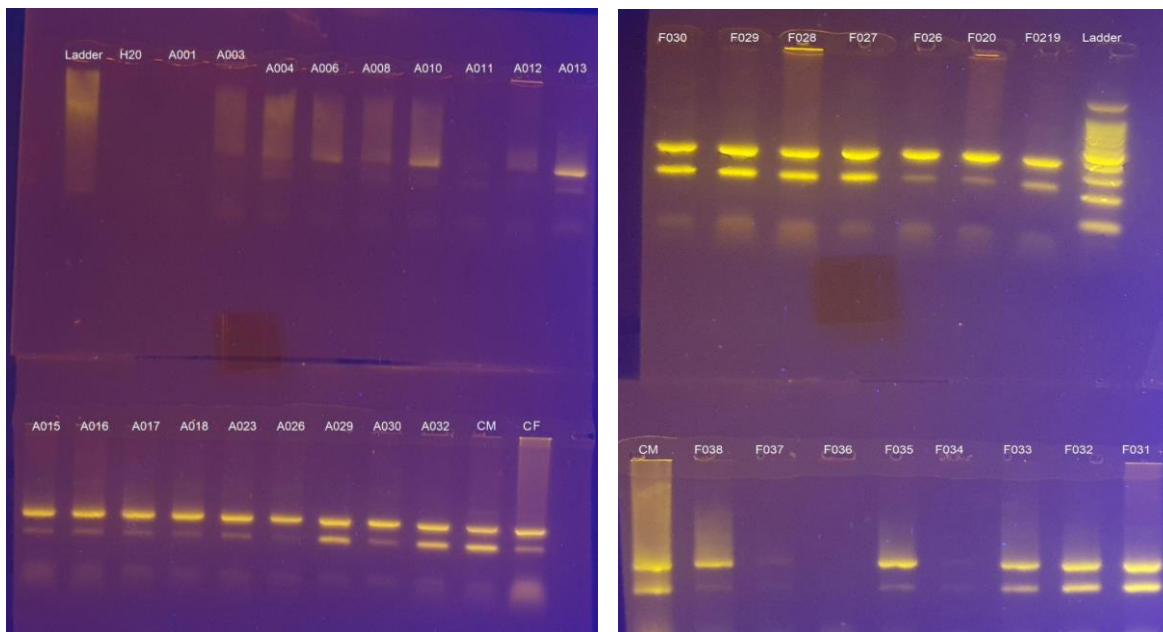


Figure 16: Picture of the gel under the UV light for 2 tests; (left) test for site Cashel Burn, number of the sample are written on the top. The top part didn't work well. (right) test for Endrick Water 1. Some samples didn't work at all (Personal source)

Figure 16 : Photo du gel d'électrophorèse sous lumière à UV bleu, pour 2 tests ; (gauche) test pour le site Cashel Burn, numéro des échantillons écrits au-dessus. La partie haute de la photo n'a pas fonctionné très bien. (droite) Test pour le site Endrick 1. Certains échantillons n'ont pas fonctionné du tout

3.4.2. Geometric morphometric analysis – quantify fish morphology

Once the sex had been determined in the laboratory, the morphometric analysis could begin. First, all the photos had to be sorted and renamed in the same way, i.e., 545 photos of trout and salmon. Those that did not meet the criteria had to be excluded, i.e.: good quality photo, fish as straight as possible, mouth closed, markings visible, etc. The names of the photos were of the type "trout" or "salmon". The names of the photos were of the type "001_En1_S_F"; in other words, the unique number of the photo which generally corresponds to the number of the fin sample tube; the site with the first three letters or the first two and the number if there are several sites in the same stream, although after the analysis they were grouped together; the species, S for Atlantic Salmon and T for Brown Trout; finally the sex, F for female and M for male. The use of Ant Rename software was very useful for this renaming. Once the photos had been selected, it was decided that a maximum of 30 photos per site was sufficient. So, when there were more than 30 individuals, a random selection was made. In the end, 192 fish were selected for the analyses, the distribution of the number of individuals was as shown in the Table 4.

The morphometric analysis begins by marking the individuals on the photos. This marking is carried out using TPSdig2 software (Rohlf, 2017). This program is used to digitise reference points and contours for geometric morphometric analysis. It should also be noted that importing photos into the software could degrade quality, so some photos had to be excluded again. Table 4 takes account of this deletion. The various marking points were chosen to obtain a general shape for the fish, that considers the main measurements of the individual: its head, eyes, body thickness, tail, etc. Figure 17 shows these landmarks (in red) on a photo.

Table 4: Sites with numbers of Atlantic salmon and brown trout included in the analysis
 Table 4 : Sites avec le nombre de saumon et de truites inclus dans les analyses

| Sites | Number of salmon | Number of trout |
|--------------------------|------------------|-----------------|
| Cashel Burn | 1 | 10 |
| Kirkhouse Burn (1 & 2) | 0 | 16 |
| Blane Water (1 & 2) | 1 | 27 |
| Endrick Water (1, 2 & 3) | 22 | 22 |
| Walton Burn | 0 | 21 |
| Balmenoch Burn | 0 | 18 |
| Leven Water | 27 | 0 |
| Boquhan Burn | 8 | 19 |
| Total | 59 | 133 |

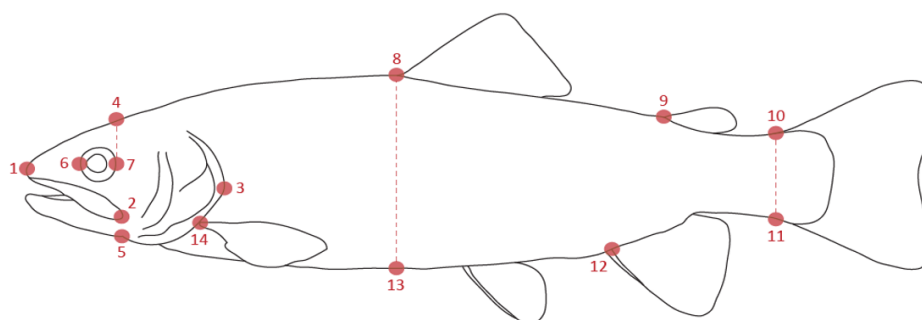


Figure 1. Landmarks used for geometric morphometric analysis of *S. trutta* shape: 1. tip of snout; 2. posterior of maxilla; 3. posterior tip of operculum; 4. dorsal surface perpendicular to (7); 5. joint of lower jaw; 6.–7. anterior and posterior of eye; 8. anterior insertion of dorsal fin; 9. anterior insertion of adipose fin; 10. dorsal junction of caudal fin; 11. ventral surface perpendicular to (10); 12. anterior insertion of anal fin; 13. ventral surface perpendicular to (8); 14. anterior insertion of pectoral fin.

Figure 17: (top) Salmon with the 14 landmarks position and the shape frame for the analysis (blue). (bottom) scheme of a fish with landmarks and the explanation of the position (Source: personal and Peter Koene)

Figure 17 : (haut) Saumon avec les 14 points de marquages et la forme de référence pour les analyses (bleu). (bas) Schéma d'un poisson avec les points de marquage et les explication des positions

After marking the 192 fish with these 14 points, a file containing the 28 coordinates (x and y coordinates for the 14 landmarks) was obtained. The second stage was then carried out using MorphoJ software. This is a geometric morphometric analysis program for two- and three-dimensional marker data (Klingenberg, 2011). Initially, to standardise the data along the main axes and eliminate differences in size and orientation between fish, which allows comparisons of shape only, a Procrustes analysis was performed. This enables all the landmarks to be observed, along with any individual variations among the different individuals, and to see whether any major marking errors have been made (Appendix 12). It is also useful to look at potential outliers. To do this, the software calculates the cumulative distribution of the distances of individual specimens in relation to the mean shape of the data set (Appendix 13). This average is given by the Mahalanobis distances. Mahalanobis distance provides an indication of how unusual an individual is relative to the others in the sample. In this analysis, 10 individuals had to be excluded in order to minimise their outliers on the mean (these have also already been counted in table 4). Then, using the markers and the program, an average frame of the shape of the fish is created (Figure 18, also drawn in the previous figure 17). This shape will be used to visualise the variations in the different classes.

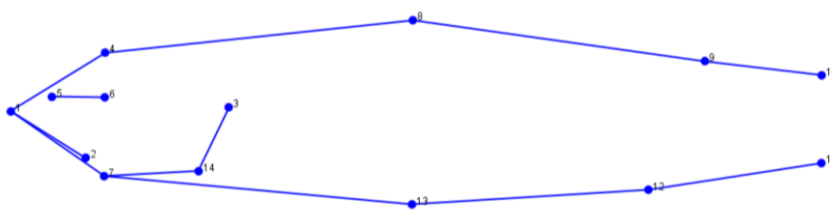


Figure 18: Shape frame created with MorphoJ, linking the landmark in order to have a kind of fish shape (Source: MorphoJ software)

Figure 18 : Forme 'cadre' de référence créée avec MorphoJ, liant les points de marquages afin d'obtenir une forme de poisson

Analysis groups are also created according to the desired comparisons. In this study several classes were created: one according to species (2 groups), one according to site (8 groups), another according to sex (2 groups), according to species and sex (4 groups), and a final one according to species and site (12 groups) (Appendix 14).

After this preliminary work, the statistical analyses were quickly carried out using MorphoJ. Residuals from a linear regression of Procrustes coordinates on centroid size were used in subsequent analyses to eliminate the effects of the age and growth rate allometry of the individuals, as it is not certain that all are exactly the same age and that their growth rate is the same. Next, a principal component analysis (PCA) was performed. PCA was used to observe differences in shape across the dataset. The arrangement obtained with the different components gave a visual and quantified idea of the morphological differences. The MorphoJ software provides 3 different graphs during a PCA: a principal component (PC) shape change, the Eigenvalues for every PC and a graph PC score. The first is particularly useful in our study to get a general idea of the shape of fish depending on the PC score. This graph shows the shape associated with maximum PC score (dark blue) superimposed over the consensus (mean PC score) shape (light blue) (example appendix 18). The second gives the percentage contribution of variation of each component and the last, the scatter plot of PC scores, called "morphospace" (Klingenberg, 2011).

Once we have an overall idea of the variations, a Canonical Variate Analysis (CVA) and/or Discriminant Function Analysis (DFA) are useful. These are analyses of the morphological variation between the different classes that have been predefined. They will therefore give a more specific idea of each group studied. Like PCA, they organise the data according to axes (in this case called canonical variates (CV) or discriminant functions (DF)). But the difference is that the differentiating characteristics will be more specific to the classes and not to all the individuals in general. The CVA will therefore be used to differentiate between classes. The DFA concerns classes with only two groups and will therefore be used for the species and sex classes. It can also be used if there is a need to compare specific groups in other classes more closely. The CVA reveals two graphs: one for the shape changes associated with the canonical variates, the second a scatter plot of the CV scores. As for the DFA, it gives three graphs, the same as the PCA but corresponding to the two groups studied.

Accompanying the graphs, quantified results are given; the different scores of the principal components for each landmark, as well as for the canonical variables or the discriminant functions. In addition, the Procrustes distances between each group and their corresponding p-values are given for the CVA and DFA. These must be looked at to conclude on the significance of the results. A Procrustes distance is a measure of how different the two groups being compared are, based on a Procrustes analysis.

To complete the morphometric analyses and find out how is the variation within each group, the composite shape score (CSS) was calculated for each individual, followed by a standard deviation (SD) and an average for each group, species class and site (using Excel software).

Here, the first four principal component (PC) scores were used for the CSS formula:

$$CSS = (PC1 * PVE1) + (PC2 * PVE2) + (PC3 * PVE3) + (PC4 * PVE4)$$

With PCx for the PC score; PVE_x for the percentage variation explained by the PC_x

3.4.3. Environmental effect on the morphology with a statistical analysis

Finally, after the morphometric analyses, the environmental data were analysed. This was done in two stages: an initial analysis of the water sample, followed by a statistical analysis. A freshwater chemistry test kit containing around ten reagents was used to test water quality. To test each element, all that had to be done was to follow the protocol provided by the kit, generally pour a certain number of drops of the reagent into 5 or 10 mL of water to be tested, wait the indicated reaction time, and then compare the colour of the sample with the colour chart (Figure 19). Appendix 15 contains the reference values given by the kit for each element. The elements tested are ammonium (NH₄), nitrite (NO₂), copper (Cu), oxygen (O₂), nitrate (NO₃), phosphate (PO₄), silicates (SiO₂), and iron (Fe). Most are important nutrients for aquatic plants and sometimes for fish too. Although the majority are not toxic to animal organisms, all have thresholds that are recommended for the proper functioning of the system (JBL GmbH & Co. KG, n.d.).

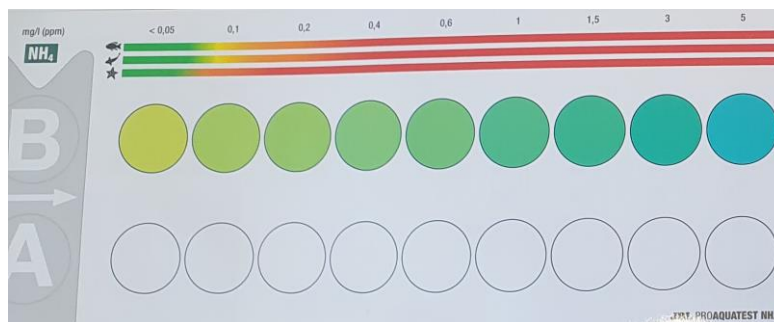


Figure 19: Colour chart for comparison of samples tested, here for the element NH₄. B = blank sample. A = sample with reagent. Comparison of the two colours of the samples following the colour chart and where the colour between the blank one and the tested one appears identical, the corresponding concentration is recorded (Source: JBL GmbH & Co. KG, n.d.)

Figure 19 : Nuancier de couleur pour comparer les échantillons testés, ici pour l'élément NH₄. B = échantillon vierge. A = échantillon avec le réactif. Comparaison des deux échantillons suivant la couleur du nuancier, quand la couleur entre le vierge et le test semble identique, la concentration correspondante est relevée

Then, in order to know if each parameter would have a significant effect on the morphologies of the individuals, a dataset with the 192 individuals was created. The variables chosen were their corresponding sites and species, their respective CSS values and their CV1 and CV2 values, and then each environmental parameter corresponding to their collection site (Mean depth, Mean width, Temperature, Conductivity, pH, Dissolved Oxygen, NH₄, NO₂, Cu, O₂, NO₃, PO₄, SiO₂, Fe) (Appendix 16). For the Kirkhouse, Blane and Endrick sites, as several collection points were carried out, several data sets were obtained. As all the data were grouped under a single site, the averages of all the data were calculated. Using R Studio statistical software, a linear model and ANOVA were

performed between each parameter and the three values representing morphology (CSS, CV1 and CV2) (Script in Appendix 17). A linear model provides information on the effect of each variable on the individuals. ANOVA was then performed on this model to test the significance of the differences between the different groups.

4. Results

4.1. Description of the morphology with a principal component analysis

4.1.1. Salmon and trout - two species, two morphologies

An initial principal component analysis (PCA) was used to identify morphological trends at the level of all individuals. The first four principal components (PC) contributed to a total of 68% of the variation. After PC4, the contributions were well below 10%, which is why they are not considered. Table 5 summarises the characteristics described by each component, and appendix 18 contains the corresponding shapes compared to a consensus.

Table 5: Summary of what high scores for the PCs explain, for the first four principal components, and their percentage of variation explained

Table 5 : Résumé de ce qu'un score élevé explique pour chacune des quatre premières composantes principales, et leur pourcentage de variation expliqué correspondante

| | | |
|-----|--|----------------------|
| PC1 | Smaller eye and head - Thinner body | 26% of the variation |
| PC2 | Longer caudal peduncle (9 to 10) - A bit smaller head | 20% of the variation |
| PC3 | Smaller eye and head - Pectoral fin closer the head - Smaller caudal peduncle - A bit deeper body | 12% of the variation |
| PC4 | Very deeper body - A bit smaller eye and head - Smaller caudal peduncle | 10% of the variation |

This PCA makes it possible to make conclusions about the general shape of the fish. It is then possible, by looking at the PC score point cloud graphs, to see the trends in the fish for certain components and thus obtain a general morphology for them. This is particularly the case for the two species. The graph of PC2 as a function of PC1 shows a tendency for salmon to have a higher score for PC2 (Figure 20).

Atlantic salmon seem, therefore, to have a longer caudal peduncle. On the shape comparison plot PC2 in appendix 18, the dark blue shape showing the shape associated with maximum PC2 score, would, therefore, correspond more to the morphology of salmon than trout. This caudal peduncle length is notably an element of the fish's swimming mechanism. With a greater length, they will have better swimming muscles for long-distance swimming (Koene, pers. comm. 2023). Conversely, PC1 seems to contribute to elements of feeding mechanisms, such as vision, which is useful for seeing prey from greater or lesser distances, and body depth. Apart from this trend for PC2, the other components do not show any distinguishing trend between groups (Appendix19).

Using the PC score obtained for everyone, the composite shape score (CSS) for each individual was calculated, taking into account the first four components. A mean score of 0.00150 was obtained for salmon, while a negative one of -0.00067 was concluded for trout. Then, with a standard deviation (SD) 30% higher (SD salmon \pm 0.00548; SD trout \pm 0.00389), salmon show greater morphological variation within their species than trout. However, this result must consider the fact that salmon represent a much smaller sample than trout, 59 salmon individuals for 133 trout.

