POLYTECH TOURS

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Individual report

4th year DAE

The impacts of Threespine Stickleback on invertebrate communities, in a mesocosm experiment, Iceland

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IMA
2020-2021

From April 3rd,2021
To July 23rd, 2021

ABSTRACT

My internship was part of a research on the impacts of the threespine stickleback *(Gasterosteus aculeatus)* on its environment. This study is based on different types of stickleback existing within the same lake system: Mývatn. These different populations have developed different phenotypes depending on their habitat of origin. This study uses fish from a warm basin and a cold basin at different densities in a mesocosms experiment to reproduce the lake's ecosystem. This experiment tries to understand the short-term but also long-term effects of stickleback on their environment. My internship topic focused on the impacts of stickleback on invertebrate communities. Despite a delay in the experiment, the results obtained showed that the seeding of mesocosms from an invertebrate communities taken from a lake is possible and very effective. However, there was a loss of species diversity completely different from the initial stand. Therefore, the study aiming to reproduce the Mývatn ecosystem is not fully respected, but still represents a good approach to the study of the stickleback on its environment or on invertebrate communities.

Key worlds : Threespine Stickleback, invertebrates, adaptation, phenotype, mesocosms

RESUME

Mon stage s'est inscrit dans le cadre d'un sujet de recherche sur les impacts de l'Epinoche à trois épines (*Gasterosteus aculeatus)* sur son environnement. Cette étude se base sur différentes population d'épinoche existant au sein d'un même système lacustre : le lac Mývatn. Ces différentes populations ont développé des phénotypes différents selon leur habitats d'origines. L'étude à laquelle j'ai participé étudie des populations d'épinoches provenant d'un bassin chaud et d'un bassin froid à différentes densité dans des mésocosms visant à reproduire l'écosystème du lac. Cette expérience cherche à comprendre les effets à court terme mais également à long terme de l'épinoche sur leur environnement. Mon sujet de stage s'est centré sur les impacts de l'épinoche sur les communautés d'invertébrés. Malgré un retard pris dans l'expérience, les résultats obtenues ont montré que l'ensemencement des mésocosms à partir d'une communauté d'invertébrés pris d'un lac est possible et très efficace. Cependant, il a été observé une perte de diversité des espèces qui composées le peuplement initiale. Par conséquent, l'étude visant à reproduire l'écosystème de Mývatn n'est pas totalement respecté, mais représente tout de même une bonne approche en ce qui concerne l'étude de l'épinoche sur son environnement ou sur les communautés d'invertébrés.

Mots-clés : Epinoche à trois épines, invertébrés, adaptation, phénotype, mésocosm

ACKNOWLEDGEMENT

I want to thank my two supervisors Bjarni K. Kristjánsson and Benoît Chamiot-Clerc for giving me the chance to do my internship with them, give me the opportunity to discover the research world and specially to travel in Iceland despite the current Covid-19 epidemic. But especially a big thanks to Benoît Chamiot-Clerc for accompanying me throughout my internship, for always being there to answer my questions, for taking the time to explain to me many things in the field of ecology, evolution, development, … and for teaching me many things.

A great thanks to all the people of the department of aquaculture and fish biology that were always presented to help me, answer at my question and especially for the warm welcome they gave me. I would also like to thank all the other trainees with who I have shared very nice moments and I was happy to meet.

I thank my academic supervisor Mrs Boisneau to have been accompanied me, to have been presented to respond at my concern and steer me to continue on the good way during my internship. I want to thank Mr Bacchi that helped me and answered my specific question about invertebrates on my subject. I also thank Mrs Nour and Mrs Robin who helped me a lot in all administrative skills to make my internship in Iceland come true.

Finally, thank to my family and my friends to encourage me to make the experience and they have been a great support to me on this journey.

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GLOSSARY

Abiotic factors : refer to non-living physical and chemical elements in the ecosystem
Adaptation : actions or processes of change by which an organism or species becomes better suited to its environment
Benthic : anything associated with or occurring with the lowest ecological zone in a water body and usually involves the sediments at the floor
Biodiversity : from biological diversity refers to the variety of life at different scale, from gene to ecosystem and can encompass the evolutionary, ecological, and cultural processes that sustain life
Biotic factors: refer to living or once-living organisms in the ecosystem
Community : in ecology, a community is a group or association of populations of different species occupying the same geographical area at the same time
Ecosystem : system of biotic and abiotic components interacting with each other
Epibenthic : organisms that live on or just above the bottom sediments in a body of water
Evolutionary selection : selection process made by the environment to select the most suitable individuals by different mechanisms
Lentic : refers to standing waters such as lakes and ponds, or swamps and marshes
Lotic : refers to running water such as rivers and streams
Mesocosm : is any outdoor experimental system generally of medium size that examines the natural environment under controlled conditions
Pelagic : Zone refers to open and free waters in the body of the ocean, sea, river, lake, … that stretch between the surface and the bottom
Phenotype : set of observable characteristics of an individual resulting from the interaction of its genotype with the environment
Plasticity : the adaptability of an organism to changes in its environment or differences between various habitats
Zooplankton : small animals present in the water column

ACRONYM

DEVO : Development of organism

ECO : Ecological conditions

ECO-EVO-DEVO : Concept to understand the functioning of the environment and try to find the impacts of environment on the different processes of ecology, evolution and development but also understand the possible feedback induced on the environment. This concept includes : " In ECO-EVO, the environment influences the evolution of populations through natural selection; In EVO-ECO, evolutionary responses (i.e. phenotypic changes across generation) influence ecological processes in an ecosystem; in ECO-DEVO, the environment affects developmental processes of individual organisms (broadly encompassing any form of individual plasticity and parental effect); in DEVO-ECO, within-generation developmental responses of individuals influence the response of populations and, subsequently, ecosystems to environmental change; in EVO-DEVO, evolutionary processes across generations provide inherited signals (e.g. direct genetic and epigenetic variation) that influence phenotypic development; and in DEVO-EVO, selection acts on phenotypic variation from development. In nature, ECO-EVO-DEVO processes interact and are likely to act dynamically, that is *via* reciprocal feedback responses." (Skúlason et al., 2019)

EVO : Evolutionary

TABLE OF FIGURES AND TABLES

1. PRESENTATION OF THE ORGANISATION : Hólar University

Hólar University, in Northern Iceland is based in Hólar but also in Sauðárkrókur. Where the Department of Aquaculture and Fish Biology has its main offices and laboratories (Figure 1). Hólar is a very historical site. A school has been there since 1106, but in 1882 an agricultural school was created, which became Hólar University in 2007. The University is composed of three departments: Aquaculture and fish biology, Equine science, and Tourism studies. The University accommodates about 200 students, approximately 50 staff including 28 academic staff.

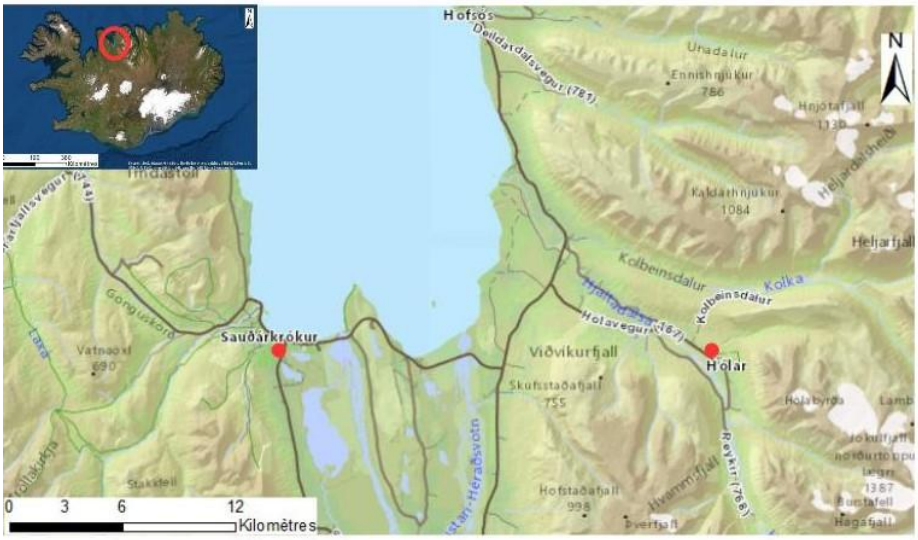

Figure 1 : Map of localisation of Hólar University and Sauðárkrókur office

Concerning the aquaculture and fish biology department, the main building is based in Sauðárkrókur at Verið located on the harbour. The building is divided into three parts including office space, laboratories, and rearing facilities for research in aquaculture, fish biology and limnology. The department is headed by Bjarni K. Kristjánsson and includes academic staff, technical staff, post-doctoral fellows, doctoral students, students in master-project and numerous interns to help in the several ongoing projects (see Appendix 1). The purpose of aquaculture and fish biology studies is to improve the knowledge in this field. Currently, researches are mainly focused on threespine stickleback (*Gasterosteus aculeatus)*, Artic charr (*Salvelinus alpinus)* concerning the fish biology studies (Figure 2A) and Atlantic salmon (*Salmo salar)* and Artic charr in aquaculture (Figure 2B).

