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Photographic identification of Arctic Charr, *Salvelinus alpinus*, based on spot patterns

A method to assess Arctic Charr populations sizes

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Key words: Arctic Charr; Spot patterns; Mývatn; Population size; Lava cave
Sauðárkrókur; Norðurland vestra; Iceland

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Abstract

Since 2012, Hólar University college researchers have been monitoring 16 isolated population of small benthic Arctic charr living in 20 water-filled lava caves near lake Mývatn, NE Iceland. The objective is to study the micro-evolutionary processes that shape phenotypic and genetic diversity in these small populations. The scientists have collected as many individuals as possible in each of those caves and fish longer than 45 mm at capture have been tagged with a PIT-tag. Unfortunately, it is quite common that the researchers encounter some fish which have lost their tags. I tested a method for reassigned fish that have lost their PIT-tag. This method is called manual photographic identification, using the natural marks present on the fish flank to identify a fish. This method was very efficient but it demanded a lot of time with larger population. The method allowed me to estimate a realistic population size in all the lava caves. Then I could verify if population size is related to local ecological factors. The results showed that there was no relationship between local ecological factors and the population sizes.

Résumé

Depuis 2012, les chercheurs de l'université de Hólar étudient 16 populations isolées d'une forme naine d'Omble chevalier vivant dans des grottes volcaniques situées à proximité du lac de Mývatn. L'objectif de l'étude est de comprendre les processus de microévolution dans ces petites populations. Les chercheurs ont collecté autant d'individus que possible dans chaque grotte et tous les poissons supérieurs à 45 mm de long ont été marqués avec des micro-puces électroniques. Mais il est fréquent de rencontrer des poissons ayant perdus leurs puces. J'ai testé une méthode pour identifier ces poissons ayant perdu leur micro-puce. Cette méthode s'appelle l'identification photographique manuelle et elle utilise les marques naturellement présentes sur le flanc du poisson pour l'identifier. Cette méthode a été très efficace mais elle a demandé beaucoup de temps sur les grandes populations. La méthode m'a permis d'avoir une réelle estimation de la taille des populations dans les grottes. Ensuite j'ai pu vérifier si la taille des populations était liée aux facteurs environnementaux locaux. Les résultats ont montré qu'il n'y avait pas de relation claire entre les facteurs environnementaux locaux et les tailles des populations.

Preamble: Short report version (French version)

Préambule : Version simplifiée du rapport (Version Française)

Identification photographique d'Omble chevalier, *Salvelinus alpinus*, à l'aide de la disposition des marques sur le flanc

Une méthode pour estimer la taille des populations

Contexte : Depuis 2012, les chercheurs de l'université de Hólar étudient 16 populations isolées d'une forme naine d'Omble, *Salvelinus alpinus*. Ces populations vivent dans un système de 20 grottes volcaniques situées à proximité du lac de Mývatn, en Islande. Le but des chercheurs est de comprendre les processus de microévolution dans ces petites populations. Ainsi, des visites dans les grottes sont effectuées chaque année afin de prélever le plus de poisson possible. Chaque poisson mesurant plus de 45 mm de long a été mesuré et marqué avec des micro-puces électroniques implantées dans le ventre du poisson. Cependant, il est fréquent que les poissons perdent leur micro-puces. Pour remédier à ce problème, les chercheurs ont développé une méthode pour identifier ces poissons. Cette méthode consiste à comparer les marques naturellement présentent sur le flanc du poisson.

L'objectif de ce stage a été d'évaluer l'efficacité de l'identification photographique manuelle des Ombles chevalier à partir des marques naturelles. Ces identifications mon permis d'avoir une réelle estimation de la population dans chaque grotte. J'ai pu ensuite vérifier si la taille de la population était liée aux facteurs environnementaux comme les paramètres physico-chimiques ou encore, la disponibilité en nourriture.

Caractéristiques du lieu d'étude : Le lac de Mývatn est situé dans le Nord-Est de l'Islande. Il a été formé il y a environ 2 300 ans à la suite d'une grande éruption volcanique. L'eau du lac provient presque entièrement de source des nappes phréatiques volcaniques, avec une température et un pH constant et une grande quantité de minéraux. Dans les alentours, il y a de nombreuses grottes volcaniques remplies d'eau. Ces grottes sont probablement apparues en même temps que le lac de Mývatn. Les bassins présents dans les grottes sont en grande partie couverts, mais des ouvertures sont présentes à ciel ouvert (Image 1). Des organismes terrestres tombent dans ces ouvertures et viennent nourrir les poissons présents. Les grottes sont habitées par une forme naine d'Omble chevalier (Image 2 et 3). Ces poissons proviennent probablement du lac de Mývatn, mais aujourd'hui, les grottes ne sont plus connectées à celui-ci.

Image 1 : Photo de l'intérieur d'une grotte volcanique

Une forme naine d'Omble chevalier : Poisson de la famille des salmonidés, l'Omble chevalier est adapté aux eaux froides. Cette espèce présente une très grande diversité et il est fréquent de trouver plusieurs formes ou morphotypes dans un même lac. Les grottes volcaniques sont habitées par une forme benthique et naine qui est adaptée à vivre dans ces conditions particulières. Ils sont en général de petite taille (15 cm maximum), présentent une sombre coloration, ont des yeux plus larges que la moyenne et une ligne latérale protubérante (Image 2 et 3).

Travaille sur le terrain : Les Ombles chevalier présents dans les grottes ont été capturés à l'aide de pièges à Vairon, de verveux et par pêche électrique. La combinaison de ces méthodes est la plus efficace pour attraper les Ombles sans les blesser ou les tuer. Les poissons sont ensuite anesthésiés, pesés, mesurés et une photo haute définition du flanc gauche est prise pour tous les poissons supérieurs ou égale à 45 mm de long.

Ensuite, chaque poisson est scanné pour savoir si une micro puce est présente ou non dans le poisson. Si c'est le cas, le poisson est relâché dans la grotte, sinon, un échantillon de nageoire caudale est prélevé et une micro puce est insérée dans le ventre du poisson, ensuite le poisson est relâché. Les facteurs environnementaux ont été mesurés avec une sonde. Des pièges placés à l'entrer des grottes ont collecté les matériaux organiques tombant dans les grottes. La morphologie des grottes a été mesurée les années précédentes.

L'identification photographique : La première étape a été de déterminer si les poissons n'ayant pas de micro puce ont été capturés avant, lors de précédentes visites. Pour cela, j'ai vérifié si la nageoire caudale a été coupée avant ou non. Si non, cela veut dire que c'est la première fois que le poisson a été capturé. Si oui, le poisson a déjà été capturé lors d'une précédente visite et il a perdu sa micro-puce électronique. Je procédais alors à l'identification photographique de ce poisson (Image 2 et 3).

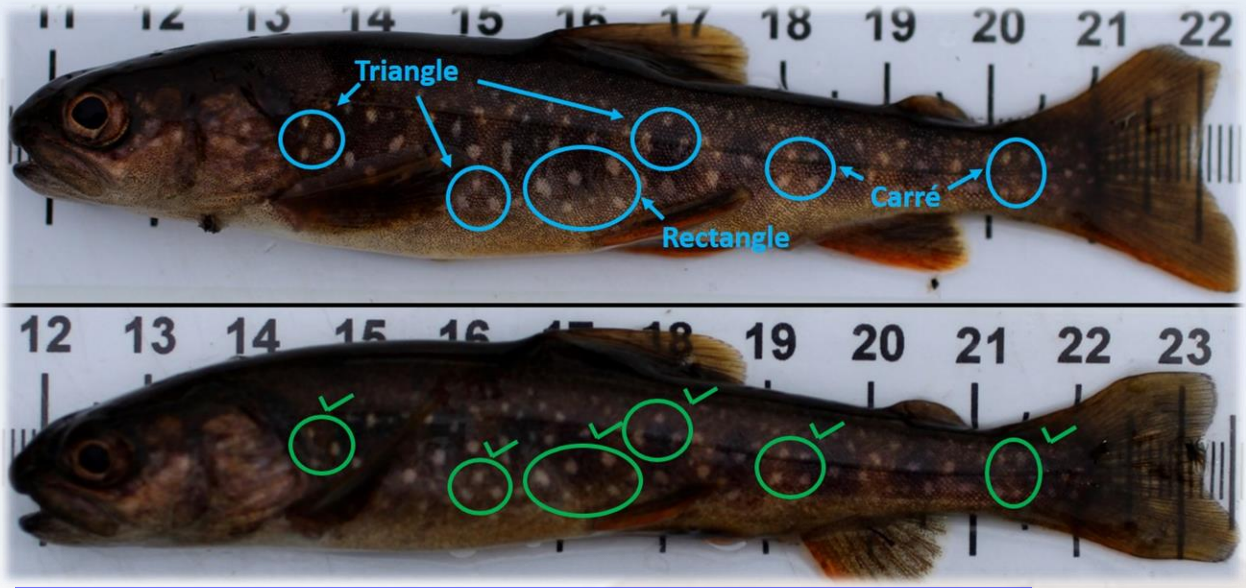

Image 2 : Identification photographique d'un Omble chevalier entre 2013 et 2012

Les marques naturelles sur le flanc des Ombles sont utilisées pour les identifier. Des formes géométriques communes peuvent être aperçus (triangle, rectangle, carré …) sur le flanc, facilitant l'identification (Image 2). Pour l'identification des plus petits poissons, j'ai utilisé les « parr marks » pour les identifier (Image 3). Les parr marks sont des taches noires présentes sur les jeunes salmonidés.

Image 3 : Identification photographique d'un Omble chevalier à l'aide des « parr marks »

L'identification a été beaucoup plus difficile pour les poissons ayant une taille comprise entre 65 et 80 mm de long car pendant cette période, les parr marks commencent à disparaitre et les points sur le flanc commencent à apparaitre. L'identification photographique a été très efficace sur les petites populations d'Omble chevalier et m'a permis d'avoir une réelle estimation de la taille des populations.

Mais lorsque les populations deviennent grandes, la méthode demande beaucoup trop de temps (environ 30 minutes pour identifier un poisson). Les tailles des populations estimées avec l'identification photographique étaient très proches de celles calculées avec d'autres méthodes (Peterson, Schnabel …).

Les facteurs écologiques : Après avoir calculé la taille des populations dans toutes les grottes de lave, j'ai vérifié si elle était liée aux paramètres environnementaux présents sut le lieu d'étude. Les résultats ont montré qu'il n'y a pas de relation claire entre les tailles des populations et les facteurs environnementaux locaux.

Ce qu'il faut retenir :

L'identification photographique manuelle de la forme naine de l'Omble chevalier à l'aide des marques naturellement présentes sur le flanc des poissons est une méthode efficace pour estimer la taille des populations. Cependant, elle demande beaucoup trop de temps sur les plus grandes populations. La mise au point d'un logiciel spécifique serait un moyen pour identifier les poissons plus rapidement.

La taille des populations ne semble pas être liée aux facteurs environnementaux étudiés ici. Il est plus probable que la taille des populations soit majoritairement liée au volume d'eau présent dans les grottes et à la disponibilité en nourriture présente dans l'eau. Des futures études permettront de tester ces hypothèse

Summary

Introduction

Since 2012, Hólar University College researchers have been monitoring sixteen isolated populations of small benthic Arctic Charr (*Salvelinus alpinus*). These fish populations inhabit in a system of twenty lava caves near lake Mývatn, in the North-Est of Iceland. This system is very peculiar because there are a high number of small and isolated populations distributed over a very small area (about 4 km^2) (pers. comm. C. Leblanc). Here, the main objective of the research project is to study the micro-evolutionary processes that shape phenotypic and genetic in these small populations. The Arctic charr, a fish of the *Salmonidae* family, is an ideal species to study these processes. Indeed, it shows a strong phenotypic, ecological and life history diversity within and among habitats (Klemetsen, 2010). One particular side of this diversity is the frequent occurrence of two or more distinct charr morphs in a same lake (Klemetsen, 2010).

The research project on these spatially replicated but unique populations of fish is divided in several parts. One important component is to look at the influence of local ecological factors on phenotype and fitness of individuals within and among caves (which in most cases are different populations). The researchers predict that the number of fish (N) and the number of breeders (*Ne*) in a given population is related to cave size and food availability (Leblanc, 2016). However, to be able to address this question (and other evolutionary questions) it is crucial to accurately estimate population size, which can be done by identification of captured and recaptured individuals in a given population.

Thus, in June and August each year, since 2012, the scientists have collected as many individuals as possible in each of those caves. Each Arctic charr longer than 45 mm at capture has been PIT (Passive Integrated Transponder)-tagged to be followed individually over time. Currently, about 1 600 individuals above 65 mm across all populations have been monitored (see appendix 3).

Unfortunately, it is quite common to encounter fish which have lost their tags. These fish are identified because their upper caudal fin has been clipped and/or a scar is visible on their flank. Larger fish seems to lose their tags at a higher rate than smaller ones. The issue of identifying individual fish that have lost their tags is not specific to this study but rather a recurrent issue in the study of population dynamics in which organisms need to be tagged and followed over a long period of time (pers. comm. Leblanc).

Because these populations are rather small in size and isolated (pers. comm. Leblanc), the researchers I have worked with are developing a method to identify fish that may have lost their tags. This method uses the patterns of spots found on the left side of the flank. Indeed, the positions and the shape of the spots are unique to each individual. Persat (1982) and Gifford (2014) have used a similar method to identify individual Grayling (*Thymallus thymallus*) and Westslope Cutthroat Trout (*Oncorhynchus clarkia lewisi*) by comparing the pattern of each natural marks presents on the flanks of the fish. This alternative method to identify a small number of fish is only applicable if the color of the spots contrasts well with the body color which is the case in small benthic Arctic charr (see method section).

