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Variation in space and time in body shape of Arctic charr *(Salvelinus alpinus)* found in lava caves near Lake Myvatn

Birault Marie

Mots clés :

Omble chevalier, *Salvelinus alpinus*, géométrie morphométrie, microévolution, adaptation, morphologie, points de repères, analyse discriminante, analyse procrustéenne généralisée

Hólar, Sauðárkrókur, Lac Mývatn, Islande

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ABSTRACT

ENGLISH VERSION

Since 2012, scientists at Hólar University College have studied microevolutionary processes in small Arctic charr (*Salvelinus alpinus*) populations that inhabit 20 lava caves near Lake Mývatn, in northeast Iceland. The main objective of the project is to increase the understanding of the importance of spatial and temporal variation in evolutionary parameters for diversity and increase the knowledge of the relationship between ecological and evolutionary factors, in maintaining and developing biological diversity. In this way, I studied the variation in space and time in body shape populations in relation to ecological factors. I worked on June 2012, 2013 and 2014 data-sets using landmarked based geometric morphometric approach and several statistical tests such as analysis of covariance (ANCOVA) and Discriminant Function Analysis (DFA). We found that there were average body shape variations in space and time and that the thickness of the fish is the most important variation. We found also that the interaction of cave and year was significant. This means that there are average fish body shape variations between years and between caves and that this variation goes in different directions. Then, we found that most of the caves that are physically and genetically connected showed differences in average body shape. It may indicate the importance of local ecological factors for fish body shape in interaction with others factors mostly heritability and evolutions processes. Our results showed that fish were finer at colder water temperatures which may results in lower fish metabolism, lower food consumption and thus finer fish. Finally, we found that food diversity (macroinvertebrates taxa diversity) did not correlate with body shape variability and that food availability and diversity did not correlate with the geomorphology of the cave.

VERSION FRANÇAISE

Depuis 2012, une équipe de chercheurs de l'Université d'Holar, en Islande, étudie les processus de microévolution au sein de populations d'Omble chevalier (*Salvelinus alpinus*) présentes dans un système de 20 caves situées à proximité du Lac Myvatn situé au Nord-est de l'Islande. Ce projet a pour objectif premier de comprendre comment les variations spatiales et temporelles influent sur les processus d'évolution et la diversité mais également leurs relations avec les paramètres écologiques du milieu. Ainsi, le but de ce stage a été d'étudier la variation morphologique dans l'espace et dans le temps des populations d'Omble chevalier résidant dans ces caves en relation avec les paramètres écologiques du milieu. Pour ce faire, nous avons utilisé les techniques de morphométrie géométriques et réalisé différents tests statistiques tels que des analyses de la covariance (ANCOVA) et discriminantes (DFA). Les données utilisées correspondent à celles collectées en juin 2012, 2013 et 2014. Nos résultats ont montré que la morphologie des populations varie significativement dans l'espace et au cours du temps, l'épaisseur des poissons étant la variation morphologique la plus notable. En outre, l'interaction entre la cave et l'année a un impact significatif sur la morphologie des poissons, chaque cave évoluant morphologiquement différemment au fil du temps. Les populations des caves connectées n'ont pas forcément des morphologies similaires, cela témoignant de l'interaction entre les paramètres écologiques du milieu et d'autres facteurs tels que l'héritabilité ou encore les processus d'évolution propre à chaque population. Enfin, il s'est avéré que plus la température de l'eau est froide, plus les poissons sont fins. On suppose que cela est dû à une diminution de leur métabolisme et donc de la quantité de nourriture consommée. Enfin, il n'y avait pas de corrélation entre la quantité de nourriture disponible et la diversité morphologique des individus, ni entre la quantité et la composition de nourriture disponible et la géomorphologie de la cave.

MOTS CLES

Omble chevalier, *Salvelinus alpinus*, géométrie morphométrie, microévolution, adaptation, morphologie, points de repères, analyse discriminante, analyse procrustéenne généralisée

PREAMBLE (FRENCH VERSION)

Contexte et objectifs :

Depuis 2012, une équipe de chercheurs de l'Université d'Holar, en Islande, étudie les processus de microévolution au sein de populations d'Omble chevalier (*Salvelinus alpinus*) présentes dans un système de 20 caves situées à proximité du Lac Myvatn (figure 1). On suppose ici que les caves sont isolées les unes des autres et que par conséquent, chaque population vit et évolue dans un environnement qui lui est propre.

Le but de ce stage est d'étudier la variation morphologique dans l'espace et dans le temps des populations d'Omble chevalier résidant dans ces caves en relation avec les paramètres écologiques du milieu.

Nos hypothèses étaient les suivantes :

- La morphologie des populations d'Omble chevalier évolue au fil du temps. La composition et la quantité de nourriture disponible variant également.
- La morphologie des populations d'Omble chevalier évolue dans l'espace (entre les caves).
    - Chaque cave présente un habitat et une géomorphologie qui lui est propre influençant la morphologie des individus.
    - La composition et la quantité de nourriture varient d'une cave à l'autre, influençant la morphologie des individus.

Nous avons utilisé les techniques de morphométrie géométriques et réalisé différents tests statistiques. Les données utilisées correspondent à celles collectées en juin 2012, 2013 et 2014.

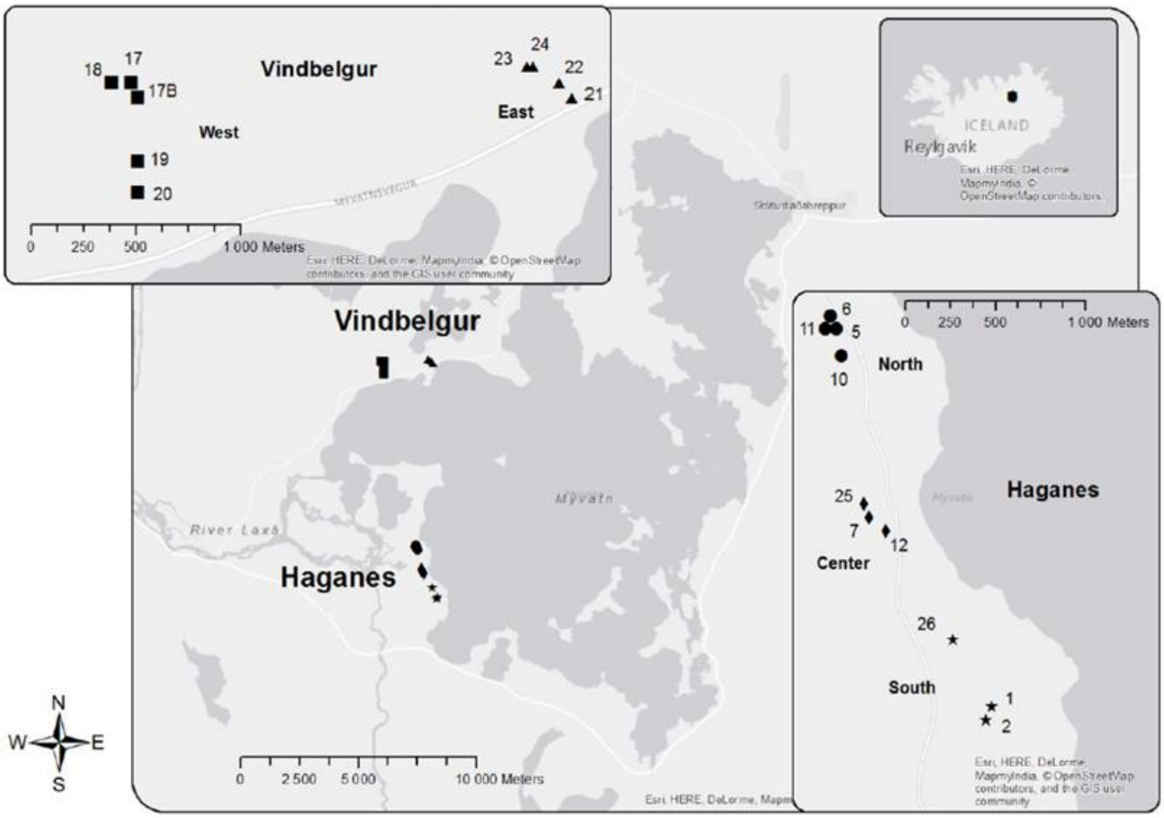

Figure 1 : Localisation et dénomination des caves volcaniques étudiées (Lherbier, 2017)

Le système d'étude :

Connu pour sa richesse et son abondance faunistique et floristique, le lac Myvatn (37km²), situé au nord-est de l'Islande, s'est formé il y a 2300 ans suite à une éruption volcanique. De nombreuses caves volcaniques ont également été formées à proximité du lac et sont aujourd'hui l'habitat de populations, isolées les unes des autres, d'Omble chevalier. Ces caves présentent des ouvertures (figure 2) qui permettent une production primaire mais également un apport alimentaire aux populations de poissons. En effet, en période estivale, de nombreux insectes ou autres organismes terrestres tombent à la surface de l'eau via ces ouvertures.

Figure 2 : Photo d'une des caves volcaniques

Appartenant à la famille des salmonidés, l'Omble chevalier est connu pour sa diversité morphologique. En effet, il n'est pas rare de trouver dans un même lac différentes formes d'Omble chevalier. Ces dernières sont principalement basées sur la ressource alimentaire utilisée. Par exemple, deux morphotypes coexistent au sein du lac Myvatn, une forme pélagique et une forme benthique.

Au sein des caves volcaniques, on retrouve seulement un petit morphotype benthique d'Omble chevalier. Il est facilement reconnaissable par sa petite taille à maturité (figure 3), sa coloration sombre, son corps trapu, sa longue nageoire pectorale ou encore ses yeux protubérants. Ces caractéristiques seraient le résultat de son adaptation à son milieu de vie ; ici les caves volcaniques.

Matériels et méthodes :

Lors des visites de terrain, qui ont lieu chaque année (en juin et en aout), les poissons sont capturés grâce à des pièges à Vairon, des pièges à anguille et également par pêche électrique. La combinaison de ces méthodes permet de capturer entre 70% et 90% de la population de chaque cave. Une fois capturés, les poissons, sous anesthésie, sont mesurés et pesés. Pour les individus de plus de 45mm, une photographie de leur flanc gauche est prise (ce sont ces photos qui seront utilisées plus tard, pour la morphométrie géométrique). Les poissons sont ensuite scannés. S'ils ne disposent pas de de puce et mesurent plus de 45mm, ils sont pucés et un échantillon de leur nageoire caudale est prélevé. Dans l'autre cas, leur numéro d'identification est scrupuleusement noté.

Les paramètres physico-chimiques des caves (température, turbidité, oxygène dissous, luminosité) sont également collectés via l'utilisation d'une sonde. La nourriture disponible pour les poissons est également estimée via l'utilisation de pièges récoltant les insectes ou autres organismes tombant à la surface de l'eau ainsi que via l'échantillonnage de la microfaune benthique.

La morphométrie géométrique est utilisée ici pour étudier la forme des poissons. Cette technique permet d'étudier la forme d'une structure biologique ou non en la caractérisant grâce à des points d'intérêts. Dans notre cas, nous avons utilisé 21 points d'intérêts dont 15 points d'intérêts fixes et 6 points d'intérêts glissants (figure 3). Ces points sont placés sur les photographies des individus prises lors des visites de terrain en utilisant la suite logiciel tps (figure 3). Les points fixes sont positionnés à des endroits précis tel que le point 4, qui est situé au niveau de l'insertion antérieur de la nageoire anale. Les points glissants ont des positions plus relatives tel que le point 16, qui est situé au milieu du museau. L'utilisation du logiciel tpsDig232 permet de relever les coordonnées de ces points d'intérêts en 2D (x et y).

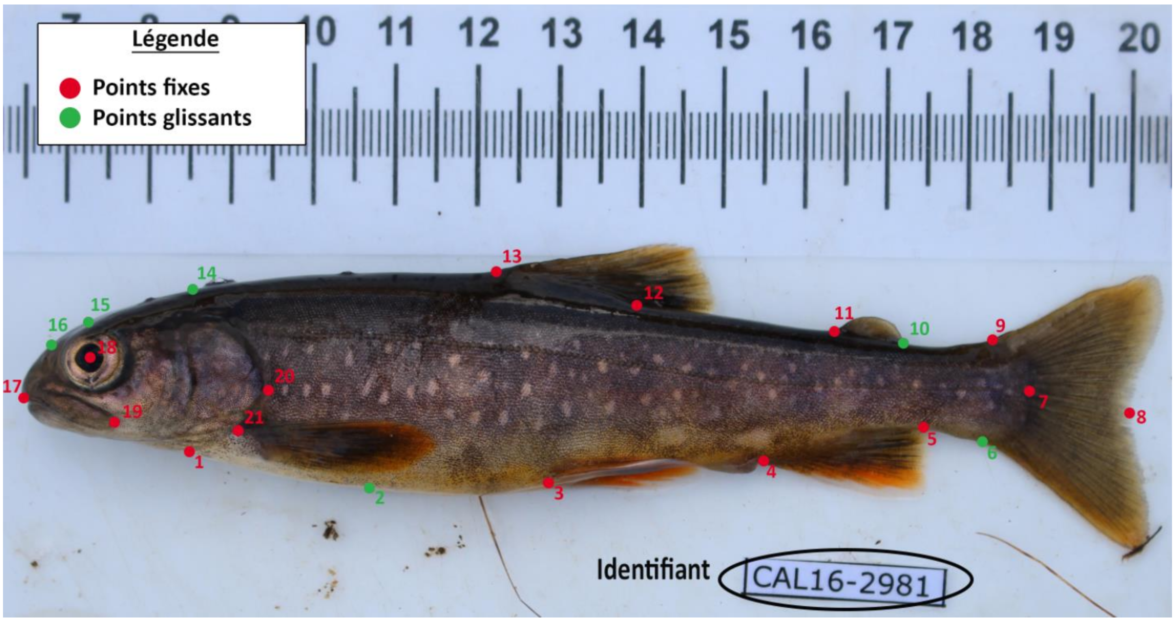

Figure 3 : Localisation des 21 points d'intérêt utilisés sur un individu d'Omble chevalier lors de l'analyse de géométrie morphométrie

Afin d'avoir des données objectivement comparables, on réalise une Analyse Procrustéenne Généralisée grâce à Rstudio. Cette étape supprime les variations autres que les variations liées à forme des individus et notamment l'effet d'échelle afin d'aboutir à une configuration consensus.