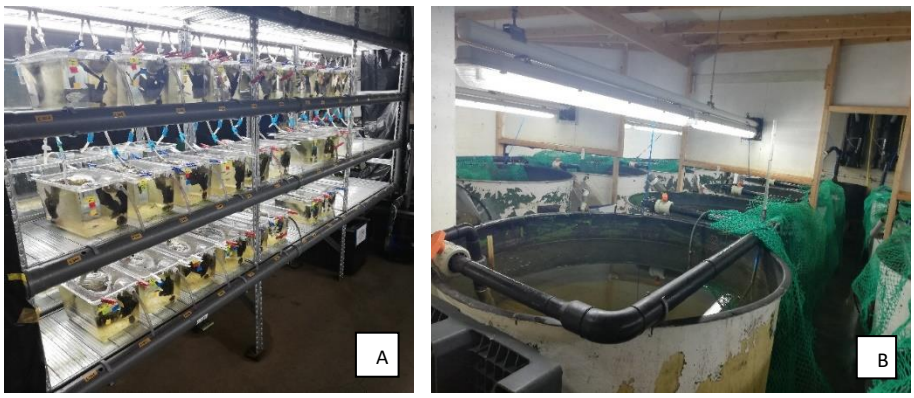

Figure 2 : Picture of experimental setup for biology (A) and on aquaculture studies (B)

2. PRESENTATION OF TASKS

In my internship at Hólar University I assisted Benoît Chamiot-Clerc, a doctoral student, who is currently working on his thesis on the ECO-EVO-DEVO concept and more precisely he studies the impacts that threespine stickleback have on their environment. To do this, a mesocosm experiment and a laboratory experiment have been put in place to test the impacts of different threespine stickleback populations (cold shore habitat and warm spring shore habitat) from lake Mývatn, with different density of fish (low and high density) on the environment (*more details are available in cf.4 concerning the study system and the experiment)*. The mesocosm experiment takes place in three phases to test short- and long-term effect on the environment. A first phase to set up the ecosystem in place. A second phase with adult stickleback is kept in the tanks with different treatments : cold or warm water origin and at high or low density with a control, where there is no stickleback. This allows us to see the direct impact of the stickleback on the system. A third phase with juveniles to see how the adults may impact the conditions for the next generation of fish is realized. This experiment shows the direct impact of stickleback on their environment. We also decided to set up a second experiment in the laboratory to see the indirect impact of fish on their environment.

My work focused on the two first phase of mesocosm experiments, and focused, mainly, on the impacts of stickleback on the invertebrate communities. Here my background in the aquatic field helps me to approach questions of ecology, the studies of fish but also the studies of invertebrates. Three other interns worked with me: Pierre de Broissia worked on developing protocol and technical point of the subject, Flora Baudart worked on the on the impacts of stickleback on the water chemistry and Lucas Pulcini helps us for two weeks.

In this internship my first task was a bibliography study to find literature that related to my internship subject, but also to find solutions and to develop the sampling protocol of invertebrates during the mesocosm experiment. My second task was to put in place the experimental setup in the mesocosms and apply other protocols to put in place correctly the mesocosm experiment. My third main task has been to monitor the experiment during the setting up phase by taking physical and chemistry measures, sediment samples and invertebrate samples. At the same time, I had to take care of fish which will be used in the laboratory experiment. My fourth task was to identify and count invertebrates from previous samples to make some diversity and density analyses in the software R. My last task was to write a report on my subject : the impacts of stickleback on the invertebrate communities, with the setting up phase of the mesocosm experiment and the results obtained.

3. WORKFLOW OF TASKS

3.1. Bibliographic research on my subject

During my internship, I did a lot of different type of work. The first month I mainly did bibliography in link with my subject. I read some articles on notion and concept in relation to my subject, for example the ECO-EVO-DEVO framework, phenotypic and genetic plasticity, the concept of adaptation. Following this, I focused my theoretical research on my specific subject about the impacts of stickleback on invertebrate communities. I read some articles on Lake Mývatn to learn more about the functioning and the ecology of the lake, but also the potential invertebrate communities that I could find in my experiment. I read on the adaptation and phenotypic plasticity of stickleback, their diet, but also the possible invertebrate's phenotypic variation in the presence of stickleback. This was to better understand the potential changes in those invertebrate population that I can expect to have in the mesocosms. And finally, I tried to find different methods to sample different communities of invertebrates (pelagic, epibenthic and benthic) in the mesocosms, without disturbing the ecosystem in place, mainly without destabilising the substrate, which is very easily suspended.

3.2. Protocol for sampling and treating invertebrate communities

During the phase of bibliographic research and before the experiment was put in place, I had to create a protocol of sampling and treating invertebrate communities (Appendix 2).

In the mesocosm experiment, the aim was to collect the invertebrates without resuspending the substrate to avoiding disturbing the ecosystem in place. For that, we knew that the two major communities of invertebrate were pelagic and benthic.

For pelagic invertebrates, the major methods that I found consisted to take a sample of water column with a pipe (shows in Figure 3A) and concentrate the invertebrates with sieve (Des Roches et al., 2013; Jakobsen et al., 2003; Matthews et al., 2016). This method has the advantage to avoid disturbing the sediment.

The most inconvenient to sample is the benthic invertebrates because the main method of sampling consist of collecting the invertebrates from the sediments using a net (Matthews et al., 2016; Tourville Poitier, 2009), but this method destroys the ecosystem. To minimize the effect, I decided to use the idea of another intern (Pierre De Broissia) by using a trap. These traps put in place before the sediment and can be collect using a magnet at any time after (see Figure 3B). The magnet closes tightly the trap and d minimize ecosystem disturbances. However, the traps need to be collected gently. However, with this method, we collect only benthic invertebrates that do not move much.

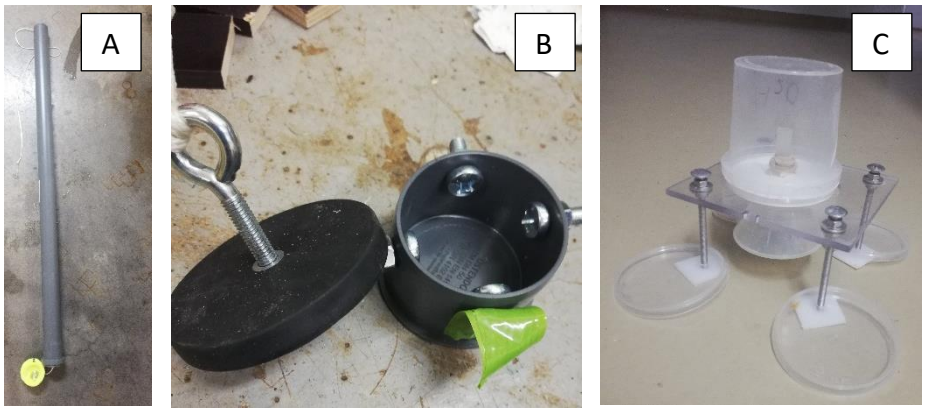

Figure 3 : Samplers for pelagic invertebrates (A), benthic invertebrates (B) and epibenthic invertebrates (C) using during the mesocosm experiment

For these reasons I made more research on method to collect epibenthic invertebrates, and the majority of methods use a trap (Bo et al., 2007; Nickel & Moore, n.d.; Scatolini & Zedler, 1996). However to avoid to disturb the sediments in place I used the technic developed by Combot, 2018, that exploits the movement of the epibenthic invertebrates with a trap which can be put in place and stays for 12 hours to capture invertebrates (illustrates in Figure 3C). This technique avoids disturbing the sediments because that it does not need a mechanical movement to collect invertebrate, but they need to be put in place gently to avoid re-suspending too much of sediment.