The objective of my internship was to evaluate the efficiency of a manual identification of small benthic charr based on their spots patterns, from photographs. This was done in two selected populations that vary in size (two rather small connected caves, and two larger connected caves). This was primarily done on fish that were larger than 65mm (PIT-tagged fish). However, I also tested if similar method could be used on smaller fish (i.e. juvenile larger or equal to 45mm). If the method is efficient, it will allow me and the researchers to have a realistic estimate of population size in the caves. A second objective of my study was to study how population size in these lava caves is maybe related to local ecological factors.

The relationship between habitat variability and population size in fragmented habitats is poorly understood, but might have important evolutionary consequences (Wood et al., 2014). Wood et al (2014) found that means habitat parameters were significantly related to N and *Ne* in several populations of Brook trout (*Salvelinus fontinalis*). Similar results may be found for Arctic charr populations near lake Mývatn, and therefore could better explain local adaption of these small populations.

The first part of this report will present briefly the Hólar University College and the study system (lake and species). The second part will describe the material and the methods used in this study. Results will be presented for each of the research questions and objectives. Finally, I discuss the results and make recommendations about the use of this method for this research project and beyond.

1 - Presentation of the internship's place and of the small benthic Arctic Charr

1.1- Hólar University college (source: www.holar.is)

Hólar University college (HUC) is located in the valley of Hjaltadalur, in the mid-northern part of Iceland (Figure 1). The nearest town is Sauðárkrókur, located 32 km from Hólar, this is where the department of aquaculture and fish biology is located. The university is 322 km from Reykjavik, the Iceland's capital.

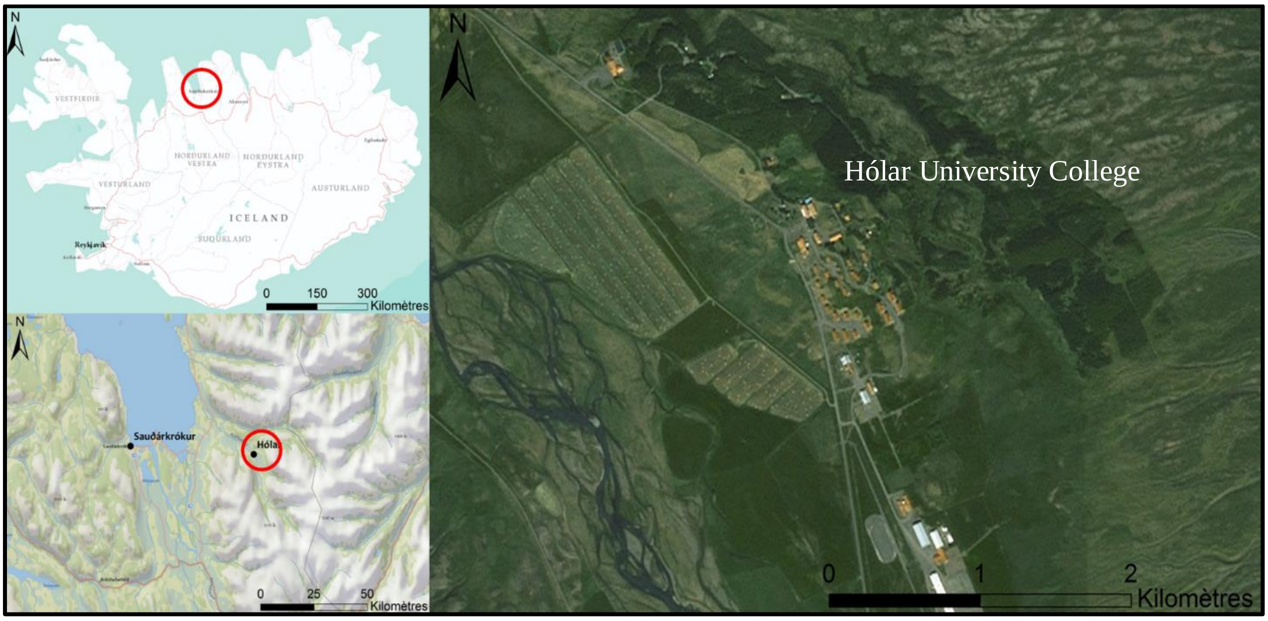

Figure 1: Location of Hólar University College (Source of the maps: National Geographic World Map, World Imagery and World Terrain Base)

The name Hólar means hills. The school was established in 1106, date of the foundation of Bishop Jón Ögmundsson's cathedral. This makes HUC one of the oldest school in Europe (pers. comm. Leblanc). In 1550, the school was converted to a Latin school that remained in operation until 1801. In 1882, an agricultural school was established at Hólar. During the past 20 years, the school at Hólar has developed from a conventional agricultural school to a modern university-level institution.

In 2003, the College was granted permission to graduate students with an undergraduate and graduate degrees, and on 1 July 2007, Hólar University College formally commenced operations. There are three academic departments in Hólar, the department of aquaculture and fish biology, the department of equine studies and the department of rural tourism.

I did my internship in the department of aquaculture and fish biology. These department facilities are mostly located in Sauðárkrókur and is composed of office space and fish rearing facilities. The main goal of this department is to gather and disseminate knowledge in the fields of aquatic biology, aquaculture, and fish biology. The staff is composed of academic researchers, technical and administrative employees, a total of 11 persons. More than 15 graduates' students and approximately 7 summer interns are also working in the department in summer 2016.

1.2- The lake Mývatn and the lava caves

The lake Mývatn (37 km²) is a eutrophic lake located in the North-East of Iceland (Figure 2). The lake is divided into two main basins, the North Basin (8.5 km²) and the South Basin (29.2 km²) The depth in the South Basin is between 3 and 4 m deep whereas in the North Basin is between 2 and 5.5 m deep (Einarson et al, 2004). The shape of the lake as we know it today was formed about 2300 years ago following a major volcanic eruption (Thorarinsson, 1951; Einarsson, 1982; Sæmundsson, 1991). A larger lake existed before the eruption but the lava covered a big part of the previous lake (Einarson et al, 2004).

Water enters the lake almost exclusively from springs along its east shore. Most of the spring are about 5°C but spring in the North Basin are warmer (up to 30°C). They have a high pH, relatively high concentrations of phosphate, some nitrate and high concentration of silicate. The groundwater, with its constant flow and temperature, acts as a stable source of dissolved constituents (Einarson et al, 2004).

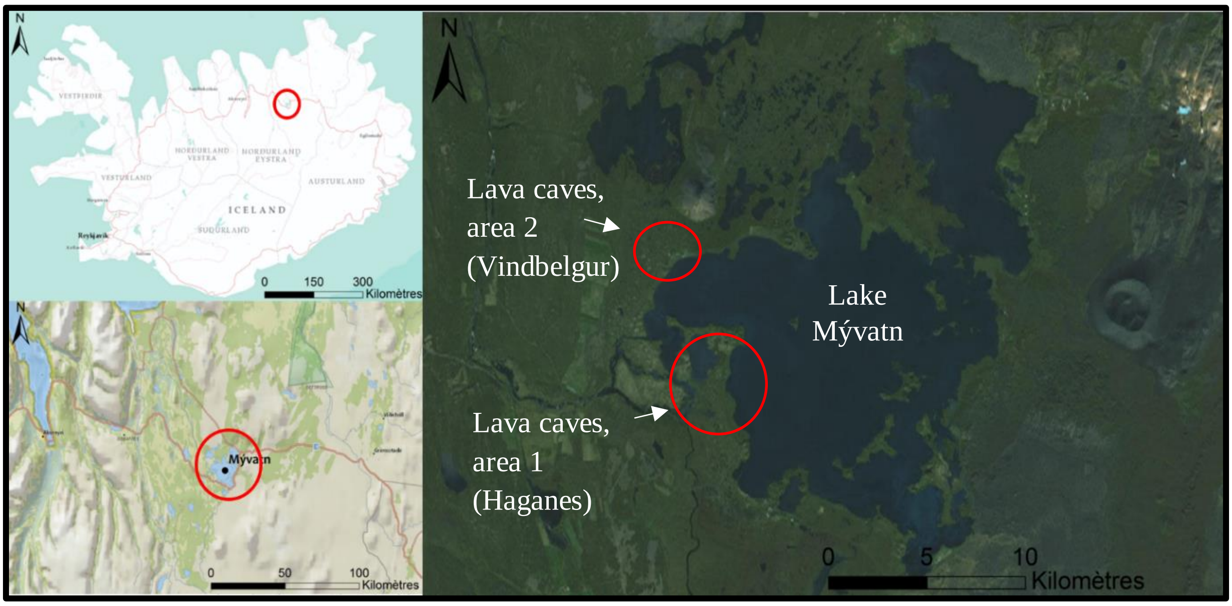

Figure 2: Lake Mývatn and its location in northern Iceland. We can see on the map the location of lava caves' studied. (Source of the maps: National Geographic World Map, World Imagery and World Terrain Base)

The high concentration of nutriment allows a high primary and secondary production. The benthic community is dominated by chironomids larvae, which are the principal food of the wildfowl and fish populations. There are three species of fish in the lake, the three-spine stickleback (*Gasterosteus aculeatus*), Arctic charr and the brown trout (*Salmo trutta*) (Einarson et al, 2004). Two morphs of Arctic charr are found in the lake, one larger fish and one bulkier and shorter fish called the Krús (local name). The larger is found all over the lake, whereas the Krús morph is found where springs emerge at the basalt shoreline (pers. comm. Leblanc).

In the surrounding lava fields, there are many ground water-filled lava caves, although the density of caves is higher in two main areas. The pond within each cave is covered by a lava roof, but commonly, some parts of the pond are open to the sky (Figure 3). In these openings, terrestrial organisms fall to the surface of the water. The light through the openings allows for primary production (Leblanc, 2016).

The water-filled lava caves were created at the same time that the lake Mývatn, about 2300 years ago after the volcanic eruption. Nowadays, most of these caves are not connected to the lake (pers. Comm. Leblanc), but they were probably connected to the lake in the past, allowing their colonisation of this system by Arctic charr and in some cases by three-spine sticklebacks.

Figure 3: Photograph of the inside of a water-filled lava cave. We can see that the water in the cave is very clear and it is rather shallow. A minnow trap is present too (Source: G. Chevalier).

The water in the lava caves comes almost exclusively from groundwater. The groundwater springs are usually constant in water temperature and have a relatively high mineral content (Cantonati et al, 2006). This is the case with the water in the lava caves studied, but some variation can occur. They have a constant temperature (about 6,7 °C), pH (about 8,7), conductivity (about 121 ùS/cm) and O_2 dissolved (about 7,6 mg/l). The water in the lava caves has a very low turbidity (about 9 NTU max) and are shallow (Figure 3). The lava caves are diverse in size and shape. The surface area of water exposed to open sky vary between 0 and 37 m².

1.3- The small benthic Arctic Charr (*Salvelinus alpinus*)

The Arctic charr is a fish of the Salmonidae family. It is adapted to cold and cool water and is widely distributed in Arctic and subarctic regions around the world, at higher altitude in mountains further south and in some temperate lowland lakes (Klemetsen, 2010; Johnson, 1980). The Arctic charr shows a strong phenotypic, ecological and life history diversity throughout its range. The diversity is so large that it has been asked "if the arctic charr is the most variable of all vertebrates?". This polymorphism has been termed "the charr problem" (Klemetsen, 2010; Johnson, 1980).

In Iceland, it is common to find two or more distinct charr morphs in the same lake (e.g. Mývatn). The highest diversity can be found in lake Thingvallavatn that contains four morphs of Arctic charr: the dwarf benthic (DB), large benthic (LB), planktivorous (PL) and piscivorous (PI) (Figure 4).

Benthic morphs of charr are relatively common (Kristjansson et al., 2012) and have trophic specialisations for bottom feeding and can be identified by having blunt snouts, a sub-terminal mouth and large pectoral fins. (Johnston et al, 2004).

Figure 4: Four sympatric morphs of Arctic charr from Thingvallavatn, Iceland. From top to bottom, they are: Piscivorous (PI), small benthivorous (DB), planktivorous (PL) and large benthivorous (LB) (modified from D. L. G. Noakes, 2008) (photograph by David Noakes).

The small benthic morph can effectively exploit the interstitial habitat of lava fissures at the bottom of the lake for food and shelter. Small benthic Arctic charr are found in a number of location throughout Iceland, most likely in response to specific lava habitats (Kristjánsson et al, 2012; Noakes, 2008). Several morphological characteristics have been used to describe the small benthic Arctic charr: they are small but robust in body shape, with a subterminal mouth and dark coloration (Figure 5) (Kristjánsson et al, 2012). Kapralova et al (2011) have recently indicated that these populations have evolved independently and rapidly.

The Arctic charr found in the water-filled lava caves is a small benthic morph. It feeds on invertebrates present in the benthic zone and on invertebrate falling into the water. Individuals have also been observed feeding at the surface. However, it is unknown if all individuals are able to feed at all locations or if some individuals have specialised feeding on certain prey types. Fish in the caves may show some morphological adaptations to low light conditions. In Mexico, a cave form of *Astyanax mexicanus* lives without eyes in full darkness (Jeffery et al, 2003). In our case, Hólar's researcher have observed that some fish in the caves may have larger eyes and a protuberant lateral line (Figure 5).