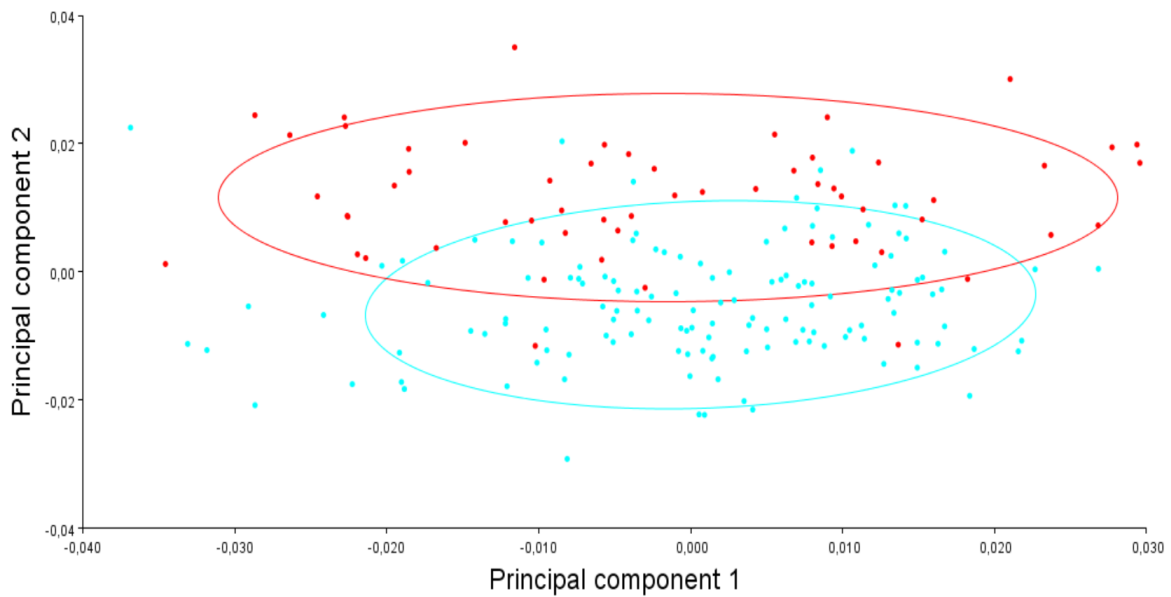


Figure 20: Scatter plot of PC2 and PC1. Red dot: Atlantic salmon. Blue dot: Brown trout. Confidence ellipse 0.8. (Source: MorphoJ software)

Figure 20 : Scatter plot de la CP2 en fonction de la CP1. Point rouge : saumon Atlantique. Point bleu : truite brune. Ellipse de confiance à 80%

4.1.2. Salmon and trout – Different populations, different morphologies

A second PCA on the species/sites class provides a breakdown of the groups according to the components and an idea of the average shapes of the individuals within the different groups. For this analysis, the two groups salmon/Cashel (S/Ca) and salmon/Blane (S/Bl) were excluded because they contained only a single individual. As before, the first four principal components are of interest. They describe 91% of the variation. The results confirm the tendency of salmon to have a longer caudal peduncle than trout (Appendices 20 and 21). They also provide different information on the general trends of the groups. For example, the group of Boquhan salmon (S/Bo) appears to have large mean differences compared to the other salmon (S/Le and S/En), as it is always further apart on the shape comparison plots. The PC1 score of S/Bo is also relatively high, so these salmon have thinner bodies and smaller eyes and heads. The case of the Balmenoch and Walton trout groups should also be noted (T/Ba and T/Wa). Their separation in morphospace from the other trout groups will be discussed again later. Finally, the Cashel trout group (T/Ca) appears to be differentiated at PC3, with a low score. Or the Blane and Boquhan trout groups (T/Bl and T/Bo) show opposition according to PC4. These observations of morphospace plots can be related to the shape plots, so it is possible to obtain a general idea of the typical variation between the different species/site groups.

As before, calculations of the mean CSS and standard deviations for each group show greater variation for salmon than for trout, where this is comparable, i.e., for the Boquhan and Endrick groups. Among the trout, Balmenoch appears to have the greatest variation (SD T/Ba \pm 0.0055) and Cashel the smallest (SD T/Ca \pm 0.0021). But overall, the standard deviations are close (Table 6). The groups therefore appear to have similar amplitudes of variation between species.

Table 6: Mean Composite Shape Score (CSS) and the Standard Deviation (SD) for every group Species/Sites
 Table 6 : Score de composition de forme moyen et l'écart type, pour chaque groupe espèce/site

| Groups Species/Sites | Mean CSS | SD |
|------------------------|-------------------|------------------|
| Salmon/Leven (S/Le) | -0.00071949110939 | 0.00367819111929 |
| Salmon/Boquhan (S/Bo) | 0.00680741733117 | 0.00464404898238 |
| Salmon/Endrick (S/En) | 0.00117292925668 | 0.00523010808025 |
| Trout/Boquhan (T/Bo) | -0.00131910723337 | 0.00334887350169 |
| Trout/Endrick (T/En) | -0.00153989228578 | 0.00365751872055 |
| Trout/Blane (T/Bl) | 0.00084513877419 | 0.00314175814375 |
| Trout/Cashel (T/Ca) | 0.00031078232344 | 0.00206480385258 |
| Trout/Kirkhouse (T/Ki) | 0.00133332513041 | 0.00432691694813 |
| Trout/Balmenoch (T/Ba) | -0.00241935961811 | 0.00546769715433 |
| Trout/Walton (T/Wa) | -0.00158439493972 | 0.00313566637106 |

4.2. Intraspecific variation within species, with a canonical variate analysis

The results obtained with the canonical variate analysis (CVA) according to the species/sites class (without the two groups S/Bl and S/Ca) show the significant differences between the groups. The first three canonical variates describe 79% of the variance within the class. This provides a good explanation of morphology trends and variations between groups.

Table 7: Summary of what describe a high score for the three first canonical variates (CV) from the species/site class analysis, and their percentage of variation explained

Table 7 : Résumé de ce que décrit un score élevé pour les trois premières variables canoniques pour les groupes espèce/site, et le pourcentage de variation expliqué associé

| | | |
|-----|--------------------------------|----------------------|
| CV1 | Shorter caudal peduncle length | 40% of the variation |
| CV2 | Very deeper body - Deeper head | 23% of the variation |
| CV3 | Very deeper eye and head | 16% of the variation |

Using Table 7 and figures 21 and 22, it is possible to describe the morphologies of each group. It seems that trout possess a higher CV1 score than salmon. This corresponds to the description made earlier with PC2; Salmon have a longer caudal peduncle. They also appear to have a thinner body overall than trout. The dark blue shape on the shape comparison plot of CV1, in appendix 22, therefore shows the approximate morphology of brown trout.

Then, CV2 shows a slight distinction from the Boquhan site, for both trout (turquoise blue) and salmon (red). The fish have a smaller head and a slightly slimmer body at this site. The trout at Cashel Burn also seem to fit this description. Trout from Balmenoch Burn and Walton Burn have a high CV2 score. Conversely, they have a large, thick body and a larger head.

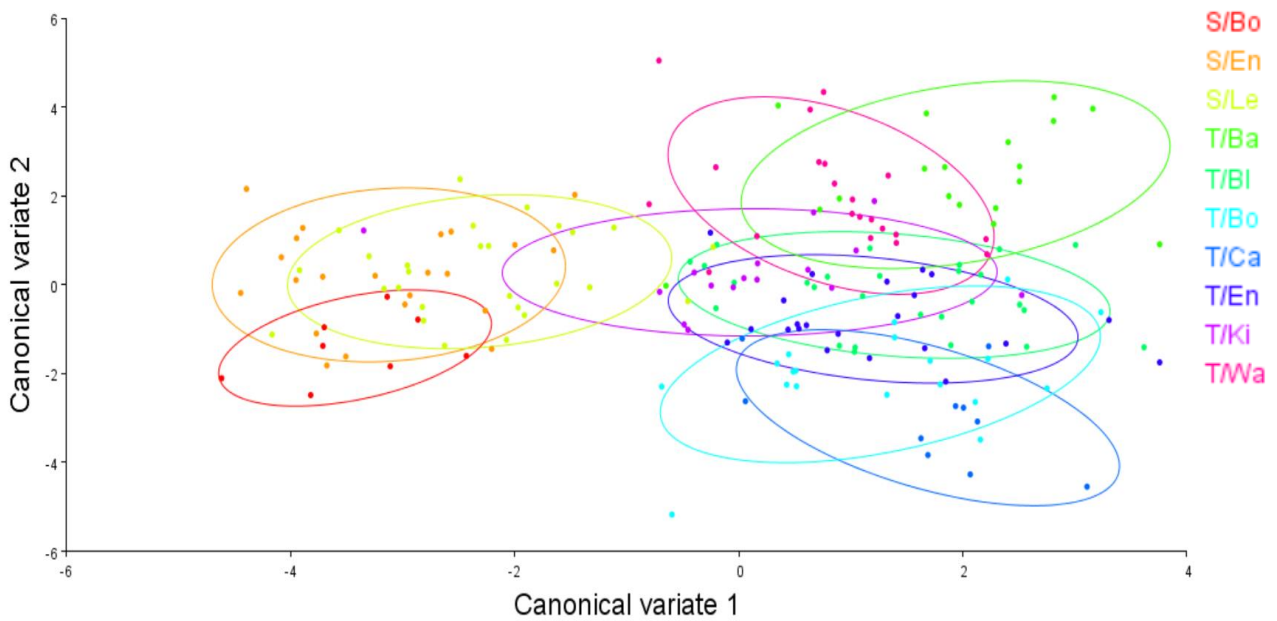


Figure 21: Scatter plot of CV2 and CV1. Color is different for each group. Confidence ellipse 0.8. (Source: MorphoJ software)

Figure 21 : Scatter plot de CV2 en fonction de CV1. Couleurs différentes pour chaque groupes. Ellipse de confiance à 80%

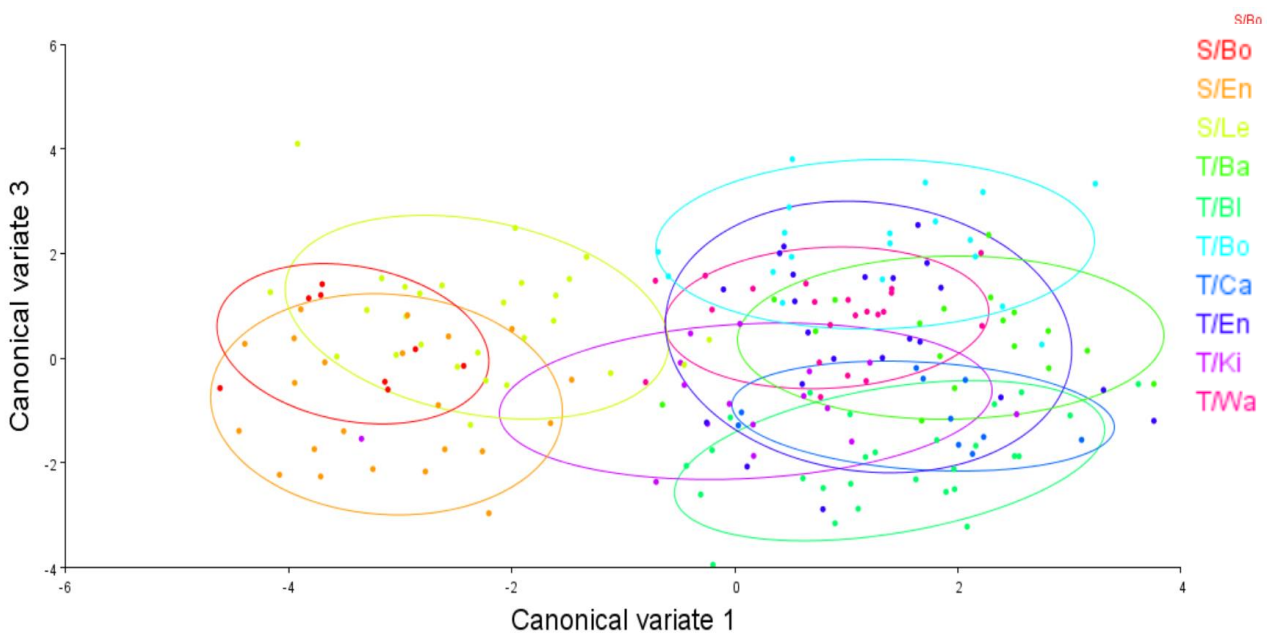


Figure 22: Scatter plot of CV3 and CV1. Color is different for each group. Confidence ellipse 0.8. (Source: MorphoJ software)

Figure 22 : Scatter plot de CV2 en fonction de CV1. Couleurs différentes pour chaque groupes. Ellipse de confiance à 80%

In terms of the CV3 description, the Blane, Cashel and Kirkhouse trout groups appear to have smaller heads and eyes, in contrast to the Boquhan trout group. For a clear summary of the approximate descriptions of each group based on the CV scores, see appendix 23. Another result that the CVA show, is the fact that the site does not seem have an impact on the morphology of the fish. In site with both salmon and trout (Boquhan, Blane and Endrick) the CV2 and CV3 do not distinguish the groups. The environment seems influencing both species in a similar way.

The ellipses on the result graphs, above, show an 80% likelihood of the individuals to be within it. The Procrustes distances, and their corresponding p-values, provided by the CVA show the significance of these different groups in relation to each other (Figure 23). Except for one pair of salmon (Endrick and Boquhan) and one pair of trout (Balmenoch and Walton), all the comparisons between the different population show significant result (p-values < 0.05). Populations are morphologically different.

| Procrustes distances among groups: | | | | | | | | | |
|------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | S/Bo | S/En | S/Le | T/Ba | T/Bl | T/Bo | T/Ca | T/En | T/Ki |
| S/En | 0,0106 | | | | | | | | |
| S/Le | 0,0168 | 0,012 | | | | | | | |
| T/Ba | 0,0277 | 0,0239 | 0,0176 | | | | | | |
| T/Bl | 0,0219 | 0,0235 | 0,0238 | 0,0215 | | | | | |
| T/Bo | 0,0214 | 0,0246 | 0,0224 | 0,0217 | 0,0201 | | | | |
| T/Ca | 0,0191 | 0,0241 | 0,0267 | 0,0287 | 0,0148 | 0,0195 | | | |
| T/En | 0,0204 | 0,0223 | 0,019 | 0,0169 | 0,0125 | 0,0114 | 0,0154 | | |
| T/Ki | 0,0176 | 0,0192 | 0,021 | 0,0205 | 0,0144 | 0,0162 | 0,0194 | 0,015 | |
| T/Wa | 0,0248 | 0,0219 | 0,0153 | 0,0075 | 0,0215 | 0,0186 | 0,0268 | 0,0147 | 0,0187 |

| P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups: | | | | | | | | | |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | S/Bo | S/En | S/Le | T/Ba | T/Bl | T/Bo | T/Ca | T/En | T/Ki |
| S/En | 0,3556 | | | | | | | | |
| S/Le | 0,0011 | 0,006 | | | | | | | |
| T/Ba | 0,0006 | 0,0001 | <.0001 | | | | | | |
| T/Bl | <.0001 | <.0001 | <.0001 | <.0001 | | | | | |
| T/Bo | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | | | | |
| T/Ca | 0,0011 | <.0001 | <.0001 | <.0001 | 0,0008 | <.0001 | | | |
| T/En | <.0001 | <.0001 | <.0001 | 0,0002 | <.0001 | 0,002 | 0,0015 | | |
| T/Ki | 0,0022 | <.0001 | <.0001 | 0,0003 | 0,0002 | <.0001 | 0,0001 | <.0001 | |
| T/Wa | <.0001 | <.0001 | <.0001 | 0,3679 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |

Figure 23: Procrustes distances among groups of the class Species/Sites, and p-values corresponding. Red; no significant value. Orange highlight; salmon, Green highlight; trout. No comparisons are done between salmon groups and trout groups (white boxes). (Source MorphoJ software)

Figure 23 : Distance Procruste entre les groupes de la classe espèce/site, et les p-value associés. Rouge : pas de significativité. Surligné orange : saumon. Surligné vert : truite. Pas de comparaison faite entre les saumons et les truites directement (cases blanches)

4.3. Juvenile female and male – no significant difference

To finish with the morphologies, a Discriminant Function Analysis (DFA) was carried out for the sex class. The results were as expected, with no significant difference between females and males (Procruste distance 0.00899215; p-value > 0.05). This applies to both species combined. But the same is true for a comparison within species (CVA Species/Sex). In salmon, as in trout, females and males showed no significant variation in morphology (Figure 24).

| Procrustes distances among groups: | | | |
|------------------------------------|--------|--------|-------|
| | S_F | S_M | T_F |
| S_M | 0,0201 | | |
| T_F | 0,0273 | 0,0225 | |
| T_M | 0,0244 | 0,0238 | 0,012 |

| P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups: | | | |
|---|--------|--------|--------|
| | S_F | S_M | T_F |
| S_M | 0,2423 | | |
| T_F | 0,0014 | 0,0012 | |
| T_M | 0,007 | 0,0008 | 0,1611 |

Figure 24: Procrustes distances among groups of the class Species/Sex, and p-values corresponding. Red; no significant value. Orange highlight; salmon, Green highlight; trout. No comparisons were done between salmon groups and trout groups (white boxes). (Source: MorphoJ software)

Figure 24 : Distance Procruste entre les groupes de la classe espèce/sexe, et les p-value associés. Rouge : pas de significativité. Surligné orange : saumon. Surligné vert : truite. Pas de comparaison faite entre les saumons et les truites directement (cases blanches)

4.4. Effect of environmental factors on the morphology

4.4.1. Raw data – not a lot of difference

The environmental data collected in the field were used to obtain averages for all the parameters. For sites with several collection points, an average was calculated for all the data taken together. For the basic parameters relating to the watercourse (depth, width, etc.), the values are variable or very similar between sites (Figure 25). It is difficult to conclude significant differences between sites from these simple data. It is therefore assumed that each site is different because its values are different.

| Name | Mean depth (cm) | Mean width (m) | Temperature (°C) | Conductivity (µS) | pH | Dissolved Oxygen (mg/L) |
|--------------------|-----------------|----------------|------------------|-------------------|-------|-------------------------|
| Cashel | 33,780 | 4,620 | 15,160 | 15,780 | 6,910 | 10,020 |
| Kirkhouse (1 & 2) | 25,250 | 2,110 | 15,890 | 61,110 | 8,295 | 10,110 |
| Blane (1 & 2) | 23,440 | 4,990 | 18,250 | 133,500 | 8,000 | 9,735 |
| Endrick (1, 2 & 3) | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 |
| Walton | 23,360 | 4,900 | 14,150 | 75,000 | 7,820 | 9,730 |
| Balmenoch | 20,870 | 7,700 | 15,000 | 67,000 | 7,480 | 9,640 |
| Leven | 27,300 | 31,000 | 16,480 | 65,200 | 7,150 | 10,510 |
| Boquhan | n/a | n/a | n/a | n/a | n/a | n/a |

Figure 25: Values of every environmental parameter for the watercourse, for every site. n/a; no data. The value 31 means above 30 m, and has been chosen to enable the analysis on R

Figure 25 : Valeur de chaque paramètre environnemental des cours d'eau pour chaque site étudié. n/a ; pas de donnée. La valeur 31 signifie au-dessus de 30 m, et a été choisi afin de pouvoir faire les analyses sur R

The data on the vegetation of the watercourse and its surroundings do not appear to be very different (Figure 26). Perhaps, apart from Walton and Balmenoch which do not have large substrates (cobble type > 70mm), nor a very diverse bank. The River Leven is also certainly worth noting, as it is the only watercourse to be found in the middle of a town.

| Name | Substrat type | Bank river | Bank river top | Bank structure |
|--------------------|-------------------------------|--------------------------------------|--|----------------|
| Cashel | Boulder, Cobble, Gravel | Boulder, Cobble, Gravel, Sand | Tree row, Hedge, Scrub, Tall herbs, Rock | Complex |
| Kirkhouse (1 & 2) | Boulder, Cobble, Gravel, Sand | Cobble, Gravel, Sand, Earth | Tree row, Hedge, Scrub, Tall herbs, Moorland, Pasture | Complex |
| Blane (1 & 2) | Cobble, Gravel, Sand | Boulder, Cobble, Gravel, Sand, Earth | Tree row, Scrub, Tall herbs, Moorland?, Pasture? | Complex |
| Endrick (1, 2 & 3) | Cobble, Gravel, Sand | Cobble, Gravel, Sand, Earth | Tree row, Scrub, Tall herbs, Moorland, Pasture, Tilled land? | Complex |
| Walton | Sand, Silt, Mud, Clay | Earth | Tall herbs, Moorland, Pasture, Tilled land, Crop | Simple |
| Balmenoch | Gravel, Sand, Silt, Mud | Gravel, Sand, Earth, Sticky clay? | Scrub, Tall herbs, Moorland, Pasture | Simple |
| Leven | Cobble, Gravel | Cobble, Gravel, Sand, Earth | Tree row, Hedge, Scrub, Urban | Complex |
| Boquhan | n/a | n/a | n/a | n/a |

Figure 26: Vegetation type collecting for every site. n/a; no data

Figure 26 : Type de végétation relevé pour chaque site.