Pour tester nos hypothèses, on réalise ensuite différent tests statistiques. L'analyse de la covariance nous a permis d'identifier quelles variables (l'année, la cave, la taille ou encore la combinaison de l'année et de la cave) influent le plus la morphologie de nos populations. Enfin, des analyses discriminantes nous ont permis d'une part d'étudier comment la morphologie des poissons diffère entre les caves, les années et leur combinaison. D'autre part, elles nous ont permis de tester l'exactitude de la classification, c'est-à-dire à quel point les morphologies de nos populations diffèrent ou non les unes des autres. Enfin, on a réalisé des tests de corrélations de Pearson afin d'étudier premièrement la corrélation entre les paramètres géomorphologiques des caves (nombre d'ouvertures, surface des ouvertures) et la quantité et composition de nourriture disponible mais aussi la corrélation (ou non) entre le nombre de poissons capturés par cave et la diversité morphologique par cave.

Aboutissements :

Premièrement, nos résultats ont démontré que la morphologie des populations varie significativement dans l'espace (entre les caves) et au cours du temps. Il s'est avéré que l'épaisseur des poissons était la variation morphologique la plus importante.

Nous avons remarqué que plus la température moyenne annuelle de l'eau est froide, plus les poissons sont fins. On suppose que cela est dû à une diminution de leur métabolisme et donc de la quantité de nourriture consommée. La température affecte également la distribution et le pic d'émerge des chironomidea et par conséquent la quantité et la composition de nourriture disponible.

De plus, nous avons remarqué que les caves les plus proches géographiquement ont des habitats semblables. Cela pourrait expliquer pourquoi leurs populations ont des morphologies similaires.

En outre, nous avons trouvé que l'interaction entre la cave et l'année a un impact significatif sur la morphologie des populations. Ainsi, la morphologie des populations de chaque cave évolue différemment au fil du temps.

Les populations des caves connectées (physiquement et génétiquement) n'ont pas forcément des morphologies similaires. Cela témoigne de l'interaction entre les paramètres écologiques du milieu et d'autres facteurs tels que l'héritabilité ou encore les processus d'évolution propre à chaque population.

Enfin, nos résultats ont montré qu'il n'y avait pas de corrélation entre la quantité de nourriture disponible et la diversité morphologique des individus, ni entre la quantité et la composition de nourriture disponible et la géomorphologie de la cave.

SUMMARY

INTRODUCTION

Since 2012, scientists at Hólar University College have studied microevolutionary processes in small Arctic charr *(Salvelinus alpinus*) populations that inhabit lava caves near Lake Mývatn, in northeast Iceland.

The main objective of the project is to increase the understanding of the importance of spatial and temporal variation in evolutionary parameters for diversity and increase the knowledge of the relationship between ecological and evolutionary factors, in maintaining and developing biological diversity (Leblanc, 2016). The current study is divided into three parts:
The first one is based on study of the variation in space and time of phenotype among caves in relation to local ecological factors. The main hypothesis is that phenotype such as body shape, growth, sensory organs and body colour varies among caves and is connected to important ecological factors (Leblanc, 2016). In this way, water temperature, size of the cave and food availability are expected to have an important impact on the body shape of Arctic charr populations.
The second part's objective is to study the influence of local ecological factors and phenotype on fitness of individual Arctic charr within and among the cave (Leblanc, 2016). That is mean the fate of individuals, their reproductive success and survival related to ecological factors and phenotype.
The third part is based on the study of microevolution, natural selection and genetic drift. The aim of this part is to study the role of genetic drift, adaptability and inbreeding in shaping phenotypic variation among individuals in small populations.
Here, I studied the variation in space and time in body shape among populations in relation to ecological factors, which is an important component of the first part of the study. This work will allow us to have a first overview of body shape variation among years, and among the caves, and will increases our understanding of how body shape may varies with ecological factors. No analyses have been conducted yet using this data. Our results will guide further research on this system.

Arctic charr is an ideal system to study the relation between ecology and microevolutionary processes (Leblanc, 2016). It is partly due to the fact that Arctic charr is believed to be one of the most phenotypically variable vertebrate species in the world (Klemetsen, 2013). It presents a high degree of variability in behaviour, growth and life-history traits. This ample phenotypic variation depends mainly on the environment it inhabits and on resource availability. For example, four resource-based Artcic charr morphs have been described in lake Thingvallvatn in Iceland including small a benthivorous Arctic charr which have retained juvenile characteristics. Similar population of charr can be found throughout Iceland,mainly in habitats with lava and ground water (Kristjansson, 2005). These habitats are characterized by a relatively stable water regime and temperature and are often rich in nutrients (Cantonati *et al.,* 2006; Karst-Riddoch *et al.,* 2009). Lava provides also a three-dimensional substrate and is thus an unique benthic habitat (Kristjansson *et al.,* 2011). Lava caves near Lake Mývatn, are one of the habitats where these small benthic charr can be found in. They are small, robust and dark in body coloration. However, we predict that as the caves (most of them) are isolated from each other and that each fish population evolve independently, the fish in each cave will vary in phenotype, mainly in body shape.
Lava caves near Lake Mývatn, were formed 2300 years ago by a volcanic eruption (Einarsson, 2004a). The Arctic charr is nowadays, the main fish species inhabiting the caves. The caves are particularly interesting for studying evolutionary, adaptation processes and phenotype and genetic diversity at small spatial scale. Indeed, it has been found that almost a unique population is found in each cave. As due to local ecological diversity of the caves, it will be possible to study apart the importance of small variation in ecological factors for fish morphology, growth and survival and also to test which key parameter(s) may promote evolution of these populations

To answer the above objectives, Holar University College's researchers study sixteen isolated populations of small benthic Arctic charr that are living in a system of twenty lava caves near Lake Mývatn. Yearly since 2012 these populations have been monitored in June and in August. Individuals above 45mm were PIT-tagged and measured (weight, length, photograph). Between 2012 and 2016 around 1600 individuals were tagged and it is estimated that between 70% to 90% of all adults in each cave are part of the monitoring project (Leblanc, 2016).

My part in this project was to study average body shape variation in space (among the caves) and time (over the years). Secondly, I tested relationship between morphological variation (if it exists) and ecological factors such as food availability, cave morphology (number and size of opening) or water physical and chemical parameters.

This study focused on June 2012, 2013 and 2014 data-sets using landmarked based geometric morphometric approach and several statistical tests. The main goal of morphometric is to study how shape varies and its covariance with other variables (Gelsvartas, 2010), which fit well with our objectives.

Our main hypothesis was that there is significant variation in body shape in connection with local ecological factors in each cave at a specific time. Even if sampling was conducted at the same season each year, we expected that food availability and composition may vary from one year to another. Indeed, Gardarsson *et al.* (2004), showed fluctuations of chironomid and diptera populations of the Lake Mývatn between 1977 and 1996. Chirnomid larvas found in the caves are the offpsring of the midges that have emerged from the lake. Therefore there are similar fluctuations in larvae numbers and diversity in the caves.

Our hypothesis related to body shape variation among caves is based on the following predictions:

- Habitats and morphology of the caves differ and consequently, there are differences in fish average body shape among populations;

- Food availability (e.g. terrestrial food availability) and diversity (e.g. macroinvertebrates taxa diversity) vary from a cave to another. This factor has an impact on fish body shape. We predict that:
    - Food diversity will result in increased body shape variability;
    - Caves with fewer or smaller openings have less food availability and diversity, resulting in thinner fish.

The first part of this report will introduce the host University and the study system. Then, the second part will explain materials and methods used, followed by results and discussion.

1 PRESENTATION OF THE HOST UNIVERSITY AND THE STUDY SYSTEM – CONTEXT

As I have already said it before, the first objective is to study the variation in space and time in body shape of Arctic charr *(Salvelinus alpinus)* found in lava caves near Lake Myvatn. But, before getting at the heart of matter, I will first give a slight overview of Holar University College, where my internship took place. Then, I will give more information about Arctic charr, Lake Myvatn that are both great study system.

1.1 HOLAR UNIVERSITY COLLEGE, ONE OF THE OLDEST SCHOOL IN EUROPE

Holar University College (HUC) is one of the oldest schools in Europe. In 1106, Bishop Jón Ögmundsson's cathedral school was founded. However, it was converted into a latin school from 1550 until 1801. In 1882 Holar Agricultural was founded that became Holar University College in 2007. Since 2007, the school has developed from a conventional agricultural school to a modern university-level institution (Hólar University College, 2017) and considering all departments, there are currently about 270 students enrolled at the school.

There are three main departments: Aquaculture and Fish Biology, Equine Studies and Rural Tourism. During my internship, I worked in the first one. The main objective of the Department of Aquaculture and Fish Biology « is to gather and disseminate knowledge in the fields of aquatic biology, aquaculture and fish biology » (Hólar University College, 2017) and to « promote the professional development of aquaculture in the spirit of sustainable development » (Hólar University College, 2017). Currently the staff of the department is composed of 11 employees (considering researchers, technical and administrative employees) (Hólar University College, 2017). They are also 13 doctoral students, 4 master's students (Hólar University College, 2017) and 8 summer interns.

The main campus of HUC is located in Holar, in « the valley Hjaltadalur in the Skagafjörður district of the mid-northern part of Iceland » (Hólar University College, 2017). This is « a farming district, renowned for horse breeding and training, history, heritage tourism and it´s tradition of choirs » (Hólar University College, 2017). The main campus is in Hólar, but the department of Aquaculture and Fish Biology is situated in Sauðárkrókur (figure 4), which is about 35km from Hólar.

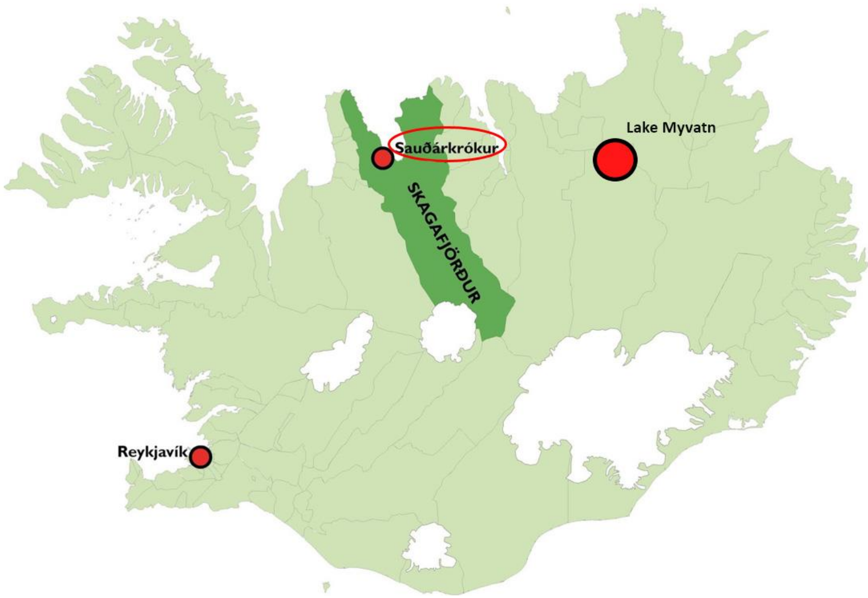

Figure 4 : Location of the Department of Aquaculture and Fish Biology (visitskagafjordur.is)

1.2 THE LAKE MYVATN, A WETLAND OF INTERNATIONAL IMPORTANCE

Known for its beauty and for its rich and abundant fauna and flora, The Lake Myvatn (location figure 1) was designated as a Nature Reserve in 1974 and has been a Ramsar Site[1] since 1978 (Einarsson, 2004b).

This large, shallow and eutrophic lake is currently about 37km² and it is divided into two main basins which are all feeding by artesian springs; the North and the South Basin (figure 5).