I have noticed that the maximal length of the fish in the lava caves is about 15 cm, but the average length is about 9.5 cm (with a standard error of 0.7 cm). The Arctic charrs in the caves probably originates from the lake Mývatn. However, the caves may not all have been invaded at the same time (pers. comm. Leblanc). Future genetic studies will be able to address that issue.

Here, my main research question was to evaluate if we could use the natural marks present on the fish flanks (spot patterns and parr marks) to reassign fish that have lost their tags. If this is possible, it will allow me to estimate population sizes in these caves. Thus I would be able to check if the populations size in the lava caves is connected to local ecological factors (water parameters and cave morphology).

Figure 5: Photograph of a small benthic Arctic charr found in a lava cave near lake Mývatn. We can see that the fish has a large eye, a subterminal mouth and a protuberant lateral line.

2- Materials and methods

2.1- Fieldwork in Mývatn

2.1.1- Fishing

Arctic charr were caught using minnow traps (Figure 6), fyke traps (Figure 7) and by electrofishing. Minnow traps are cages made from steel fine mesh and with an opening in the form of funnel at each end of the trap. With this shape, the fish can easily find the opening but cannot find the exit. Each trap has a leash that I secured on the shore while fishing. The harvest is done by opening the trap in the centre. To be efficient, the trap has to lay horizontally flat, has to be immerged and the openings at each side free from obstacles (Figure 3). These traps are not offensive and not stressful for the fish (Guillermin, 2014).

Figure 6: Photograph of a minnow trap laying vertically on the shore. (Source: G. Chevalier)

Fyke traps are one-meter-high vertical net in front of a series of smaller and smaller openings in the form of funnel. The wing is situated at the opening of the net to guide the fish inside. Like the minnow trap, the fish can find the opening but not the exit. The traps are fixed with a rock or a rod at both ends. This kind of traps are used to catch fish above 8 to 9 cm whereas minnow traps can catch much smaller benthic charr, including young of the year.

Figure 7: Photograph of a fyke trap deployed in cave 7 (Source: C. Leblanc)

Traps were commonly set for a 12-hour fishing effort. Several minnow traps and fyke traps were used in each cave. The number of traps in each cave was based on cave size, the goal being to catch the maximum number of fish. Finally, to capture a high proportion of the fish in each cave, we proceeded to electrofishing after the traps were taken out of the water.

The equipment used in the fieldwork for the electrofishing was a gas-powered generator which provides a voltage of 220 V. A cathode and an anode are connected to a control box, that gives 300 V and a current of 0,1 to 0,6 A and when they were in the water, an electric field is created. When fishing, if a fish was situated nearby the anode, the fish is partly paralyzed and pulled by the electricity towards the anode.

Then it could be easily fished out using a hand dipnet. Each cave was first electrofished from the shore where a large number of fish are usually found in the lava cracks, and then electrofishing took place in the cave itself.

The combination of these fishing methods is currently the optimal strategy used to exhaustively fish the caves without harming or killing the fish. Collected fish are kept in a large bucket with cold and oxygenated freshwater, prior to processing and tagging.

2.1.2- Fish processing

Fish were anaesthetized using a 3ppm 2-phenoxyethanol solution before processing. Each fish was weighed to the nearest 0.1 g and its body size measured with a ruler (fork length, to the nearest mm). A high resolution photograph of the left side is taken on all fish above or equal to 45 mm (Figure 5). The photographs were taken using a high definition camera fixed on a tripod.

The photographs are useful to study variations in body shape. Furthermore, they allowed me to identify individuals and thus better estimate population size. Finally, each fish was scanned by a PIT-tag reader (Figure 8). Then two scenarios were possible.

- The reader detects a PIT-tag, it means that the fish was sampled and tagged before. In this case, no more operation is done. The ID or PIT tag number of the fish is carefully written down and the figure labelled using that number.

- The reader does not detect a PIT-tag, it means that the fish was not sampled or tagged before, or that the fish has lost its PIT-tag. We could see if the fish had been sampled before by checking the upper part of the caudal fin for remains of a fin-clip (Figure 9) and/or by checking for scar on the fish's belly (the presence of scar means that the fish had been tagged before). In both cases, the upper part of the caudal fin was clipped for DNA analysis and a PIT-tag is implanted in the anterior part of the body cavity. Then the fish was allowed to recover in oxygenated freshwater before being released in the lava cave.

Any particular information (Fin-clipped, scare, male, female …) were carefully written in a fieldbook. All DNA samples were stored in cold 96 % ethanol and frozen as quickly as possible. The PIT-tagging allow the identification of individual fish and the construction of longitudinal life history records. PIT-tag is composed of a microchip and an antenna locked in glass capsule. The tag does not have a battery and therefore is very light and lasts for a long time.

Two types of PIT tags were used in this study, depending on body size of fish. Fish above 65mm and > 3gr were PIT-tagged with 12mm HDX PIT tags (weight 0.1gr Oregon RFID), and fish between 45 and 65mm and at least 1gr were tagged with RFID NONATEC 6mm tags (Lutronic International) in 2015 and 2016. In 2012, 2013 and 2014, fish between 45 and 65mm were tagged with colour tag.

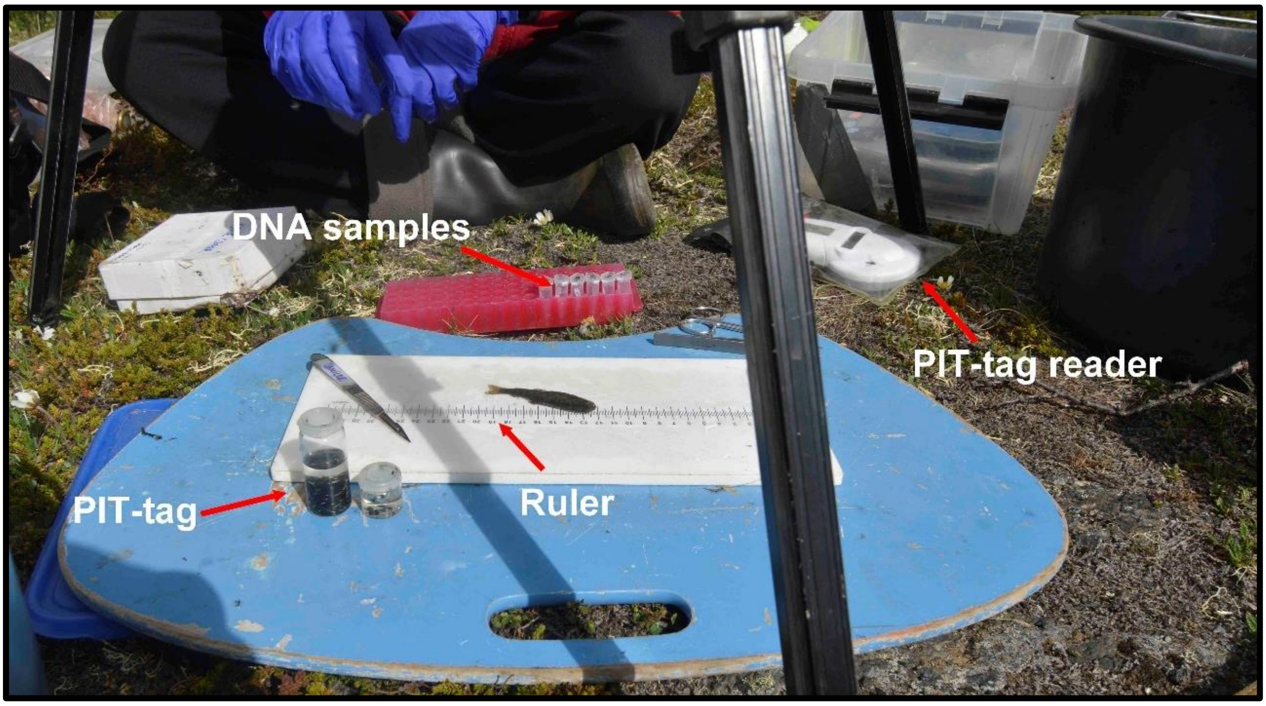

Figure 8: Photograph of an Arctic charr's sampling procedure as in this project (Source: M. SIMONSEN).

All the data on individual fish were carefully registered in a fieldbook and later entered into a database in excel.

This sampling has taken place in June and August each year starting in June 2012. At each season, each of the twenty caves is visited twice as it has proven that we capture/recapture more individuals by resampling a cave at a week's interval. A crew of four to five people is needed including one or two referent scientists, student and interns. I participated in the fieldwork for two weeks in June 2016.

2.1.3- Ecological factors

HOBO data loggers (UA-002-64, ONSET Corporation) recorded water temperature (in °C). Physical and biological parameters of the water were measured at each visit using a calibrated HydroLab probe HL3. Hólar's researchers measured temperature, pH, dissolved oxygen (in mg/l), conductivity (in µS/cm) and turbidity (in NTU).

Fall down traps have been developed and set up at the opening(s) of each cave to collect terrestrial material falling into the caves. Traps were emptied at each visit, allowing quantitative estimation of terrestrial input. All the samples were preserved in 70 % ethanol. The area of water exposed to open sky has been characterized in previous years.

2.2- Photographic identification

Manual identification from photographs requires very little equipment. A high definition camera and a computer with a large screen are necessary. The photographs were taken in the field (see above), therefore light conditions may be different among caves and visits.

Before proceeding with photographic identification of individuals, I identified fish labelled with lost PIT tags (fin clipped and/or scar visible on the flank; see figure 9) from the data base from caves 1, 2, 7 and 25. This information were carefully entered in the data base along with the individual's data. For all "new" fish above 45mm and collected since august 2012, I consistently checked the pictures from caves 1, 2, 7 and 25 for presence of a clip at the caudal fin or for a scar.

If fin clip or scar were present, it meant the fish had been sampled before. If not, it means that the fish had been sampled for the first time. If the fish had been seen before, I proceeded to its photographic identification by comparing its actual spot patterns to those of all fish seen in that particular cave before that particular visit.

From the photographs, it was possible to see if the upper caudal fin has been clipped before (Figure 9). A fish that was not sampling present no anomaly on its caudal fin and symmetric lower and upper caudal fins (Figure 9-A). A fish which had been sampled the same year has a clear cut on its caudal fin (Figure 9-B), and on a fish which had been sampled at least one year before, the caudal fin has curves and/or a darker shade (Figure 9-C). After two or three years it is usually difficult to detect if the fish has been fin clipped, especially if the fish was fin clipped at young age.

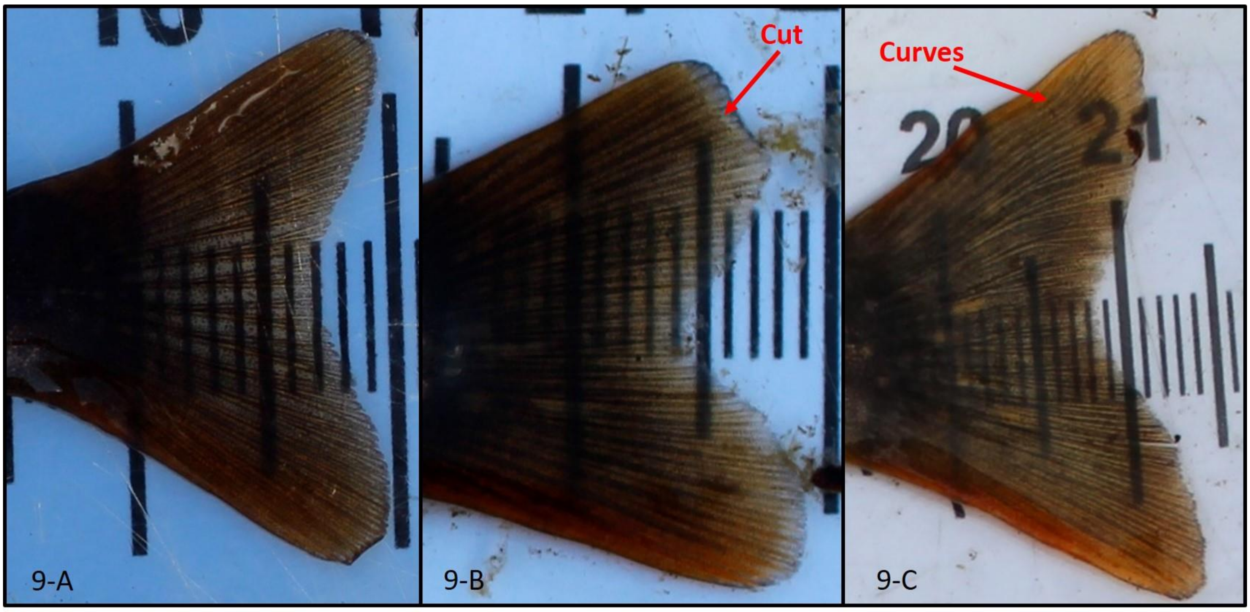

Figure 9: Several enlargements of Arctic charr caudal fins. The photograph 9-A show a caudal fin that was not clipped before, the photograph 9-B show a caudal fin which was clipped the previous year and the photographs 9-C show a caudal fin which was clipped at least one year ago.

After identifying all fish that lost their PIT-tag, I proceeded to their manual identification.

If we know that the fish was sampled during the year (clear cut of the upper caudal; Figure 9-B), I compared all the pictures taken that same year, in the same cave. But, if we know that the fish was sampled at least one year before based on the cut of the fin clip (Figure 9-C), I compared all the pictures taken the previous years, in the same cave.

Practically, I placed the picture of the fish with a missing ID on the right side of the computer screen and I put the photographs taken during the precedent visits on the left side of the computer screen to compare their pictures. We could also print all the pictures and lay them on the wall to compare them, but I did not try that method.