The data on land use around the sites did not show any real differences either. Simply the same observations made in the field; that most of the surrounding area is farmland and woodland, and that the River Leven is in an urban environment. As the variations between the sites do not appear to be important, it was expected that the water sample analysis values would be similar too. This is indeed the case (Figure 27). One surprising point is that Kirkhouse is next to a sewage treatment plant, and although the vegetation and substrate are the same as the other sites, it is surprising that there is no distinction in the water chemistry due to this.

So, there is clearly no difference for some of the chemical elements analysed: ammonium, copper, oxygen and phosphate. Nitrite should also be added to the above list, as the variations are largely negligible. Especially as the NO₂ value for Endrick is due to just one of the three values (that of Endrick 3), which was a difference of 0.009. For the other elements, we need to take a closer look at the importance of the elements for the watercourses and for living organisms, to see whether their concentration has a real impact or not. However, the aim of the study with these data is to identify a

potential effect of parameters on morphologies. The primary analysis of these results therefore did not go any further.

| Name | NH4 | NO2 | Cu | O2 | NO3 | PO4 | SiO2 | Fe |
|--------------------|-------|-------|-------|--------|-------|-------|-------|-------|
| Cashel | 0,049 | 0,009 | 0,049 | 10,000 | 0,490 | 0,019 | 2,000 | 0,150 |
| Kirkhouse (1 & 2) | 0,049 | 0,009 | 0,049 | 10,000 | 0,745 | 0,019 | 4,550 | 0,060 |
| Blane (1 & 2) | 0,049 | 0,009 | 0,049 | 10,000 | 0,745 | 0,110 | 6,100 | 0,028 |
| Endrick (1, 2 & 3) | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| Walton | 0,049 | 0,010 | 0,049 | 10,000 | 0,490 | 0,019 | 6,100 | 0,050 |
| Balmenoch | 0,049 | 0,009 | 0,049 | 10,000 | 0,490 | 0,019 | 6,100 | 0,050 |
| Leven | 0,049 | 0,009 | 0,049 | 10,000 | 0,500 | 0,019 | 0,800 | 0,019 |
| Boquhan | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |

Figure 27: Results of the chemistry water analysis for every site. n/a; no data. Highlight meaning (cf. appendix 9): Green, good value; Yellow, limit value; Red, bad value. The value 0,049 means below 0,05 and has been chosen to enable the analysis on R. 0,009 for below 0,01. 0,049 for below 0,5. 0,019 for below 0,02. And 6,1 for below 6

Figure 27 : Résultats de l'analyse chimique de l'eau pour chaque site. n/a ; pas de données. Signification des surlignements (cf. annexe 9) : Vert, bonne valeur ; Jaune, valeur limite ; Rouge, mauvaise valeur. La valeur 0,049 signifie inférieure à 0,05 et a été choisie pour permettre l'analyse sur R. 0,009 pour inférieure à 0,01. 0,049 pour une valeur inférieure à 0,5. 0,019 pour une valeur inférieure à 0,02. Et 6,1 pour plus de 6.