After having determined the methods of sampling I needed to decide on intervals between samples. To see the impacts of the stickleback on invertebrate communities we need to collect samples of invertebrates at different time period during the running of the experiment. It is important to obtain for each phase (setting up, adult and juvenile phases) a starting point and a final point, thus four sampling at least. However, as we are observing changing communities, where it may be important to follow the communities frequently, but not too often to avoid direct human impact on communities. The final plan was thus to do a sampling every three or four weeks, depending on the length of the

adult phase with 10L of water column, 3 traps of benthic invertebrates and 2 traps of epibenthic invertebrates.

But after some calculation the number of samplings would have been of 4 or 5 sampling for the entire duration of mesocosm experiment, and by consequent the number of samples would have been too much important to treat the 40 mesocosms on at least two months of experimentation. For these reasons only sampling at the beginning and the ending of each phase was chosen, with 10L of water column, 2 traps of epibenthic invertebrates and 2 benthic traps by sampling to avoid having a human impact.

NOTE : Some points of the duration and methods of sampling have been discussed a lot (sometimes sources of conflict or confusion). In fact, concerning the epibenthic trap, it was initially planned to suspend the trap at a certain height without the lids that serve as feet to avoid putting the trap on the floor and thus suspending the sediment when we remove the trap. But the trap has finally been deposited on the floor to respect the protocols for using these kinds of traps usually used in other experiments by Hólar University. By consequence, we have known in advance that this trap would disturbed the ecosystem and required delicate handling to reduce the impact.
The duration between sampling and the number of benthic traps has also been discussed a lot. The final number of replicates for benthic traps have been fixed to two and all sampling have been fixed at the beginning and the ending of each phase to limit the human impact but also to decrease the number of samplings for technical and economic reasons. By consequence, we have only an overview of the experiment with an "before-after" impacts of the stickleback, and not a real evolution or adaptation along experiences.
The question about the quantities of sediment when we take sample have been also discussed, because with the protocol presented before, the sediment will be taken with the trap but not put back into the mesocosms. By consequence, with many traps the quantity of sediments would have decreased of 2/3 at the end of the experiment. We have thought about reputing sediment when we collect trap, but this solution would have disturbed the ecosystem at each time. It is for that reason; we have decided to decrease the number of traps to avoid removing too much sediment and to avoid having to reapply some sediment into the mesocosms.

After collecting, the samples were filtered through a 63µm sieve and invertebrates collected preserved in 95% ethanol. Following, the invertebrates will be identified to lowest possible level, counted and their body length measured, with spines for *Daphnia longispina,* for 30 individuals at least. Diversity, density, body length, defence structure of invertebrates will be analysed for each treatment using RStudio.

3.3. Mesocosm experiment

The aim of this mesocosm experiment (see Appendix 3 for different phases) is to see the direct effect stickleback have on the invertebrate communities. For that, we used 40 mesocosms made out of plastic tanks of $1m^3$ (1m*1m*1m), obtained from various production companies who use them as storage containers. The first step consisted of cutting off the top and cleaning them with hot water.

The mesocosms were put in place and made level. They were filled with water from a nearby river using a pump. We placed cleaned, to remove salt, marine sand as a base layer (20L per mesocosm).

Once the tanks had be put in place, we created the benthic invertebrate and sediments traps using different size of pipes. For invertebrate traps we used 240 plastic pipes of 3cm x 4.5cm Ø (6 pipes per mesocosm) with 4 screws on each side to allow the traps to sink when they are set but also to be able to recover them using a magnet. We also added two stick ruban on two sides to allow us to see the

traps to recover them. For sediments traps we used 240 plastic pipes of 4cm x 3.5cm Ø with a flat washer fix on the top to allow the traps to sink when they are set but also to be able to recover them with a magnet. We then placed six invertebrate traps and six sediments traps in a random way (mesocosm was divided into 36 possible places) in each mesocosms.

The next step consisted of going to lake Mývatn to take sediments (see map in Appendix 4) using a net of 100µm. The sediment was transferred to the experimental site in Hólar and 20L of sediment placed in each mesocosms. Furthermore, we collected invertebrate communities with a plankton net of 100 µm. We added to each mesocosms 1L of water containing the community and took additionally three samples of 1L to serve as reference in the invertebrate analyses. We collected macrophyte like *Cladophora* or *Myriophyllum spicatum* using a 100 µm net and placed some plants into the mesocosms. In Mývatn we also collected fish using minnow traps (Figure 4), laid at least 12 hours.
The fish have been, or will be, collected twice, first to collect breeders, to create offspring for the third phase of the mesocosm experiment and a second time to collect adults for the second phase of the experiment. In both cases, the same number of fish were collected at both the warm and cold habitat.

Figure 4 : Picture of minnow trap (Steve A. Price)

When selecting breeders, we selected fish with no visible parasites Figure 5A and were sexually mature (presence of eggs in the female by a swollen abdomen Figure 5B, and nuptial colour obvious blue eyes and orange-red throat in the male Figure 5C). We made sure to collect additional female to decrease problems caused by misinterpretation related to parasitism, which increases the size of the abdomen that can look like female containing eggs, but also additional male not presenting nuptial colours.

Figure 5 : Threespine stickleback with parasite (A) female (B) and male (C) (Source : Steve A. Price)

For the second fishing, 240 fish will be collected for the adult phase of mesocosm experiment (16 mesocosms x 10 fish for high density treatment and 16 mesocosms x 5 fish for low density treatment). all fish must be over 40mm long, have no visible parasites and not be sexually mature to avoid having a varying number of fish during the adult phase and not have any reproduction that will compromise the juvenile phase that follows.

During the breeding phase, the fish were put in buckets with water at 17°C (see Figure 6A) until the crossing and were feed with bloodworms. The females were separated from the males in addition to separate fish from cold basin and warm basin. The buckets that contain fish, had a hole in the centre at the bottom and with a long tube connected with 4 holes in the bottom to filter the water and a filter around this pipe to avoid the fish to escape (see Figure 6B).

The females were checked daily to see if they were ready to lay eggs. When females were ready, we proceed to the breeding protocol available in Appendix 5, consisting of removing the testes of males by surgery and cross sperms with eggs previously removed from females. The fertilize eggs were then raised in special buckets with mesh (see Figure 6C). Eggs are monitored until they hatch and dead eggs are removed to avoid developing fungi that can contaminating other viable eggs. After hatching the fish larvae are fed with bloodworms that have been chopped down.

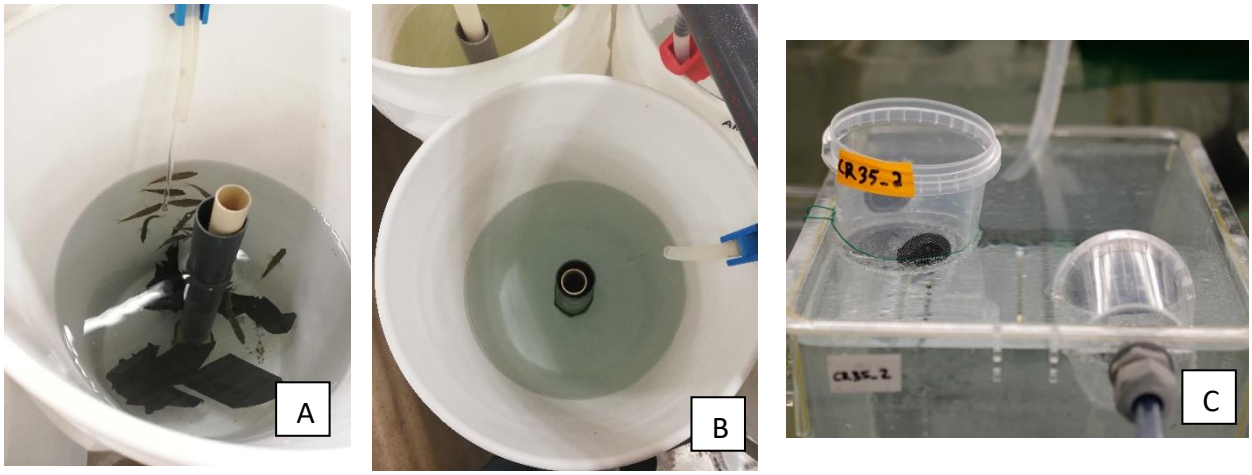

Figure 6 : Pictures of buckets experiment of stickleback with (A) and without fish (B) and for babies fish (C)

During the phase of setting up, several trials have been done to determine the time that take the water chemistry measurement and the sampling of pelagic invertebrate. For invertebrates, the water column sampler was previously clean, then put in different area of the mesocosm to collect samples, later poured into a clean bucket and repeated until 10L were collected. The invertebrates in the water were sieved through a 63µm sieve and preserved in 95% ethanol in 50 ml falcon tube. This procedure was repeated for each mesocosms and provides an overview of the development of invertebrate communities since their seeding.