I used two types of spots to identify fish. If the fish was at least 65 mm, I used the patterns of clear spots on the left side of the fish (Figure 10). If the fish length was 45 to 65 mm, I used the patterns of parr marks on the left side of the fish (Figure 11). This is possible as the spot or parr mark position and shape are unique to each individual (see results section).

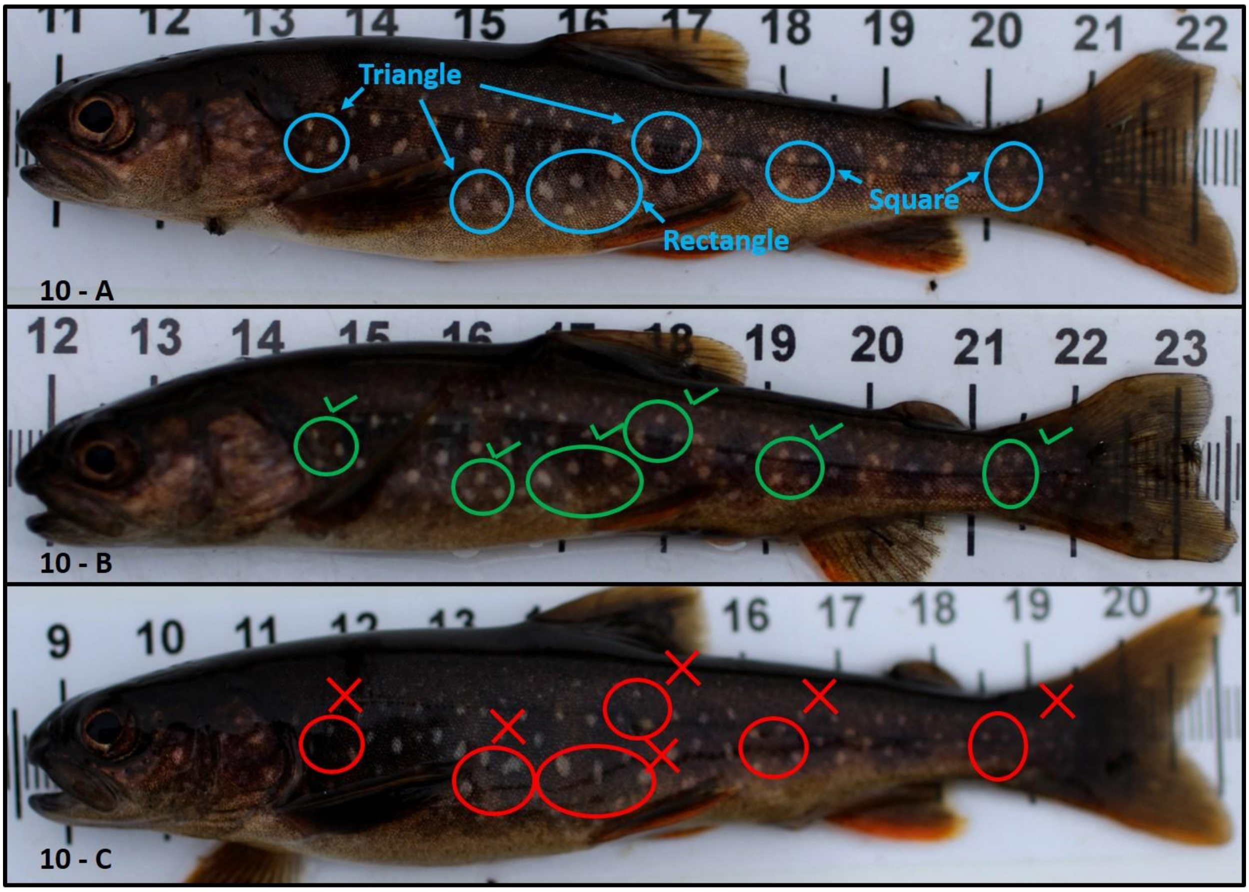

Figure 10: Photographs of the fish 4ED5E5 in August 2012 (10 – A) and in August 2013 (10-B), the spot patterns is exactly the same. The photograph 10 – C show another fish; some spots alignments have similar shape but are not found in the same location on the flank.

To identify a larger fish (≥ 65 mm), I compared first the spot pattern near the pectoral fin (figure 10). If the patterns from both fish were similar, then I compared the rest of the fish flank. Certain geometric form could be easily detected and compared as square, triangle, rectangle, line, circle of spots (Figure 10). I noticed that almost all the areas on the fish flank can be used to identify the fish. Figure 10 show that the areas near the pectoral, ventral and caudal fin but also near the lateral line present characteristic spot patterns (square, triangle …). If the spots were in the same position and in the same shape, it meant the fish was the same individual (usually, I used 6 spot forms minimum to identify a fish) (Figure 10).

To identify a smaller fish (< 65 mm), I compared all the parr marks present on the fish flank (figure 11). If all the parr marks were in the same position and had a very similar shape, then the fish was identified as the same individual. As the fish grew, it was harder to identify it because parr marks fades away in these species and lighter spots appear (see figure 13).

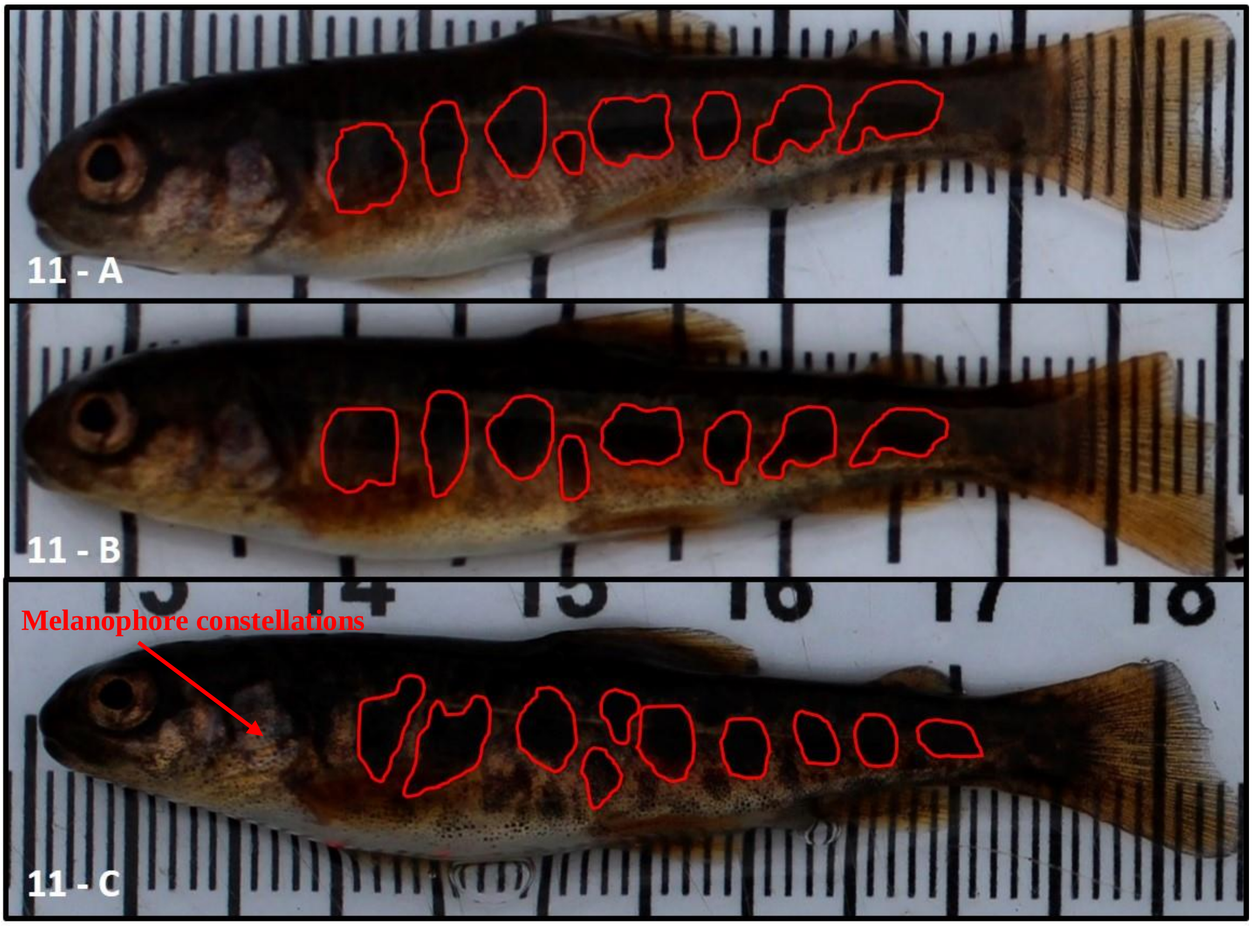

Figure 11: Photographs of the fish 13153 in June 2013 (11-A) and in August 2013 (11-B). The parr marks are in the same position and shape. The photograph 11 – C show another fish; parr marks position and shape are totally different.

I tried to use melanophore constellations near the head (Figure 11) to identify small fish. Donaghy (2005) has used this method to identify wild young Atlantic salmon (*Salmo salar*). But in our case, the photographs were sometime of low quality and this method was not always applicable.

I looked for all the fish with lost PIT-tags in caves 1 and 2 (two connected small caves) and in caves 7 and 25 (the cave 7 is of medium size but the cave 25 is the largest cave studied, both are connected). These two sets of two caves form two independent populations (based on a population genetic study of all these caves (pers. comm. Leblanc). To evaluate the efficiency of the manual photographic identification of individuals, I recorded the necessary time to find a fish and calculated the percentage of fish reassigned and not reassigned in each cave.

2.3- Population sizes

2.3.1- Estimation based on photographic identification

Once all fish were reassigned using the manual identification method from photographs, we obtained a number of individual in the two populations. Since June 2012, 17 visits or fishing effort have taken place in the caves, who are a closed environment. Thus, we can make the hypothesis that almost all the fish in the caves have been captured at least once since 2012. Based on capture-recapture data, we can estimate the fish population sizes N in these caves

The population in the caves can be calculated using the following formula:

$$N = Nb\ individual - (Nb\ dead\ fish + Nb\ fish\ not\ reassigned)$$

Nb individual: Number of individual ≥ 65 mm obtained after the manual identification.
Nb fish dead: Number of fish ≥ 65 mm accidentally killed during the processing.
Nb fish not reassigned: Number of fish ≥ 65 mm not reassigned with the manual identification.
N: Population size of fish ≥ 65 mm

We have to subtract the number of fish not reassigned to an ID if we know that this fish was seen before (i.e. there are two different ID code for one fish). This formula gives an over-estimation of the real population size because it does not take into account the number of that may have died of natural causes (predation, senescence).

2.3.2- Estimations of population sizes using three different statistical methods: Schumacher-Eschmeyer, Schnabel and Peterson.

When there are several population censuses (like in our case), three methods can be used to estimate population size N. Strictly speaking, the methods require that populations are constant, with no recruitment and no mortality during the experiment; but it is often accepted that these conditions are only approximately satisfied (Ricker, 1975). The following data is necessary to test all three methods; I have used all the fish ≥ 65 mm length:

M*t*: total marked fish in the cave at the start of the *t* th day, i.e. the number previously marked minus any dead fish if know.

C*t*: total number of fish caught taken on day *t*.

R*t*: number of recaptures in the sample Ct.

R: total recaptures during the experiment (∑ R*t*).

m: number of visit.

t: corresponding to m – 1 degrees of freedom from student's table (t-table).

Ricker (1975) explained how to use for fish populations. The Petersen method is the simplest mark-and-recapture method. It gives a population size (N) and a variance (S^2) estimations at each visit. The mean of these Peterson estimates is the estimate of N and S^2. The Schnabel method is an extension of the Peterson method (Krebs, 1999). The Schumacher and Eschmeyer method judge that the estimation of 1/N is the slope of the straight line Rt/Ct plotted against Mt, with the restriction that the straight line goes through the origin (Ricker, 1975).

Petersen: $N = \frac{Mt*Ct}{R} CI \left(\frac{1}{N}\right) = \frac{1}{N} \pm t * \sqrt{\frac{Rt*(Ct-Rt)}{M^2*C^3}} = \frac{1}{N} \pm t * \sqrt{S^2}$

Schnabel: $N = \frac{\sum(Ct*Mt)}{R}$ $CI \left(\frac{1}{N}\right) = \frac{1}{N} \pm t * \sqrt{\frac{R}{(\sum Ct*Mt)^2}}$

Schumacher and Eschmeyer: $\frac{1}{N} = \frac{\sum (Mt*Rt)}{\sum(Ct*Mt^2)}$

$$CI \left(\frac{1}{N}\right) = \frac{1}{N} \pm \sqrt{\frac{\sum(Rt^2/Ct) - (\sum Rt * Mt)^2/\sum(Ct * Mt^2)}{(m-1) * \sum(Ct * Mt^2)}}$$

3- Results

3.1- Manual photographic identification

3.1.1- Feasibility of the method

The manual photographic identification of small benthic Arctic charr is a feasible and reliable method for Arctic charr found in the lava caves. The figures 10, 12 and 13 show recaptured fish identified with PIT-tag where no confusion is possible about their identities. This prove that each small benthic Arctic charr have unique combination of spot patterns. Several spot patterns make them unique and allow the identification of an individual (Figure 10, 12 and 13). The figure 12 show that all fish larger than 65 mm keep their spot patterns over time (at least four years), but I have seen that the some of the spots sometimes fading or sometimes are darker according to time or light conditions. (Figure 12 and 13).