The Lake Myvatn was created 2300 years ago because of a volcanic eruption (Einarsson, 2004a). Prior to this, another bigger lake but was mostly destroyed by the lava (Einarsson, 2004a).

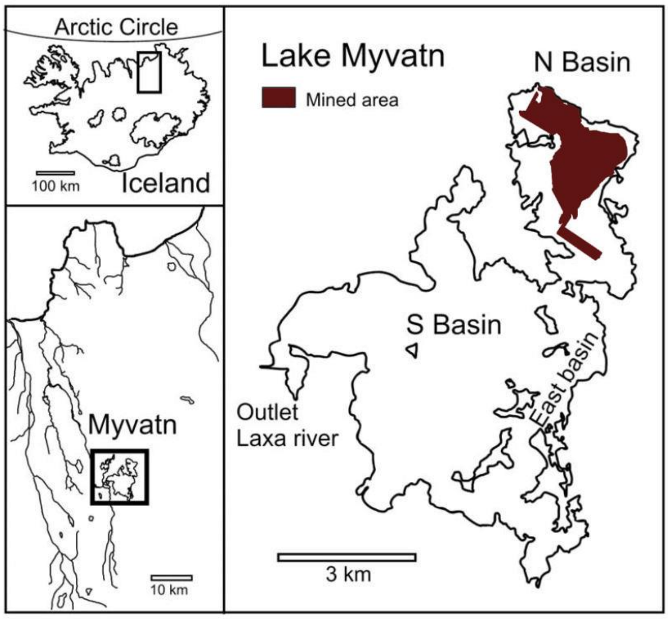

Figure 5 : Location of the Lake Myvatn (Einarsson, 2004a)

The North basin is about 8.5km² with a depth between 3 and 4 meters and the South Basin is about 28.2km² with a depth between 2 and 5.5 meters (Einarsson, 2004a). The North basin is deeper due to dragging activities (diatomite mining) which started in 1967 but has now stopped. The lake is spring fed. Springs have a high pH, high phosphate and silicate concentrations and some nitrates. Furthermore, the spring water discharge is very stable (Einarsson, 2004a). Temperature of the largest spring is around 5°C. There is a temperature gradient from the south to north with the warmest springs reaching 30°C. The lake drains through the Laxa River, which flows into the Arctic Ocean 61km away.

In the lake, there is a high primary and secondary production. The secondary production can be attributed mainly to chironomids species which dominate the benthic community. Fluctuations of these chironomids populations (and of Cladocera populations) are correlated with variations of their vertebrate predators (such as the Arctic charr), body condition and fish mortality (Eirnasson, 2004a).

[1] « Ramsar Sites are designated because they meet the Criteria for identifying Wetlands of International Importance. The first criterion refers to Sites containing representative, rare or unique wetland types, and the other eight cover Sites of international importance for conserving biological diversity. These criteria emphasize the importance the Convention places on sustaining biodiversity.

The Contracting Parties confirmed in 2005 that their vision for the Ramsar List is "to develop and maintain an international network of wetlands which are important for the conservation of global biological diversity and for sustaining human life through the maintenance of their ecosystem components, processes and benefits/services » (Ramsar, 2017)

The most abundant fish species in the lake is three-spine stickleback (*Gasterosteus aculeatus*). However, there are also two main salmonid species; Arctic charr (*Salvelunus alpinus*) (two morphs) and brown trout (*Salmo trutta*) (Eirnasson, 2004a)

The Lake is surrounded by lava fields where is some locations we can find lava caves (figure 6). Commonly these caves are inhabited by Arctic charr (these populations are the subject of this study). These caves are also spring fed and parts of the roofs collapsed, often creating several openings (figure 6).

Figure 6 : Example of one of the lava caves near the Lake Myvatn

Since 2012, researchers at Holar University Collegge sampled 20 caves twice each year in June and August. These caves are largely covered by lava roof (figure 6) but have openings where primary production occurs. During summer, flies and terrestrial invertebrates fall on the surface of the water, bringing important food source in to the caves. During wintertime the openings are mostly covered by snow and the caves in total darkness. It is thus believed that food is scares during that time period.
The sampling is conducted in June and August to capture fish at the beginning of the growing season and well into the season.

The monitored caves are in two areas, in Haganes on the Western shore and Vindbelgur on the North-Western shore of the lake (figure 7). Within a given are caves are commonly in close proximity to each other. Each cave is environmentally unique; they vary in size, water volume, external exposure, water temperature and food availability. The charr in the caves are commonly small in body size and the populations are small, and have evolved independently. According to the first results of the study, based on the tagged-fish movements, caves 5 and 11, 7 and 25, 17 and 18, and 19 and 20 are connected.
It is likely that these caves were settled by charr from the lake shortly after the formation of the caves, 2300 years ago.

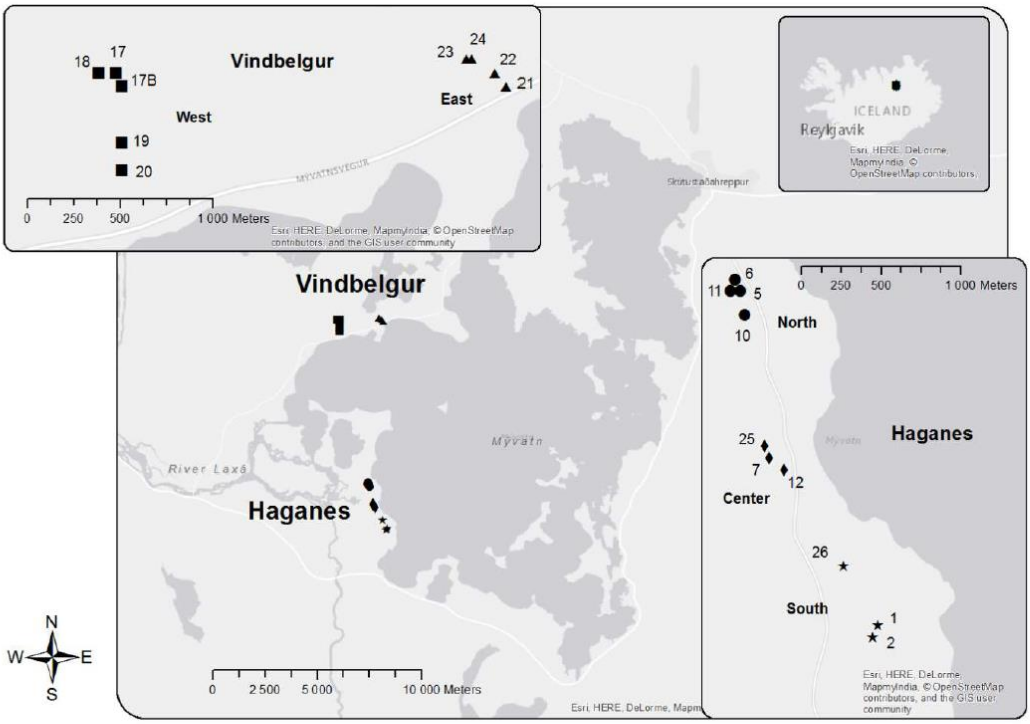

Figure 7 : Study site location near Lake Myvatn: several clusters of caves found in two main areas (Haganes and Vindblgur) dwelling unique populations of small benthic Arctic charr (Lherbier, 2017, unpublished)

Nowadays, the Lake's catchment, which is estimated to 1400km², is impacted by human activities. First, there are numbers of farms along the shorelines of the lake which are mainly based on sheep farming and fishing. Moreover, for the past thirty years, human population and tourism activities have increased greatly. There is relatively recent village (Reykjahlíð) in the north of the lake (480 inhabitants). All these activities have led to maintain an open landscape and may have contributed to excessive soil erosion and run off (Einarsson, 2004a).

1.3 THE SMALL BENTHIC ARCTIC CHARR, THE RESULT OF GENETIC CONNECTIVITY ADAPTATION AND NATURAL SELECTION

Arctic Charr is a salmonid fish with the northern most circumpolar distribution. Furthermore, populations of this species can be anadromous or residential (Jonsson, 1999). This species is found in cold-water, e.g alpines lakes and Arctic and Subarctic coastal waters. The species is a generalist feeder and commonly eats chironomid larvae, pupae, benthic/planktonic cladocera, snails and other fishes, e.g. stickleback (Jonsson, 1999). Across the distribution of Arctic charr it is common that two or three morphs coexist in the same water body (Klemetsen *et al*. 2013).

In Iceland, the Arctic charr populations vary extensively in body size, head morphology, growth rate, behaviour and life history (Jonsson *et al*., 1988). For example, four different Arctic charr morph coexist in Lake Thingvallavatn: planktivorous morph (PL), piscivorous morph (PI), small benthivorous morph (DB) and large benthivorous morph (LB) (Jonsson *et al*., 1988). The LB and DB are benthic morphs whereas the PI and PL are more limnetic morphs. These morphs differ both genetically and phenotypically, which is likely the result off natural selection, representing adaptation to location and resources (Jonsson *et al.* 1988). Benthic morphs are commonly suited for stony littoral zones and feeding mainly on benthic invertebrates. Consequently, they have stocky body, long pectoral fins, blunt snout and a subterminal mouth (Skulason et *al.,* 1989). Pelagic morphs are adapted to prolonged swimming and feeding in the water column, they have more streamlined body are more terminal snout and mouth (Skulason et *al.,* 1989).

Figure 8 : Example of phenotypic diversity of Icelandic Arctic charr from Lake Vatnshlidarvatn (larger fish are called silver Arctic charr, more generalist, and smaller fish are brown morphs which are small and benthic fish, more specialized on benthic food) (Leblanc, 2014)

In Lake Myvatn, two Arctic charr morphs coexist, a small benthic and a generalist morphs. Small morph is commonly found in spring habitat within or near lava fields, which can be isolated from other water bodies (such as the lava near lake Myvatn) (Kristjansson, 2005). The small benthic morph is characterized by small size at maturity, and a juvenile appearance. The small size and often high-density population may be the result of adaptation towards spring water habitats, low temperatures and special structure (Kristjansson, 2005) e.g. lava habitats. Small benthic charr are commonly dark in colour, have stocky body, long pectoral fins, blunt snout and often short lower jaw (Kristjansson, 2005). This morph shows marked differences in foraging morphology depending on variation in ecological factors (Kristjansson, 2005).

In the lava caves, we can find small benthic morph. However, fish within the lava caves are darker and more robust than the small morph found in Lake Myvatn (pers. comm. Leblanc). These characteristics are likely the results of their adaptation to their living conditions.

2 MATERIALS AND METHODS

At the beginning of the project, in 2012, the morphology and important ecological factors of 20 caves, which have been selected for the project (figure 7) was mapped. Since then, each year, each cave is visited twice in June and twice in August. Caves are visited twice in each month to maximize the number of fish caught.

In 2012, Arctic charr population from the Lake Myvatn were also sampled, corresponding to number 30 and 40 in the following data-set.

The project is divided into two main parts; fields trip and data analysis. The collection of data and its analyse will be explained below.

<u>Remark:</u> At the beginning of the project, 25 caves were chosen. However, due to too small fish populations, accessibilities issues or because it was too difficult to conduct the electrofishing, five caves were removed, those are caves 3,4,8,9 in 2013 and cave 13 was removed in 2014. Nevertheless, body shape data were available at one point in time then these data where analysed for the analysis comparing body size variation in space.

2.1 MATERIALS AND METHODS USED DURING THE FIELD TRIPS

During the field trips, data has been collected on fish and on the ecology of the caves, e.g. physical characters, cave structure, invertebrate community and the availability of food (LeBlanc, 2016). I was part of the crew for field trips in 2017.

2.1.1 MATERIALS AND METHODS FOR FISHING

To conduct an exhaustive fishing, where the aim is to catch all the fish in each cave, minnow traps, eel traps and electrofishing were used. First, minnow (figure 9) and eel traps were set in the cave for at least 12 hours. In eel traps, we mainly captured fish with an average length between 8 and 9 cm whereas minnow traps can capture smaller fish. The number of traps set in each cave is related to its size and to the number of traps available.

Figure 9 : Picture of a minnow trap used on the field

Once traps have been removed from the water, we electrofished the cave. This step was conducted by one person. We used a gas-powered generator which provides 220 V (Chevalier, G., 2016) and the current given was about 300 V and between 0.1 to 0.6 A. Once fish were paralyzed, we caught them with a hand held net. There is no common rule about how to electrofishing the caves because each one has different shape and accessibilities. Nevertheless, we usually start from the shores and then, we go in the cave itself.

2.1.2 MATERIALS AND METHODS FOR THE FISH PROCESSING

After fishing, fishes were kept in a bucket witch cold and oxygenated freshwater (which comes from the corresponding cave). They were then, anesthetized with 3ppm 2-phenoxyethanol.
Once they were asleep, we measured (fork length, nearest mm) and weighed (body mass, nearest 0.1g) (Leblanc, 2016) each individual and a high-resolution and focused photograph was taken of the left side. On the pictures, fish must be straight placed and must have their mouth closed. These are these pictures that we will use for the geometric morphometric analysis.