NOTE : Originally, the aim of my internship was to study the impacts of stickleback on invertebrates and to see the evolution of communities in terms of density, diversity and phenotypic variation. Unfortunately, the mesocosm experiment took a lot of late due to administrative, communications problems and seasonal late of Mývatn lake. By consequence, the communities of invertebrates were complete and ready for the mesocosm experiment at the end of June. The seeding of the communities in the mesocosms was delayed and could not be done before, also delayed the start of the set-up phase. The seasonal late of the lake has also disturbed the sexual maturity of fish which postponed the start of the breeding that served for the juvenile phase and thus postponed the start of the adult phase. However, the first phase of fishing for reproduction was carried out at the end of June to obtain a few sexually mature individuals to be used for breeding. Unfortunately, many gravid females have either lost their eggs or have resorbed them through stress delaying again all phases of the experiment. To overcome this problem, another breeding attempt was made in mid-July directly in the field using a portable laboratory to eliminate the stress of transport and the risk of losing the eggs. This method allowed the recovery of many eggs. By consequence, with all this late in the mesocosm experiment I have only seen the setting up phase and not the adult and juvenile phase that initially I would have studied. Concerning the samples, I would have to study the pelagic, benthic and epibenthic invertebrates but finally I have only the three references that we took after the invertebrates collecting at Mývatn and the 40 trials samples of water column that we took during the setting up phase.

During my last two weeks of internship, I had to go through the 40 invertebrate samples of water columns and the three reference samples of communities took at Mývatn. This treatment consisted of identifying the invertebrates to the lowest taxonomy level and count them. The biggest individuals like

adult *copepods, Chironomidae* and *Daphnia longispina* are isolated for further phenotypic analyse after into Eppendorf tubes. The indeterminate individuals are also separated into Eppendorf tube. All the Eppendorf samples are conserved with 95% ethanol. Concerning the smallest individuals like *nauplii copepods* and *Rotifers* they are not isolated, because they were presented in large density in samples and their collect was too complex to collect them one by one without take other species around. When their density was too important sub-samples of 10ml on the initial 50ml are made after homogenisation. A factor of 5 is applied at all individuals counting into sub-samples to estimate the diversity and the density of invertebrate sample.
Unfortunately, I had not enough time to go through the 40 mesocosm and reference samples. I went through only 22 invertebrate samples from water column of mesocosm and only two reference samples.

Concerning the treatment of invertebrate data collection, a first step consisted into organize the data table with species in column and the mesocosm number or reference number in line.
After that some calculations were done to have the total invertebrate number of each sample and the number of species found. These results allowed me to calculate the density of invertebrates based on study system of mesocosm:

- For reference sample : the number of invertebrates found into the 1L of water that serve to seed the mesocosms are thus the number of invertebrates into 1000L of mesocosm content.
- For water column sample : the number of invertebrates found into the 10L sampling was multiplied by 100 to obtain the density of invertebrates into 1000L of mesocosm content.

The Shannon diversity index was calculated to express the quantitative arrangement of species that constitute the populating.
After some statistic tests were done on the density and the diversity to first see distribution of data, and then to determine statistical tests that will serve to answer the different predictions about the impacts of threespine stickleback on invertebrate communities.

NOTE : Only the treating data are presented in this report, but the raw data are not integrated to respect the confidentiality of the data before the editing of the thesis. But the procedure and the specific statical test are presented in part 4.2.4 of this report but only part of it was achieved in the time I had left. The results about invertebrate density, diversity and abundance are available in the part 4.3 and several hypotheses of results have been made concerning the expectations on the adult phase and the juvenile phase, that finally I did not see.

3.4. Laboratory experiment

During my internship and before the start of the laboratory experiment, I had to take care of several fish from a previous experiment, focusing on the diet of fish from warm and cold habitats in lake Mývatn. There were 46 buckets to feed with different density of fish. Some were fed only benthic invertebrates like bloodworm and others fed pelagic invertebrates like *Cladocera* or *Artemia*. All problems in buckets were recorded like the death of fish and the causes (fungi, fight, digestive problem, …)

The laboratory experiment is a supplement to the mesocosm experiment, to see in a control environment the indirect effect of the stickleback on invertebrate communities. To see that, we tested the same treatments with warm and cold basin origin and with low or high density of fish.

NOTE : I did not have the chance to see this experiment before the end of my internship, but it was initially planned for the month of July. Unfortunately, all the problems on the main mesocosm study required all the attention to solve them, thus postponing the secondary experiment in the laboratory.

3.5. Other tasks

During all my internship I made weekly meeting (Monday and sometimes Friday) to be aware of the other people projects, some lecture of articles, and talked about the advancement of my project with the whole team or just with my supervisors Benoît Chamiot-Clerc and Bjarni K. Kristjánsson.

4. REPORT FOR THE ORGANISATION : Scientific article on my subject

4.1. Introduction and subject context

An ecosystem is a system or a group of interconnected elements, formed by the interaction of a community of organisms with their environment in a given space and at a given time (Blew, 1996). The environment can put constraints on those communities living in a given ecosystem, promoting adaptations. Such adaptation can be seen in morphology, diet or behaviour modifications and can sometimes increase fitness (e.g., survival, reproduction, transmission of genes) within populations, resulting in population evolution. It is well known that the genetic plays a key role in the adaptation process but phenotypic plasticity of organisms may be important for adaptation that allows for rapid phenotypic responses which may allow organisms to improve their fitness in a changing environment (Bolnick & Ballare, 2020).

Communities change because of the evolutionary response of populations within them. Those evolutionary processes are well known to be influenced by interacting abiotics and biotics factors which can shape the divergence of populations and the diversity of species (Swingland, 2001). We know that abiotic factors driving variation in species communities include chemistry and physical components (e.g., pH, conductivity, nutrient concentrations, temperature, hydraulic parameters, ...)(Bartrons et al., 2015). As well as the abiotic components, the biotic factors are known to be driven by interactions between species like bottom-up factors involving resources and top-down factors such as predation (Bartrons et al., 2015). These environmental conditions are highly variable among habitats and can lead to the natural selection process and promote adaptation of populations and sometimes lead to ecological speciation (Millet et al., 2013). This process is the result of an interaction of ecological conditions (ECO) and the evolutionary processes (EVO) that is strongly related to biological diversity.

However Hendry, (2016) and Matthews et al., (2016) have shown that the development (DEVO) of organisms can drive the plasticity of organisms and thus allow populations to be adapted to their environment. Thanks to organism's plasticity, populations that appear in first view to be ecologically generalist are in reality heterogeneous assemblages of specialised individuals and can be observable by phenotypic diversity in a given population. These specialised individuals are the results of the population adaptation at biotic and abiotic components in constant variation thanks to genetic, behaviour changes or morphological plasticity (Bolnick & Ballare, 2020).

Diet specialisation is a good example of specialised individuals within populations. Some studies have been done on the impacts of populations' diet and the diet variation within the same populations to understand the specialized individuals and variation of plasticity in link with this process (Bolnick & Ballare, 2020; Day et al., 1994; Lavin & McPhail, 1986). It has been proved that a diet variation within a population can increase population stability in using all sources of energy, promote coexistence by reducing the competition on the same resource, and thus reduce extinction risk if a population is less adapted. Such diet variation and specialization may alter ecosystem properties (Bolnick & Ballare,

2020). Consequently, plasticity plays a key role in the adaptation of species in evolution but also in the environment modification.

To take in account all interactions between ecology, evolution and development processes, an ECO-EVO-DEVO concept was created to gain better understanding on the environment functioning. This concept tries to understand the impacts of the environment on the different processes of ecology, evolution and development but also seeks to understand the possible link between each process and allow the modelisation of the environment in turn (shown in Figure 7) (Skúlason et al., 2019). To find that, the ECO-EVO-DEVO concept studies the possible abiotic-biotic links in the environment and the type of these links, for example direct or indirect.