The parr marks patterns are good landmarks to identified fish between 45 and 65 mm. The figure 11 and 13 show recaptured fish identified with colour tag and PIT-tag where no confusion is possible about their identities. This show that the parr marks pattern are unique to each small benthic Arctic charr. Their position is always the same on the fish flank, but the shape can change a bit (Figure 11 and 13). It is when the fish length is between 65 and 80 mm that the manual photographic identification become difficult to use. Indeed, during this period, the parr marks begin to disappear and the spot patterns begin to appear (Figure 13).

Figure 12:Photographs of the fish C98DDD in 2012 and in 2016. The spot patterns are exactly the same. The fish was identified with PIT-tag; no confusion is possible about the identity. Sometimes, the spot patterns are fading are less visible because of the light condition

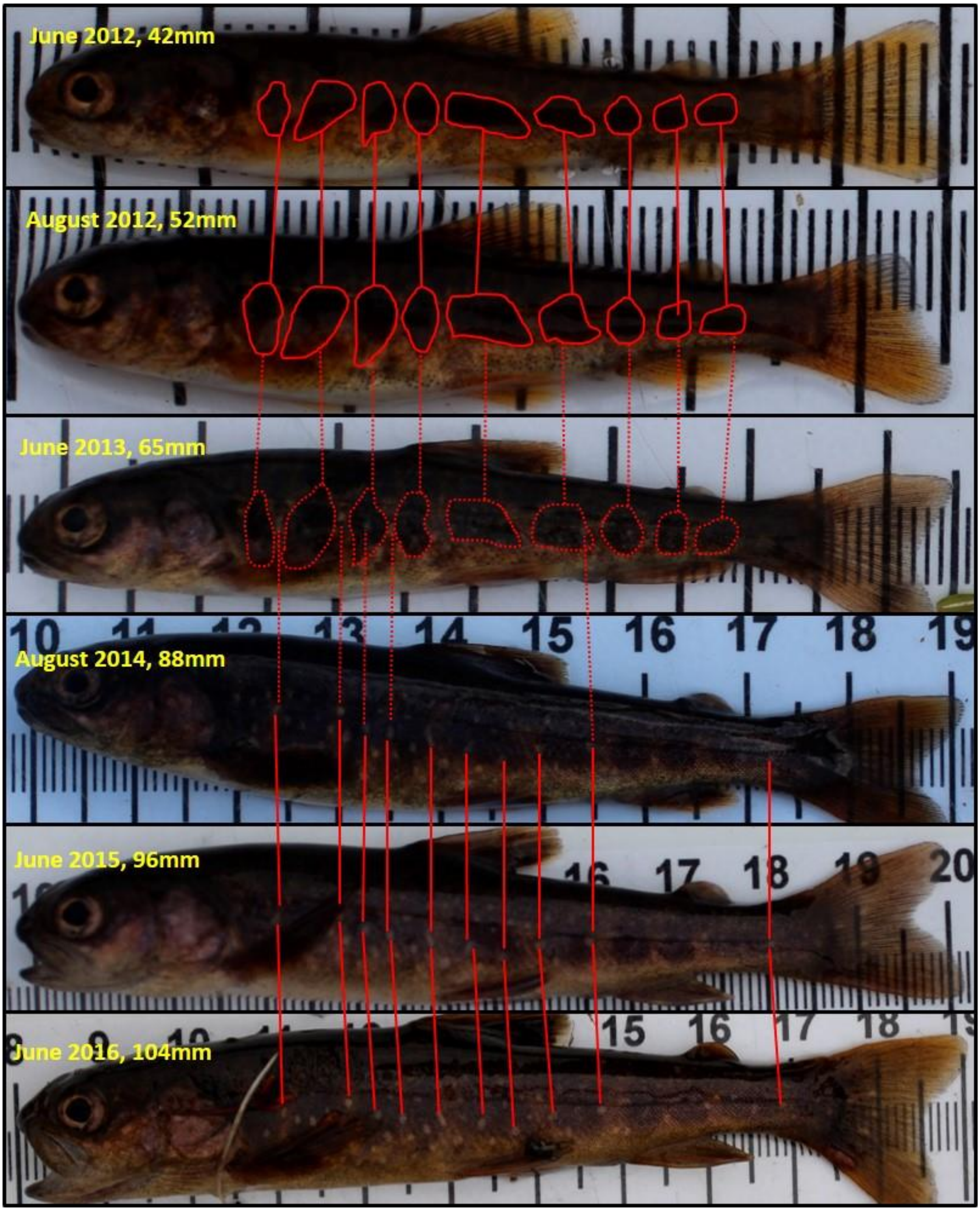

Figure 13: Photographs of the fish 4EF225 from 2012 to 2016. The fish was identified with PIT and colour tag (except for June 2012); no confusion is possible about the identity. Sometimes, the spot patterns are fading or are less visible.

3.1.2- Efficiency of the method

Using a manual photographic identification based on the unique spot patterns present on fish flank, I have reassigned a total of 57 fish in caves 1 and 2 and 165 fish in caves 7 and 25 (Table 1).

Table 1: Efficiency of the photographic identification in the caves 1 and 2 and in the caves 7and 25

	Cave 1 and 2	Cave 7 and 25
Number of fish reassigned to an ID (%)	57 (97 %)	165 (86 %)
Number of fish NOT reassigned to an ID (%)	2 (3 %)	26 (14 %)
Total time spent (h)	9	93
Average time spent per fish (min)	9	29

I needed about one week of work to reassign all fish with missing ID from caves 1 and 2; i.e. average time of 9 minutes per fish (Table 1, Figure 14). I needed considerably more time to proceed with caves 7 and 25 (Table 1, Figure 14), which are the largest caves and very likely the largest population of all caves studied. I spent in average 29 minutes to reassign each fish, but a lot of variation was seen among individual (Table 1, Figure 14).

Indeed, I could identify a fish in 10 seconds whereas I spent up to 2h to identify one individual. This time depended on the number of photographs to be compared with. For example, if a fish-found in 2016 needed to be reassigned, they were about 1600 photographs to compare with since June 2012. A total of four weeks was needed to reassign all fish in caves 7 and 25, representing approximately 93 hours of work (Table 1).

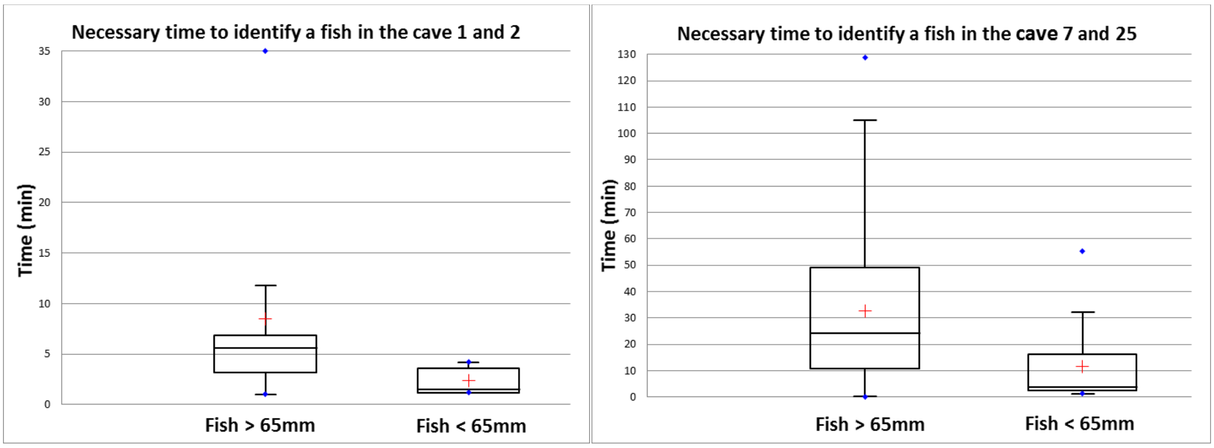

Figure 14: Necessary time to identify a fish in the cave 1 and 2 and in the cave 7 and 25. The red points represent the average and the blue points represent the extreme values. The line in the boxes represents the median, the upper lines the 9th decile and the lower lines the 1st decile.

Using a t-test, there was no difference between the average time to identify fish smaller than 65 mm and the average time to identify a fish longer than 65 mm in caves 1 and 2 (t-test t= - 1.308 and P value = 0.207).

But in the caves 7 and 25, there is a very strong difference between both average, the necessary time to identify a fish is shorter when the fish is smaller than 65 mm whereas it took longer time to reassign a fish longer than 65 mm (t-test t= -3.193 and P value = 0.002; Figure 14). This can be due to the number of photographs to compare with that was lower for smaller fish. Indeed, the number of fish under 65 mm found at each visit was generally lower than the fish above 65 mm.

In the caves 1-2 and 7-25, the proportion of fish that lost their PIT-tag was dominated by large fish from 113 to 123 mm in fork length (12 fish in caves 1 and 2 and 27 fish in caves 7 and 25; figure 15 and 16 respectively). These values were larger than the average length in both populations (97 mm in the caves 1-2 and 92 mm in the caves 7-25; figure 15 and 16 respectively)

In the caves 7 and 25, among the few fish that could be sexed (i.e. these fish gave eggs or sperm at least once in August visits), more females had lost their tags: eight females against one male. When looking at fish that retained their tags and could be sexed, 16 males and only 7 females kept the tag (Figure 17). Mature adults were not observed in caves 1 and 2 to compare with caves 7 and 25.

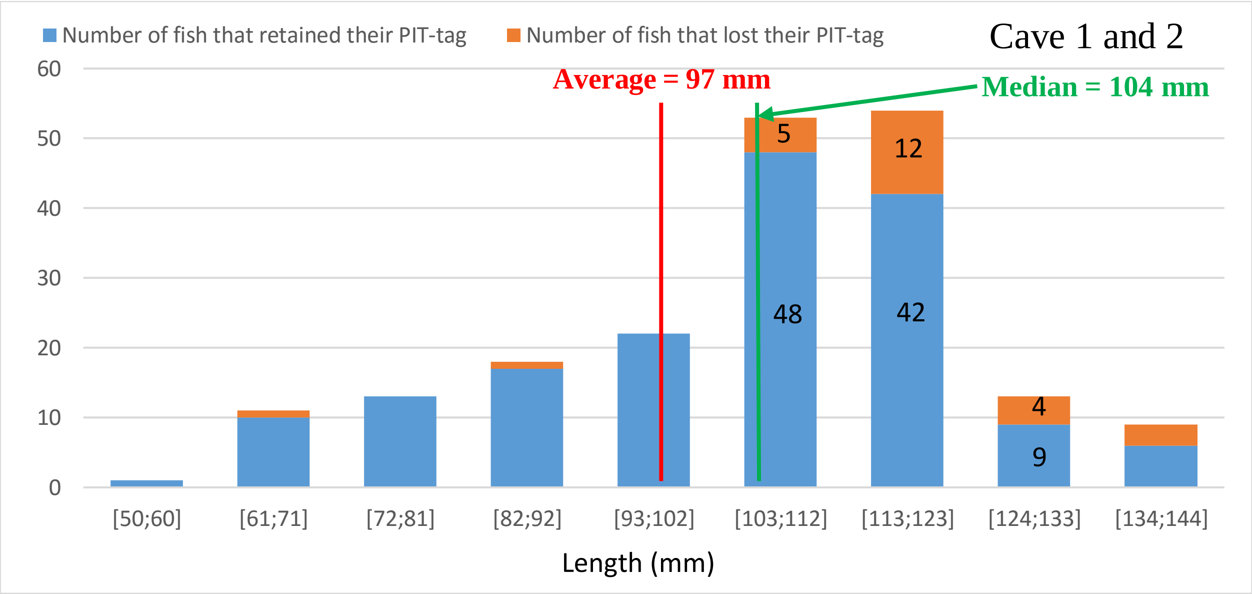

Figure 15: Proportion of fish that retained or lost their PIT-tag in the caves 1 and 2.

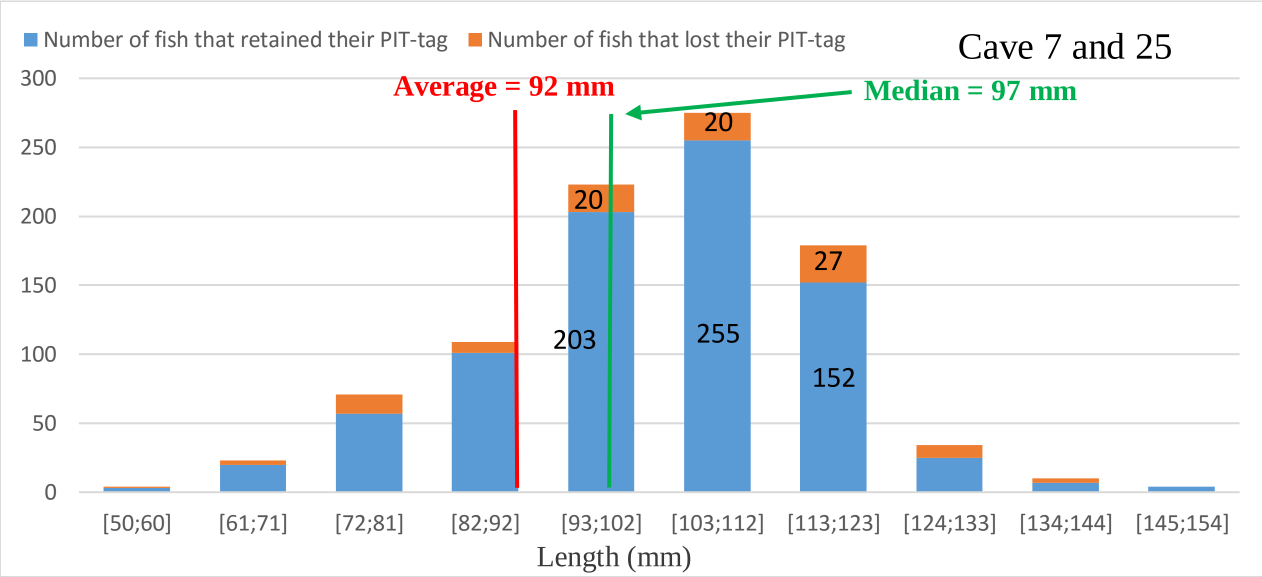

Figure 16: Proportion of fish that retained or lost their PIT-tag in the caves 7 and 25.