We also checked if the individual was known, that is mean if it has already been PIT-tagged. If not, individuals with a length above 45mm were tagged and identified with RFID NONATED tags (6mm, Lutronic International) and individuals above 65mm with a HDX PIT-tag (12mm, 0.1g, Oregon RFID) that is implanted in the anterior part of the body cavity. Then, a fin clip was taken for DNA analysis and DNA samples were stored in 96% ethanol and frozen as soon as possible (Leblanc, 2016).

There are a number of reasons why a fish caught can be classified as unknown. They may be young of the year, they may never have been caught before or they may have lost their tag. In the last case, they may still have the scar of the fin clip (figure 11) and they may be identified later by DNA or by manual photographic identification (Chevalier, G, 2016).

During the processing, all data and all-important information were written in a field book. Two persons were needed for the whole processing, one for writing and one to process the fish. The data were then entered in excel as soon as possible.

After the processing, fishes were put in a recovery bucket with oxygenated freshwater and then, they were released in the lava cave they originated from.

2.1.3 THE COLLECTION OF ECOLOGICAL VARIABLES

In each cave, HOBO data loggers (UA-002-64, ONSET Corporation) were set and recorded the variation of temperature (°C) and light (LUX) few times a day and all year around. We collected their data at each sampling time.

During the first visit in June and August, three rocks were taken from each cave and brushed to collect benthic macroinvertebrates (sieve size 125 microns). This step allows us to determine their biomass in each cave and to identify and classify to their order or genus macroinvertebrates in each cave. Samples were stored in 70% ethanol.

Terrestrial materials falling into the caves were also recorded using fall down traps that were set up above the surface of the water in each cave opening (figure 10). They contain half water and half propylene glycol plus a drop of soap. These buckets were emptied during the second visit both in June and August and as for benthic macroinvertebrates, samples were stored in 70% ethanol. It allows us to estimate qualitatively and quantitatively the terrestrial input in each cave at two season (winter, i.e. September until June, and summer i.e. June till August).

Figure 10 : Example of a fall down trap used to collect terrestrial materials falling at the surface of a lava cave

During the second visit, both in June and August, we also collected physical and chemical parameters by using a calibrated sonde HydroLab probe HL3. It allowed for measurements of temperature (°C), pH (NTU), conductivity (µS/cm), turbidity (NTU), dissolved oxygen (mg/L) and the Photosynthetically Active Radiation (PAR).
PAR represents the fraction of sunlight (LI-COR, 2017). It allows to "gauge of how much light is available to unicellular and multicellular phototrophic organisms such as algae, aquatic plants, protists, and phytoplankton" (LI-COR, 2017).

2.2 Data processing; geometric morphometric and statistical analysis

For this part, pictures of fish that were taken during field trips in June 2012, 2013 and 2014 were used. Landmarks were placed on pictures using tps software. This step corresponds to the first step of the geometric morphometric approach. It allowed characterizing fish body shape and turning visual data into numerical data. Then, we applied statistical tests such as an Analysis of Covariance and a Discriminant Function Analysis.

Landmark-based geometric morphometric uses a set of landmarks to describe shape (Gelsvartas, 2010). Landmark is, in our case, a two-dimensional point. Of course, quality of the results depends greatly on the quality of the landmarks (Gelsvartas, 2010) and the quality of the photographs. Landmarks have thus to be chosen carefully and have a biological significance (Gelsvartas, 2010).

2.2.1 Geometric morphometric analysis with tipsUtil64 and tpsDIG232

The first step consists of assessing morphological variation with geometric morphometric by using landmarks to characterize individual body shape. We used the programs tpsUtil64 and tpsDIG232 to place landmarks on each photograph.

In tpsDig232 we placed 21 landmarks capturing the whole morphology of the fish (figure 11, table 1) on the fish body. The positions of landmarks were determined to capture both trophic morphology of the fish and important factors in the overall body morphology. We chose to put 21 landmarks, but their number can vary according to the aims of the study and the species studied. The most important thing is to use enough landmarks to adequately sample the shape (Indiana University, 2012). Landmarks should be repeatable (i.e. each landmark must be present on every studied organism, Gelsvartas, 2010) and always must be placed in the same order, which is described on table 1.

In our case, we used two kinds of landmarks; 15 fixed landmarks (also called true landmarks) and 6 sliding semi-landmarks (also called sliding-landmarks). The first type is situated on a fixed structure and has a biological significance (Gelsvartas, 2010) and the second type is defined by a location relative to other landmarks and can move slightly from one fish to another. Sliding landmarks minimize how individuals deviate from the mean shape of the population. They have lighter weight in the analysis and lighter biological significance. For each fish, we inform its ID and the scale of the picture.

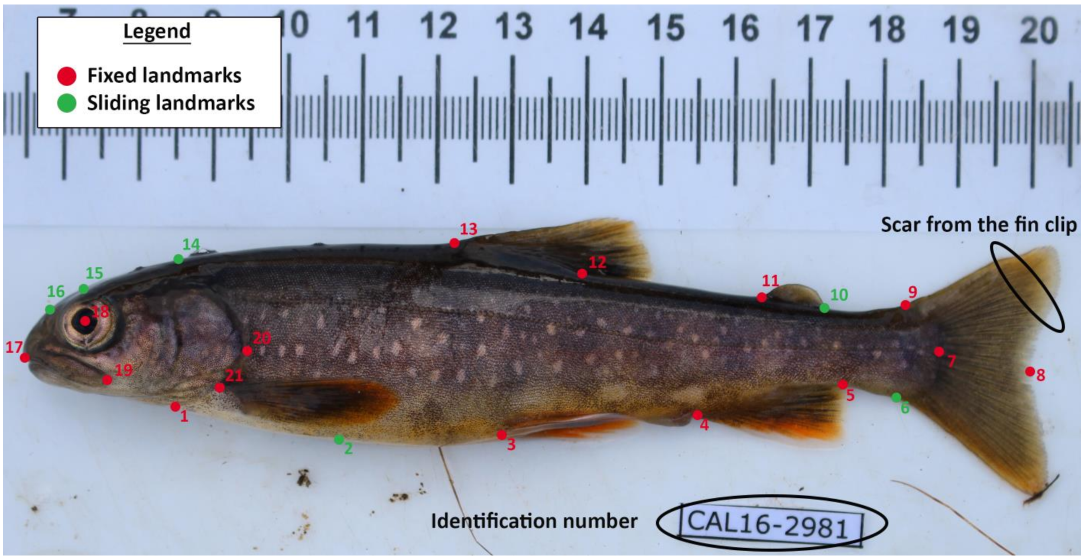

Figure 11 : Location of the 21 landmarks used on an Arctic charr for the geometric morphometric approach using tpsDig232

Table 1 : Anatomical description of landmarks and semi landmarks used (Modified from Skoglund et al. 2015; and Krisjánsson et al. 2012)

Landmark	Anatomical description
1	Ventral point of the opercular opening
2	Half distance between landmarks 1 and 3
3	Anterior insertion of the pelvic fin
4	Anterior insertion of the anal fin
5	Posterior insertion of the anal fin
6	Ventral insertion of the caudal fin
7	Posterior point of the hypural bone at the lateral midline
8	Fork of the caudal fin
9	Dorsal insertion of the caudal fin
10	Posterior insertion of adipose fin
11	Anterior insertion of adipose fin
12	Posterior insertion of the dorsal fin
13	Anterior insertion of dorsal fin
14	Posterior edge of the cranium
15	Top of cranium at midpoint of eye
16	Middle of snout
17	Upper tip of the snout
18	Centre of the bony orbit of eye
19	Posterior point of the maxilla
20	The most posterior point on the curve of the operculum
21	Anterior insertion of the pectoral fin

The aim of this operation consists of turning visual data into numerical data for statistical analysis, and using R for that matter. Thus, the software assigned 2D coordinates (x and y) at each landmark. In this way, we use location of landmarks instead of distances.

We corrected the data for up and down bending using TpsUtil64. We use the landmarks 17, 20, 7 and 8 (figure 11) as the rectilinear base for the landmarks coordinates. Then, the software corrected by itself landmarks coordinates to minimize up and down bending of each fish.

2.2.2 DATA PROCESSING WITH R STUDIO

We analysed the data using R Studio. The objective of this step was to compare and analyse data set to test for our hypothesis. However, the first step consists in making our data set comparable by defining sliding landmarks and doing a Generalized Procrustes Analysis. Then we conducted an analysis of covariance (ANCOVA) to assess which variables influence fish body shape. To finish, we ran a Discriminant Function Analysis (DFA) allowing us first to visualize how the pattern of shape differs between the caves, the years and the caves*year. And to see if we could accurately classify the fish into cave, year and cave by year.

2.2.2.1 THE DEFINITION OF SLIDING LANDMARKS

Once data are implemented into R, the first step consisted of defining sliding landmarks using the function “define.landmarks”. When we put landmarks using tps software, we did not define which one were sliding landmarks. It is important to do it here, because sliding landmarks do not have the same influence and weight as fixed landmarks. As a reminder, sliding landmarks are landmarks 2,5,10 14,15 and 16 (figure 12).

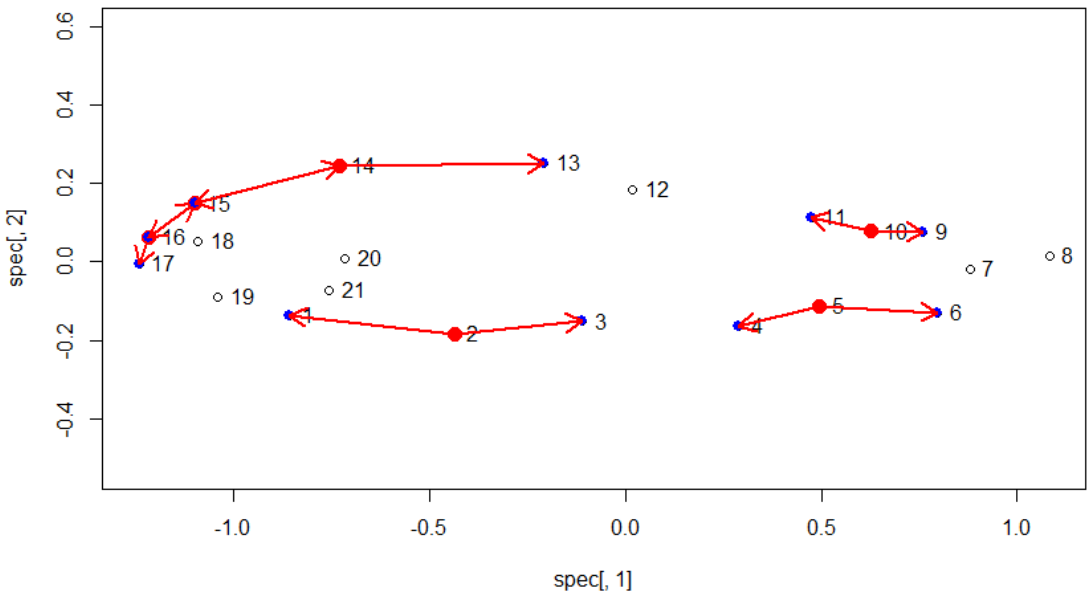

Figure 12 : Scheme of the definition of sliding landmarks using R

2.2.2.2 THE GENERALIZED PROCRUSTES ANALYSIS

Extracted landmark data has a lot of variation in position, orientation and scale between specimens (Gelsvartas, 2010). To remove these non-shape variations and make data comparable, we did a Generalized Procrustes Analysis (GPA) (using the function "gpagen"). First it consisted of calculating mean shape and centroid size. Then, it removes non-shape variations using rescaling, translation, rotation and reflection.

The centroid size is the most common measure of size used in geometric morphometric (Mitteroecker *et al.,* 2013). It corresponds to the square root of the summed squared distances between all landmarks and their centroid (Mitteroecker *et al.,* 2013) (figure 13). The centroid of a landmark configuration is the average of all landmarks (Mitteroecker *et al.,* 2013). The centroid size is correlated with shape (Bookstein, 1991).