4.4.2. Significant effect of the habitat on the morphology

The results of the individual linear models conclude that only two of the fourteen independent variables have an impact. Temperature and phosphate were the only variables to have a significant effect on the Composite Shape Score (CSS) of the fish (p-value < 0.05) (Figure 28). However, for both, the F-value is not very high. Generally, the higher the F-value, the more the independent variable (in this case temperature or phosphate) explains the variance in the dependent variable (CSS). Furthermore, a closer look at the PO4 values for each site shows that only Blane has a different value (0.110 mg/L), while the other six sites all have 0.019 mg/L PO4. Blane's value corresponds to the average of the site's two collection points, which were 0.019 and 0.200. It is therefore unwise to consider this significance, which is based on a single divergent value.

```
> anova(PO4)
Analysis of Variance Table

Response: Data$`Composite shape score`
      Df Sum Sq Mean Sq F value Pr(>F)
Data$PO4  1 0.00007874 7.8735e-05 4.1502 0.04325 *
Residuals 163 0.00309232 1.8971e-05
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Temp)
Analysis of Variance Table

Response: Data$`Composite shape score`
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Temperature  1 9.507e-05 9.5075e-05 5.0381 0.02614 *
Residuals      163 3.076e-03 1.8871e-05
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 28: ANOVA result for the linear model of the variable PO4 and Temperature on the Composite Shape Score (Source: R Studio)

Figure 28 : résultat de l'ANOVA sur le modèle linéaire des variables indépendantes 'PO4' et 'Température' sur la variable dépendante 'le score de composition de forme'

Two complex linear models were also run. One shows the effect of all the variables related to the watercourse (mean depth, mean width, temperature, pH, conductivity, and dissolved oxygen) on morphology (represented by the CSS). The second is done with water chemistry variables, also on morphology (Appendix 24). While the first model confirms that temperature is the only one to show a significant effect, the second model presents a completely different result. It concludes that there is a significant effect of the element NO3 (F-value 8.9484; p-value 0.00322) on morphology and a slight

effect of Iron (F-value 3.0186; p-value 0.08425). However, when tested individually, they no longer had any effect. Ammonium and iron might have an impact on fish morphology when they are not considered alone.

With the exception of temperature, ammonium and iron (if the second complex model is considered) it seems that overall the environmental parameters do not have a important effect on the morphology of the fish, a morphology represented by the general shape score (CSS) which takes into account the first four PC scores.

The same analyses were carried out to see the effects on the CV1 and CV2 scores. The results show that several parameters have an effect on the CV scores (Appendices 25 and 26). Depth, width, pH and dissolved oxygen appear to have a significant effect on CV1, which describes the size of the caudal peduncle length (Table 7). Whereas for body size (explain with CV2), only depth, temperature and dissolved oxygen show an effect. Surprisingly, all the chemical elements tested had a significant effect on the scores. These different ANOVAs therefore make it possible to observe which environmental parameters have an effect on fish morphology.

5. Discussion and limits

Thus, as expected, juvenile salmon and trout are morphologically different and this is consistent with the literature (Alexander & Adams, 2000; Bush & Adams, 2007; Ferguson, 1989). What is interesting to note from this study is that Atlantic salmon show greater intraspecific variation than their sympatric brown trout.

The main aim of this study was to compare populations between sites. The results show significant differences between eight of the ten groups in the species/site class. This is consistent with studies showing divergent morphologies between allopatric and sympatric populations (Fraser et al., 2011; Koene et al., 2020; Piggott et al., 2018). Genetic analysis was not carried out in this study, but previous work on brown trout and Atlantic salmon in tributaries of Loch Lomond has concluded that there are significant genetic divergences between populations in different streams (Coulson et al., 2013; Rodger, pers. comm., 2023) It is therefore interesting to note that variation between populations in the Loch Lomond catchment is not only genetic but also phenotypic. However, the sample sizes were very different, with only 59 salmon and 133 trout. This result, therefore, needs to be put into perspective, and a bigger sample size for salmon might be better.

The groups that did not show any significance could be explained by the very short distance between the sites, such as the Balmenoch Burn and Walton Burn which are only 250 metres apart. Perhaps they could have been grouped together at the start of the analyses. Or by the fact that most of the streams in the study are tributaries of the River Endrick (Figure 15). But, in this study it was assumed that all individuals were from the location in which they were caught. It is therefore not surprising that Boquhan salmon do not show significant differences from Endrick salmon. Conversely, it may be surprising that the other tributaries of this river are significant (Walton and Balmenoch or Blane). However, despite the significance of the Procrustes distances, this link between the streams can be seen in the fact that the River Endrick has the smallest distance values with all the other groups (Figure 23). This finding should also be qualified because Endrick is one of the streams with the largest sample of individuals, salmon and trout combined.

Regarding the Balmenoch and Walton streams, it was noted in the results that they often appear apart from the other trout populations. This is not surprising, since when the data was collected, the overall size of the fish was noted to be much larger than the others. It was therefore expected to observe this. In addition to size, the question of age also arose for these individuals. The parr marks, the main sign of the juvenile stage of the trout, seemed not to be clearly visible, almost disappearing on most of the fish in these two streams. To see if the results were different if these two sites were removed from the analysis, a CVA without them was carried out. As the Procrustes distances and their p-values did not

vary significantly, it was concluded that with or without Balmenoch and Walton the results were similar (Appendix 27).

Analysis of the canonical variates allows us to characterise the types of morphology for each species and site. In addition, for the study sites with salmon and trout, it was found that the environment had a similar effect on both species. This result shows that at the juvenile stage, the fish habitat does not exert a different effect on the two salmonids, especially when they live in the same environment. This is what the study by Fraser et al (2011) seems to confirm by concluding that between salmonid populations that are geographically close, selection regimes can be similar. In this report, the study takes place on a small geographical scale (that of Loch Lomond and its environs), so this conclusion seems to accord with the literature. However, it might be worth adding a quantification of the effect of the environment on variations. Here, the analysis of environmental data was only preliminary. The aim was simply to see whether the observed parameters had an effect on the overall morphology. This was done using linear models on the composite shape score (CSS). To take this analysis further, it would be necessary to increase the number of study sites containing the two species, and also to increase the collection of environmental data. In this study, the time limit imposed, the material constraints and the lack of precision in the data collection did not allow us to go much further in the analysis. But this result does raise questions about why the site does not seem to distinguish between salmon and trout and exerts the same effect on morphology. A closer study of the magnitude of the effect of certain environmental parameters on population morphologies could be interesting.

Temperature is the only parameter to have shown significant effects on morphology. However, in this study, the temperature data are not representative of the temperature across seasons or across the lifetimes of the fish, as it was collected at a precise moment in time. Furthermore, the difference between sites cannot be considered constant throughout the year. But numerous studies have demonstrated the significant impact of this parameter on the life cycle of salmonids. Their growth from the egg stage to adulthood, their chances of survival during migration, their reproductive success, etc. Temperature is widely known to be a determining factor in most of the key moments in the life of fish (Enders & Boisclair, 2016; Ferguson et al., 2019; Mobley et al., 2021; Réalis-Doyelle et al., 2016; Sloat & Reeves, 2014; Thorstad et al., 2012). What is perhaps surprising is that it seems to have an impact on general morphology (with CSS), but on more precise characteristics, such as the size of the caudal peduncle distance (CV1), or body size (CV2), temperature shows a different effect. The tail is used for the swimming movements of the fish, but temperature does not seem to have a direct influence on these movements. It has more to do with metabolism than with physical mechanisms. Of course, temperature does have an effect indirectly, as it is a key element in energy metabolism and therefore in the energy used for movement (Lee et al., 2003). But overall, this would explain the result that temperature has no significant effect on CV1 but does have an effect on the morphology score.

The chemical elements analysed in the water are mainly due to the degradation of organic matter or rocks in the habitat. They are also very often essential nutrients for living organisms and therefore contribute to the quality of the aquatic environment. They can also be added in varying quantities by the surrounding environment. Water pollution, agriculture, pesticides or even rain can change their concentration (Brian Moss, 1998). In this study, they do not show a very significant effect on average morphology but seem to have a greater influence on the characteristics described by CV1 and CV2. In any case, at the concentration at which they are found. These nutrients are mainly used by plants, so it is normal for their effects to be minimal. Perhaps they are more significant than the CV scores individually than on the overall fitness score because they affect the body's metabolism and not morphology directly. Phosphate is essential for energy metabolism, for the production of *e.g.* adenosine triphosphate (ATP), and iron for *e.g.* the transport of oxygen and carbon dioxide throughout the body.

Another expected result was that the morphological difference between individuals according to their sex was not significant. It is known that there is sexual dimorphism in adult salmonids. Females and males of reproductive age, when maturation begins, have different physical traits (Hayashida et al.,