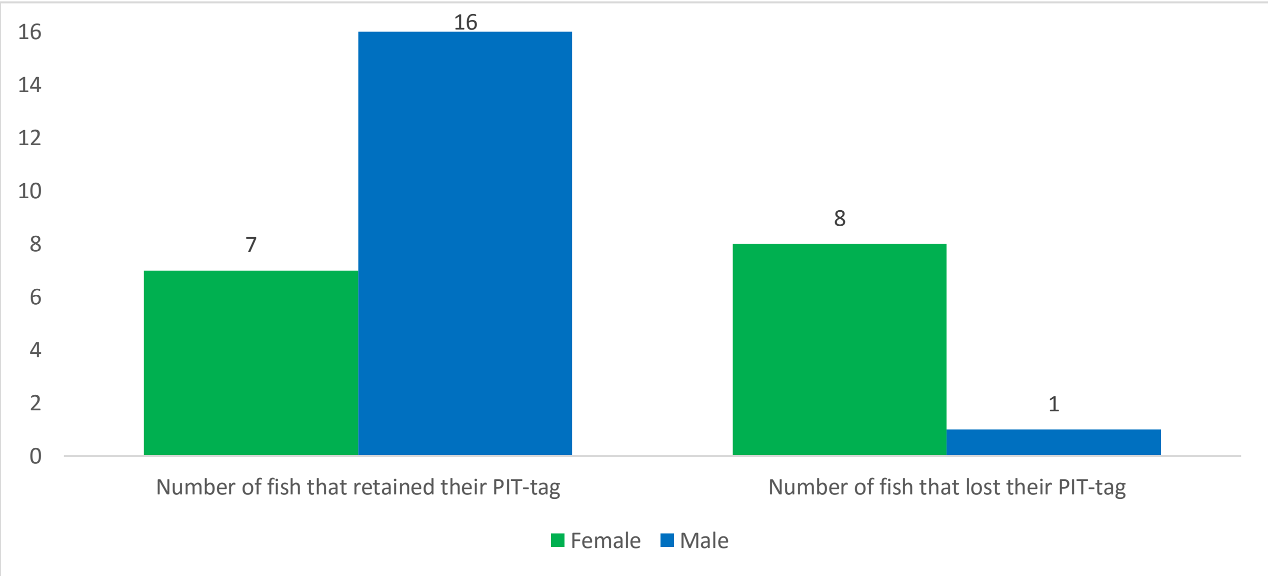

Figure 17: Proportion of females and males that have retained or lost their PIT-tag in caves 7 and 25. These numbers are based on the few individuals that could be sexed based on their maturity in August visits each year. Note that only a small proportion of adult fish have been sexed.

A high number of small fish needed to be reassign to an ID in the caves 7 and 25. Indeed, in these caves, a large recruitment of young fish has been noticed. In addition, for some visits, fish between 45 and 65 mm were not tagged because they were too many, but they were nevertheless fin-clipped.

In four caves, I have noticed that 54 % of fish not reassigned to an ID are fish between 66 and 81 mm fork length (Figure 18). These size corresponds to the moment when the parr marks begin to disappear and the spot patterns begin to appear (Figure 13).

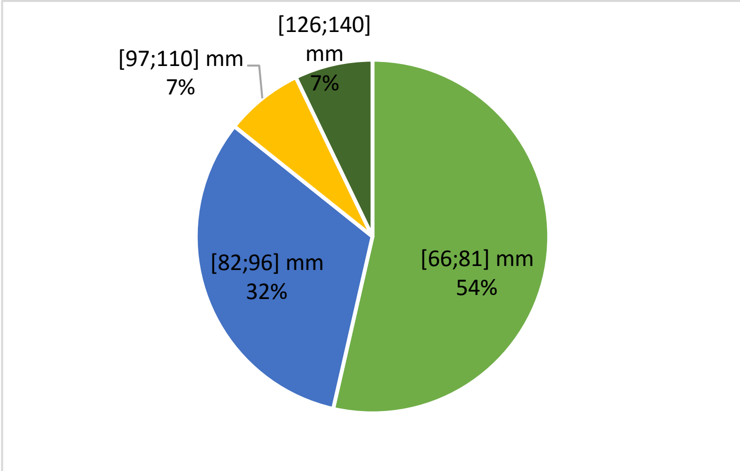

Figure 18: Percentage of fish not reassigned to an ID in all caves

3.2- Population sizes

Using photographic identification method, I have estimated a population of 87 fish in the caves 1 and 2 and a population of 540 fish in the caves 7 and 25 (Table 2, Appendix 1 and 2). We obtained very similar results by using the three different methods of calculations (Table 2).

Table 2 : Population size estimations (fish > 65 mm) (CI: Confidence intervals).

Cave	**1 and 2**			**7 and 25**		
Estimation	N	CI (95 %)		N	CI (95 %)	
Photographic identification	87			540		
Petersen	89	66	136	556	456	712
Schnabel	83	72	97	519	485	557
Schumacher and Eschmeyer	84	77	91	533	482	596

I had not the time to use the manual photographic identification in all caves. To estimate the populations in the others caves, I chose to use the Schumacher-Eschmeyer estimations (Appendix 3). Indeed, Schnabel and Schumacher-Eschmeyer are multiples census estimates and there are more compatible with our study. Also Schumacher-Eschmeyer estimations were closer to the photographic identification estimates than the Schnabel estimates.

The population sizes in the lava caves ranged from 17 to 393 fish above 65 mm according to this method (Appendix 3). That is if we consider that one cave is equal to one population, which we know was not necessarily the case for all caves (see caves 1 and 2 and caves 7 and 25). In fact, cave 25 was the largest of all caves with a population size of 393 individuals above 65mm.

3.3- Ecological factors

The temperature and the pH did not vary a lot among caves (between 5.3 and 7.4 °C, and 7.7 and 9 unity pH) (Figure 19). However, the conductivity is very different according to the cave's location. The conductivity in Haganes is higher (between 118 and 144 µS/cm) than in Vindbelgur (between 97 and 109 µS/cm), revealing a different water origin between both areas. In general, the turbidity in the caves was very low (less than 1 NTU), but some caves have a larger turbidity, with an extreme value of 9.1 NTU (Figure 19),

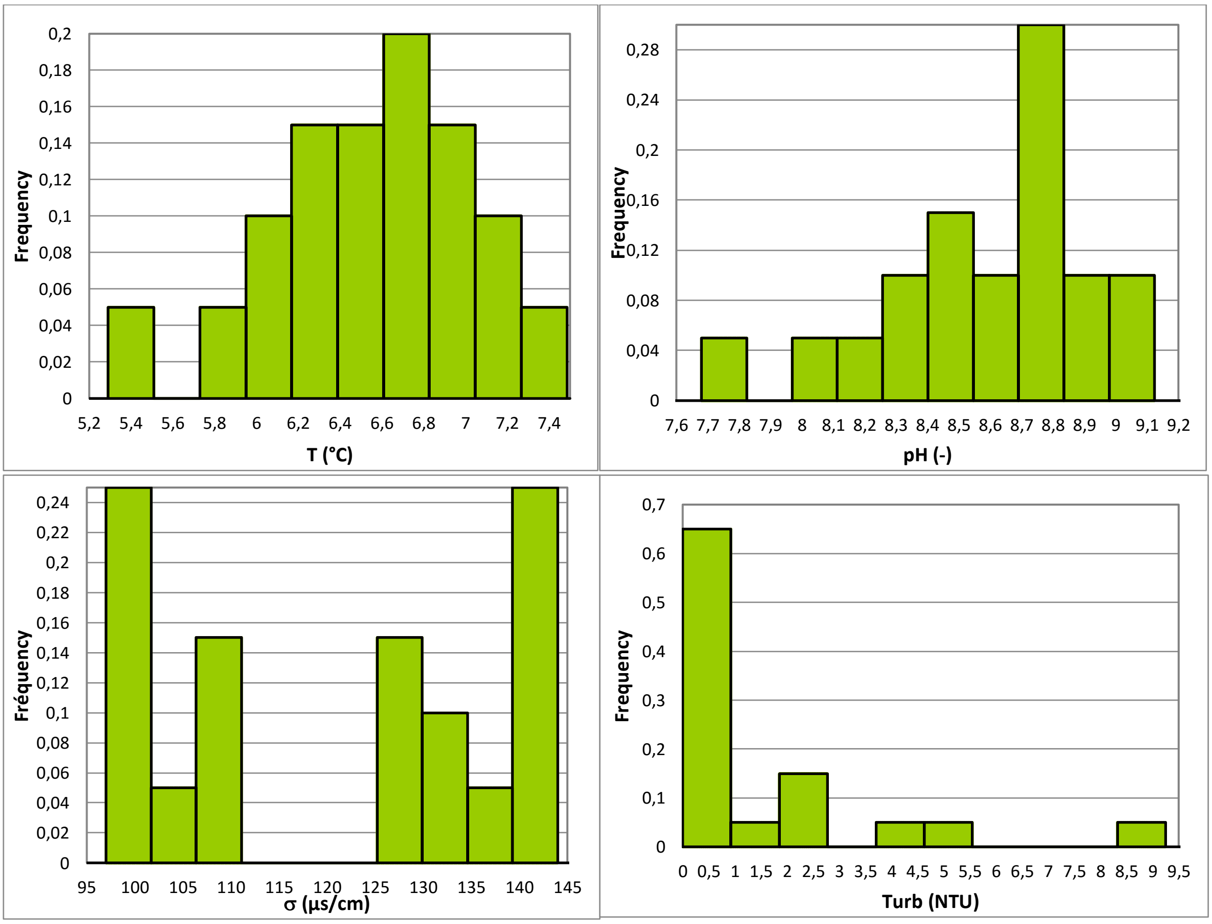

Figure 19: Range of the temperature (°C), pH (-), conductivity (µS/cm) and turbidity (NTU) in the 20 studied lave caves

The dissolved oxygen has a wider range than the other physical and chemical parameters, it varies between 5.2 °C and 11.6 °C among caves (Figure 20). Most of the caves have a surface of water open to the sky that is less than 5.0 m². An extreme value is present, corresponding to the cave 25 with an open area of 37.5 m².

The estimations of volume of organic material falling into the cave varied greatly (0.2-106 mL) (Figure 20), but data were not available for all caves, including cave 25. These volumes corresponded to the total volume of material (including macroinvertebrates) falling in the fly traps presents in the lave caves.

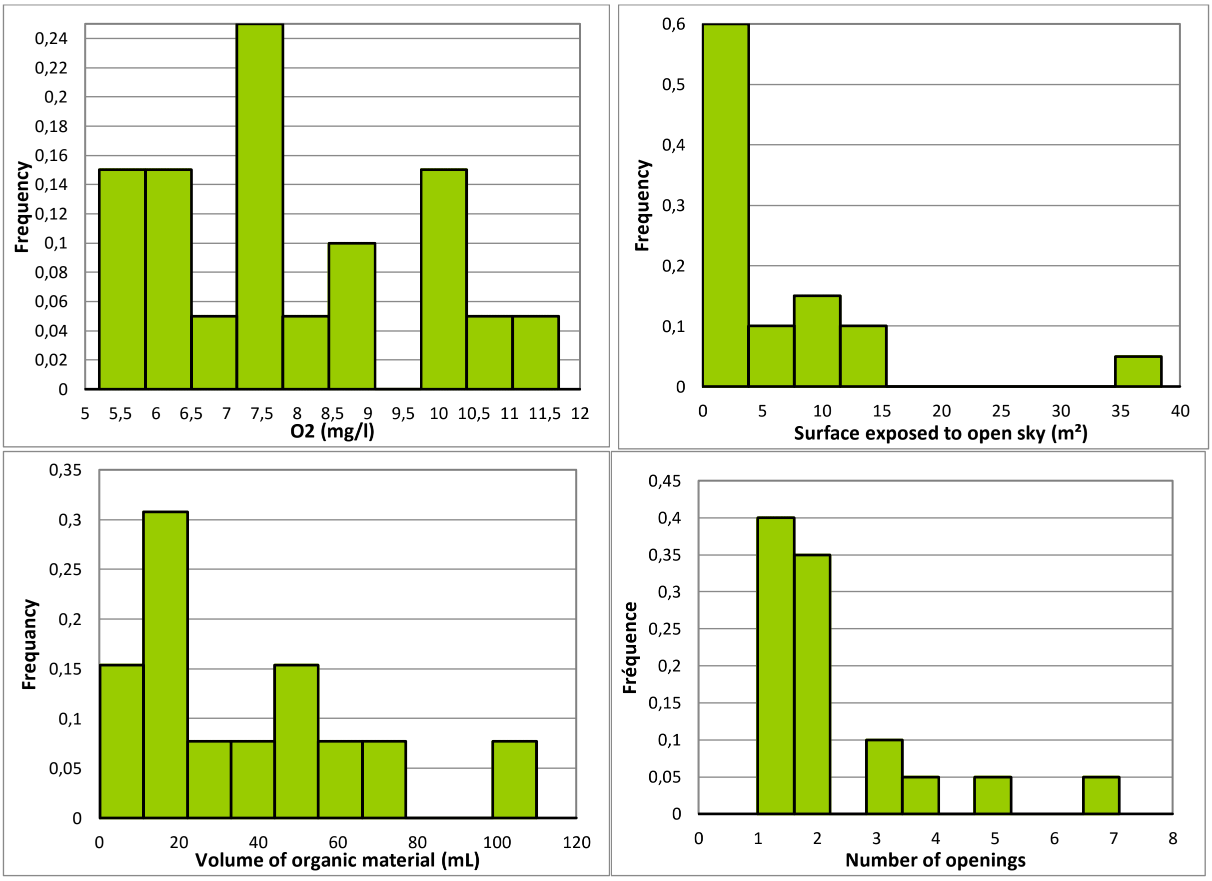

Figure 20: Distribution of several characteristic of the lava habitat found in 20 lava caves around Lake Mývatn. From left to right: dissolved oxygen (mg/l), surface of water exposed to the sky (m²), volume of organic material falling into the caves (mL) and the number of openings.