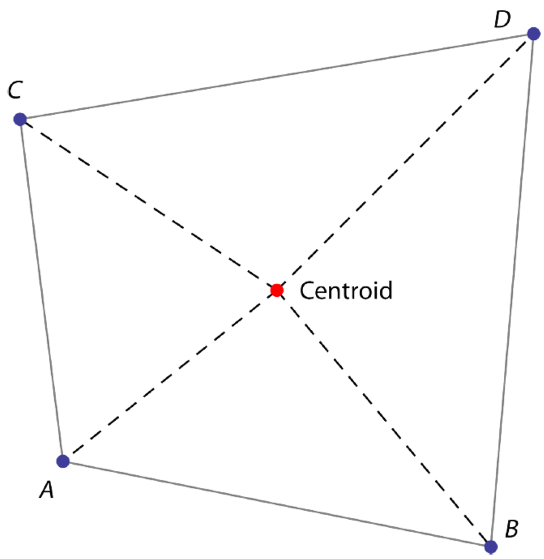

Figure 13 : A configuration of four landmarks with their centroid (Mitteroecker et al., 2013)

According to Gelsvartas, 2010, here is the explanation of the first steps;

We used k points (landmarks) in two-dimensional space, with k = the number of specimen. For each landmark, we have:

$$((x1, y1), (x2, y2), \dots (xk, yk))$$

First, it calculates the mean shape by calculating the average x,y coordinate of each landmarks:

$$\bar{x} = \frac{x1 + x2 + \cdots xk}{k} \qquad \bar{y} = \frac{y1 + y2 + \cdots yk}{k}$$

It creates then a mean shape, such as the example on figure 14

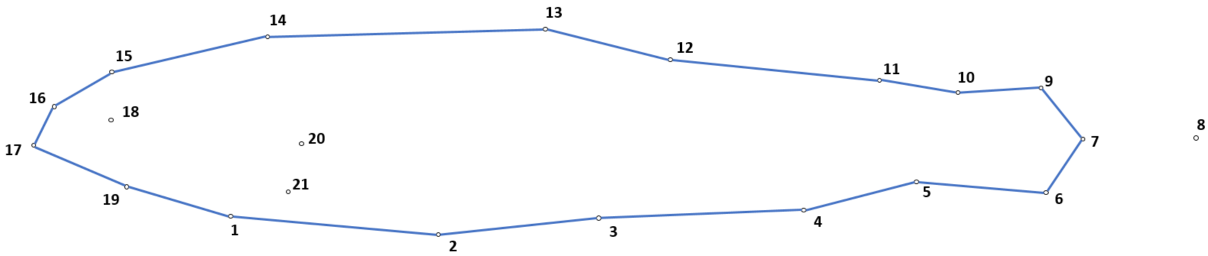

Figure 14 : Example of the result of the calculation of the mean shape

Then, next step consists of doing transformations such as translation, rotation, reflection or rescaling (figure 15) of individual data matrices to provide optimal comparability (The Society of Sensory Professional, 2017). Here are the different steps:

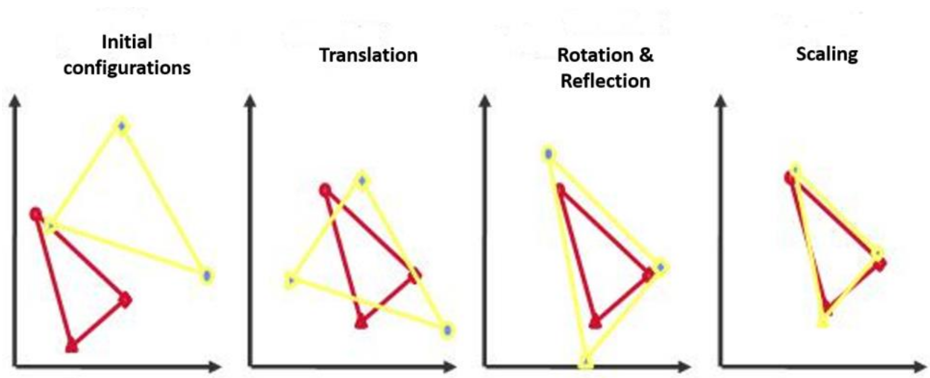

Figure 15 : Explanatory scheme of the Generalized Procrustes Analysis (The Society of Sensory Professionals, 2017)

Then, it calculates the centroid size (s), corresponding in the mean (centre) shape (Indiana University, 2012). It calculates the average x,y of all coordinates across all landmarks for each specimen and for the mean shape:

$$s = \sqrt{(x1-\bar{x})^2 + (y1-\bar{y})^2 + \cdots + (xk-\bar{x})^2 + (yk-\bar{y})^2}$$

Then all the points are scaled, using the centroid size (s) as reference:

$$\left(\left(\frac{(x1-\bar{x})}{s}, \frac{(y1-\overline{y)}}{s}\right), \ldots, \left(\frac{(xk-\bar{x})}{s}, \frac{(yk-\overline{y)}}{s}\right)\right)$$

Next, it does translations and rotations (figure 15). In our case, we did not need to do reflections because all the pictures were taken on the left size of the fish. These steps are more complicated and use more difficult mathematic formulas and will not be explained here. For more information, we can refer to Gelsvartas 2010 report.

To finish, the function returns new x,y landmarks coordinates for each individuals in a data frame. New coordinates score their deviation from the mean shape along the principal axes of variation.

At the end of the General Procrustes Analysis, data is comparable and shape differences and variations can be extracted by calculating differences between coordinates (Gelsvartas, 2010) using multivariate analysis.

2.2.2.3 THE ANALYSIS OF VARIANCE (ANOVA)/REGRESSION FOR SHAPE DATA

After the Generalized Procrustes Analysis, we ran a Procrustes ANOVA/regression for shape data using the function procD.lm, also called ANCOVA. Analysis of the covariance (ANCOVA) is an advanced form of analysis of variance that combines both ANOVA and regression analysis (keydifferences, 2017). ANCOVA is used to compare one variable in two or more populations while considering other variables. It removes the impact of one or more metric-scaled undesirable variable before undertaking research (keydifferences, 2017). Here, we used the size as covariant.

The aim of this step was to assess statistical hypotheses describing patterns of shape variation and covariation for a set of Procrustes-aligned coordinates (R, 2017). It quantifies the relative amount of shape variation attributable to one or more factors (such as year, cave, or size in our case) in a linear model and estimates the probability of this variation (R, 2017).

This step will allow us to answer the following questions:

- Which parameter influences the most average body shape?
- Do the cave*year influence average body shape?
- Does the average body shape differ between the caves?
- Does the average body shape differ between the years?
- Does the size (i.e. the centroid size) as an important effect on the average body shape?

2.2.2.4 THE DISCRIMINANT FUNCTION ANALYSIS (DFA)

Then, we did a Discriminant Function Analysis (DFA) using the function lda. DFA indicates how well the treatment groups (or study sites) differ from each other (Gagné, 2014). It provides also a classification function that determines to which groups an individual belongs (Gagné, 2014).

Thus, this step allowed us to assess whether body shape differences existed among caves, among years or among caves*years and how it differed among them. We ran three DFA using as variables cave, year and cave*year in order to visualize shape variation in space and time. We calculated DFA axes which explain different proportions of shape variation. We used the first two axes to create DFA graphs.

2.2.3 VISUALIZATION OF SHAPE DIFFERENCES

To finish, we used thin-plate spline allowing us to visualize the deformation in shape from a fish or a group of fish in relation to linear variable (here DFA axis) (Gelsvartas, 2010) using tpsRegr32 software. It calculates the transformation grid that shows how object are deformed along an axis compared with the mean shape and illustrates it (figure 16).

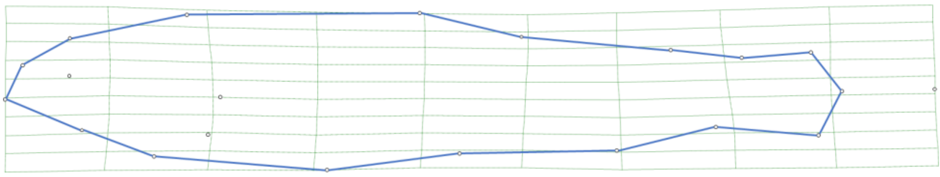

Figure 16 : Example of the transformation grid using a thin-plate spline

2.3 ECOLOGICAL DATA ANALYSIS

The second objective of this project was to study shape variation in relation to ecological factors. In this way, I used Pearson correlation tests. It allowed us to assess the linear relationship between two sets of data and to answer in particular to the following questions:

- Do the number of fish caught or food diversity in each cave are linearly correlated with fish body shape diversity?
- Does the food availability is linearly correlated with water temperature?
- Does the number of opening in each cave is linearly correlated with food diversity and availability?

We also analysed the variance (ANOVA) of cave water temperatures from 2012 to 2014 in order to assess if there were significant water temperature differences in time.

As reminder, ecological data (e.g. pH, conductivity, turbidity, dissolved oxygen concentration, macroinvertebrates taxa diversity and food availability) were recorded first in 2014 and we worked on data from 2014 to 2016. Only water temperatures were recorded for the first time in 2012. We also used geomorphological caves data (e.g. surface exposed to sky by cave, number of openings, and distance to lake). The morphologies of the caves were described in 2012.

3 RESULTS

Our main hypothesis was that there is a significant variation in body shape in connection with local ecological factors in each cave at a specific time. Even if sampling was conducted at the same season each year, we expected that food availability and composition may vary from one year to another. Indeed, Gardarsson et al. (2004), showed fluctuations of chironomid and diptera populations in Lake Mývatn between 1977 and 1996.

First, there are body shape variations among years (table 2, figure 18). Indeed we could separate the fish quite well according to their own year (table 3). In 2014, fish body shape was very different from 2012 and 2013, resulting in a finer and with a more streamlined average body shape and a more a blunt snout in all caves (figure 18). In 2012 and 2013, fish body shape was more similar, the anterior part of body was stockier, fish had a sharper snout and a more tarpered head. These characteristics were more pronounced in 2013 resulting in a little bit finer fish in 2012.

Moreover, according to the ANOVA, water temperatures were significantly different in 2014 than in 2012 and 2013 (table 4). Indeed, water temperatures were lower in 2014 (2012 (p-value <0.0001) = 2013 (p-value = 0.0001)>2014 (p-value<0.001) (see figure 17). There were no significant differences 2012 and 2013 water temperatures (table 4). Thus, at colder temperature fish were more skinny.

We can also noticed that the combination of the cave and the year had a significant effect on water temperature. Which mean that water temperatures in the caves vary in different directions.

Table 2 : Results of the Procrustes ANOVA ran with Rstudio with data from June 2012 to 2014. This analysis accounted for centroid size (factor "size"). Significant results are highlighted in bold.

	Df	SS	MS	F	Pr(>F)
Size	1	0.280	0.280	456.529	**0.001**
Cave	26	0.144	0.006	9.045	**0.001**
Year	2	0.108	0.054	88.057	**0.001**
Cave*Year	37	0.077	0.002	3.398	**0.001**
Residuals	1728	1.060	0.001		
Total	1794	1.669			

Table 3 : Results of the DFA - Percentage of fish well classify using the year as variable

Predict year	Year of origin		
	2012	2013	2014
2012	446	61	0
2013	72	538	0
2014	0	0	678
Total	518	599	678
fish well classify (%)	86%	90%	100%

Table 4 : Results of the ANOVA run with STATISTICA on water temperatures data from 2012 to 2014. Significant results are highlighted in bold.

	Df	SS	MS	F	Pr(>F)
Intercept	1	6251.880	6251.880	6271.019	**0.0001**
Année	2	10.866	5.433	5.450	**0.0001**
Cave(Année)	48	238.605	4.971	4.986	**0.0001**
Error	294	293.103	0.997		

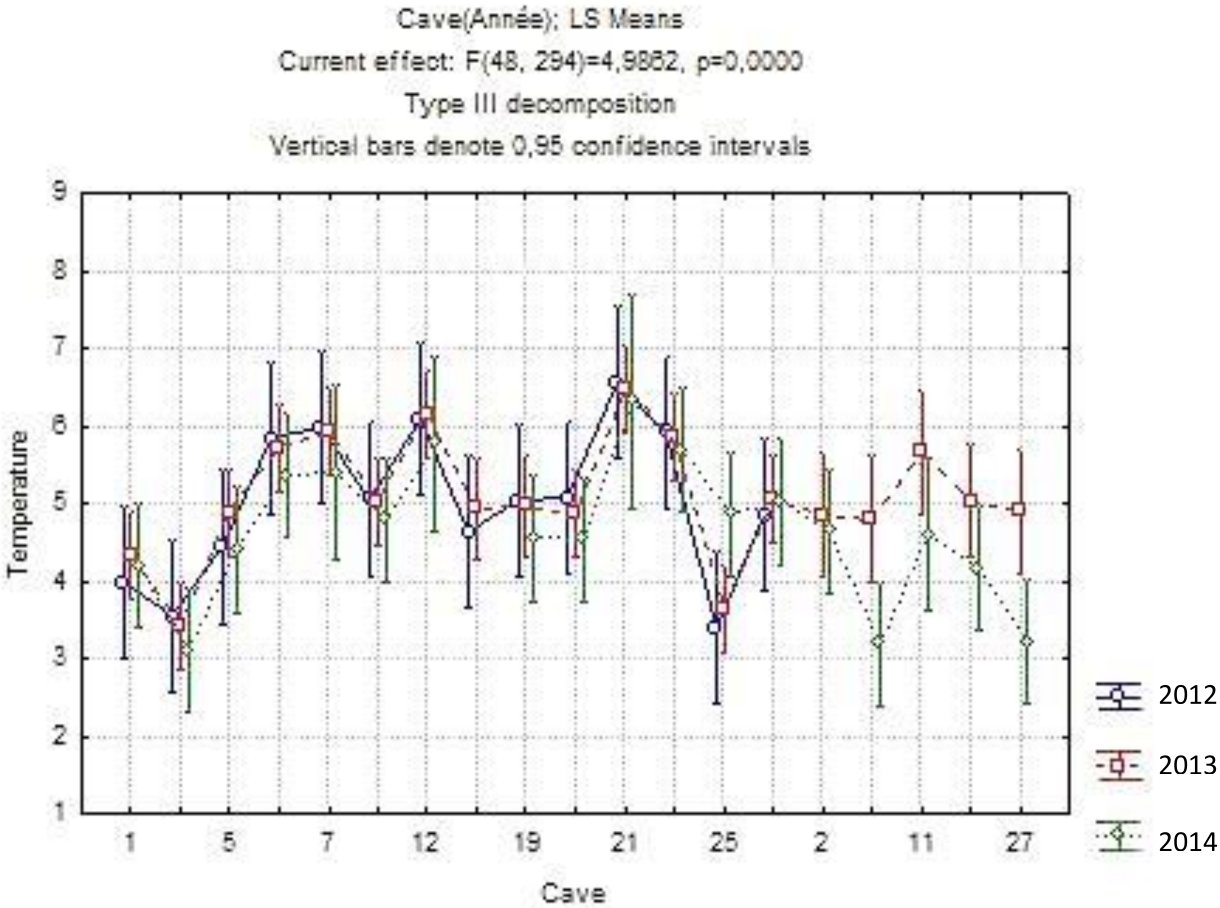

Figure 17 : Caves water temperatures from 2012 to 2014 – Graph of the ANOVA run with STATISTICA