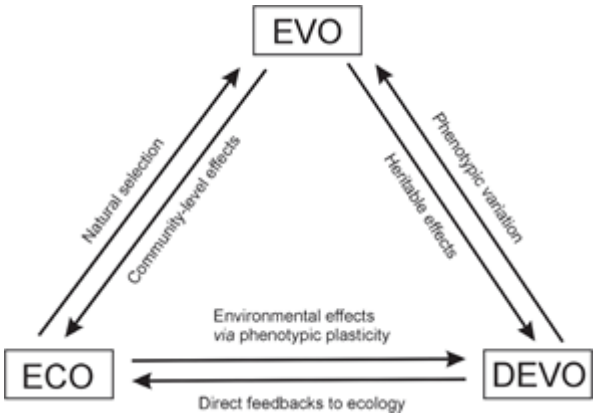

Figure 7 : The model of ECO-EVO-DEVO concept (Skúlason et al., 2019)

To better understand this framework of ECO-EVO-DEVO we choose to study the populations of threespine stickleback (*Gasterosteus aculeatus),* because this fish shows a high phenotypic plasticity, a quick rate of evolution and the ability to colonize a great diversity of habitats, both marine and freshwater, lotic or lentic (Millet et al., 2013). This species also has a wide range of morphology depending on its diet, the temperature of water, the hydraulic parameter or predator pressure (Bolnick & Ballare, 2020; Day et al., 1994; Lavin & McPhail, 1986). For example, it is possible to observe stickleback with a streamlined body when they feed on zooplankton or a wider mouth and a larger body when they feed on benthic invertebrates or again long spines to protect themselves against predators (Bolnick & Ballare, 2020; Lavin & McPhail, 1986). Moreover, the threespine stickleback is a meso-predator eating a variety of invertebrates, including primary consumers, but being hunted by other predators. Thus, its presence can impact communities on different scales within ecosystems, making it a good species to study the relationship between ecological, evolutionary and developmental processes.

Here we will study threespine stickleback from lake Mývatn in Iceland (Figure 8A), because there is a considerable phenotypic variation among stickleback populations, promoted by an important variety of habitats in the same lake. The lake was created about 2,300 years ago by volcanic activity and was thus recently colonized by the stickleback (Bartrons et al., 2015; Einarsson et al., 2004; Millet et al., 2013). The lake is divided into two basins North and South. In the smaller (8.5km²) but deeper (5.5m maximum) North basin, hot springs (up to 30°C) can be found. The basin is characterised by lava rocks, silica mud and covered by *Potamogeton filiformis.* Some part of the northern basin has undergone mining activities forming localised deep areas with anoxic substrates without vegetation. The southern basin is larger (28.2km²) but shallower (4m maximum). The main flow into the south basin are cold springs (down to 5°C) and characterised by vegetation composed of *Cladophorales* algae (Figure 8B). This diversity of habitats within the same lake has likely led to phenotypic and behavioural diversification of stickleback populations without leading to speciation (Millet et al., 2013).

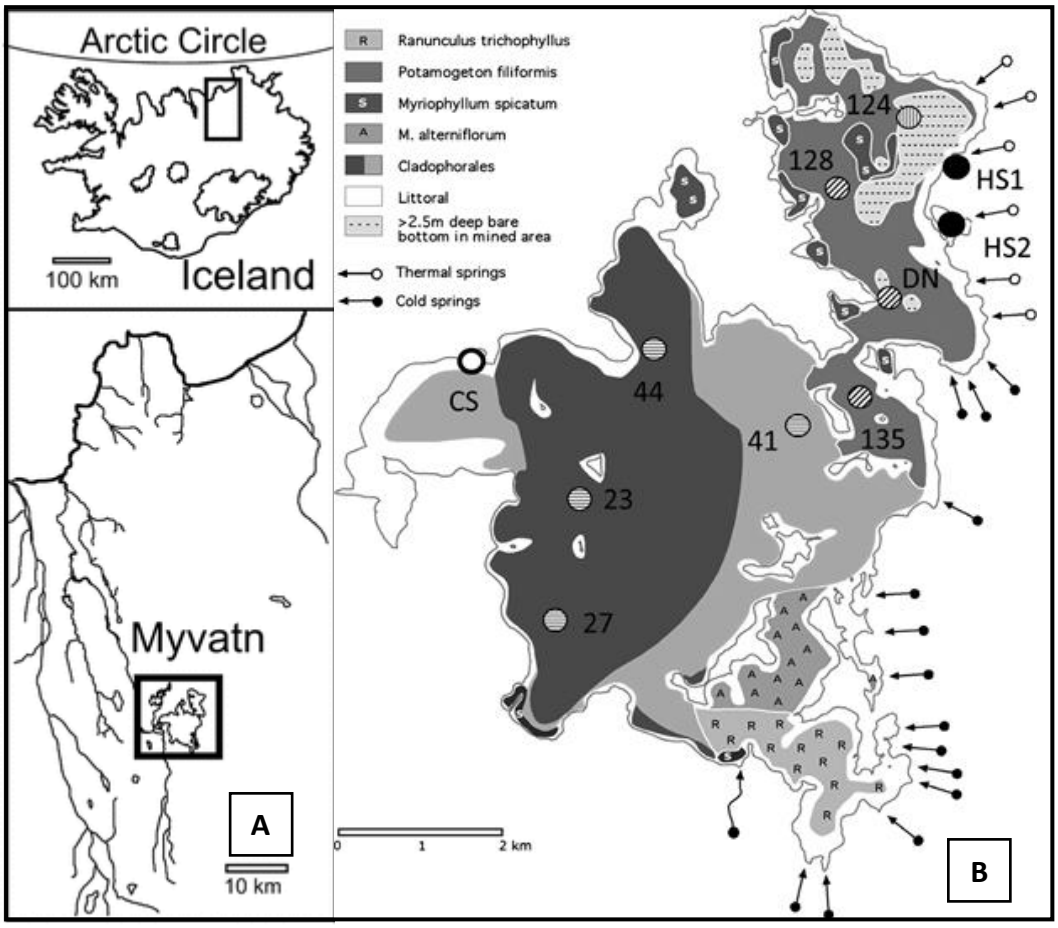

Figure 8 : Location map of Mývatn lake (A), the dominating benthic macrophytes and spring water inflows (B) (Einarsson et al., 2004)

Moreover, it has been observed in the northern basin of the lake that the populations of stickleback are 10-fold higher density, are larger with longer spines and narrower gaps between fewer gill rakers than fish in the southern basin. By consequence, the diet of stickleback in the northern basin is mainly composed of zooplankton, in contrast to the southern basin, where the diet is composed more of benthic invertebrates (Millet et al., 2013). Moreover, it was also observed that predation by other fish (*Salvelinus alpinus* and *Salmo trutta)* and by birds on stickleback was higher in the northern basin. In response, the northern stickleback has longer spine than the southern basin fish. (Millet et al., 2013).

Consequently, different stickleback populations are observed in the same lake with different density, morphs and use of resources that can have different impacts on the environment but also on lower trophic communities like invertebrates. However, the environment can change and the

impacted communities can also adapt in response to the impacts caused by the stickleback. Consequently, invertebrate populations may adapt to the pressures induced by the stickleback and develop different phenotypes to survive and have effect on the environment in return. Here we ask whether the threespine stickleback from different origin and at different density can impact invertebrate abundance, composition or phenotypic diversity. In this study, we will try to understand with a mesocosm and laboratory experiments what the impacts of stickleback on invertebrate communities are. We will try to understand the density and diversity of invertebrate communities in addition to their phenotypic variation in response to the stickleback pressure.

We expected that there will be a decrease in zooplankton populations with fish of warm water origin and a decrease in benthic invertebrate populations with fish of cold water origin according to the observation of (Millet et al., 2013) in Mývatn. Moreover, we predict that increased density of fish will result in lower density of invertebrates (Jakobsen et al., 2003). We also predict a phenotypic variation of invertebrates with smaller individuals to avoid being too much visible with the presence of stickleback or on the contrary bigger individuals to avoid to be easily eat by the fish (Bo et al., 2007; Campbell, 1991; Des Roches et al., 2013; Jakobsen et al., 2003), but also an improvement of their structure defences like long spine, thicker carapaces (Engel et al., 2014; Stoks et al., 2016) or gelatinous sheath (Campbell, 1991). I also expected an augmentation of invertebrates which are not predated by stickleback such as *Copepod, Nauplii* and *Rotifers* due to the decrease of competition by the other invertebrates eaten by the fish (Des Roches et al., 2013).