Using Pearson regressions when the variables are normally distributed, and Spearman regressions when the variables are not normally distributed, I did not find significant relationships between ecological factors and fish population size in the caves (appendices 4 and 6). Similar results were found if we don't take into account the cave 25, which showed extreme values for several of the parameters (appendices 4 and 5). Indeed, all the coefficients of regression (R^2) were near 0 and all the P-values were above 0,05 (appendix 4).

4- Discussion

4.1- Photographic identification

The results show that the photographic identification of individual Arctic charr is very efficient on a small and isolated population as in the caves 1 and 2, where there is in average 23 fish caught per visit (standard deviation = 10). Indeed, the percentage of fish reassigned to an ID is near 100 %, and the necessary time to identify a fish is rather short, until 2016.

But, when the population is larger, as in the caves 7 and 25 where there is in average 105 fish caught per visit (standard deviation = 25), the feasibility of using this method is very low. Indeed, the percentage of fish identification is still high (86 %), but the necessary time to find a fish became very long (about 30 minutes per fish) and the total time to reassign all fish is very high. Sometimes, it is possible to spend two hours to identify one fish and then fail.

It was relatively easy to reassign a small fish (≤ 65 mm) thanks to the parr marks (figure 11 and 13) and identify a large fish (≥ 80 mm) thanks to the spots patterns is feasible and reliable (figure 10, 12 and 13) if several areas on the flank are used. I have shown that the parr marks or the spots patterns are always in the same place on the fish flank, regardless of age or size. But I have noticed that the spots and/or the flanks are sometimes changing in colours or the light conditions are very different.

When the fork length was between 65 and 80 mm that the identification became very tedious. Indeed, during this period, the parr marks begin to disappear and the dots on the flank begin to appear (figure 13). The parr marks and the dots patterns were usually not visible enough to identify the fish reliably.

Another potential reliable landmark is the melanophore constellations present near the head of the fish (M. J. Donaghy et al, 2005). In our case, the photographs qualities were not good enough to allow identification using the melanophore constellations. It will be interesting to test this method on small benthic Arctic charr with high photograph resolution focusing on fish head.

I have also noticed that two different fish can have very similar spot patterns, but a location on their flank is different (Figure 10). Maybe the spots patterns on the fish flank are related to the genetic code of the individuals. Fish with more similar patterns are maybe genetically more related. Future genetic analyses maybe able to test this hypothesis.

The populations in the caves are quite small and inbreeding may be frequent, explaining some similarities in spot patterns between individuals. I have seen on some fish a spot that could be connected with a line to another one and sometime not, according to time (Figure 21). I have not explanation about this strange phenomenon.

Figure 21: Photographs of the fish 053595 in June 2012 (14-A) and in June 2014 (14-B). We can see a line between two dot in 2012 but this link is not present in 2014.

We saw that fish can lose their tag. Tag loss can occur soon after injection if the tag exits through the opening caused by the tag injection (Feldheim et al, 2002. J. Whitfield Gibbons and K. M. Andrews, 2004), or be expelled later in life. This can often be prevented by applying a topical substance on the injection wound to speed healing (Whitfield and Andrews, 2004). A loss can happen when the animal's body recognizes the tag as a foreign object and rejects it (Whitfield and Andrews, 2004). I have noticed that females fish seem to lose their PIT-tag more easily than males fish, whereas males fish seem to keep their PIT-tag more easily than females.

An explanation on why larger fish lose their tag more is that the tag does not get embedded in the conjunctive tissue around the organs whereas it is the case in younger/smaller fish (pers. comm. Leblanc). The recommended procedure to inject a tag in the animal body is to use a PIT-tag injector, which is a specific needle to inject the tag (Columbia Basin Fish and Wildlife Authority, 1999).

In our case, the tag injection was done by making a little hole in the fish belly with a scalpel and then the tag was manually inserted in the anterior part of the body cavity. Maybe the utilisation of a PIT-tag injector will reduce the number of fish that have lost their tag. However, this will have to be tested in laboratory conditions on similar size fish. In the other hand, we did not observed mortality linked to tagging when standard procedure was applied.

I recommend to use the manual photographic identification on small population size (≤ 100 individuals). When the population size is larger, the manual photographic identification demands too much time for one person. The method can be used on large population if several persons work on it.

The manual photographic identification can be used on a variety of another species, especially fish of the Salmonidae family. Indeed, Salmon (*Salmo salar*), Trout (*Salmo trutta*), Canadian Brook Trout (*Salvelinus fontinalis*), Rainbow Trout (*Salmo gairdneri)* and others salmonids have, like the Arctic charr, dots on their flank that can be used for a manual photographic identification.

For example, Persat (1982) and Gifford (2014) have used the manual photographic identification on Grayling (*Thymallus thymallus*) and Westslope Cutthroat Trout (*Oncorhynchus clarkia lewisi*) by comparing the natural marks presents on their flank. And I think that the manual photographic identification can be used on any animals that have permanent and unique marks which contrasts well with the body color.

Marshall and Pierce (2012) used the photographic identification on sharks and rays and advised this method to assess population size. Adams and al (2006) used the photographic identification on marine mammal (Dolphin and Whale), but they used a specific software (Finbase) that is a database system designed to facilitate entry and analysis, expedite the matching and cataloguing processes. This software use fin attributes for identified a marine mammal (for example, notch or skin disorder on the dorsal fin).

Finbase is not adapted in our case because the Arctic charr's fins are not significant different attributes among individuals. The ideal software for our study would be a software that could recognize spots patterns among individuals.

4.2 Ecological factors

The ecological parameters in the lava caves did not seem to affect population sizes of Arctic charr above 65 mm. However, the only data made available were from one-time point (June 2014), and the range of values for those parameters were small. Also for the quantity of organic material falling in the caves, some data were missing for a few caves. Since 2014, the use of robust falling traps will improve the quality of the data set for that variable.

Interestingly, I have noticed that there was no positive correlation between the surface of water exposed to the sky and the quantity of organic material falling in the caves ($R^2 = 0.021$ and p-value = 0.653). It means that the food availability from the outside is not larger when the surface of opening exposed to the sky is also larger.

In my opinion, the population sizes in the lava caves would be more related to the volume of water in each cave (i.e. cave size), and to the quantity of macroinvertebrates in the benthic zone than other ecological factors.

We could have an estimation of the volume of water inside the lava cave by measuring the surface area of the pond in the cave and by measuring the average depth. The average depth can be calculated by measuring the depth in several points in the lava cave (for example, one measurement every 50 cm, figure 22). Then, we just have to multiply the surface area by the average length to have an estimation of the volume of water.

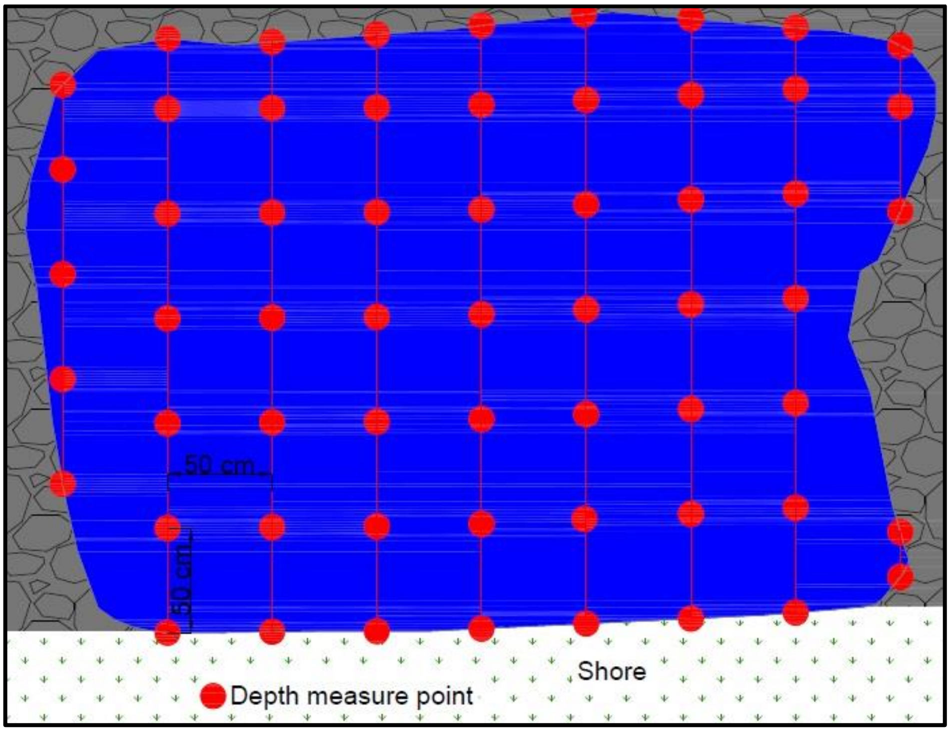

Figure 22: Example of depth measurements in a lava cave for estimate the volume of water

Conclusion

The objectives of my internship was to evaluate the efficiency of the manual photographic identification of small benthic Arctic charr based on their natural marks present on their flank. These was done in 2 selected populations that vary in size (two rather small connected caves, and two larger connected caves). The method was very efficient and it allowed me to have a realistic estimate of population sizes in the caves. A second objective of my study was to study how population size in these lava caves would be related to local ecological factors.

My results showed that small benthic Arctic charr has unique spot patterns and that their position and shape was unique to each individual. I also showed that the parr marks was a good landmark to identify young fish because there are also unique to each individual. However, similar patterns and location could be identical among fish from a same cave and this may indicate genetic connection between these individuals.

The manual photographic identification of small benthic Arctic was a reliable method to reassign fish that had lost their PIT-tag. The method was very efficient on small population size (approximately 80 individuals). With larger population size, the efficiency was still high but the necessary time to reassign all fish was very high. The method allowed me to have a realistic population size. I noticed that it was harder to reassign fish between 65 and 80 mm because the parr marks ware beginning disappearing and the spot patterns were appearing.

The Schumacher-Eschmeyer method gave very similar population sizes than the photographic identification, and I used it to calculate the population size in all the caves. The ecological factors did not significantly correlate with population sizes.

In my opinion, melanophore constellations near the head of the fish will be a good landmark to reassign Arctic charr between 65 and 80 mm. A specific software identifying the spot patterns will be a good tool to identify fish faster. I think that the population size is more related to the volume of water in each cave and to the quantity of macroinvertebrates present in the benthic zone than the parameters studied here. The continuation of the study will allow to test theses hypothesis.