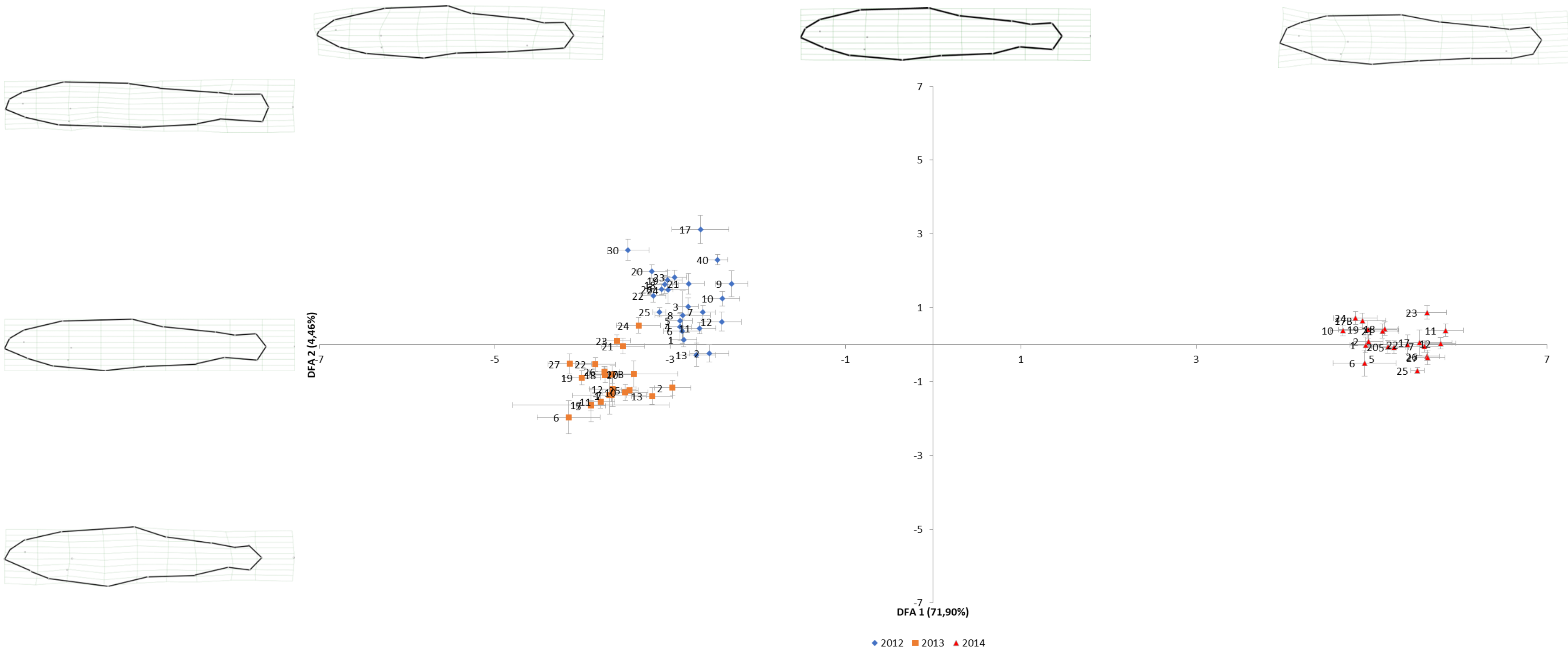

*Figure 18 : Discriminant Function Analysis results using cave*year as variable (function 1 explains 71.90% and function 2 explains 4.46% of the variation*

Average body shape between caves varied more in 2012 than in 2013. In 2014, we saw the least variation in average body shape between caves (table 3). We did not have corresponding data concerning the diversity of terrestrial matters falling into the caves for this period therefore we could not check if average body shape was connected to terrestrial food availability at this time. Nevertheless, we tested a potential correlation between the average temperature and the number of flies caught in the fall down traps from 2014 to 2016. This correlation was not significant (table 5).

Table 5 : Pearson correlation matrix between the volume of terrestrial material matter in each trap and the average water temperature from 2014 to 2016. Significant results are highlighted in bold.

		Volume of terristrial organisms in each trap (ml/trap)	Average temperature from 2014 to 2016
Volume of terristrial matter in each trap (ml/trap)	Pearson correlation (r)	**1**	
	p-values	**0**	
	R^2	**1**	0,147
Average temperature from 2014 to 2016	Pearson correlation (r)	-0,383	**1**
	p-values	0,617	**0**
	R^2	0,147	**1**

We predicted that there will be average body shape variation in time. Habitats and morphology of the caves differ and consequently, there are differences in fish average body shape among populations.

We found that there are also significant variation in body shape in space (e.g. between the caves figure 18, table 2) and that the thickness of the fish is the most important variation.

Fish populations that are geographically the closest also showed more similar average body shape (e.g. caves 18-19, figure 18). Nonetheless, each year, caves with similar body shape were not the same (e.g. caves 23 and 21 had similar average body shape in 2013 but in 2014 average body shape in cave 21 were closer to cave 18 (figure 18).

We found also that the interaction of cave and year was significant (table 2). This means that there are average fish body shape variations between years and between caves and that this variation goes in different directions (figure 18).

In cave 7, 40% of fish were classified in cave 25, confirming that cave 7 and 25 are physically and genetically connected (Leblanc *et al*. 2017, in prep), and that fish from cave 7 and cave 25 have very similar body shape.

Cave 25 has the best classification score (75%) but also this is the population with the highest number of sampled individuals (table 6). This indicates that fish from cave 25 have less variation in fish body shape (see SD in figure X). Nevertheless many fish from other caves were assigned to cave 25. There are then caves 23 (66%), 1 (58%) and 2 (57%).

Table 6 : DFA results – Percentage of fish well classified in their cave of origin based on their individual body shape

Theoritical cave	Original cave																										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**17**	**17B**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**30**	**40**
1	26	11		1					1			1					1	1					1	2			
2	7	43	1	2	2		1			1	2	2				2		3	2				8	3	1		
3			7																1					1		1	
4				10	59																		1	3	10		
5		1			4	3	4			10	8	2	1		2	4		3	1		3		8		2		
6	1					13				2	4											1	4				
7							31			4	3			1	1	2				1	2		8	1			
8		2		1				3	1		1					1	1							1			1
9								1	6	1											1						
10	1				7	3				26	4	1			2		1	2		2			1	2			
11		2			5	1	4		1	2	13	3	1			2		1			1	1	5	2			
12			1		1		3			1		21	1			1				1	1		5				1
13	1				2		1			2	3	1	6										2				1
17														2		1							1				
17B							1			1				1	3		3	4						1			
18	1	3			1		1				4	1		4	5	42	2	6	2	1	5	3	4	2		1	
19					5									1	1	1	17	5	1	3	1		3	2	1	1	1
20	1	1				1	2			2	3			1	2	5	6	40		4	2	1	3	2	2		
21	1	2			3		2	1						1		1	1		39	7	1	5	4	1			
22							3				5					2	2	1	7	46	1	6	6	6		1	
23		1	1		2		3				1	3		2		4	3	3	4	7	71	2	11	5	1		
24											2	16	1			1			2	2	3	19	4				
25	5	5	2	1	19	6	41			12	28		3	4	3	13	8	10	5	4	8	8	283	16	6		
26	1	4			6		5		1	1	2			1	3	7		1	1	2	5	3	14	71	2		1
27										2								3	1	1	1		2	2	24		
30														2		1					1			1		5	1
40		1			1							1					3		1	1			1				42
Total	45	76	12	15	117	27	102	5	10	67	83	52	13	20	22	90	48	83	67	82	107	49	379	124	49	9	48
Fish well classify	58%	57%	58%	67%	50%	48%	30%	60%	60%	39%	16%	40%	46%	10%	14%	47%	35%	48%	58%	56%	66%	39%	75%	57%	49%	56%	88%

In the other hand, some cave had very low classification score such as caves 7, 11, 17B and 17 with respectively 30%, 16%, 14% and 10% (table 6). This indicates that they are more fish wrongly classified for those populations. We checked if there was a possible correlation between the total number of fish caught in each cave and the classification efficiency but there was no significant correlation (table 7 and 8). This mean that the number of fish caught do not affect the observed average body shape.

Due to missing data caves 3,4,9 and 13, and from the lake have a great percentage of fish well classified (table 6).

Table 7 : Pearson correlation matrix between the number of fish caught and the percentage of fish well classified. Significant results are highlighted in bold

		Number of fish caught	Fish well classify (%)
Number of fish caught	Pearson correlation (r)	**1**	0,105
	p-values	**0**	0,408
	R^2	**1**	0,011
Fish well classify (%)	Pearson correlation (r)	0,105	**1**
	p-values	0,408	**0**
	R^2	0,011	**1**

*Table 8 : DFA results - Percentage of fish well classified using cave*year as variable*

Year	Cave	Fish well classify (%)	Number of fish caught	Year	Cave	Fish well classify (%)	Number of fish caught
2012	1	72%	18	2013	13	100%	3
2012	2	56%	18	2013	17	25%	4
2012	3	75%	12	2013	18	43%	28
2012	4	53%	15	2013	19	38%	16
2012	5	46%	28	2013	20	52%	27
2012	6	58%	12	2013	21	50%	18
2012	7	68%	31	2013	22	65%	26
2012	8	80%	5	2013	23	62%	52
2012	9	60%	10	2013	24	53%	15
2012	10	50%	22	2013	25	66%	151
2012	11	55%	20	2013	26	58%	38
2012	12	53%	17	2013	27	76%	17
2012	13	31%	10	2013	17B	40%	10
2012	17	71%	7	2014	1	79%	19
2012	18	52%	25	2014	2	67%	27
2012	19	26%	19	2014	5	50%	34
2012	20	73%	26	2014	6	57%	7
2012	21	50%	22	2014	7	59%	39
2012	22	69%	35	2014	10	56%	25
2012	23	87%	23	2014	11	61%	31
2012	24	89%	9	2014	12	67%	27
2012	25	71%	52	2014	17	56%	9
2012	26	88%	25	2014	18	68%	37
2012	30	56%	9	2014	19	85%	13
2012	40	79%	48	2014	20	53%	30
2013	1	38%	8	2014	21	70%	27
2013	2	71%	31	2014	22	76%	21
2013	5	47%	55	2014	23	75%	32
2013	6	63%	8	2014	24	52%	25
2013	7	16%	32	2014	25	79%	176
2013	10	35%	20	2014	26	85%	61
2013	11	41%	32	2014	27	62%	26
2013	12	50%	8	2014	17B	42%	12

We also predicted that food availability and diversity vary from a cave to another. This factor has an impact on fish body shape. We predicted that:

- **Food diversity will result in increased body shape variability;**
- **Caves with fewer or smaller openings have less food availability and diversity, resulting in thinner fish.**

Table 9 : Results of the Pearson test between food diversity and body shape variability

		Macroinvertebrates taxa diversity	Fish well classify (%)
Macroinvertebrates taxa diversity	Pearson correlation (r)	**1**	-0,007
	p-values	**0**	0,979
	R^2	**1**	0,000
Fish well classify (%)	Pearson correlation (r)	-0,007	**1**
	p-values	0,979	**0**
	R^2	0,000	**1**

We found that food diversity did not correlate with body shape variability. Indeed, there was no significant linear correlation between the number of wish well classified in each cave and the number of taxa found at the bottom of each cave (table 9).

Table 10 : Results of the Pearson correlation matrix between local ecological factors and food diversity as the number of taxa found at the bottom of the cave. Significant results are highlighted in bold

Variables	Terrestrial organisms (ml/trap)	Number of openings	Vertical opening (m²)	Surface exposed to sky (m²)	Macroinvertebrates taxa diversity
Terrestrial organisms (ml/trap)	**1**	-0,275	-0,327	0,298	0,003
Number of openings	-0,275	**1**	0,382	-0,406	0,367
Vertical opening (m²)	-0,327	0,382	**1**	-0,052	0,198
surface exposed to sky (m²)	0,298	-0,406	-0,052	**1**	-0,459
Macroinvertebrates taxa diversity	0,003	0,367	0,198	-0,459	**1**

Furthermore, we found that food availability and diversity did not correlate with the geomorphology of the cave (table 10) (e.g. number of openings, vertical opening and surface exposed to sky), meaning; caves with fewer or smaller openings did not have less food availability and diversity. There was no significant correlation between body shape and the tested ecological factors.