4.2. Material and methods

4.2.1. Study system

4.2.1.1. Mývatn lake

The subject of the study is about the threespine stickleback of lake Mývatn, which is a eutrophic shallow lake formed about 2 300 years ago by volcanic activity. This lake of 37 km² is located at 277m altitude in the North-East of Iceland and is covered by ice 6 months in the year causing stratification in winter. The lake is supplied by spring water, Laxa river is the outflow and is composed of 2 basins one in the North and the other one in the South. The northern basin is supplied by mostly warm water (30°C) whereas the southern basin is supplied by cold water (5°C) forming a variety of habitats (Millet et al., 2013). Consequently, this diversity of habitats contains a lot of birds and three fish species : threespine stickleback, arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) and numerous species of invertebrates dominated by *Chironomidae* and *Cladocera* (Bartrons et al., 2015).

4.2.1.2. Mesocosm experiment

Our experiment used the stickleback populations of two different basins of Mývatn because they present a great phenotype difference between each other. In summer of 2021, 300 wild stickleback not sexually mature were collected (150 females and 150 males) from warm water (W) and cold water (C) (see Appendix 4). The fish were collected with minnow traps (Dynamic Aqua-Supply Ltd, Surrey, BC, Canada, mesh size 3.2cm), let at least 12 hours into the water before collecting. During the collecting, all fish were measured and weighed before and after the mesocosm experiment.

The fish were used in an outdoor experiment with 40 mesocosms ecosystems (1000L ~ 1 x 1 x 1m) and the experiment was run in three phases. The first phase was a phase of setting up after putting in each mesocosms : 20L of pre-washed sand to serve as a base on the ground ; 20L of sediments taken into Mývatn lake extracted with 100µm nets ; Some macrophytes composed of *Cladophora* and *Myriophyllum spicatum* fixed with a rock have been added into the mesocosms also taken into Mývatn ; 1L of water containing invertebrates communities take at Mývatn with a plankton net of 100µm ; and

fill the mesocosms with water took from the nearby river. This phase was running without fish to let the ecosystem build itself during approximately four weeks.

The second phase is the adult fish phase conducted over three weeks, that use adult fish over 4mm long but not sexually mature. This phase will be a complete randomized block design with eight replicate blocks of five mesocosms, consisting of one mesocosm with no fish (NF treatment) and four mesocosms with factorial treatment combinations WH, CH, WL and CL to represent warm origin fish (W), cold origin fish(C), with either high density (H) composed of 10 fish or low density (L) composed of 5 fish. Dead individuals will be replaced as quickly as possible to avoid changing the chemistry of the water and therefore disturb the ecosystem. At the end of the experiment, all fish will be collected and euthanised in Phenoxy ethanol solution. The fish will be measured and weighed before to be conserved into bucket of 95% ethanol for further stomach contents and morphological analysis.

The third phase is the juvenile phase conducted over eight weeks, which uses juvenile fish previously bred from adults respectively from cold and warm origin. This phase uses the previous mesocosms adult treatment. All the mesocosms of WH, CH, WL, CL and 4 NF of adult treatment are used and in each 36 juveniles was put (18 from cold and 18 from warm origin parents) after remove all adult fish. 4 no juvenile fish (NF treatment) are let without fish to serve as control. Dead individuals will be replaced as quickly as possible to avoid changing the chemistry of the water and therefore disturb the ecosystem.

4.2.2. Invertebrates sampling during mesocosm experiment

To study all the communities of invertebrates three variables will be sampled, the zooplankton in the water column, the epibenthic invertebrates and benthic invertebrates.
Concerning the zooplankton, we made a sampling of each mesocosms at the beginning and the ending of each phase. We used a pipe of 1.5m x 4.5cm of diameter which can close by a tennis ball thanks to a string to collect the water column, at different locations into the mesocosm until having 10L of water. After the collection, the water is filtered through a sieve of 63µm, and the sample conserved with 95% ethanol.

For the epibenthic invertebrates, we made a sampling of each mesocosms at the beginning and the ending of each phase. We used two epibenthic traps for invertebrates (Combot, 2018), composed of a plexiglas plate (140mm*145mm*4mm) with a hole (ø 80mm) in the centre, four carriage bolts of 100mm for the legs, a transparent plastic bucket fixed upside down on the plexiglas plate by thick rubber band with a hole (ø 8mm) in the centre of the lid and a funnel slide in with the narrow opening (ø 8mm) towards the bucket and the wide opening (ø 75mm) towards the ground fixed by rubber band. The funnel is fixed at the plate and the bucket thanks to the rubber band and fixed to the system to leave 30mm between the floor and the funnel entrance (Combot, 2018). Two traps per mesocosm have been put on the water by letting the trap submerge “upside down”, with funnel facing the water surface and after it’s full, turn the trap underwater with funnel facing the sediment to avoid that the sieved water in the container from seeping out when the trap was deployed. The traps have been gently placed at the bottom of the mesocosms on the substrate and let 12 hours before collecting. After, the bucket content is filtered through a sieve of 63µm, and the sample conserved with 95% ethanol.

Concerning the benthic invertebrate, we made a sampling of each mesocosms at the beginning and the ending of each phase. We used benthic traps, put in place before putting the sediment into the mesocosms, to let developed benthic invertebrates into the trap. For that the traps are composed of a little pipe of 3cm x 4.5cm of diameter (to collect approximately 37.8-47.7 cm3 of sediment per trap), with four screws on each side to allow the traps to sink when they are set but also to be able to collect

them with a magnet. Two stick ruban on two sides have been added to allow us to see the trap when we wanted to recover them. During the sampling phase two traps were collected by mesocosm to make the sample. After, the trap content is cleaned and filtered through a sieve of 63µm, and the sample conserved in 95% ethanol.

4.2.3. Processing

All samples of invertebrates were processed in the laboratory facilities of Hólar University at Verið. Concerning the dead fish, they are reused to study their stomach contents and see which invertebrate species are predated. For that, fish are dissected in petri dishes with 95% ethanol, the belly and stomach are opened, and stomach content are spread in the dish. The invertebrate residues are identified at the lowest taxonomic level and counted using a microscope Leica M165C.

Concerning the invertebrate samples, each sample was transferred in a petri dish and processed using a microscope Olympus SZX7. Invertebrates are identified at the lowest taxonomic level, counted and sorted into Eppendorf tubes for larger *Cladocera* individuals, adult *Copepods* and unidentified invertebrates. when the density of small invertebrates is too high, samples are split and/or the sample size is reduced during counting. A factor is applied based on the initial split volum to estimate the number of invertebrates of each species in the sample. Further measurement on body-length and spine length for *Daphnia longispina* have been done on 30 individuals at least, to see the phenotype variation between the different phases and between the different mesocosm treatment.

4.2.4. Statistical analyses

All statistical analyses were performed in R using RStudio (Version 4.1.0. © 2021 The R Foundation for Statistical Computing

The invertebrate data collection allowed to calculate the total invertebrate number of each sample and the number of species found. These results allowed to calculate the density of invertebrates based on study system of mesocosm and have a density of ind/1000L of water.
For that, the number of invertebrates into the 1L of water serving as references sample used to seed the mesocosms are the same number into 1000L of mesocosm content.
For water column sample, the number of invertebrates found into the 10L of water was multiplied by 100 to obtain the density of invertebrates into 1000L of mesocosm content.
For benthic sample, the number of invertebrates found into ~ 38cm^3 of sediment was multiplied by 2E+4cm3 to obtain the density of invertebrates in each mesocosm.
For epibenthic sample, the relative density of invertebrates was estimated regarding to epibenthic trap.

The Shannon diversity index was calculated using the following formula :

$$\text{Shannon diversity index} = -\sum_{i=1}^{n} Pi \times \log_2(Pi) \quad \text{and Pi=(ni/N)}$$

with N = ∑ni sum of ni individuals of S species that compose the sample and Pi the relative abundance of i species.

The normality distribution of invertebrate density and diversity was checked with a *Shapiro test*. Concerning the invertebrate density as the p-value = 0.1415, by consequence the density does not follow a normal distribution. And concerning the invertebrate diversity the p-value =0.005, by consequence the diversity follows a normal distribution.

After some statistic tests (see Table 1) have been done on invertebrate density and diversity to answer the different expectations about the impacts of threespine stickleback on invertebrate communities.