2013; Mobley et al., 2021). One of the main characteristics is their different colouring as adults (Figure 2 and 4). However, in the juvenile stages, before they begin to mature, salmon and trout do not display sex-dependent phenotypes, although their genotype is clearly identified (Hayashida et al., 2013). This was confirmed in this study.

Finally, obtaining the values of the canonical variates (CV) gives an average for Atlantic salmon and another for brown trout. A sort of form score scale could be obtained. It would then be possible to position hybrid individuals according to this scale of values. Depending on their morphometric analyses, they could be identified as resembling one of the species or not. The results of the study clearly distinguish salmon from trout. It should, therefore, be possible to create this type of scale. However, a larger sample of Atlantic salmon would be more favourable in order to guarantee the significance and generalisation of the results. Also, in view of the scores obtained, it would seem that assuming the absence of hybrids from the start of the study was correct. Indeed, in the results obtained, no group of individuals seems to be morphologically between the two species, at least not distinctly (Figure 21). The question of hybridisation had arisen for individuals from the Endrick River. These were finally classified as salmon. In the end, this group (S/En) showed clear and distinct results, which seemed to validate that the individuals in question belonged to the chosen species.

6. Perspective of the study

In the context of studying the spread of hybrids within Loch Lomond, the study presented in this report shows satisfactory results. What could be taken a step further is the genetic identification of potential hybrids. Although it is assumed that there are none in the sample of salmon and trout, genetic confirmation would be a plus. In the event that some are found to be hybrids, it would be interesting to take a closer look at the morphometric analyses and identify the different CV scores, or trends, that these individuals show compared with the pure species. They could then be placed on the scale of morphological values, which would refine the scale and make it easier to place other future individuals. If this is not the case, it might be wise to continue the morphometric study of juveniles and increase the sample sizes of salmon and trout in order to increase the quality of the final results.

If the study is to be continued or repeated, it may be necessary to review certain aspects of the method, such as the choice of sites. It would be more interesting to have sites containing both salmon and trout. It would therefore be necessary either to study the density of populations in previous years and choose according to these statistics, or to have more time and make more collection sites. Sex identification could be an analysis to be left aside, but the collection of adipose fin remains a key point. Even so, it will enable us to obtain the DNA of the individual and identify hybrids genetically, using a different technique of identifying known differences in microsatellites, if necessary (Perrier et al., 2011). It would also be a plus to take environmental parameters further. First of all, the precision of the data collection would have to be reviewed. In this study, it is unfortunately not possible to say that all the sites were collected in the same way. The number of data points collected per site, in order to calculate averages afterwards, was not the same for all of them. For example, for some sites, the average depth was calculated from around ten readings, while others had only three or four. In view of the results of the chemical analysis of the water samples, it may not be necessary to repeat it. Especially as the tests also had certain accuracy limitations. The tests were based on the person's ability to differentiate between the shades of colour in the colour charts, using the naked eye. Although the results remain approximations, the reading error could be easy and different if the light in the room changed, for example. The results linked with the water chemistry are interesting, but how is the influence on morphology is unclear. It might be useful to go further in the usefulness of every element of the water on the fish's life to understand their effect. Also, to examining the water quality of Scotland's streams and their impact on fish morphology, this analysis would be relevant. Aquatic environments such as rivers are highly changeable. It has also been shown that the impact of climate change on these habitats is significant and represents a major problem. Salmon and trout are

already subject to several pressures throughout their lives, particularly during migration (Figure 5). They already must adapt to many changes. The quality of their living environment is therefore probably a key factor in their survival. A closer look at how pollutants in the water, agricultural residues, or human waste impact the morphology of fish would be interesting and could provide information on the possible adaptations of species to environmental changes. In a context where water resources are increasingly under threat, taking a closer look at how fish adapt behaviourally to this is a pertinent question. More so for salmon and trout, which are key economic species in Scotland.

Conclusion

In conclusion, there is a significant difference in morphology between juvenile Atlantic salmon and brown trout. More importantly, between 8 streams in the south and south-east of the Loch Lomond catchment, individuals of the same species have different physiques. This rapid and effective morphometric study shows the variations that exist between species in different rivers. It allows conclusions to be drawn about the average shape of populations and comparisons to be made between them. Within trout, this variation, although small, remains significant. Except for two very similar streams, trout populations are morphologically different depending on their stream. The same is true of Atlantic salmon. However, as the sample of Atlantic salmon was very small, the conclusion can only be drawn from a comparison of three streams. It is nevertheless possible to conclude that, on a small scale, characteristic population-level variations exist within these two species. The morphology of the fish is potentially linked to the effects of the environment, particularly temperature, but a more detailed study of these effects would be interesting in order to conclude with certainty the causes of these phenotypes and possibly to compare these effects on different populations at different sites.

Conclusion from a personal point of view

As part of this job, I have been lucky enough to experience many things. First of all, working in research. Three years ago, I said I did not want to do research. I did not really know what it meant. So, these six months in this field have given me a good insight into a lot of the work that can be done and have proved me wrong. At SCENE, the projects are diverse and varied, and the working environment and atmosphere are perfect. After this experience, I would say that research is also about sharing ideas, helping each other, and working hard on the challenges of tomorrow. I was lucky enough to do a study on my own. With the right support and help, I learnt how to set up a project. I can now say that continuing in this field would be a possibility.

Then I developed and improved a lot of professional and personal skills and knowledge. I took on a project for which I had no background. I knew nothing about salmon and trout or Scotland. I knew that adapting wouldn't be a problem, as I had already spent seven months abroad last year. What probably took the most effort during the first few weeks was learning the basic vocabulary in the area so that I could communicate with everyone. Or getting used to communicating in English every day again, and getting used to my new daily life in the Scottish countryside, with no car to get around in. But thanks to my ability to adapt and my strength of character, I think I have succeeded in this challenge. Especially as living in the Scottish countryside, between Loch Lomond and the mountains, is not the worst of daily lives!

Finally, my apprenticeship and my engineering skills will help me to understand the environmental conservation issues that I want to defend. This end-of-study work placement has largely confirmed my desire to work internationally and in species conservation. What is more, thanks to this work placement, I have had the opportunity to discover a number of scientific projects for the preservation of the environment, including Darwin200, which I am lucky enough to be taking part in soon. This six-month work placement has been a great conclusion, both professionally and personally.

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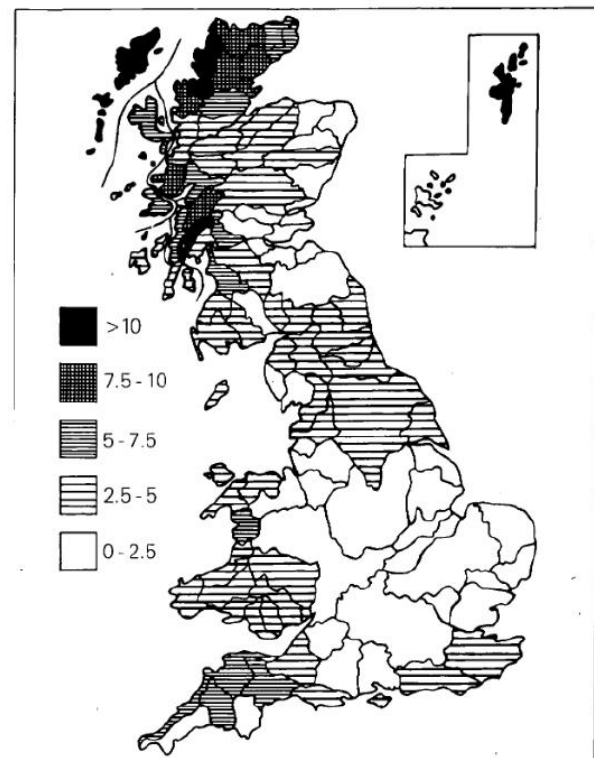
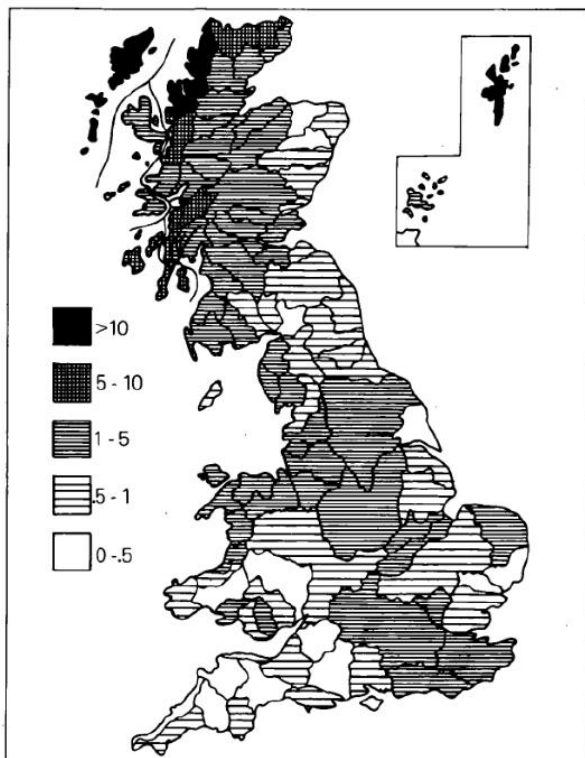
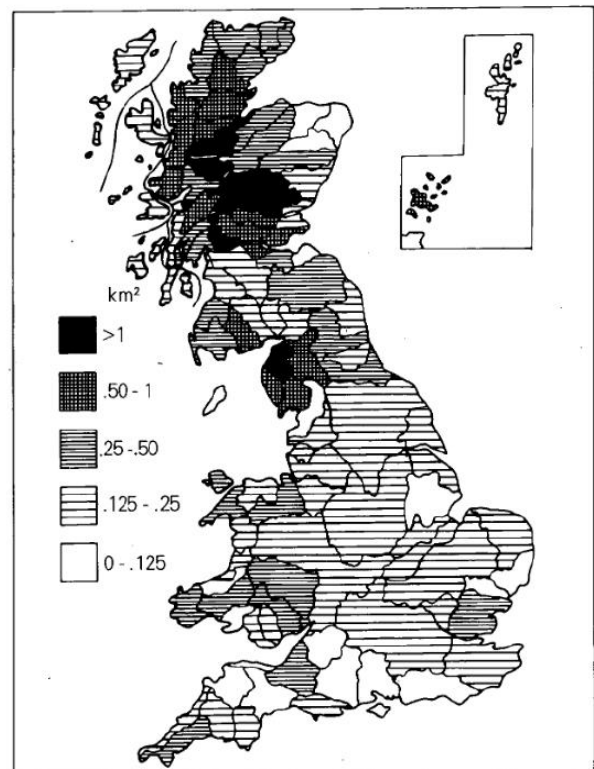
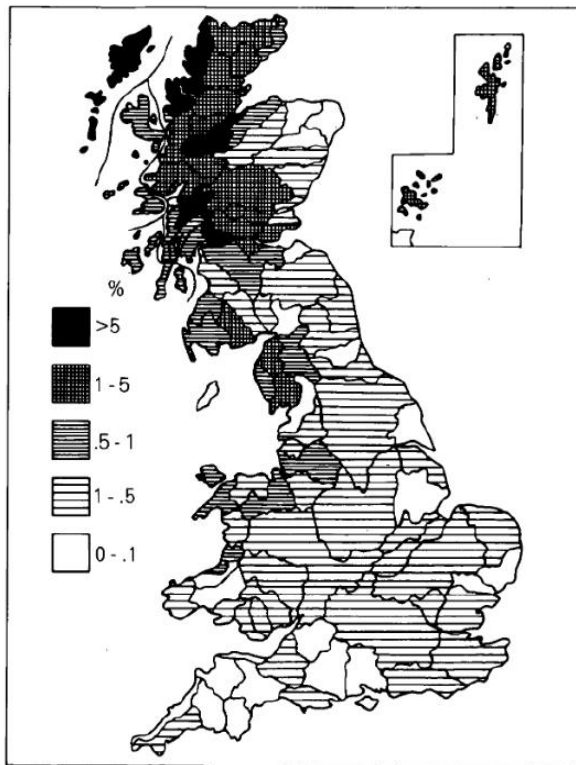
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Appendix

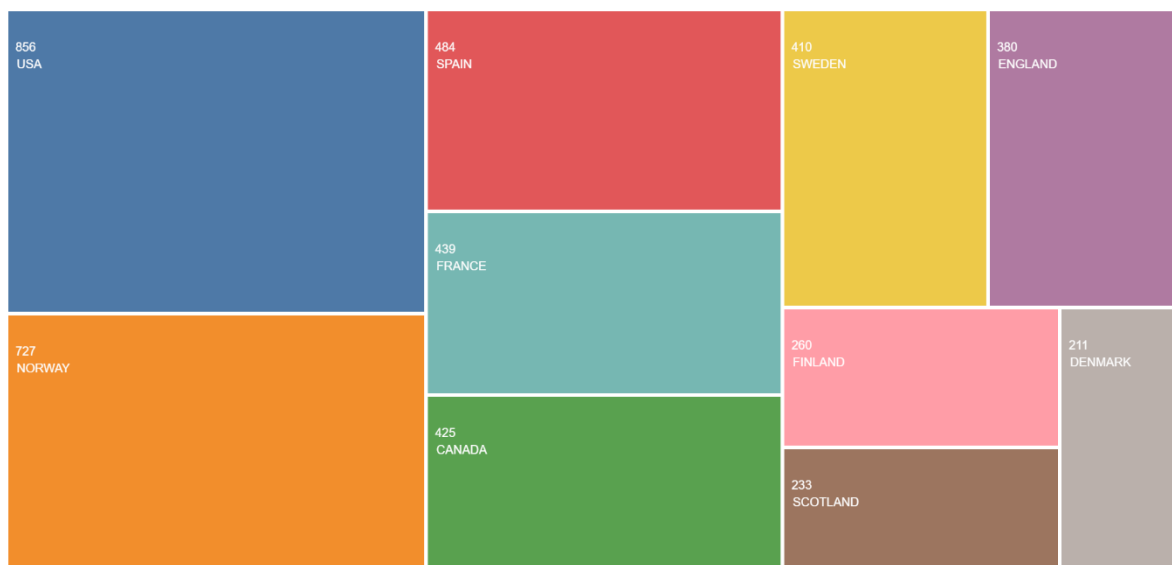
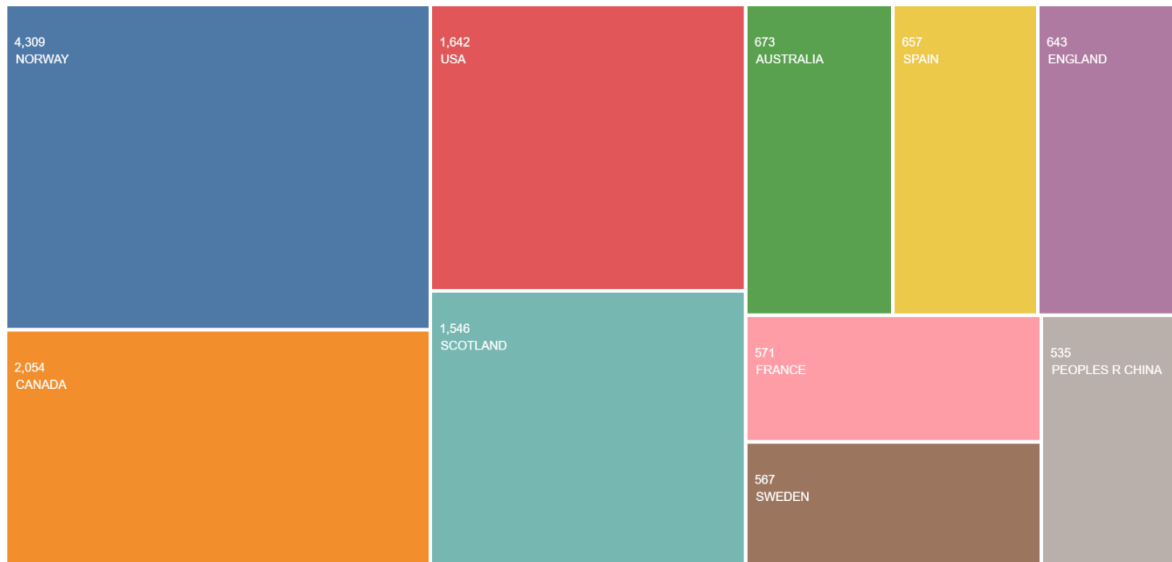
Appendix 1: The extent and distribution of freshwater by Hydrometric Area in Great Britain (top left) The number of standing water per 100 km²; (top right) The number of streams per 100 km²; (bottom left) The percentage area of standing water; (bottom right) The mean area of standing water (Source: Smith & Lyle, 1979)

Annexe 1 : L'étendue et la distribution de l'eau douce par zone hydrométrique en Grande-Bretagne (en haut à gauche) Le nombre d'eaux stagnantes par 100 km²; (en haut à droite) Le nombre de cours d'eau par 100 km²; (en bas à gauche) Le pourcentage de surface d'eau stagnante; (en bas à droite) La surface moyenne d'eau stagnante



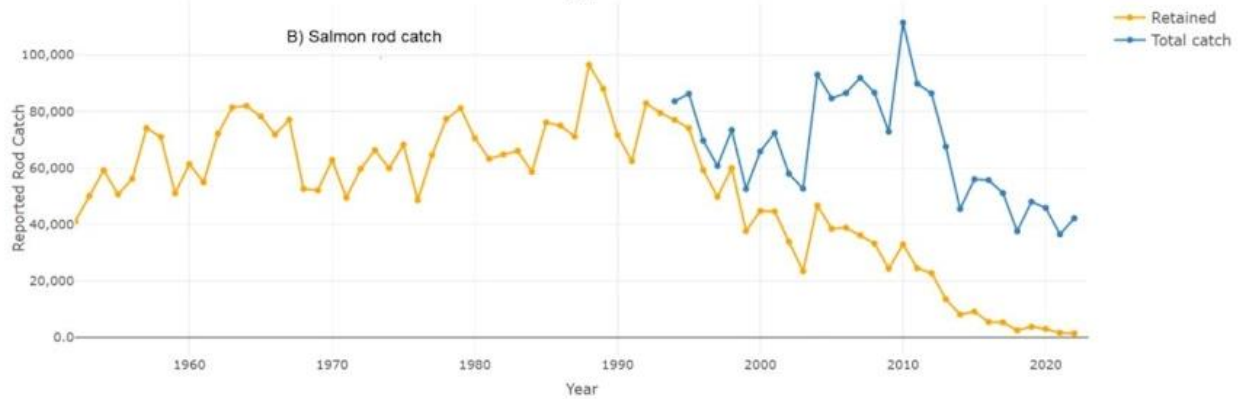
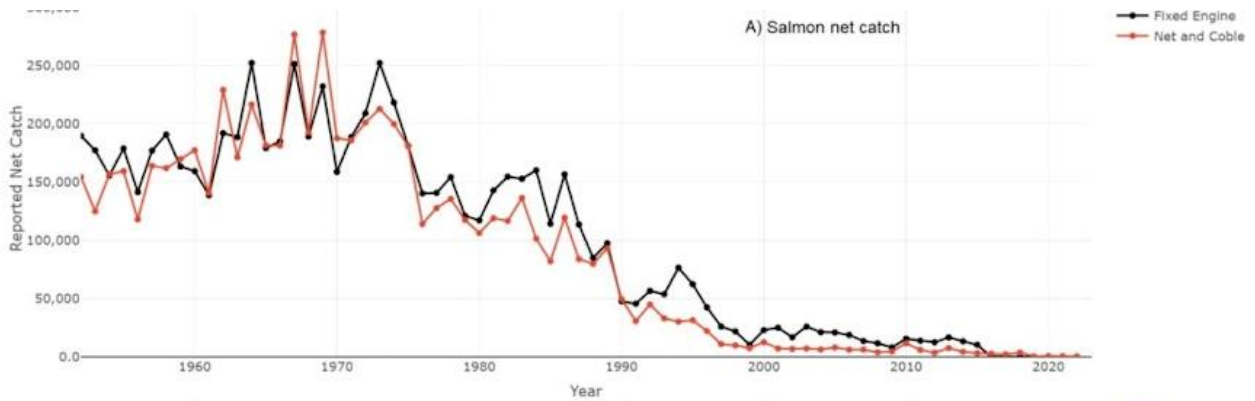
Appendix 2: (top) TreeMap diagram showing the top 10 countries/regions publishing on sea trout, *Salmo trutta*. (bottom) TreeMap diagram showing the top 10 countries/regions publishing on Atlantic salmon, *Salmo salar* (source: Web of Science)

Annexe 2 : (en haut) Diagramme TreeMap montrant les 10 premiers pays/régions publiant sur la truite de mer, *Salmo trutta*. (en bas) Diagramme arborescent montrant les 10 premiers pays/régions publiant sur le saumon de l'Atlantique, *Salmo salar*

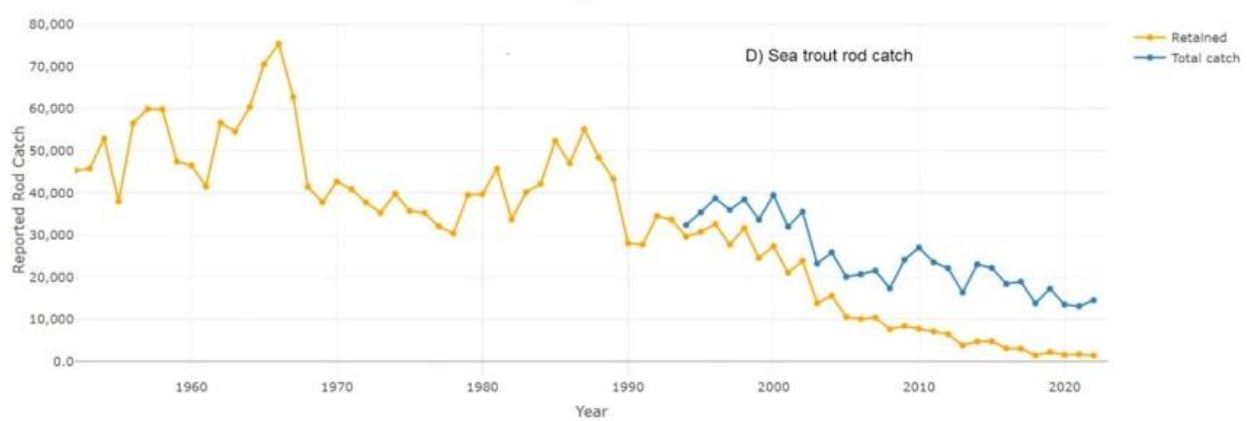
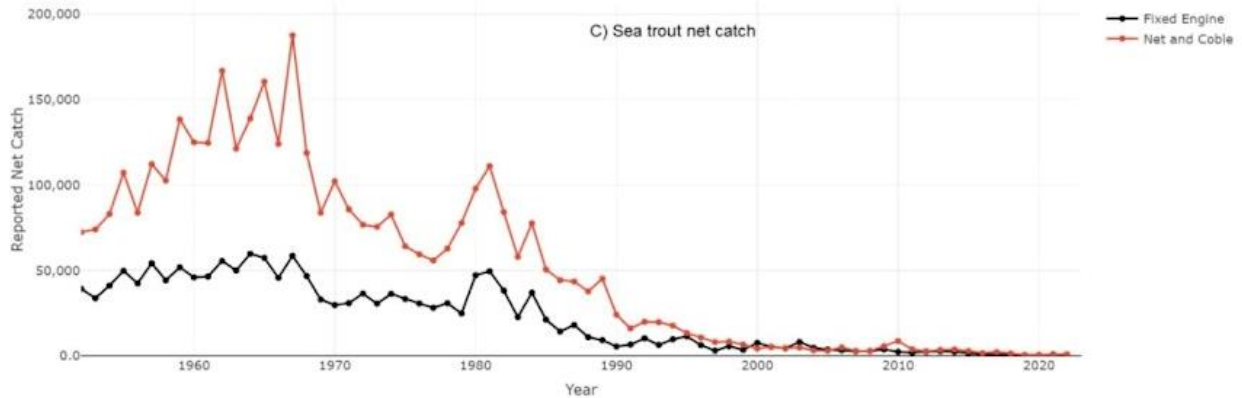


Appendix 3: Number of Atlantic salmon, *Salmo salar*, caught by net (A) and rod (B) and of Sea Trout, *Salmo trutta*, caught by net (C) and rod (D) between 1950 and 2020 (source: Marine Scotland)

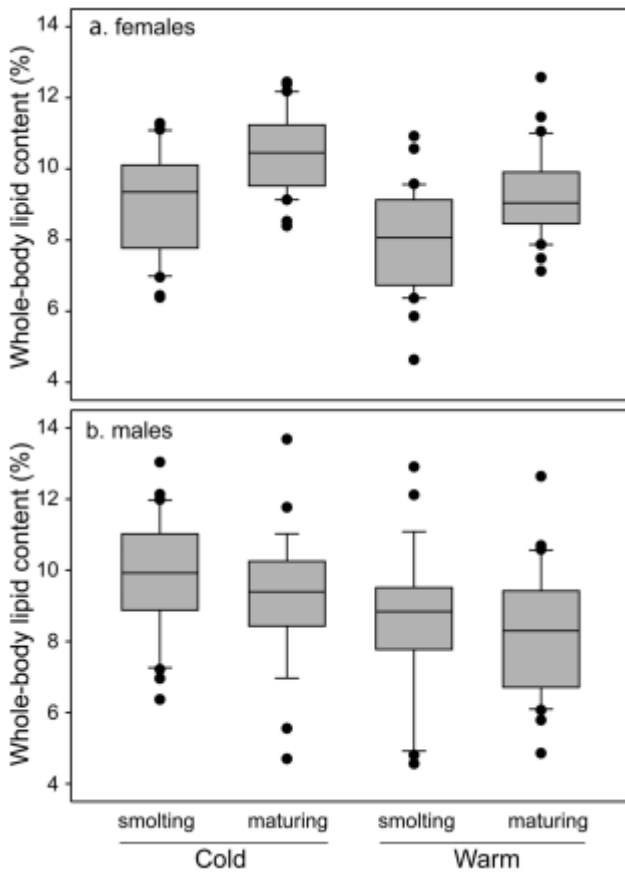
Annexe 3 : Nombre de saumons atlantiques, *Salmo salar*, capturés au filet (A) et à la canne (B) et de truites de mer, *Salmo trutta*, capturés au filet (C) et à la canne (D) entre 1950 et 2020 (source : Marine Scotland)



Difference between total catch and retained = numbers released

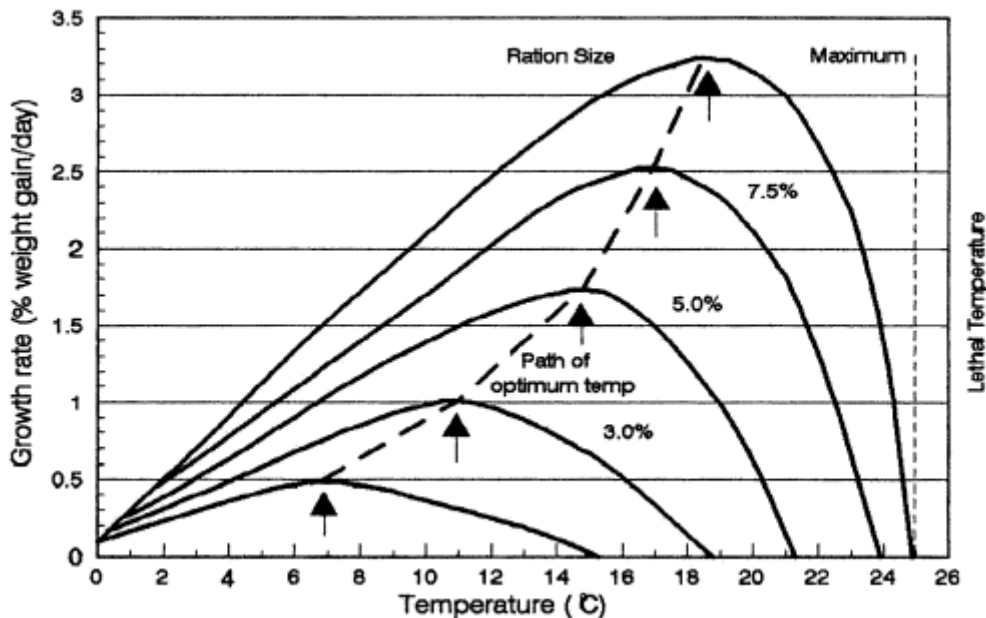


Difference between total catch and retained = numbers released



Appendix 4: Whole-body lipid content for smolting and maturing (a) female and (b) male *O. mykiss* reared under a cold and warm thermal regime. Whiskers represent lines to data that are no more than 1.5 times the interquartile range. Top lines of boxes denote the 75th percentile, bottom line the 25th percentile, and middle line the means. Filled circles denote outliers (Source: Sloat and Reeves, 2014)