4 DISCUSSION

The first objective of this project was to study average body shape variation in space (among the caves) and time (over the years). Secondly, it was to test the relationship between morphological variation (if it exists) and ecological factors such as food availability and diversity (macroinvertebrates taxa diversity), cave morphology (number and size of opening) or water physical and chemical parameters.

Our main hypothesis was that there is significant variation in body shape in connection with local ecological factors in each cave at a specific time. Then, we forecast that habitats and morphology of the caves differ and consequently, there are differences in fish average body shape among populations. To finish, we supposed that food availability and diversity vary from a cave to another impacting fish body shape.

4.1 METHODOLOGICAL APPROACHES AND RECOMMENDATIONS

First of all, I remarked that several fish had their mouth opened on the pictures I used for the landmarks based geometric morphometric approach impacting the position of landmarks 17 (located in the upper tip of the snout) and 19 (located in the posterior point of the maxilla) mostly. Even if fish were unbended, it could have affected the fish body shape, mostly snout shape and maybe the fish classification according to the Discriminant Function Analysis. From my experience, I know that it is sometimes complicated due to the meteorological conditions and demands a lot of patience to have a good fish picture with a mouth closed. I would suggest being even more meticulous during this step to ensure that all fish have a close mouth in order to assess head shape. Nevertheless, I analysed the first years of the study and the protocol to take picture is getting better with the experience of the researchers.

Then, it was sometimes complicated to put landmarks 6 and 9 (ventral and dorsal insertion of the caudal fin). On some pictures, it was quite easily to put precisely these landmarks due to the high luminosity of the picture or because the fish were mature and consequently their caudal fin were coloured. Nevertheless, most of the time it is not easier to find the insertion of the caudal fin and it required me a lot of training to become precise.

We did the analysis with individuals 45mm and above, which remains small fish. I noticed that the smaller the fish the blurrier the picture was (at least most of the time). Therefore, I would expect that the smaller fish the less reliable the landmarks are. Water around small fish may also be an additional issue and participate in less precision in landmark placing. Cave 25 had a lot of small fish, and its average body shape could have been impacted by the fact that this population may have more young fish than other populations. This could also explain why so many other fish from other caves were wrongly classified into that particular cave. Moreover, we can forecast that fish of this size have not their adult size yet or that their body shape is different than bigger or mature fish. In any case, I recommend to take closer (and therefore more focused) picture of the younger fish to ensure that the picture quality is as good as with larger fish. Using an adjustable lens and not a fixed lens maybe the best option.

To me, the number of blurred pictures (mostly photographs of fish with a length between 45 mm and 665mm) or pictures of fish with the mouth opened could have impacted the body shape of individuals and therefore the percentage of fish well classified in their originated cave. Thus, I recommend adding a column on the ID list with the picture quality and the size of the fish. For the next studies it will be thus easier to remove bad pictures and/or smaller fish from the geometric morphometric data-sets or to analyse if they have a significant impact on the average fish body shape during analysis.

It should be also noticed that landmarks were put on pictures by three different persons (one person by year, I did the year 2013). Even if the position of landmarks should be repeatable, differences between the years could be due to operators switching. That is why it will be better if the whole landmarks were put by the same person. Then, it could have some differences in the accuracy of putting landmarks on the pictures according to the experience of the operator and for how he is doing it. For the first pictures I digitized, due to my lack of experience, I could have made some mistakes. For the last pictures I digitized, I was much more experienced but also less concentrated. It is important to not digitize too much pictures in a raw and during the day in order to stay focus and put landmarks very precisely.

I also noticed some obvious shape differences concerning the anal fin among fish. This characteristic cannot be visualized with the geometric morphometric approach used here. Indeed, here we marked only the posterior and ventral insertion of the anal fin and differences came mainly from the total shape of the fin.

We could have not run statistical analysis using directly geometric morphometric data. This step demanded must more practice and skill on R with the geometric morphometric package. Moreover, none researcher were familiar with it.

Then, from 2012 to 2014 there are a lot of missing data. Only temperature was recorded since 2012 while other ecological data were recorded for the first time in 2014. That is why we could not study local ecological factors variation among these years. Moreover, it missed some data for some caves (e.g. the surface exposed to sky and the surface of the vertical openings of 26) resulting in statistical tests that did not take into account all the caves. It may have affected our results. Consequently, there were too much missing data to answer completely to our hypothesis.

4.2 VARIATION IN BODY SHAPE IN SHAPE AND TIME

First of all, we found that variations in space and time in fish body shape were mostly variations in the thickness of fish. Thus, shape variations may result in diet variations in time and space.

Fish were stockier in 2013 and 2012 and thinner in 2014. From 2012 to 2014 we had only temperature data, nevertheless, we found that average water temperatures were also higher in 2013 and lower in 2014 and according to Luoto *et al*. (2014) work, water temperature is a key variable in determining chironomids distribution.
Moreover, we know that temperature affects the metabolic rate of ectothermic animals (e.g. fish). Metabolic rate is low at cold temperature and high at hot temperature (Campbell, 2017). At warmer temperatures (e.g. in 2013), fish metabolic rate is higher which may result in higher consumption of food and in stockier fish.

Nevertheless, field trips were not realized at the same time each year. Indeed, in 2012 and 2013 they were conducted quite at the same time, during the third and the fourth weeks of June, while in 2014 they were conducted during the first and the last weeks of June. Field trips in 2014 were maybe too early in the season and food availability was maybe not as available as in 2012. There are also meteorological condition variations among and over years. It could have impacted chironomids growing time and consequently food availability in the caves and also fish growth. To me, it is very important to do the field trips at a similar time from the peak of midges which obviously varies every year with the environmental conditions. We know that if there is no food (e.g. no flies at the surface of the water), it is very hard to catch the fish. Fish do not move, so the indication that we catch fish means that fish are feeding on terrestrial food (pers. comm. Leblanc).

According to the ecological data, caves that are closed to each other have more similar local habitats, which may result in similar fish body shape in those populations. We can suppose that the whole habitat influence fish body shape. Habitats rarely differ in only a single ecological factor and various aspects of fish's local environment can be agents of selection on body shape (Landy and Travis, 2015).

We found also that the interaction of cave and year was significant. Which means that there are average fish body shape variation between years and between caves and that this variation goes in different directions. This may indicates the importance of local ecological factors for fish body shape in interaction with others factors (heritability of body shape, evolutionary forces at play within each population).

Fall down traps allowed us only to compare how many terrestrial organisms fall in the surface of the water at each opening. It is an interesting data to compare food availability and distribution among years. Nonetheless, it does not allow us to compare food availability among caves. We found that there is no significant correlation between the number of openings and the surface exposed to cave with the number of terrestrial organisms caught into the trap. But, it does not mean that caves with more openings have more food availability (or controversy). The number of terrestrial organisms falling into the surface of the water could be also related to the exposition of the openings (e.g. vertical or horizontal openings or if it is located in a windy area or not). In my opinion, and further to the field trips, more flies are flying around openings less windy and more flies fall at the surface of the water.

Macroinvertebrates diversity, corresponding to our food diversity data-sets (taxa diversity) were recorded only on stone that we brushed. Thus, only one substrate presents in the cave is represented. It could be interesting to sampled also benthic macroinvertebrates on the others substrates to have an overview of the food diversity more completed. Thus, I recommend to sample the bottom of the cave and the vegetation.

In each cave, the number of fish fished over the three years varies drastically. For example, in total 379 fish were sampled in caves 25 and 107 in cave 23. For comparison, 20 and 45 fish were sampled in caves 17 and 1. On the first hand, we can forecast that it affects the mean shape of caves with less data and that it does not represent totally the population. But we know that the number of fish catch is positively correlated with estimates of population sizes (Leblanc, 2016), and thus, we are capturing a large amount of each population.

Then, caves studied in this project have been selected to represent their diversity in size and morphology. It is possible that caves are connected to caves not studied here. Consequently, it could explain why some populations have more body shape diversity. Caves are difficult study systems. Indeed, their entire geomorphologies have still not been mapped and it looks complicated to do it. Caves may have passages or areas that we cannot see and properly map.

Our analysis of spatial variation in body shape revealed that fish from caves 7 and 25, which we knew were physically connected (fish movement between the two), did not differ in average body shape. However this was not the case for the other physically and genetically connected caves. We knew that caves 1-2, 5-11, 17-18 and 19-20 are connected but we still show differences in average body shape between these pair of caves.

This may reveal that only a few of individuals pass from one cave to another. The majority of the fish are resident into both caves, and therefore the average fish body shape still differs between the two caves. This may indicate again the importance of local ecological factors for fish body shape in interaction with others factors.

CONCLUSION

The main objective of my project was to study average body shape variation of Arctic charr populations in space (among the caves) and time (over the years). Secondly, it was to test the relationship between morphological variation (if it exists) and ecological factors such as food availability, cave morphology (number and size of opening) or water physical and chemical parameters.

This study was part of a bigger one which is running at Holar University College. Its aim project is to increase the understanding of the importance of spatial and temporal variation in evolutionary parameters for diversity and increase the knowledge of the relationship between ecological and evolutionary factors, in maintaining and developing biological diversity (Leblanc, 2016).

We found that there is a significant variation in average body shape among caves and years and that the thickness of the fish is the most important variation.
Concerning time variation, we found that fish were finer at colder temperatures (and controversially). Colder temperatures may results in lower fish metabolism, lower food consumption and thus, finer fish. Temperature variations could also have impacted chironomids growth and distribution.
Then, we found also that caves that are closed to each other have more similar local habitats, which may result in similar fish body shape in those populations.
Moreover, we found that the interaction of cave and year was significant. This means that there are average fish body shape variations between years and between caves and that this variation goes in different directions. Then, we found that most of the caves that are physically and genetically connected showed differences in average body shape. It may indicates the importance of local ecological factors for fish body shape in interaction with others factors
Finally, we found that food diversity (macroinvertebrates taxa diversity) did not correlate with body shape variability and that food availability and diversity did not correlate with the geomorphology of the cave.

This work allowed us to have a first overview of body shape variation among years, and among the caves, and increased our understanding of how body shape varies with ecological factors. This first analyse and these first results will guide further research on this system. We know now, that the thickness of the fish is the most important shape variations and it will take into account for the further analysis. Shape study and the geometric morphometric approach are quite new in ecological research resulting in new study possibilities and discoveries.

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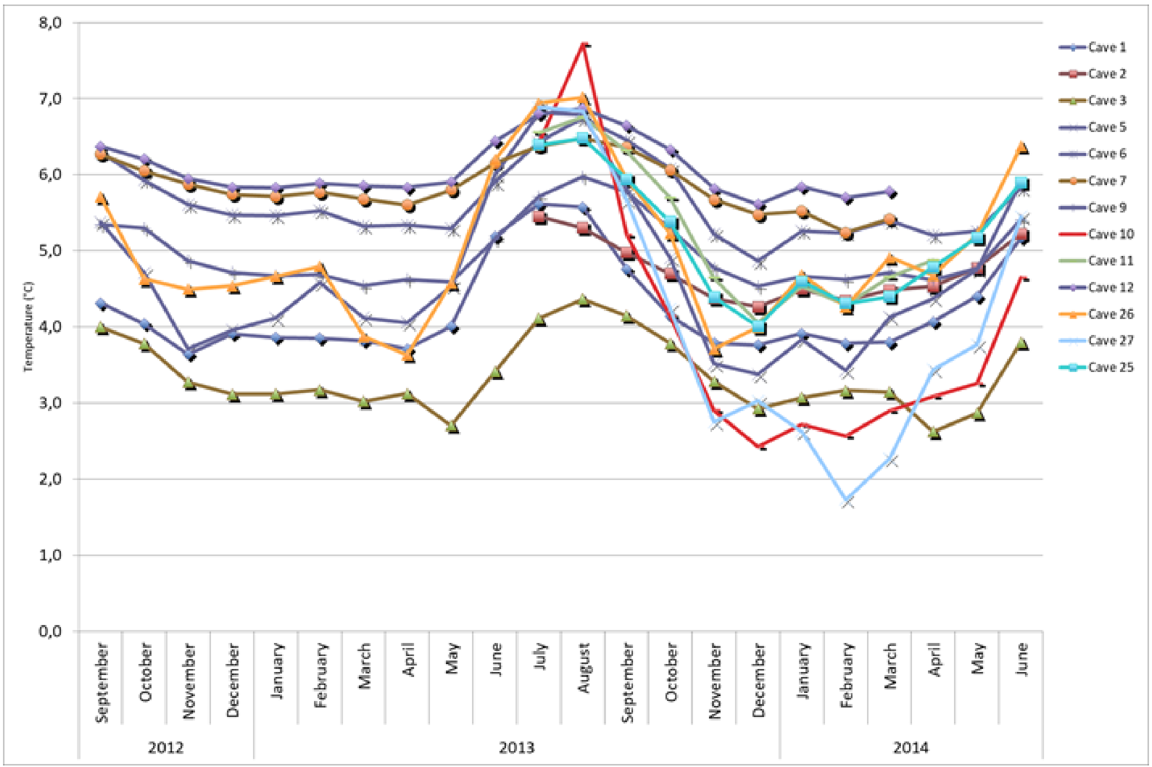
LIST OF THE FIGURES