Table 1 : Statistical tests applied for the study of stickleback impacts on invertebrate communities

Test	R code example	Aim for studies
Linear model (normal distribution)	- Lm(data$B_div ~ F_origin*F_density*F_age) - Lm(data$EpiB_div ~ F_origin*F_density*F_age) - Lm(data$P_div ~ F_origin*F_density*F_age)	See the type of invertebrates consumed by fish depending on fish origin, fish density
Generalize Linear model (no normal distribution)	- Glm(data$den ~ F_density)	See if the fish density has effect on invertebrate density
Generalize Linear model (no normal distribution)	- Glm(data$sp1 ~ F_origin*F_density*F_age - Glm(data$sp2 ~ F_origin*F_density*F_age … - Glm(data$spN ~ F_origin*F_density*F_age	See the impacts of stickleback on each invertebrate species density, and see whether smaller individuals are favoured at the expense of larger ones or vice versa See whether species not eaten by fish are dominant species in the stand or not
Linear model (normal distribution)	- Lm(data$LspineDaph ~ F_origin*F_density*F_age)	See the stickleback influences on phenotypic variation like spine length *Daphnia longispina*
Non-Metric Multidimensional Scaling	-PCA with I_den, F_origin, F_density, F_age -PCA with I_den, F_origin, F_density, F_age	See which invertebrate densities are link with the density, origin and age of fish or are not link with
Pearson correlation	-cor.test (variable1,variable2)	Estimate the degrees of link between visually related variables by PCA

NOTE : Accronym: B = Benthic ; EpiB = Epibenthic ; P = Pelagic ; F = fish ; Div = invertebrate diversity ; Den = invertebrate density ; LspineDaph = Daphnia longispina Spine Length ; I = Invertebrate; age = juvenile or adult fish.
If data number are not sufficient the code can be simplifying by added "+" that treat independently variables instead of "" that treat independently and the interaction between variables.*

4.3. Results

The experiment was finally delayed and only reference invertebrate samples and water column samples during the set-up phase of the mesocosms were collected and processed. However, expectations of possible results the adult and juvenile phase will be integrated in the result section.

The reference samples of invertebrate communities presented between 21 and 23 different species as shown Figure 9 and that is composed of *Tanypodinae, Orthocladinae, Chironominii, Tanytarsini, Exuvie, Pupea, Chironomidae adult, Cladoceran Eggs, Alona affinis, Alonella nana, Eurycercus lamellatus, Chydorus sphaericus, Acroperus harpae, Daphnia longispina, Cyclops, Nauplii, Brachionus, Keratella, Asplanchna, Filinia, Ostracod, Bivalve* and *No determinated.*

The water column sample took during the setting up presented between and 4 and 8 different species as shown Figure 9 and composed of *Alona affinis, Eurycercus lamellatus, Chydorus sphaericus, Daphnia longispina, Cyclops, Nauplii, Keratella, Asplanchna, Ostracod* and *No determinated.*

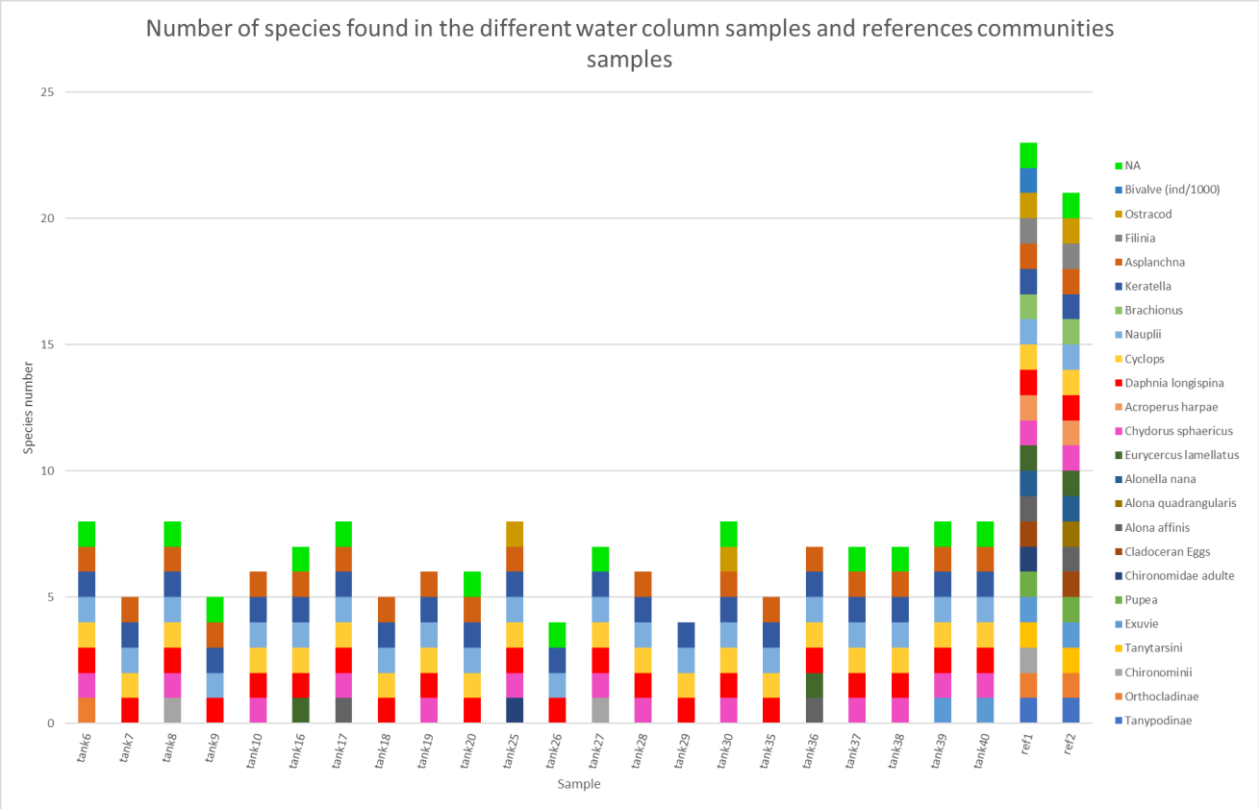

Figure 9 : Number of invertebrate species found in the different water column samples and references samples

However, the Figure 9 shows an important difference between the species diversity of the reference invertebrate communities and that found in the mesocosms after seeding. In the treatment of invertebrate samples, *Rotifers* dominated both references and water column samples, but only the *Asplancha* and *Keratella* genus were presented in mesocosms after seeding, whereas *Fillinia* and *Brachionus* were initially present. The adult *Copepods, Nauplii* and *Daphnia longispina* were presented in both type of samples but are the dominant population into mesocosms.

The invertebrates counting of each species allowed to calculate the total density into each mesocosms and their diversity. As shown in the Table 2 and following the previous observations, the Shannon diversity is higher in reference samples (2.35±0.09) than in the mesocosms samples (1.01±0.42). Showing that the diversity of species presents in the mesocosms compared to their number, is much lower than they were seeded with the reference. Moreover, this diversity also varies greatly between the mesocosms and can be poor in some mesocosms or rich in others, depending on the number of species found and their density.

In fact, we can see clearly in the Table 2 that total density of invertebrates varies greatly between each mesocosms (96350±42625 ind/1000L) but is very weak in the mesocosm 9 (2500 ind/1000L), altering the diversity result. Moreover, we can clearly see that invertebrate density of mesocosms is higher than invertebrate density of reference samples (5560 ±2857 ind/1000L), showing a good seeding of mesocosms with the reference communities.

Table 2 : Number of invertebrate species and invertebrate density, diversity found in samples

Sample	Species numbers	Density (ind/1000l)	Diversity
tank6	8	97200	0.94
tank7	5	71800	0.51
tank8	8	212700	1.13
tank9	5	2500	2.10
tank10	6	135100	0.78
tank16	7	78700	0.57
tank17	8	148400	1.10
tank18	5	63200	1.16
tank19	6	98000	1.28
tank20	6	165800	0.94
tank25	8	99400	1.14
tank26	4	56500	0.70
tank27	7	79800	1.07
tank28	6	103100	0.92
tank29	4	90300	0.62
tank30	8	98700	0.95
tank35	5	83500	0.71
tank36	7	50100	0.53
tank37	7	84400	1.65
tank38	7	101500	1.76
tank39	8	87100	1.12
tank40	8	111900	0.53
ref1	23	7580	2.41
ref2	21	3540	2.29

Concerning the expected results if the experiment had gone well, we could have a variation of invertebrate density and diversities depending on the fish age (juvenile or adult), the fish origin (cold or warm) and fish density (high or low), or maybe a link between these different variables.