Annexe 4 : Teneur en lipides du corps entier pour les femelles (a) et les mâles (b) *O. mykiss* en période de smolting et de maturation, élevés sous un régime thermique froid et chaud. Les moustaches représentent les lignes vers les données qui ne sont pas plus de 1,5 fois l'intervalle interquartile. Les lignes supérieures des boîtes indiquent le 75e percentile, la ligne inférieure le 25e percentile et la ligne centrale les moyennes. Les cercles remplis indiquent les contours



Appendix 5: Scope for growth of chinook salmon at different ration sizes and environmental temperatures (Source: Brannon et al. 2004)

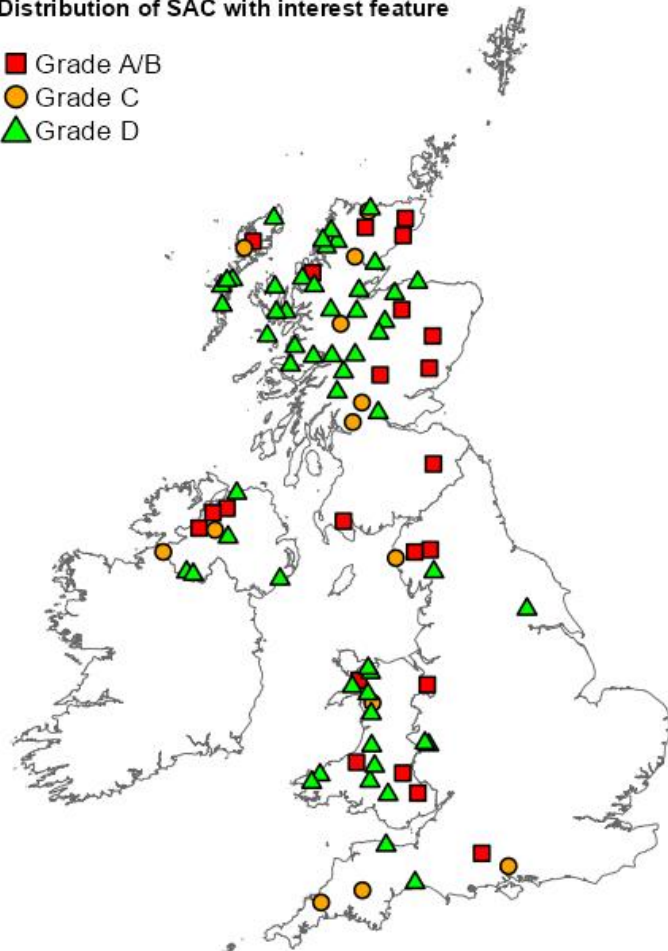
Annexe 5 : Possibilité de croissance du saumon quinnat à différentes tailles de rations et températures environnementales (Source : Brannon et al. 2004)

Appendix 6: Distribution of Special Area for Conservation (SAC) containing species *Salmo salar*. A = Outstanding examples of the feature in a European context. B = Excellent examples of the feature, significantly above the threshold for SSSI/ASSI notification but of somewhat lower value than grade A sites. C = Examples of feature which are of at least national importance (i.e. usually above the threshold for SSSI/ASSI notification on terrestrial sites) but not significantly above this. These features are not primary reason for SACs being selected. D = Features of below SSSI quality occurring on SACs. These are non-qualifying features ("non-significant presence"), indicated by a letter D, but this is not a formal global grade (Source JNCC, n.d.)

Annexe 6 : Répartition des zones spéciales de conservation (ZSC) contenant l'espèce *Salmo salar*. A = Exemples exceptionnels de la caractéristique dans un contexte européen. B = Excellents exemples de la caractéristique, nettement supérieurs au seuil de notification des SSSI/ASSI, mais d'une valeur légèrement inférieure à celle des sites de catégorie A. C = Exemples d'éléments qui sont au moins d'importance nationale (c'est-à-dire généralement au-dessus du seuil de notification SSSI/ASSI pour les sites terrestres), mais pas de manière significative. Ces caractéristiques ne sont pas la raison principale de la sélection des ZSC. D = Caractéristiques d'une qualité inférieure à celle d'une SSSI présentes sur les ZSC. Il s'agit de caractéristiques non qualifiantes ("présence non significative"), indiquées par la lettre D, mais il ne s'agit pas d'une note globale formelle

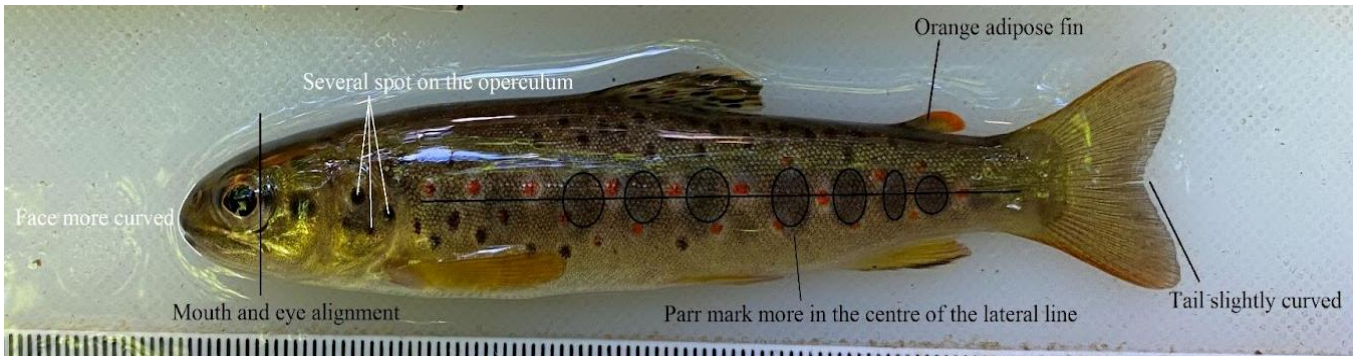
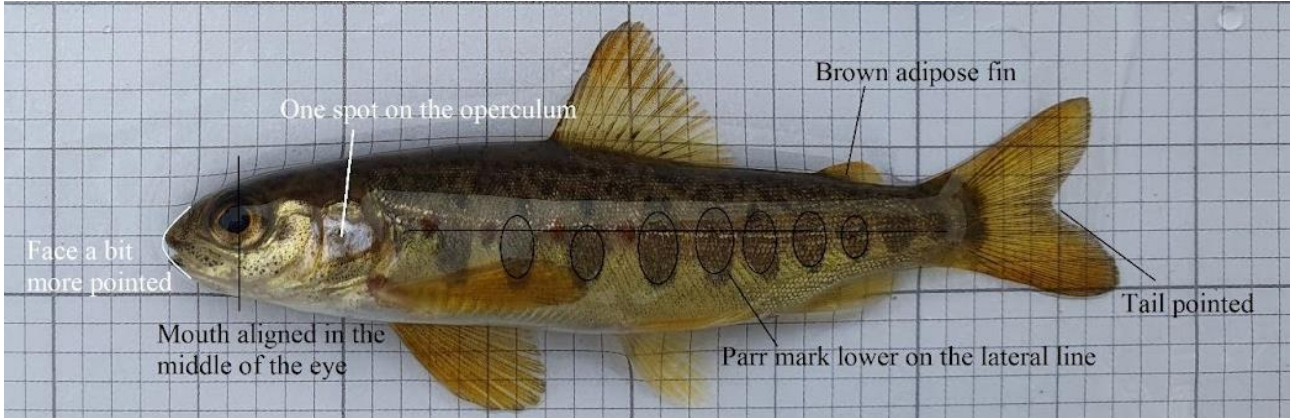
Distribution of SAC with interest feature

- Grade A/B
- Grade C
- ▲ Grade D



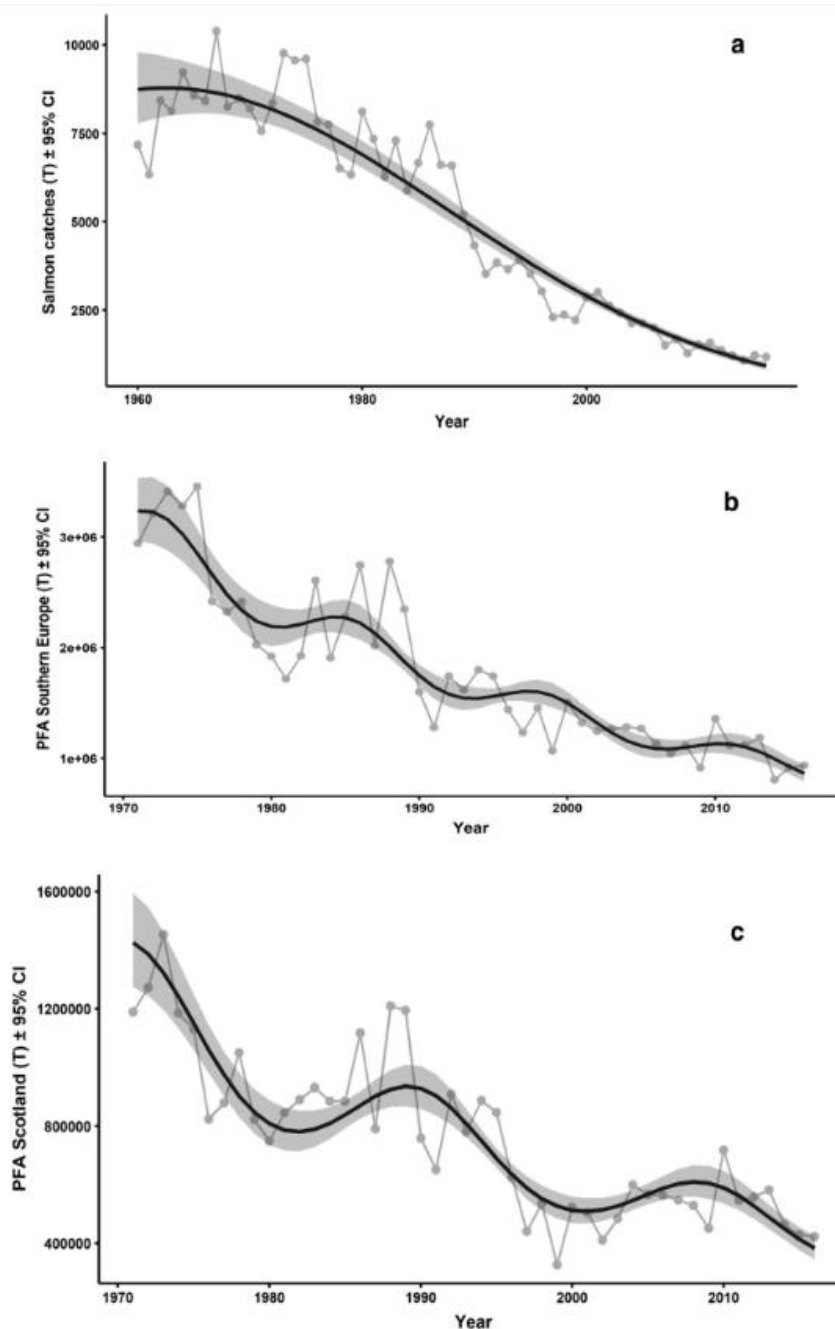
Appendix 7: Captioned photo to differentiate between juvenile salmon and trout. Top: juvenile Atlantic salmon. Bottom: juvenile Brown trout (Personal source)

Annexe 7 : Photo légendée pour différencier les juvéniles de saumon et de truite. En haut : saumon atlantique juvénile. En bas : truite brune juvénile



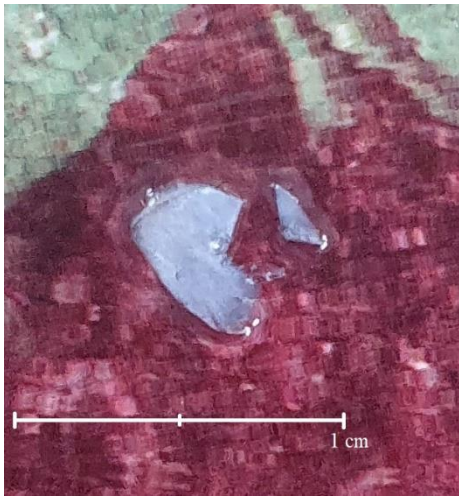
Appendix 8: Changes in the Atlantic salmon population metrics over different geographical areas. Grey points and the solid line are the uncorrected annual catch data on year; solid dark line is the fitted polynomial model with grey shading the 95% CI, (data source ICES WGNAS 2017). (a) The change in national reported catches (in tonnes) across all ICES WGNAS reporting countries (Canada, USA, Norway, Russia, Iceland, Sweden, Denmark, Finland, Ireland, England, Wales, Scotland, Northern Ireland, France and Spain) between 1960 and 2016; (b) Pre-fishery abundance (PFA) estimates (fish number) across ICES WGNAS reporting countries for southern Europe (Scotland, Northern Ireland, England & Wales (combined), Ireland, France and south & west Iceland) between 1972 and 2016; (c) PFA estimates for Atlantic salmon for Scotland between 1960 and 2016 (Source: Adams et al. 2022)

Annexe 8 : Changements dans les métriques de la population de saumon atlantique dans différentes zones géographiques. Les points gris et la ligne continue sont les données de captures annuelles non corrigées par année ; la ligne continue foncée est le modèle polynomial ajusté, l'IC à 95 % étant ombré en gris (source de données CIEM WGNAS 2017). (a) L'évolution des captures déclarées au niveau national (en tonnes) dans tous les pays déclarants du CIEM WGNAS (Canada, États-Unis, Norvège, Russie, Islande, Suède, Danemark, Finlande, Irlande, Angleterre, Pays de Galles, Écosse, Irlande du Nord, France et Espagne) entre 1960 et 2016 ; (b) Estimations de l'abondance avant la pêche (PFA) (nombre de poissons) dans les pays membres du CIEM WGNAS pour l'Europe du Sud (Écosse, Irlande du Nord, Angleterre et Pays de Galles (combinés), Irlande, France et sud et ouest de l'Islande) entre 1972 et 2016 ; (c) Estimations de la PFA pour le saumon de l'Atlantique en Écosse entre 1960 et 2016 (Source : Adams et al. 2022)



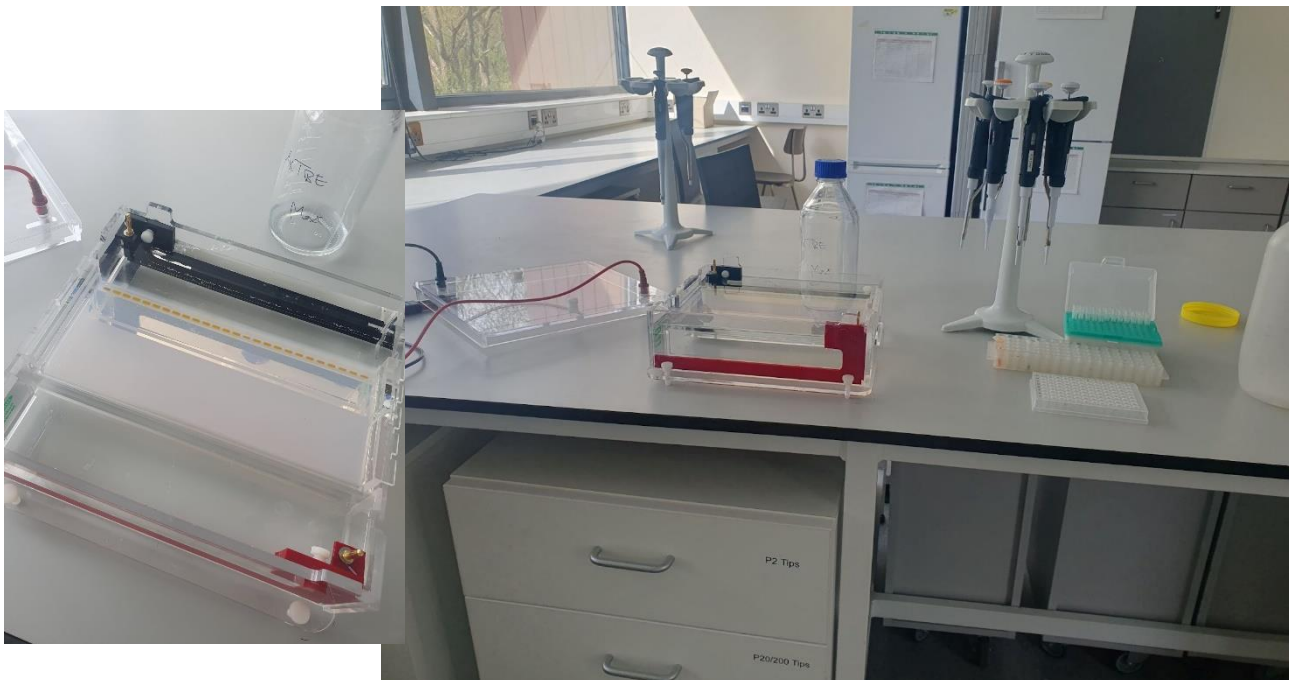
Appendix 10: (left) picture of a fin clip with the small piece of 1-2 mm used for the analysis. (right) picture of the material and worktable for step 1 (Personal source)

Annexe 10 : (à gauche) image d'un clip de nageoire avec le petit morceau de 1-2 mm utilisé pour l'analyse. (à droite) photo du matériel et de la table de travail pour l'étape 1



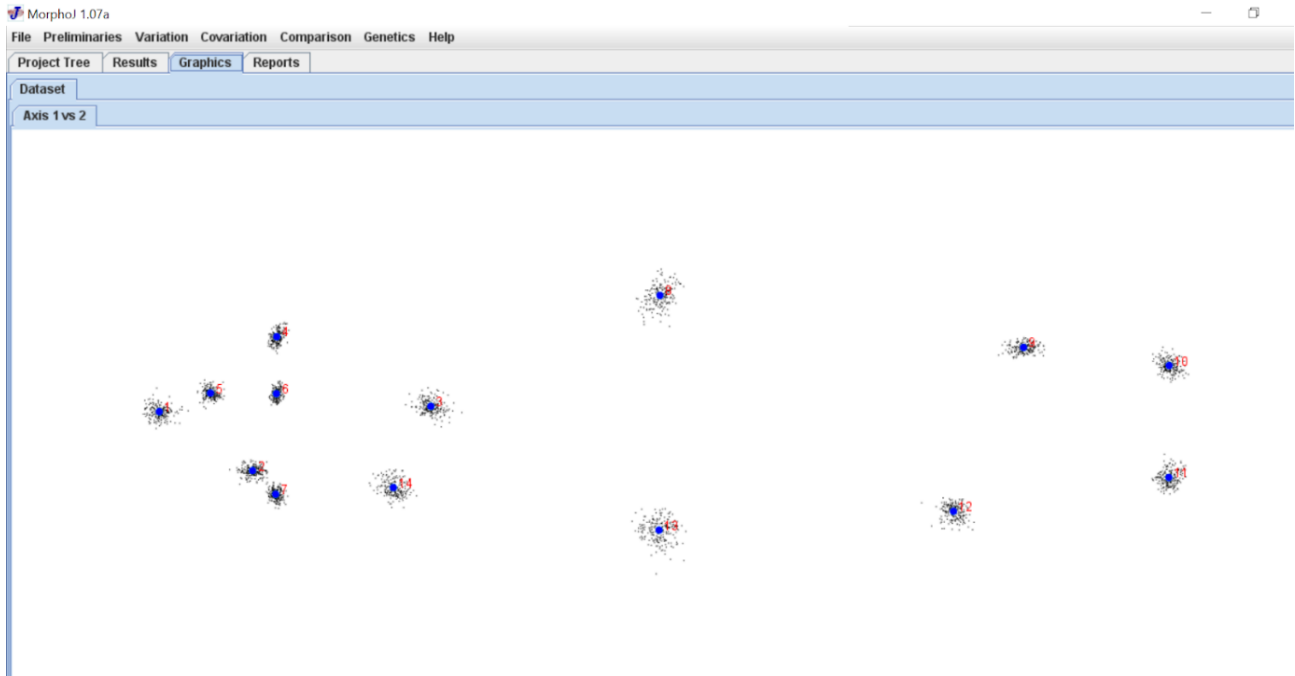
Appendix 11: (left) picture of the electrophoresis machine with filled wells. (right) picture of the material (machine and reusable 96-well plate, and pipettes) and work table for step 3 (Personal source)

Annexe 11 : (gauche) photo de la machine d'électrophorèse avec des puits remplis. (à droite) photo du matériel (machine et plaque réutilisable à 96 puits, et pipettes) et de la table de travail pour l'étape 3



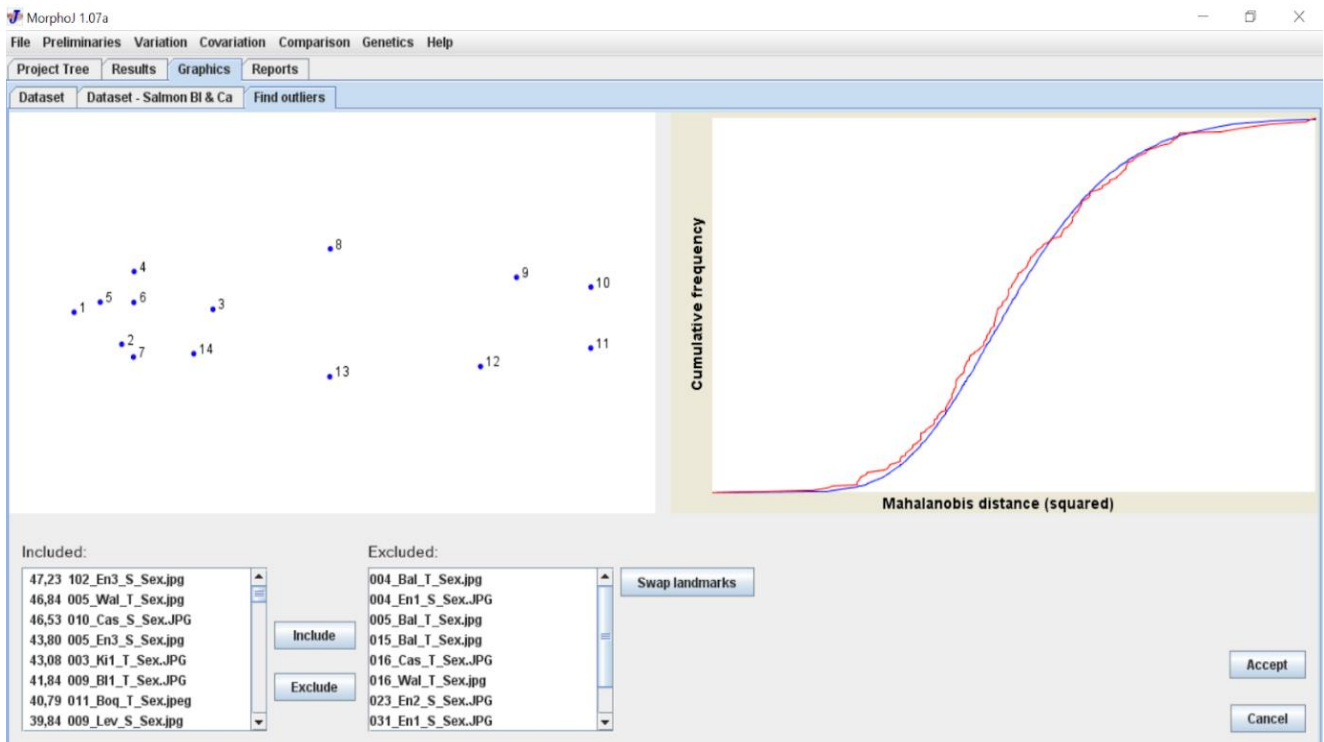
Appendix 12: Graphic with the 14 landmarks mean (blue dots) and the individuals variation around (Source: MorphoJ software)

Annexe 12 : Graphique avec la moyenne des 14 points de repère (points bleus) et la variation des individus autour



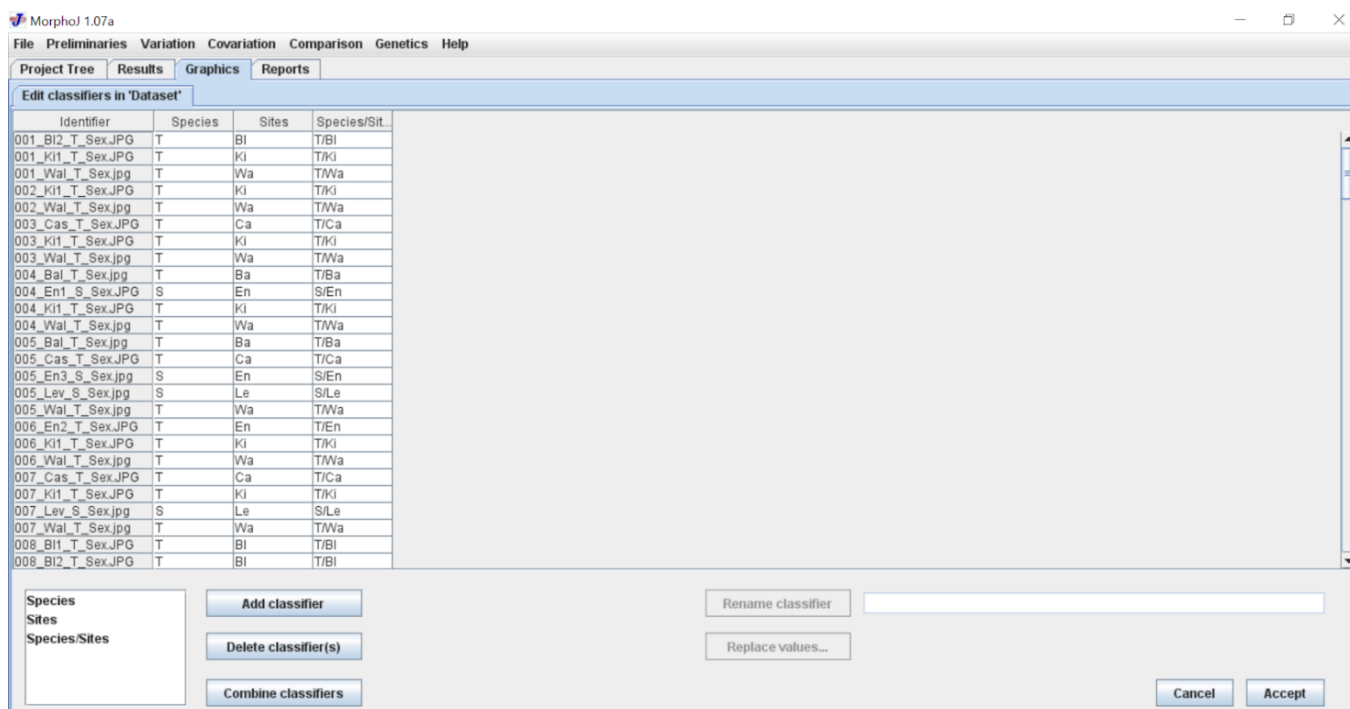
Appendix 13: Find outliers within the dataset (Source: MorphoJ software)

Annexe 13 : Recherche de valeurs aberrantes dans l'ensemble de données



Appendix 14: Three of the class created for the analysis on MorphoJ (Source: MorphoJ software)

Annexe 14 : Trois des classes créées pour l'analyse sur MorphoJ



Appendix 15: Reference value for elements analysed with the water chemistry kit (Source: JBL GmbH & Co. KG, n.d.)

Annexe 15 : Valeur de référence pour les éléments analysés avec le kit de chimie de l'eau

| Element | Correct value | Limit value | Bad value |
|-------------|---------------|--------------------------|-----------|
| NH4 | <0.05 | 0.1 - 0.2 | 0.4 - 5 |
| NO2 | <0.01 - 0.1 | 0.1 - 0.4 | 0.4 - 1.0 |
| NO3 | 1 - 15 | < 0.5 - 1 / 15 - 30 | 60 - 200 |
| PO4 | < 0.02 - 0.2 | 0.4 - 0.6 | 0.6 - 1.8 |
| SiO2 | < 0.1 - 0.8 | 0.8 - 2.0 | 2.0 - > 6 |
| Fe | 0.1 - 0.4 | < 0.02 - 0.1 / 0.4 - 0.6 | 0.8 - 1.5 |
| Cu | < 0.05 | x | 0.1 - 1.6 |
| O2 | 6 - 10 | 2 - 6 | < 0.2 - 2 |

| Id | Sp | Sites | Sp/Sites | CSS | CV1 | CV2 | Mean depth | Mean width | Temp | Cond | pH | DO | NH4 | NO2 | Cu | O2 | NO3 | PO4 | SiO2 | Fe |
|--------------------|----|-------|----------|--------|--------|--------|------------|------------|--------|---------|-------|--------|-------|-------|-------|--------|-------|-------|-------|-------|
| 028_Bl1_S_Sex.JPG | S | Bl | S/Bl | 0,015 | -2,766 | -0,563 | 23,440 | 4,990 | 18,250 | 133,500 | 8,000 | 9,735 | 0,049 | 0,009 | 0,049 | 10,000 | 0,745 | 0,110 | 6,100 | 0,028 |
| 028_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,009 | -2,069 | -1,320 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 052_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,006 | -2,824 | -0,028 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 074_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,000 | -3,488 | -2,025 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 092_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,011 | -4,611 | -1,904 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 160_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,003 | -3,558 | -0,976 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 174_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,013 | -2,513 | -0,510 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 200_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,010 | -2,889 | -1,609 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 215_Boq_S_Sex.jpeg | S | Bo | S/Bo | 0,003 | -3,100 | -0,365 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 010_Cas_S_Sex.JPG | S | Ca | S/Ca | 0,013 | -5,849 | -1,713 | 33,780 | 4,620 | 15,160 | 15,780 | 6,910 | 10,020 | 0,049 | 0,009 | 0,049 | 10,000 | 0,490 | 0,019 | 2,000 | 0,150 |
| 005_En3_S_Sex.jpg | S | En | S/En | -0,011 | -2,001 | 1,051 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 008_En2_S_Sex.JPG | S | En | S/En | 0,005 | -3,053 | -1,878 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 012_En1_S_Sex.JPG | S | En | S/En | -0,007 | -1,976 | 0,646 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 012_En3_S_Sex.jpg | S | En | S/En | -0,001 | -4,414 | 2,365 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 015_En1_S_Sex.JPG | S | En | S/En | 0,007 | -4,415 | 0,528 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 016_En1_S_Sex.JPG | S | En | S/En | -0,003 | -4,703 | -0,131 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 022_En1_S_Sex.JPG | S | En | S/En | 0,004 | -4,104 | 0,066 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 024_En1_S_Sex.JPG | S | En | S/En | 0,010 | -3,829 | -1,590 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 029_En1_S_Sex.JPG | S | En | S/En | 0,005 | -2,629 | 1,226 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 030_En2_S_Sex.JPG | S | En | S/En | 0,001 | -3,152 | -0,306 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 034_En3_S_Sex.jpg | S | En | S/En | -0,005 | -1,493 | 2,034 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 036_En1_S_Sex.JPG | S | En | S/En | 0,006 | -3,216 | 0,066 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 037_En2_S_Sex.JPG | S | En | S/En | 0,002 | -3,139 | 0,002 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 039_En2_S_Sex.JPG | S | En | S/En | 0,002 | -4,044 | 1,179 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 042_En3_S_Sex.jpg | S | En | S/En | 0,006 | -3,449 | -1,501 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 048_En2_S_Sex.JPG | S | En | S/En | 0,002 | -4,147 | -1,121 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 053_En2_S_Sex.JPG | S | En | S/En | 0,006 | -2,694 | -0,763 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 059_En3_S_Sex.jpg | S | En | S/En | 0,000 | -2,431 | 1,409 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 068_En3_S_Sex.jpg | S | En | S/En | 0,007 | -3,292 | 0,135 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 082_En3_S_Sex.jpg | S | En | S/En | -0,002 | -2,797 | 0,062 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 101_En3_S_Sex.jpg | S | En | S/En | -0,002 | -4,100 | 0,278 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |
| 102_En3_S_Sex.jpg | S | En | S/En | -0,004 | -3,562 | 1,714 | 31,250 | 10,680 | 14,850 | 126,833 | 7,883 | 10,067 | 0,049 | 0,039 | 0,049 | 10,000 | 1,000 | 0,019 | 5,567 | 0,025 |

Appendix 17: Script R studio