LIST OF THE TABLES

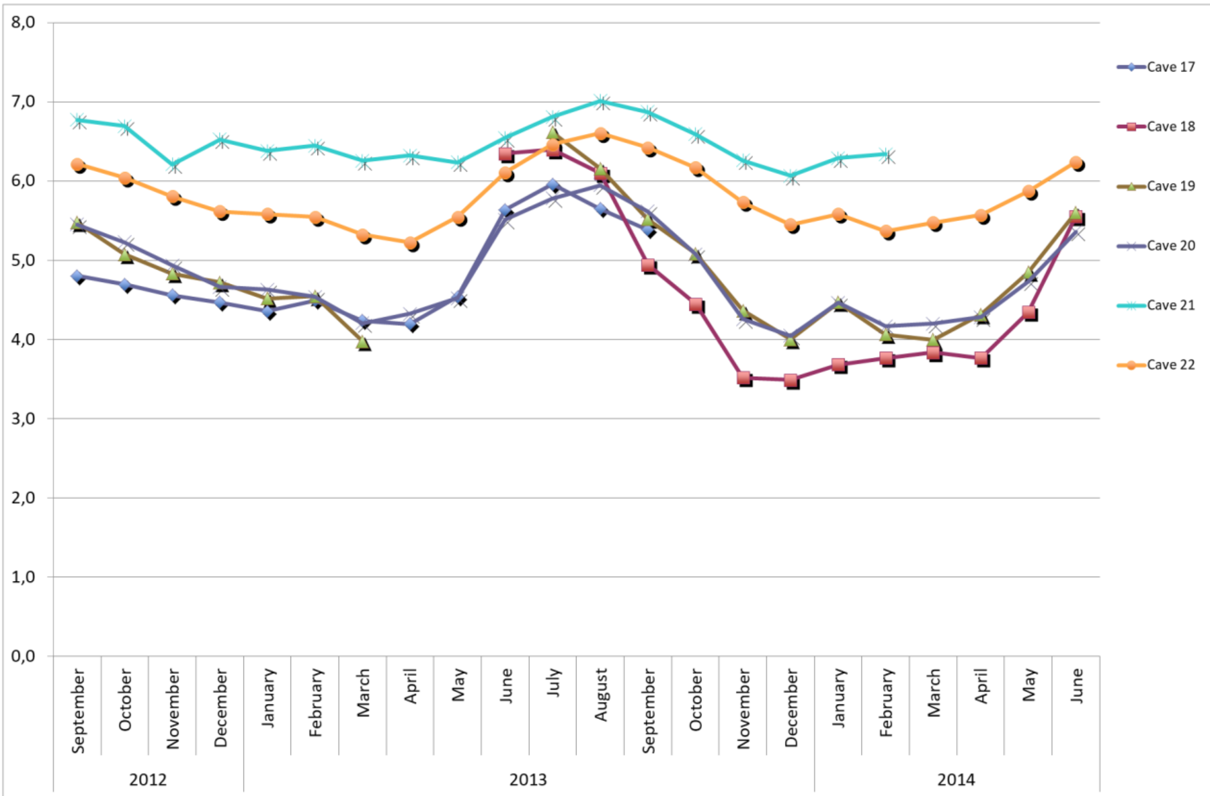
APPENDIX

DFA axes	Amount of shape variation attributable
1	0,719085746
2	0,446619021
3	0,031435184
4	0,027730944
5	0,025049681
6	0,019674141
7	0,016649181
8	0,015432913
9	0,013175819
10	0,010471408
11	0,008386039
12	0,006788013
13	0,006429403
14	0,005856773
15	0,00520077
16	0,004757045
17	0,004417309
18	0,003996655
19	0,003407498
20	0,00327685
21	0,002702776
22	0,002487096
23	0,002310965
24	0,002110473
25	0,001916562
26	0,001787029
27	0,001787029
28	0,00158244
29	0,001358613
30	0,00120633
31	0,00110076
32	0,001020651
33	0,000502587
34	0,000626229
35	0,000638468
36	0,00054817
37	0,000463167
38	0,000428124
39	0,000373282
40	0,000311352
41	0,000284108

*Appendix1: Shape variables explaining shape variation using cave*year e as variable*



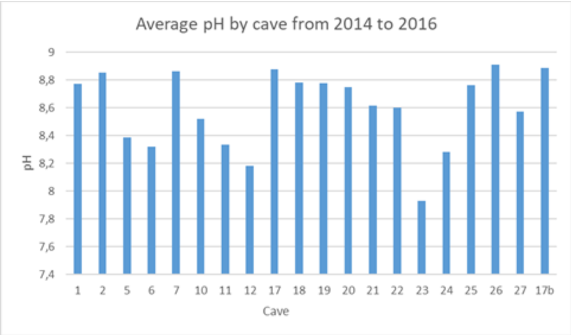
Appendix 2 : Average water temperature in lava cave in Haganes from 2012 to 2014. Data were obtained with a HOBO data logger recording daily temperature all year around (Leblanc, 2016)



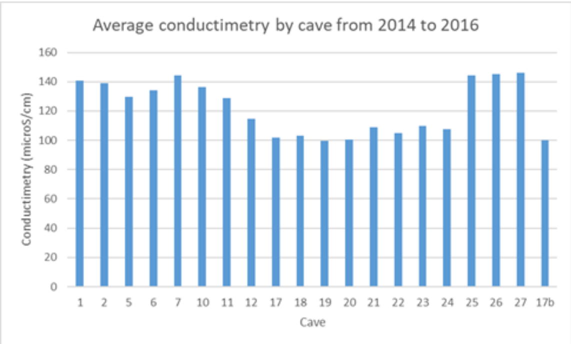
Appendix 3 : Average water temperature in lava cave in Vindbelgur from 2012 to 2014. Data were obtained with a HOBO data logger recording daily temperature all year around (Leblanc, 2016)

Cave	Temperature (°C)	pH	Conductivity (S/com)	Turbidity (NTU)	Dissolved oxygen (mg/L)	Surface exposed to sky (m²)	Number of openings	distance to the lake (m)	Flies caught (%)	Microinvertebrates density (ind/m²)	Location
1	7,10	8,77	140,88	0,08	8,29	0,04	2	170	68,5	4,8	Haganes
2	5,84	8,85	139,06	0,00	7,86	1,80	1	174	50,9	3,6	Haganes
5	7,08	8,39	129,75	6,36	7,60	0,36	5	110	62,3	6,2	Haganes
6	6,75	8,32	134,30	1,83	5,27	0,00	1	102	58,7	9,5	Haganes
7	6,42	8,86	144,10	5,10	5,59	8,85	1	117	72,4	5,8	Haganes
10	6,91	8,52	136,35	61,61	8,11	1,41	7	166	52,2	8,7	Haganes
11	6,53	8,33	128,71	6,11	7,11	0,08	2	139	62,4	6,8	Haganes
12	7,20	8,18	114,74	150,10	6,51	7,83	1	90	50,7	11,4	Haganes
17	6,03	8,88	101,99	5,18	10,95	2,50	1	378	43,9		Vindbelgur
18	6,46	8,78	103,14	8,41	10,23	8,84	3	406	45,2	8,4	Vindbelgur
19	6,19	8,78	99,47	2,71	10,17	2,55	2	236	62,2	9,5	Vindbelgur
20	6,57	8,75	100,50	13,53	10,63	0,78	4	172	47,1	5,4	Vindbelgur
21	7,31	8,61	109,05	0,00	7,48	11,64	1	61	47,4	9,7	Vindbelgur
22	6,64	8,60	104,89	7,95	7,60	6,04	2	110	56,9	10,3	Vindbelgur
23	7,23	7,93	109,67	5,13	7,73	2,24	2	174	51,2	14,5	Vindbelgur
24	6,64	8,28	107,44	140,63	6,38	0,80	1	162			Vindbelgur
25	6,76	8,76	144,16	4,77	6,57	37,45	2	139	58,2	11,7	Haganes
26	6,92	8,91	145,24	0,15	5,12		3	102	53,5	9,9	Haganes
27	6,44	8,57	145,93	2,95	7,67	2,66	2	57	61,8	7,8	Haganes
17b	5,69	8,89	100,18	45,24	10,24	4,89	12	1	56,6	7,0	Vindbelgur

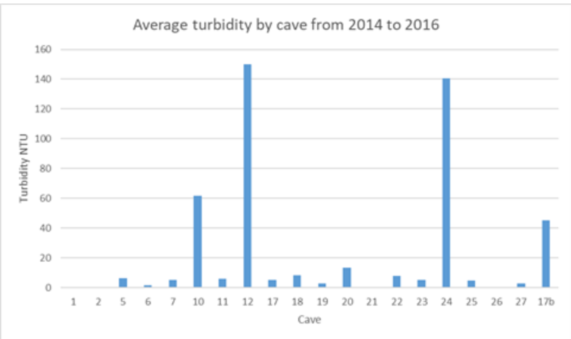
Appendix 4 : Ecological data from 2014 to 2016 and geomorphological data used. The geomorphologies of the caves were described in 2012.



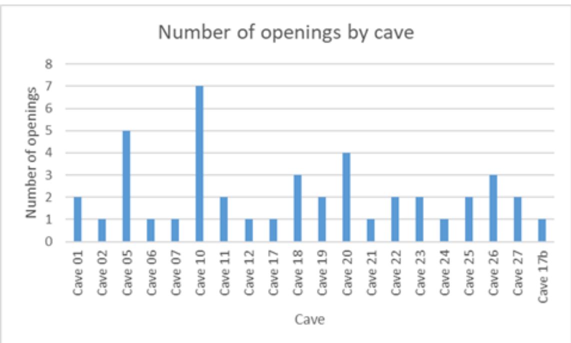
Appendix 5 : Average pH by cave from 2014 to 2016



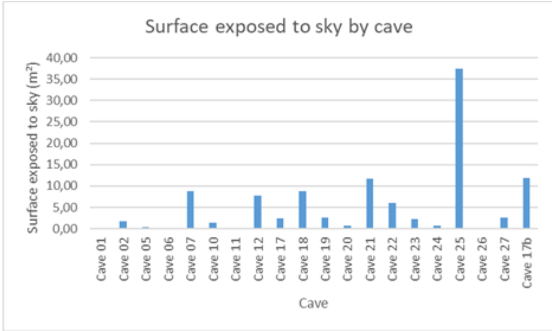
Appendix 6 : Average conductivity by cave from 2014 to 2016



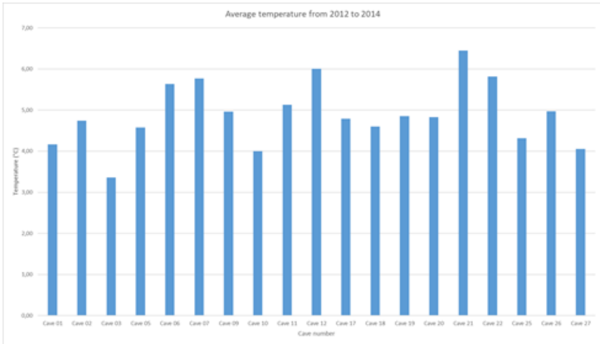
Appendix 7 : Average turbidity by cave from 2014 to 2016



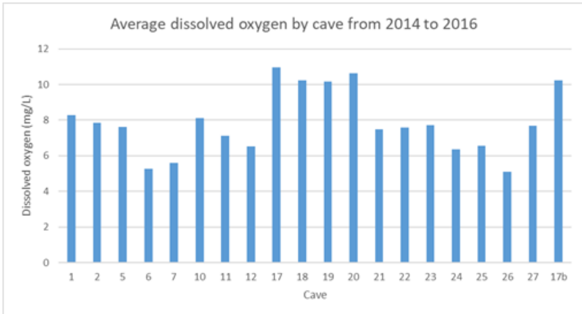
Appendix 8 : Number of openings by cave



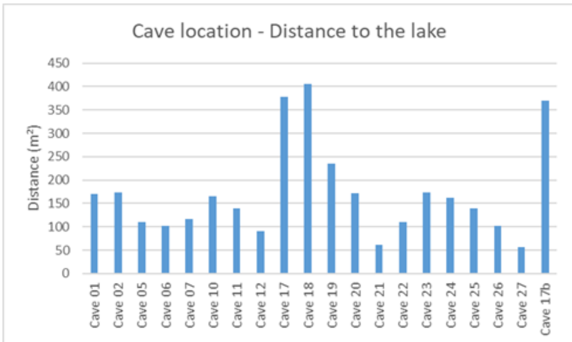
Appendix 9 : Surface exposed to sky by cave



Appendix 10 : Average water temperature by cave from 2014 to 2016



Appendix 11 : Average dissolved oxygen by cave from 2014 to 2016



Appendix 12 : Cave location – Distance from the lake

SUMMARY