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Appendices

Appendix 1: Caves 1 and 2 - Population size estimates

Visits (Caves 1 and 2)	Number caught Ct	Recaptures Rt	Number dead fish	Number tagged (less dead fish)	Tagged fish at large Mt	Mt * Rt	Ct * Mt²	Ct * Mt	Rt² / Ct	N (Petersen estimation)	S² (1/N) (Petersen)
June 2012	30	0	0	30	0	0	0	0	0.00		
August 2012_1	26	12	0	14	30	360	2.34E+04	780	5.54	65	1.06E-05
June 2013_1	8	3	0	5	44	132	1.55E+04	352	1.13	117	1.51E-05
June 2013_2	15	9	1	5	49	441	3.60E+04	735	5.40	82	6.66E-06
August 2013_1	37	28	0	9	54	1512	1.08E+05	1998	21.19	71	1.71E-06
August 2013_2	11	5	0	6	63	315	4.37E+04	693	2.27	139	5.68E-06
June 2014_1	19	13	0	6	69	897	9.05E+04	1311	8.89	101	2.39E-06
June 2014_2	20	20	0	0	75	1500	1.13E+05	1500	20.00	75	0.00E+00
August 2014_1	36	36	0	0	75	2700	2.03E+05	2700	36.00	75	0.00E+00
June 2015_1	26	25	0	1	75	1875	1.46E+05	1950	24.04	78	2.53E-07
June 2015_2	15	14	0	1	76	1064	8.66E+04	1140	13.07	81	7.18E-07
August 2015_1	23	18	0	5	77	1386	1.36E+05	1771	14.09	98	1.25E-06
August 2015_2	11	11	0	0	82	902	7.40E+04	902	11.00	82	0.00E+00
June 2016_1	6	6	0	0	82	492	4.03E+04	492	6.00	82	0.00E+00
June 2016_2	17	14	0	3	82	1148	1.14E+05	1394	11.53	100	1.27E-06
Total	300	214	1	85	1052	14724	1229786	17718	180.141611		

t 95% (t distribution table)	2.14
m	15

Schumacher and Eschmeyer estimations		
1/N		1.20E-02
N		**84**
S² (1/N)		2.24E-07
S (1/N)		4.73E-04
Confidence intervals (CI) (95%)		
CI (1/N)	1.30E-02	1.10E-02
CI (N)	**77**	**91**

Schabel estimations		
1/N		1.21E-02
N		**83**
S² (1/N)		6.82E-07
S (1/N)		8.26E-04
Confidence intervals (CI) (95%)		
CI (1/N)	1.38E-02	1.03E-02
CI (N)	**72**	**97**

Petersen estimations		
1/N		1.12E-02
N		**89**
S² (1/N)		3.26E-06
S (1/N)		1.81E-03
Confidence intervals (CI) (95 %)		
CI (1/N)	1.51E-02	7.36E-03
CI (N)	**66**	**136**

Appendix 2: Caves 7 and 25 - Population size estimates

Visits (Cave 7 and 25)	Number caught Ct	Recaptures Rt	Number dead fish	Number tagged (less dead fish)	Tagged fish at large Mt	Mt * Rt	Ct * Mt²	Ct * Mt	Rt² / Ct	N (Petersen estimation)	S² (1/N) (Petersen)
June 2012	65	0	0	65	0	0	0	0	0.00		
August 2012_1	119	16	5	98	65	1040	5.03E+05	7735	2.15	483	2.31E-07
August 2012_2	76	9	2	65	163	1467	202E+06	12388	1.07	1376	5.17E-08
June 2013_1	89	53	3	33	228	12084	4.63E+06	20292	31.56	383	5.21E-08
June 2013_2	67	48	0	19	261	12528	4.56E+06	17487	34.39	364	4.45E-08
August 2013_1	121	82	0	39	280	22960	9.49E+06	33880	55.57	413	2.30E-08
August 2013_2	54	38	0	16	319	12122	5.50E+06	17226	26.74	453	3.79E-08
June 2014_1	71	52	4	15	335	17420	7.97E+06	23785	38.08	457	2.46E-08
June 2014_2	75	63	0	12	350	22050	9.19E+06	26250	52.92	417	1.46E-08
August 2014_1	119	99	0	20	362	35838	1.56E+07	43078	82.36	435	8.97E-09
August 2014_2	75	62	1	12	382	23684	1.09E+07	28650	51.25	462	1.31E-08
June 2015_1	110	65	1	44	394	25610	1.71E+07	43340	38.41	667	1.42E-08
June 2015_2	57	47	0	10	438	20586	1.09E+07	24966	38.75	531	1.32E-08
August 2015_1	117	86	0	31	448	38528	2.35E+07	52416	63.21	609	8.29E-09
August 2015_2	107	88	0	19	479	42152	2.46E+07	51253	72.37	582	5.95E-09
June 2016_1	86	68	3	15	498	33864	2.13E+07	42828	53.77	630	7.76E-09
June 2016_2	99	81	0	18	513	41553	2.61E+07	50787	66.27	627	5.71E-09
Total	1507	957	19	531	5515	363486	193813905	496361	708.888234		

t 95% (t distribution table)	2.12
m	17

Schumacher and Eschmeyer estimations		
1/N		1.88E-03
N		**533**
S² (1/N)		8.77E-09
S (1/N)		9.36E-05
Confidence intervals (CI) (95%)		
CI (1/N)	2.07E-03	1,68E-03
CI (N)	**482**	**596**

Schabel estimations		
1/N		1.93E-03
N		**519**
S² (1/N)		3.88E-09
S (1/N)		6.23E-05
Confidence intervals (CI) (95%)		
CI (1/N)	2.06E-03	1.80E-03
CI (N)	**485**	**557**

Petersen estimations		
1/N		1.80E-03
N		**556**
S² (1/N)		3.48E-08
S (1/N)		1.87E-04
Confidence intervals (CI) (95 %)		
CI (1/N)	2.20E-03	1.40E-03
CI (N)	**456**	**712**

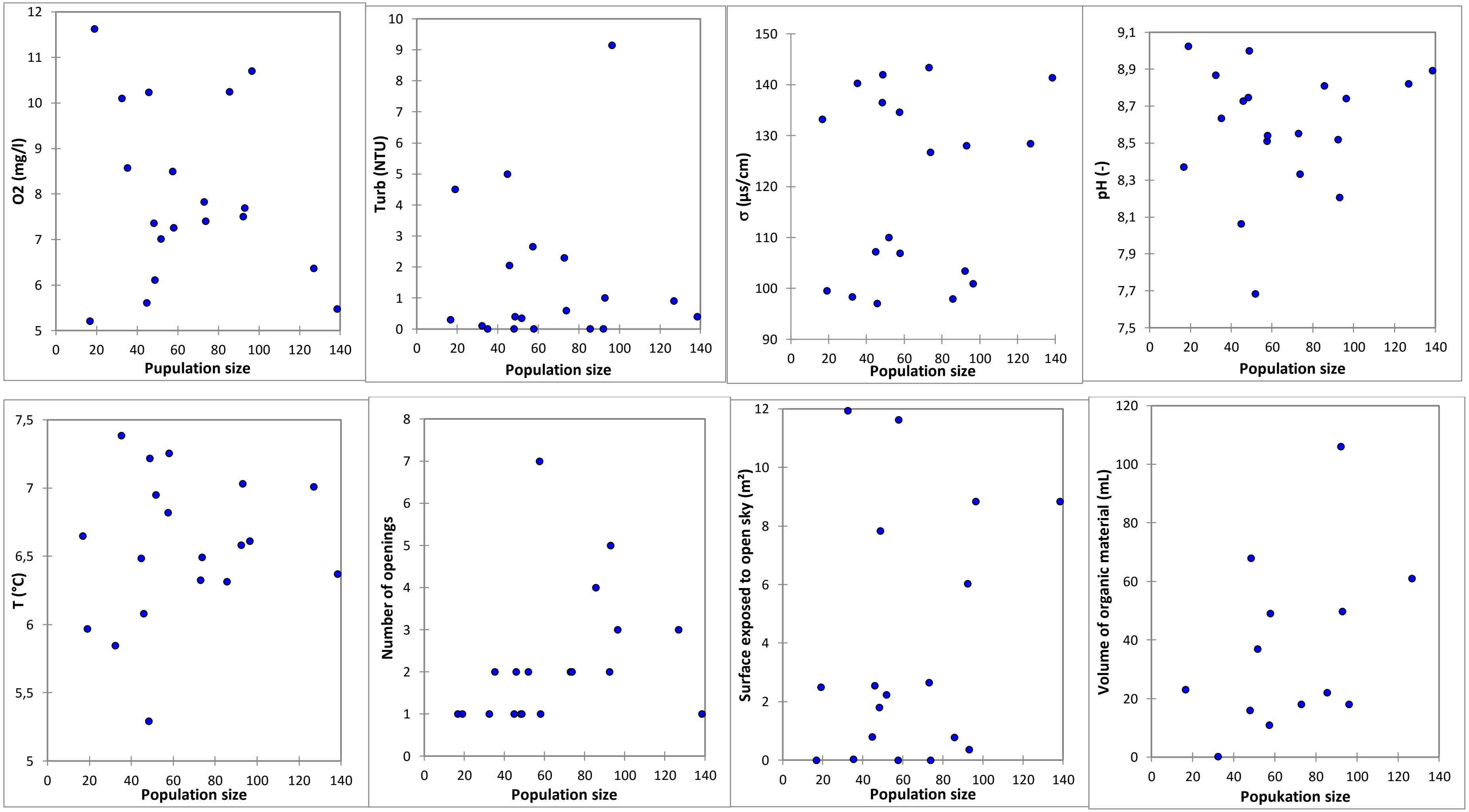
Appendix 3: Population size estimations in all the studied lava caves

Cave	N fish > 65 mm (Schumacher and Eschmeyer method)	Confidence intervals (95%)	
1	35	32	39
2	48	44	53
5	93	87	100
6	17	16	18
7	138	123	158
10	57	53	62
11	74	70	78
12	49	46	52
17	19	17	21
17b	32	30	35
18	96	92	101
19	46	41	51
20	85	78	94
21	58	54	62
22	92	81	107
23	52	48	56
24	45	41	49
25	393	354	441
26	127	120	134
27	73	64	85
Total	1629	1491	1796

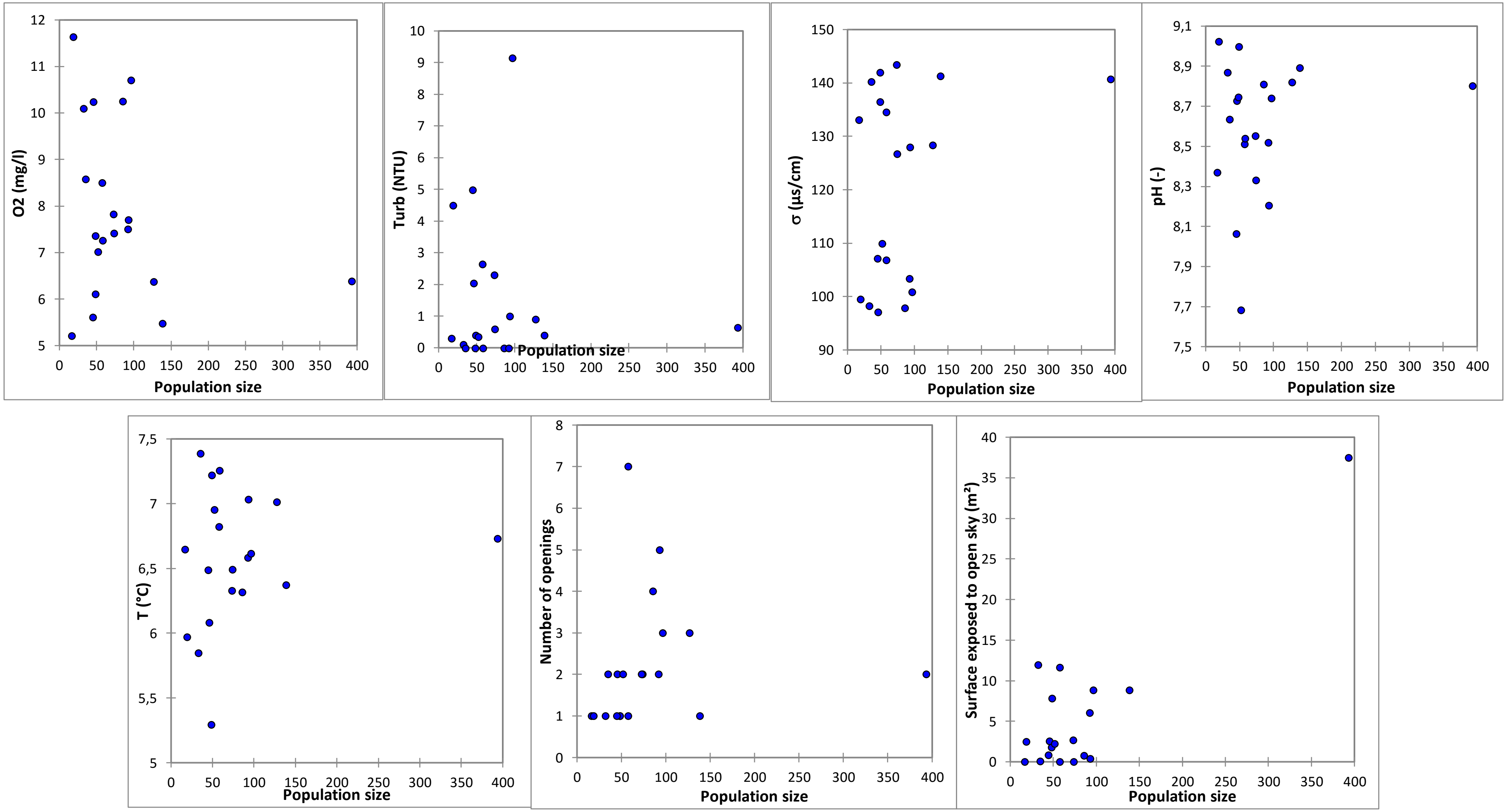
Appendix 4: Pearson and Spearman correlations

Population size plotted against:	With cave 25					Without cave 25				
	Variables normally distributed?	R^2 (Pearson)	p-value (Pearson)	R^2 (Spearman)	p-value (Spearman)	Variables normally distributed?	R^2 (Pearson)	p-value (Pearson)	R^2 (Spearman)	p-value (Spearman)
T (°C)	Yes	0.018	0.578			Yes	0.027	0.502		
pH (-)	Yes	0.032	0.45			Yes	0.012	0.658		
σ (µs/cm)	No			0.042	0.38	No			0.012	0.649
Turbidity (NTU)	No			0.01	0.674	No			0.006	0.746
O_2 (mg/l)	Yes	0.064	0.28			Yes	0.044	0.388		
Surface exposed to open sky (m^2)	No			0.102	0.18	No			0.04	0.423
Volume of organic material (mL)	Yes	0.181	0.147							
Number of openings	No			0.273	0.019	No			0.287	0.019

Appendix 5: Scatterplot of ecological factors plotted against population sizes (without the cave 25)



Appendix 6: Scatterplot of ecological factors plotted against population sizes (with the cave 25)



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