```
Data <- na.exclude(Donnees)
#Depth#####
Depth <- lm(Data$CSS ~ Data$`Mean depth`,
data= Data)
summary(Depth)
anova(Depth)

Depth2 <- lm(Data$CV1 ~ Data$`Mean depth`,
data= Data)
summary(Depth2)
anova(Depth2)

Depth3 <- lm(Data$CV2 ~ Data$`Mean depth`,
data= Data)
summary(Depth3)
anova(Depth3)

#Widht
Widht <- lm(Data$CSS ~ Data$`Mean widht`,
data= Data)
summary(Widht)
anova(Widht)

Widht2 <- lm(Data$CV1 ~ Data$`Mean widht`,
data= Data)
summary(Widht2)
anova(Widht2)

Widht3 <- lm(Data$CV2 ~ Data$`Mean widht`,
data= Data)
summary(Widht3)
anova(Widht3)

#Temperature
Temp <- lm(Data$CSS ~ Data$Temp, data=
Data)
summary(Temp)
anova(Temp)

Temp2 <- lm(Data$CV1 ~ Data$Temp, data=
Data)
summary(Temp2)
anova(Temp2)

Temp3 <- lm(Data$CV2 ~ Data$Temp , data=
Data)
summary(Temp3)
anova(Temp3)

#Conductivity
Cond <- lm(Data$CSS ~ Data$Cond, data=
Data)
summary(Cond)
anova(Cond)

Cond2 <- lm(Data$CV1 ~ Data$Cond , data=
Data)
summary(Cond2)
anova(Cond2)

Cond3 <- lm(Data$CV2 ~ Data$Cond, data=
Data)
summary(Cond3)
anova(Cond3)

#pH
pH <- lm(Data$CSS ~ Data$pH , data= Data)
summary(pH)
anova(pH)

pH2 <- lm(Data$CV1 ~ Data$pH, data= Data)
summary(pH2)
anova(pH2)

pH3 <- lm(Data$CV2 ~ Data$pH , data= Data)
summary(pH3)
anova(pH3)

#DO
DO <- lm(Data$CSS ~ Data$DO, data= Data)
summary(DO)
anova(DO)

DO2 <- lm(Data$CV1 ~ Data$DO, data= Data)
summary(DO2)
anova(DO2)

DO3 <- lm(Data$CV2 ~ Data$DO, data= Data)
summary(DO3)
anova(DO3)

#NH4
NH4 <- lm(Data$CSS ~ Data$NH4, data= Data)
summary(NH4)
anova(NH4)

NH4b <- lm(Data$CV1 ~ Data$NH4 , data=
Data)
summary(NH4b)
anova(NH4b)

NH4c <- lm(Data$CV2 ~ Data$NH4 , data=
Data)
summary(NH4c)
anova(NH4c)

#NO2
NO2 <- lm(Data$CSS~ Data$NO2 , data= Data)
summary(NO2)
anova(NO2)

NO2b <- lm(Data$CV1 ~ Data$NO2 , data=
Data)
```

```

summary(NO2b)
anova(NO2b)

NO2c <- lm(Data$CV2 ~ Data$NO2, data=
Data)
summary(NO2c)
anova(NO2c)

#Cu
Cu <- lm(Data$CSS ~ Data$Cu, data= Data)
summary(Cu)
anova(Cu)

Cu2 <- lm(Data$CV1 ~ Data$Cu, data= Data)
summary(Cu2)
anova(Cu2)

Cu3 <- lm(Data$CV2 ~ Data$Cu, data= Data)
summary(Cu3)
anova(Cu3)

#O2
O2 <- lm(Data$CSS ~ Data$O2, data= Data)
summary(O2)
anova(O2)

O2b <- lm(Data$CV1 ~ Data$O2, data= Data)
summary(O2b)
anova(O2b)

O2c <- lm(Data$CV2 ~ Data$O2, data= Data)
summary(O2c)
anova(O2c)

#NO3
NO3 <- lm(Data$CSS ~ Data$NO3, data= Data)

```

```

summary(NO3)
anova(NO3)

NO3b <- lm(Data$CV1 ~ Data$NO3, data=Data)
summary(NO3b)
anova(NO3b)

NO3c <- lm(Data$CV2 ~ Data$NO3,
data=Data)
summary(NO3c)
anova(NO3c)

#PO4
PO4 <- lm(Data$CSS ~ Data$PO4, data= Data)
summary(PO4)
anova(PO4)

PO4b <- lm(Data$CV1 ~ Data$PO4 , data=
Data)
summary(PO4b)
anova(PO4b)

PO4c <- lm(Data$CV2 ~ Data$PO4 , data=Data)
summary(PO4c)
anova(PO4c)

#SiO2
SiO2 <- lm(Data$CSS ~ Data$SiO2, data= Data)
summary(SiO2)
anova(SiO2)

SiO2b <- lm(Data$CV1 ~ Data$SiO2 , data=
Data)
summary(SiO2b)
anova(SiO2b)

```

```

SiO2c <- lm(Data$CV2 ~ Data$SiO2 , data=
Data)
summary(SiO2c)
anova(SiO2c)

#Fe
Fe <- lm(Data$CSS ~ Data$Fe, data= Data)
summary(Fe)
anova(Fe)

Fe2 <- lm(Data$CV1 ~ Data$Fe, data= Data)
summary(Fe2)
anova(Fe2)

Fe3 <- lm(Data$CV2 ~ Data$Fe, data= Data)
summary(Fe3)
anova(Fe3)

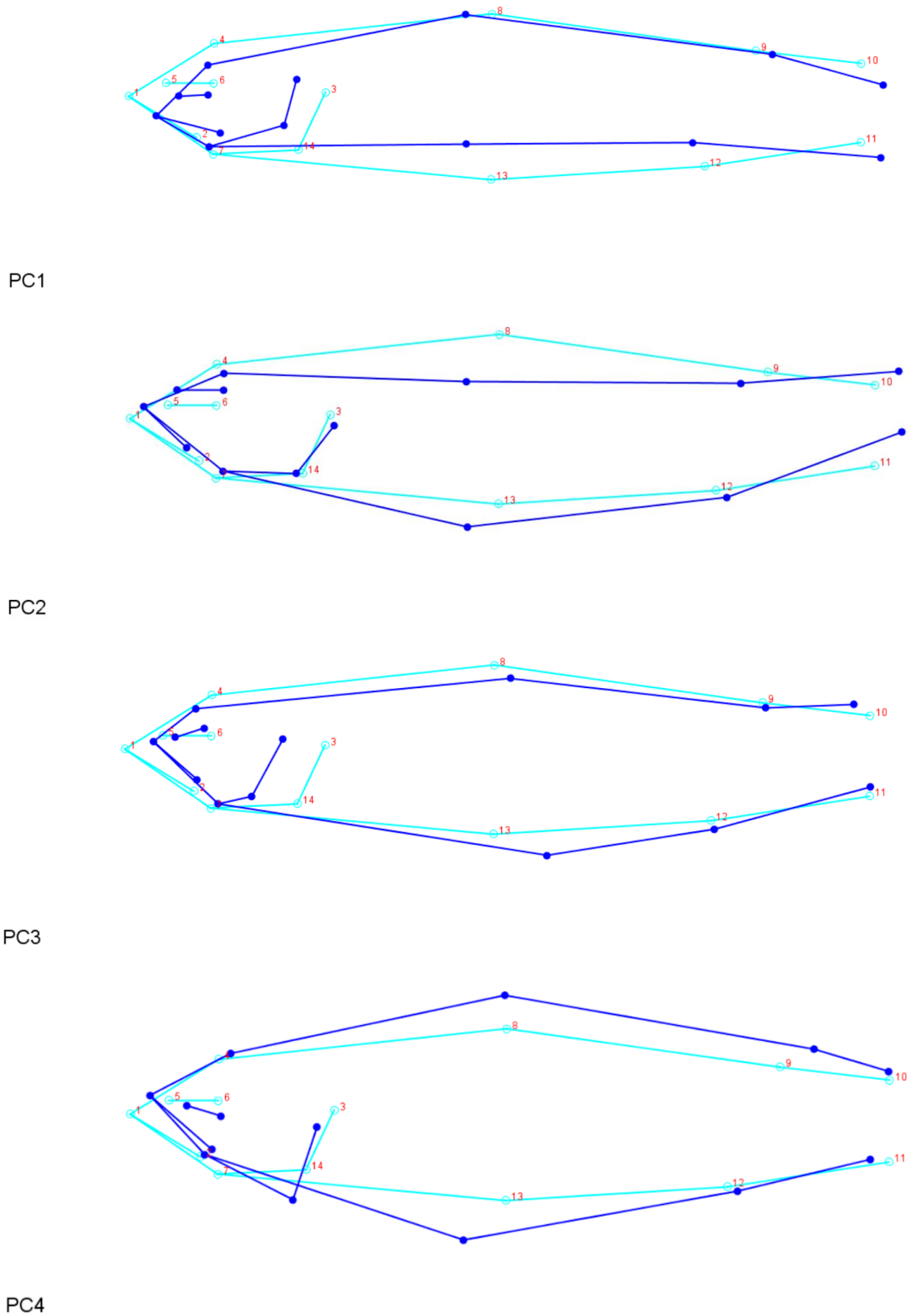
#Chemistry#
Chemistry <- lm(Data$CSS ~ Data$NH4 +
Data$NO2 + Data$Cu + Data$O2 + Data$NO3 +
Data$PO4 + Data$SiO2 + Data$Fe, data= Data)
anova(Chemistry)

#Physical data#
Physic <- lm(Data$CSS ~ Data`Mean depth` +
Data`Mean widht` + Data$Temp + Data$Cond
+ Data$pH + Data$DO)
anova(Physic)

```

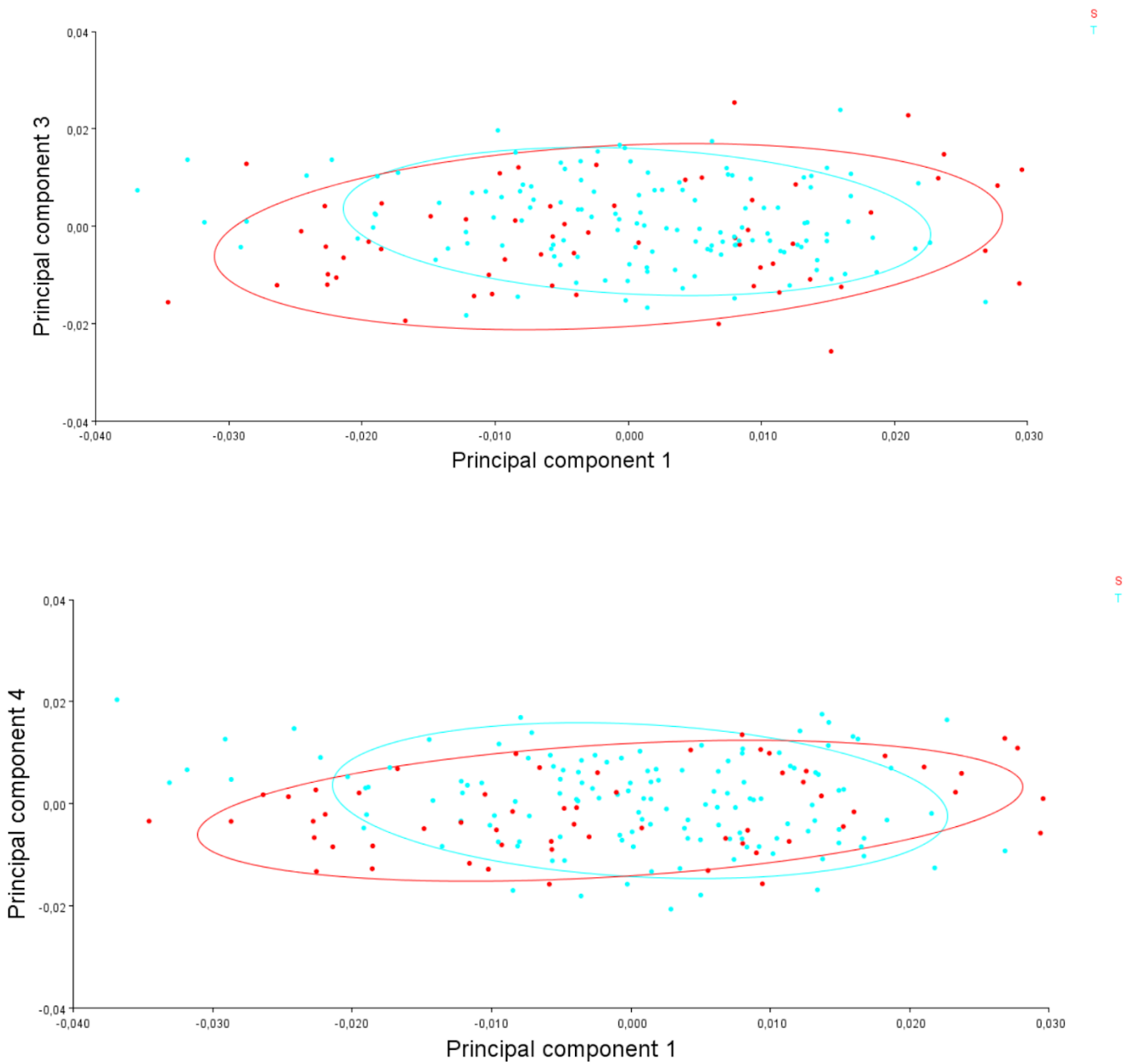
Appendix 18: PC shape change for PC1, PC2, PC3 and PC4. Light blue: mean shape. Dark blue: variations for the PCx. PCA from all individuals. Scale factor: 0.1 (Source: MorphoJ software)

Annexe 18 : Changement de forme des PC pour PC1, PC2, PC3 et PC4. Bleu clair : forme moyenne. Bleu foncé : variations pour le PCx. ACP de tous les individus. Facteur d'échelle : 0,1



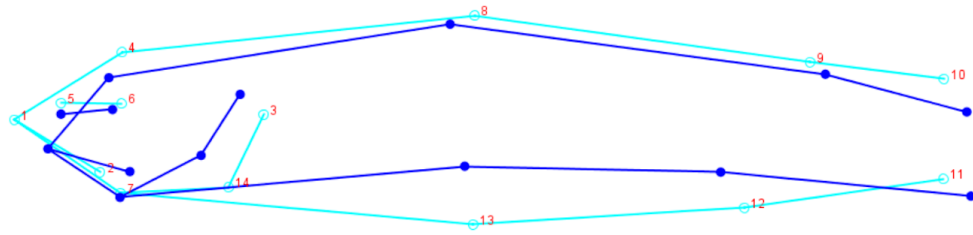
Appendix 19: Scatter plot for others principal components. PCA from all individuals. Confidence ellipse 0.8 (Source: MorphoJ software)

Annexe 19 : Diagramme de dispersion pour les autres composantes principales. ACP de tous les individus. Ellipse de confiance 0,8

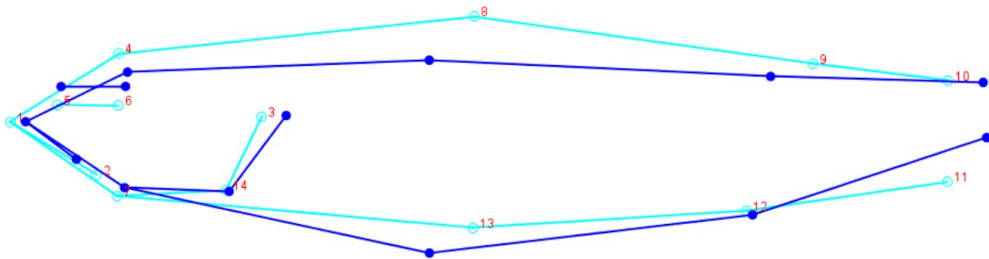


Appendix 20: PC shape change for PC1, PC2, PC3 and PC4. Light blue: mean shape. Dark blue: variations for the PCx. PCA from class Species/Sites without S/Ca and S/Bl. Scale factor: 0.1 (Source: MorphoJ software)

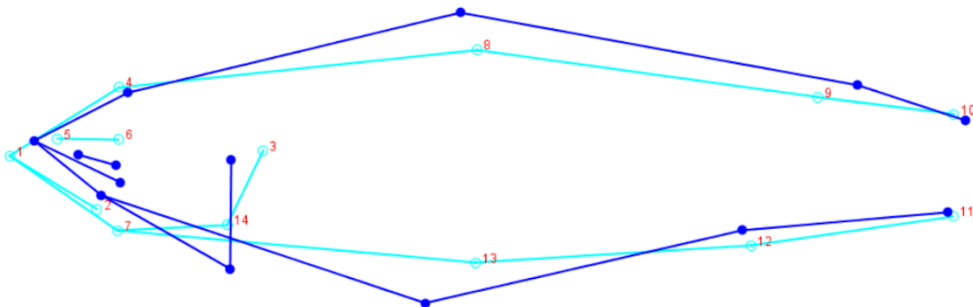
Annexe 20 : Changement de forme des PC pour PC1, PC2, PC3 et PC4. Bleu clair : forme moyenne. Bleu foncé : variations pour le PCx. ACP à partir de la classe Espèces/Sites sans S/Ca et S/Bl. Facteur d'échelle : 0,1



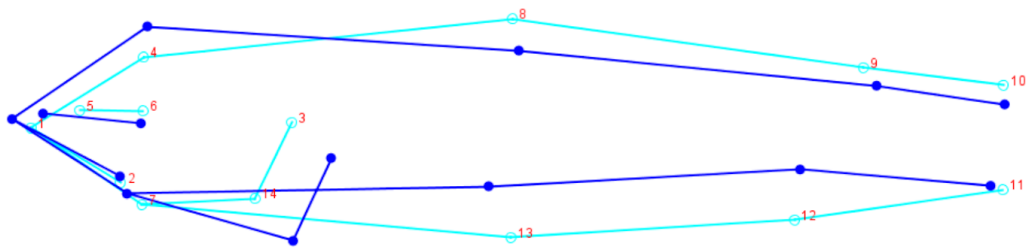
PC1



PC2



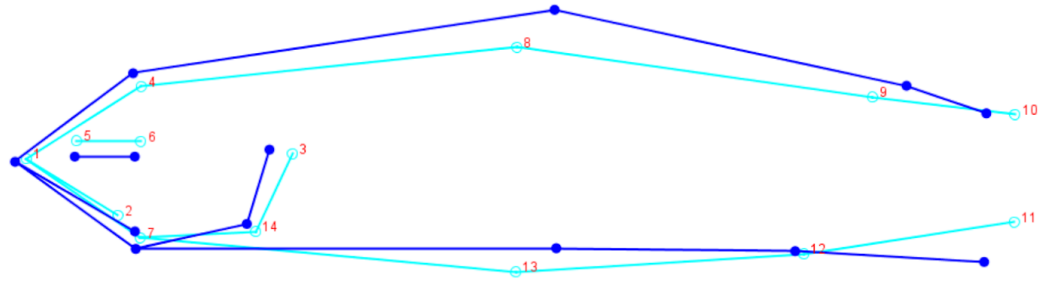
PC3



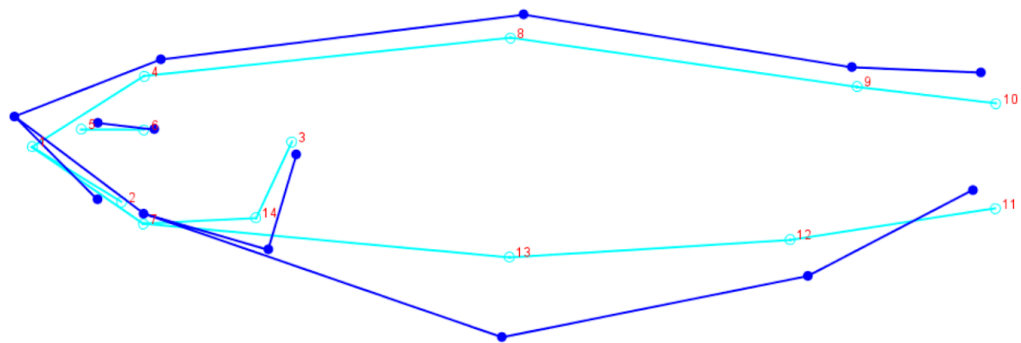
PC4

Appendix 22: CV shape change for CV1, CV2 and CV3.. Light blue: mean shape. Dark blue: variation for the CVx corresponding. CVA from class Species/Sites without S/Ca and S/Bl. Scale factor: 20 (Source: MorphoJ software)

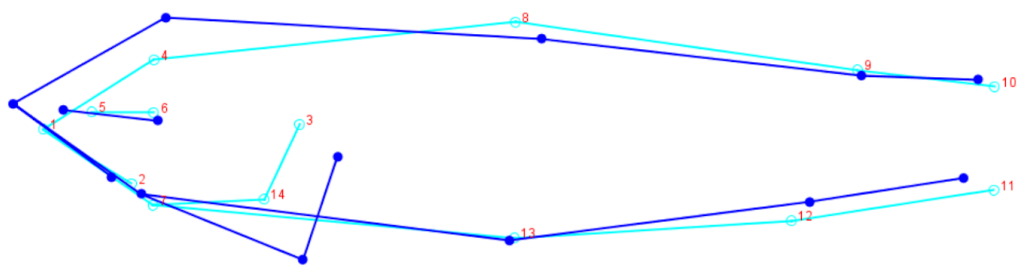
Annexe 22 : Changement de forme du CV pour CV1, CV2 et CV3. Bleu clair : forme moyenne. Bleu foncé : variation pour le CVx correspondant. CVA de la classe Espèces/Sites sans S/Ca et S/Bl. Facteur d'échelle : 20



CV1



CV2



CV3

Appendix 23: Summary of the description of every group Species/Sites for every CV score, based on the CVA. 'neutral score' means the CV score is very close to 0, so, it cannot be concluded.

Annexe 23 : Résumé de la description de chaque groupe Espèces/Sites pour chaque score de CV, sur la base de la CVA. score neutre" signifie que le score de CV est très proche de 0, et qu'il n'est donc pas possible de conclure.

| | |
|-------------|-------------------------------|
| S/Bo | CV1: Long caudal peduncle |
| | CV2: Small head - Thin body |
| | CV3: neutral score |
| S/En | CV1: Longer caudal peduncle |
| | CV2: neutral score |
| | CV3: Small eye and head |
| S/Le | CV1: Long caudal peduncle |
| | CV2: neutral score |
| | CV3: A bit small eye and head |
| T/Ba | CV1: Short caudal peduncle |
| | CV2: Deep body - Deep head |
| | CV3: neutral score |
| T/Bl | CV1: Short caudal peduncle |
| | CV2: neutral score |
| | CV3: small eye and head |
| T/Bo | CV1: Short caudal peduncle |
| | CV2: Small body - Small head |
| | CV3: Deep eye and head |
| T/Ca | CV1: Short caudal peduncle |
| | CV2: Small head - Thin body |
| | CV3: Small eye and head |
| T/En | CV1: Short caudal peduncle |
| | CV2: neutral score |
| | CV3: neutral score |
| T/Ki | CV1: Short caudal peduncle |
| | CV2: neutral score |
| | CV3: Small eye and head |
| T/Wa | CV1: Short caudal peduncle |
| | CV2: Deep body - Deep head |
| | CV3: neutral score |

Appendix 24: ANOVA result for the linear model of the multiple variable on the Composite Shape Score. Chemistry for NO₂, NO₃, O₂, PO₄, SiO₂, Fe, Cu and NH₄; Physic for Mean depth, Mean width, Temperature, Conductivity, pH and Dissolved Oxygen (Source: R Studio)

Annexe 24 : Résultat de l'ANOVA pour le modèle linéaire de la variable multiple sur le score de composition de forme. Chimie pour NO₂, NO₃, O₂, PO₄, SiO₂, Fe, Cu et NH₄ ; Physique pour Profondeur moyenne, Largeur moyenne, Température, Conductivité, pH et Oxygène dissous

```
> anova(Chemistry)
Analysis of Variance Table

Response: Data$`Composite shape score`
      Df    Sum Sq   Mean Sq F value    Pr(>F)
Data$NO2    1 0.00000005 4.6000e-08  0.0025 0.96020
Data$NO3    1 0.00016372 1.6372e-04  8.9484 0.00322 **
Data$PO4    1 0.00000001 1.2000e-08  0.0006 0.97982
Data$SiO2   1 0.00004299 4.2995e-05  2.3500 0.12727
Data$Fe     1 0.00005523 5.5228e-05  3.0186 0.08425 .
Residuals 159 0.00290905 1.8296e-05
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> anova(Physic)
Analysis of Variance Table

Response: Data$`Composite shape score`
      Df    Sum Sq   Mean Sq F value    Pr(>F)
Data$`Mean depth`    1 0.00002858 2.8578e-05  1.5567 0.213996
Data$`Mean width`    1 0.00003521 3.5214e-05  1.9182 0.168012
Data$Temperature     1 0.00017514 1.7514e-04  9.5401 0.002376 **
Data$Conductivity    1 0.00000891 8.9140e-06  0.4855 0.486948
Data$pH              1 0.00002048 2.0480e-05  1.1156 0.292483
Data$DO              1 0.00000211 2.1130e-06  0.1151 0.734863
Residuals           158 0.00290061 1.8358e-05
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix 25: ANOVA result for the linear model of the individual variable on the CVI (Source: R Studio)

Annexe 25 : Résultat de l'ANOVA pour le modèle linéaire de la variable individuelle sur le CVI

```

> anova(Cond2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Cond  1  2.24  2.2413  0.4815  0.4887
Residuals 163 758.75  4.6549
> anova(pH2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$pH  1 18.19 18.192  3.9921 0.04738 *
Residuals 163 742.80  4.557
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(DO2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$DO  1 280.06 280.06  94.919 < 2.2e-16 ***
Residuals 163 480.93  2.95
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Depth2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$`Mean depth`  1 130.55 130.553  33.755 3.195e-08 ***
Residuals 163 630.43  3.868
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(widht2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$`Mean widht`  1 187.51 187.507  53.295 1.199e-11 ***
Residuals 163 573.48  3.518
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Temp2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Temp  1  0.20  0.2004  0.0429  0.8361
Residuals 163 760.79  4.6674
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(NO2b)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$NO2  1  54.18  54.183 12.495 0.0005311 ***
Residuals 163 706.80  4.336
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(NO3b)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$NO3  1  30.40  30.4021  6.783 0.01005 *
Residuals 163 730.59  4.4821
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(P04b)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$P04  1  34.55  34.551  7.7526 0.005998 **
Residuals 163 726.44  4.457
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(SiO2b)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$SiO2  1 138.92 138.921  36.401 1.044e-08 ***
Residuals 163 622.07  3.816
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Fe2)
Analysis of Variance Table

Response: Data$CV1
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Fe  1  50.08  50.079 11.482 0.0008811 ***
Residuals 163 710.91  4.361
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Appendix 26: ANOVA result for the linear model of the individual variable on the CV2 (Source: R Studio)
 Annexe 26 : Résultat de l'ANOVA pour le modèle linéaire de la variable individuelle sur le CV2

```

> anova(Depth3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$`Mean depth` 1 152.20 152.205 75.468 3.735e-15 ***
Residuals      163 328.74  2.017
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(widht3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$`Mean widht` 1  2.57  2.5726  0.8766  0.3505
Residuals      163 478.37  2.9348
> anova(Temp3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Temp 1 34.75 34.754 12.696 0.0004808 ***
Residuals 163 446.19  2.737
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Cond3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Cond 1  1.93  1.9257  0.6553  0.4194
Residuals 163 479.02  2.9388
> anova(pH3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$pH 1  3.34  3.3416  1.1404  0.2871
Residuals 163 477.60  2.9301
> anova(DO3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$DO 1 20.86 20.8625  7.3913 0.007264 **
Residuals 163 460.08  2.8226
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(NO2c)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$NO2 1 13.61 13.6068  4.7458 0.03081 *
Residuals 163 467.34  2.8671
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(NO3c)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$NO3 1 42.18 42.181 15.67 0.0001124 ***
Residuals 163 438.76  2.692
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(PO4c)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$PO4 1 23.11 23.1089  8.2273 0.004673 **
Residuals 163 457.84  2.8088
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(SiO2c)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$SiO2 1 24.76 24.7597  8.8469 0.003382 **
Residuals 163 456.19  2.7987
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(Fe3)
Analysis of Variance Table

Response: Data$CV2
      Df Sum Sq Mean Sq F value Pr(>F)
Data$Fe 1 50.37 50.374 19.07 2.233e-05 ***
Residuals 163 430.57  2.642
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Appendix 27: Procrustes distances among groups of the class Species/Sites without Balmenoch and Walton, and p-values corresponding. Need to be compared to the figure 31 Red; no significant value. Orange highlight; salmons, Green highlight; trouts. No comparisons are done between salmon groups and trout groups (white boxes) (Source: MorphoJ software)

Annexe 27 : Distances Procrustes entre les groupes de la classe Espèces/Sites sans Balmenoch et Walton, et valeurs p correspondantes. A comparer avec la figure 31 Rouge ; pas de valeur significative. Surbrillance orange : saumons, surbrillance verte : truites. Aucune comparaison n'est faite entre les groupes de saumons et les groupes de truites (cases blanches) (Source : logiciel MorphoJ).

| Procrustes distances among groups: | | | | Without Balmenoch & Walton | | | |
|------------------------------------|--------|--------|--------|----------------------------|--------|--------|-------|
| | S/Bo | S/En | S/Le | T/Bl | T/Bo | T/Ca | T/En |
| S/En | 0,0093 | | | | | | |
| S/Le | 0,0152 | 0,0122 | | | | | |
| T/Bl | 0,0222 | 0,0233 | 0,0236 | | | | |
| T/Bo | 0,0202 | 0,0251 | 0,0226 | 0,0207 | | | |
| T/Ca | 0,0206 | 0,0242 | 0,0269 | 0,0151 | 0,0213 | | |
| T/En | 0,0196 | 0,0223 | 0,0189 | 0,0125 | 0,0119 | 0,0163 | |
| T/Ki | 0,0168 | 0,0192 | 0,0209 | 0,0144 | 0,0166 | 0,02 | 0,015 |

| P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups: | | | | | | | |
|---|--------|--------|--------|--------|--------|--------|--------|
| Without Balmenoch & Walton | | | | | | | |
| | S/Bo | S/En | S/Le | T/Bl | T/Bo | T/Ca | T/En |
| S/En | 0,5387 | | | | | | |
| S/Le | 0,0047 | 0,0048 | | | | | |
| T/Bl | <.0001 | <.0001 | <.0001 | | | | |
| T/Bo | 0,0001 | <.0001 | <.0001 | <.0001 | | | |
| T/Ca | 0,0006 | <.0001 | <.0001 | 0,001 | <.0001 | | |
| T/En | <.0001 | <.0001 | <.0001 | 0,0005 | 0,0011 | 0,0009 | |
| T/Ki | 0,0024 | <.0001 | <.0001 | 0,0001 | <.0001 | <.0001 | <.0001 |



VetAgro Sup

THOMMEREL Blandine, 2023, “Morphological variation within juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, around the Loch Lomond, Scotland”, 40 pages, mémoire de fin d’étude, soutenu à VetAgro Sup le 28 septembre 2023.

STRUCTURE D’ACCUEIL ET INSTITUTIONS ASSOCIEES :

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ENCADRANTS :

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- ◆ Tuteur pédagogique : Gilles BRUNSCHWIG

OPTION : Agriculture, Environnement, Santé et Territoire (AEST)

Résumé

Les saumons Atlantiques et les truites brunes sont des espèces de salmonidés qui partagent de nombreux points communs, dont la possibilité de se reproduire ensemble et créer des hybrides. Dans le but d'évaluer plus précisément le taux d'hybridation qui augmente en Ecosse et de comprendre comment ces individus se caractérisent, une étape de cela est d'étudier la variation morphologique au sein des populations de saumons et truites. Ces deux espèces sont connues pour posséder des différences génétiques et phénotypiques intraspécifiques. Dans ce contexte, une quantification de ces différences permettrait par la suite une reconnaissance plus facile des hybrides. C'est ce que l'étude, ici présentée, a permis de réaliser. Au sein du bassin versant du Loch Lomond, les populations de truites et de saumons sont variées. Dans cette étude, les objectifs étaient de quantifier les variations morphologiques au sein des juvéniles saumons et truites, entre différentes populations, entre les sexes et également voir l'effet des paramètres environnementaux sur les morphologies. Pour cela, huit sites différents ont été étudiés. Sur ces sites, sept populations de truites brunes (133 individus) et trois de saumons Atlantiques (59 individus) ont été collectés par pêche électrique. À l'aide de photos de chaque individu, une analyse géométrique morphométrique puis statistique ont été réalisées, avec un prélèvement de nageoire adipeuse une détermination du sexe a pu être faite, et un relevé de paramètres environnementaux basiques a permis de voir l'effet de l'habitat sur les morphologies. Les résultats de cette étude conduisent à une variation morphologique des populations à petite échelle ; sept des dix groupes de populations de saumons et truites sont significativement différents. Ces groupes possèdent des morphologies variées qui sont traduites par les scores des variables canoniques de l'analyse géométrique morphométrique. Il semblerait que pour un site contenant saumons et truites, l'environnement agit sur les deux espèces de la même manière. Il n'y a pas d'effet différent de l'habitat sur les espèces. Les variations entre les femelles et les mâles ne montrent pas de significativité. Enfin, l'analyse statistique des paramètres environnementaux montre que seulement la température a un effet significatif sur les morphologies des individus.

Mots clés : *Saumon Atlantique – Truite brune – Analyse géométrique morphométrique – Hybridation – Loch Lomond*

Abstract

Atlantic salmon and brown trout are salmonid species that share many common features, including the ability to reproduce together and create hybrids. In order to assess more precisely the rate of hybridisation that is increasing in Scotland and to understand how these individuals are characterised, one step is to study the morphological variation within salmon and trout populations. These two species are known to possess intraspecific genetic and phenotypic differences. In this context, quantifying these differences would make it easier to recognise hybrids. This is what the present study has achieved. Within the Loch Lomond catchment, trout and salmon populations are varied. In this study, the objectives were to quantify the morphological variations within juvenile salmon and trout, between different populations, between the sexes and also to see the effect of environmental parameters on morphologies. Eight different sites were studied. At these sites, seven populations of brown trout (133 individuals) and three of Atlantic salmon (59 individuals) were collected by electrofishing. Using photos of each individual, a geometric, morphometric and then statistical analysis was carried out, with adipose fin sampling to determine sex, and a survey of basic environmental parameters to see the effect of habitat on morphology. The results of this study conclude that there is morphological variation in populations on a small scale; seven of the ten groups of salmon and trout populations are significantly different. These groups have varied morphologies that are reflected in the scores of the canonical variables of the geometric morphometric analysis. It would appear that for a site containing salmon and trout, the environment affects both species in the same way. There was no different effect of habitat on the species. Variations between females and males were not significant. Finally, statistical analysis of the environmental parameters showed that only temperature had a significant effect on the morphologies of the individuals.

Keywords: *Atlantic salmon - Brown trout - Geometric morphometric analysis - Hybridisation - Loch Lomond*