Firstly, we could have a variation of invertebrate density in link with the fish density. In fact, we can expect lower density of invertebrates with higher number of fish due to the predation pressure (confirmed by stomach analyses) or any indirect impact (like water chemistry). This invertebrate density can also depend on fish age, with less invertebrates with adult fish than juvenile fish that need less food to live. Same for fish origin where northern fish are bigger than southern and by consequence can need a bigger quantity of food.

Then, we can also have a variation of invertebrate diversity due to the diet preference of fish observed according to their origin. In fact, we can expect a decrease of pelagic invertebrate like *Copepods* or *Cladocera* that are the main diet of fish from warm water origin, and vice versa, a decrease of *Chironomidae* with fish from cold water. This expectation is based on the “fish dietary habit” but we also can expect a specialisation of fish on the most nutritionally rich food like *Chironomidae.* In the case of high fish density, we could observe a distribution of type of food to avoid intra-specific competition and thus a resource repartition between fish. With that kind of behaviour, we can expect an augmentation of no consumed invertebrates like *Copepods, Nauplii* and *Rotifers* that can colonize their environment without competition of consumed invertebrates. The diversity can depend also of fish age, because the composition of diet is different between juveniles and adults. For example, juveniles will eat smaller invertebrates than adults will eat bigger individuals for energetic and ease reasons.

Concerning phenotypic variations of invertebrates with the presence of stickleback, we can expect an important number of small individuals less visible by adult fish at the end of adult phase, and contrary, an important number of big individuals too much big to be eaten by juveniles. But we can expect variations in defence structures. In particular, in *Daphnia longispina* species, that have a long spine that allow them to protect themselves. By consequence, at the end of adult phase we can expect to find more *Daphnia* with longer spine and find them also at the end of juvenile phase because the little fish cannot eat them.

Finally, we could see an evolution between the 3 phases, depending on both the diversity and density of invertebrates initially present into the mesocosms before each phase, but also, the diversity and density of invertebrates no impact by fish at the end of each phase.

4.4. Discussion

To study the ECO-EVO-DEVO framework, this mesocosm experiment about the impacts of threespine stickleback on invertebrate communities was conducted. However, this experience has had complications on its implementation. Indeed, the seasonal delay of Lake Mývatn, which serves as the study system in the experiment, has caused delays in the sexual maturation of fish and in the development of invertebrate communities. All these disturbances caused delays in the development of each phase. First, there was a delay in the set-up phase with the seeding of the invertebrate communities. Then a delay also occurred in the adult phase with the prior preparation of the offspring that would be used for the juvenile phase. This phase was also postponed due to the delay in sexual maturation of the stickleback that is necessary to reproduce them. Once the sexually mature fish had been obtained, reproductive problems occurred (gravid eggs or resorption of eggs, ...) an alternative was therefore made by breeding individuals directly in the field to limit the stress linked to capture and transport. However, the incubation of eggs for the juvenile phase also had complications due to the presence of numerous fungi, which increased the mortality of the eggs.

All these delays are problematic in Cold regions such as Iceland. Indeed, the experiment in mesocosms takes place outdoors and is exposed to meteorological and seasonal hazards. The experiment should have lasted between 3 and 4 months to see the effect of the stickleback on its environment in the short and long term, but also on its offspring. The delay in the launch of the experiment meant that the total duration of the phases had to be reduced and some phases could not be carried out to avoid the first frost or bad weather in the autumn, which would have disrupted the study.

Regarding the mesocosm experiment to see the impacts of the Stickleback on its environment, various parameters and environmental variables present in the study system are not reproduced in the experiment ; such as the presence of natural predators (other species of fish or birds), use of the same substrate and macrophytes for the study instead of taking substrate and macrophytes in function of fish origin for the studied treatments, ... These variables not considered in the experiment represent a bias in the analysis of the mesocosms compared to the lake study system where they are naturally present.

The same applies to the variables analysed during the experiment. Some variables such as bacteria, algae or other parameters such as hormones diffused by the fish ... which are present in the mesocosms but are not studied, are biases in the interpretation of the impacts of the stickleback on its environment. These unstudied variables may also play an important role and have an indirect impact on invertebrates for example. Therefore, further studies in mesocosms or controlled system in laboratory or with a larger scale experiment on macrocosms (with enclosures in the lake) are needed to verify the results obtained in this study.

In terms of results for invertebrate communities, there was a high variability between invertebrate densities and diversity between mesocosms and between water column and reference samples. The amplification of the density between the reference samples and the sampling of the water column during the set-up phase shows that the seeding of mesocosms with the community worked well and that the invertebrates are developing well.

However, it was observed a great variability in the composition of the species found in the samples with a greater abundance of some species, unlike the reference samples which do not observe any

dominance or on other species. This change in the composition of the communities shows that some species including *Rotifere Keratella* and *Asplancha, Copepods* and *Nauplii* but also *Daphnia longispina* are relatively wellacclimatized to their new environment to the detriment of others. This observation is in line with the remark previously made that the mesocosms does not actually represent Lake Mývatn, because the water column samples differ from the reference initially taken in lake.

This difference can be explained by the water chemistry that can differ between mesocosms and therefore impact the development and composition of species. Additional analyses combining water chemistry and the composition of invertebrate communities are needed to test this hypothesis.

However, this difference can also be explained by the sampling method used. Indeed, only the water column was sampled, while the reference samples were rich in substrate therefore presenting benthic invertebrates and not pelagic. To overcome this problem of sampling method, it would be necessary to carry out a sampling of benthic and epibenthic invertebrates to have the overall composition invertebrate and to be able to compare them with the reference samples.

In addition, the sampling methods are different between that carried out in mesocosms and that of recovering the communities in the lake. Indeed, for mesocosms three different methods are used (water column by method of a pipe sealed with a tennis ball, benthic traps by magnet and epibenthic trap) while the one used in the lake use an invertebrate net. This difference in sampling methods may explain the wide variability of results. further studies are needed to test the effectiveness of the methods, whether certain methods are specific to trapping certain invertebrates and, above all, whether the methods are comparable with each other.

It was also observed a very low density and diversity on mesocosm N°9. This result can be explained by a poor cleaning of the tank, thereby changing the chemistry of the water and indirectly the communities present.

4.5. Conclusion

In conclusion, although the initial experiment did not go as planned, it is important to note that invertebrate seeding of a media may be possible from a reference community. However, variations may occur in the development of the latter compared to other variables such as water chemistry.
Additional studies or analyses are needed to further integrate parameters that may impact invertebrate communities indirectly in addition to studying the direct impact of stickleback on invertebrates.

5. FEEDBACK ON MY INTERNSHIP

During my internship I was able to discover the world of research with the advantages and disadvantages that can be linked to it.

Indeed, I liked most about research was the fact that I was able to discover and study concrete cases in science that have not yet been discovered or, on the contrary, to confirm or even affirm research results that have already been tested.

I also liked to work and especially to be able to exchange thoughts and ideas with a team of researchers, professors, and even students who are specialized in other fields. These exchanges allowed me to see that research remains a world of observation and study which can give rise to completely different opinions depending on the point of view, but where the knowledge of each person makes it possible to move things forward or to find alternatives.

This internship in the field of living organisms and their environment has shown me that I like to work in this field to understand the functioning and behaviour of different species or populations but also the functioning of the system that surrounds them. However, this dynamic system also reminded me that it has many hazards, as the experiment was significantly delayed due to a seasonal shift in Lake Mývatn, also impacting all the communities that inhabit it. Other problems such as floods, administrative and communication problems also invited themselves to my internship disrupting its good running and disrupting the experience. But this taught me that it is necessary to adapt to move forward.

What I found most interesting during my internship was to set up the experiment with all the technical aspects, but also to think about all the scenarios and hazards that could happen during the experiment and to find solutions to avoid or surpass them.

This internship allowed me to see part of the job of a researcher but above all it reinforced my idea of wanting to become an engineer. I really enjoyed working with living things, studying animal and plant species, but also studying their environment. But I especially liked setting up a project and having to think at all possible scenarios to be able to draw the advantages but also to overcome the disadvantages, find solutions and sometimes innovate to create new things.

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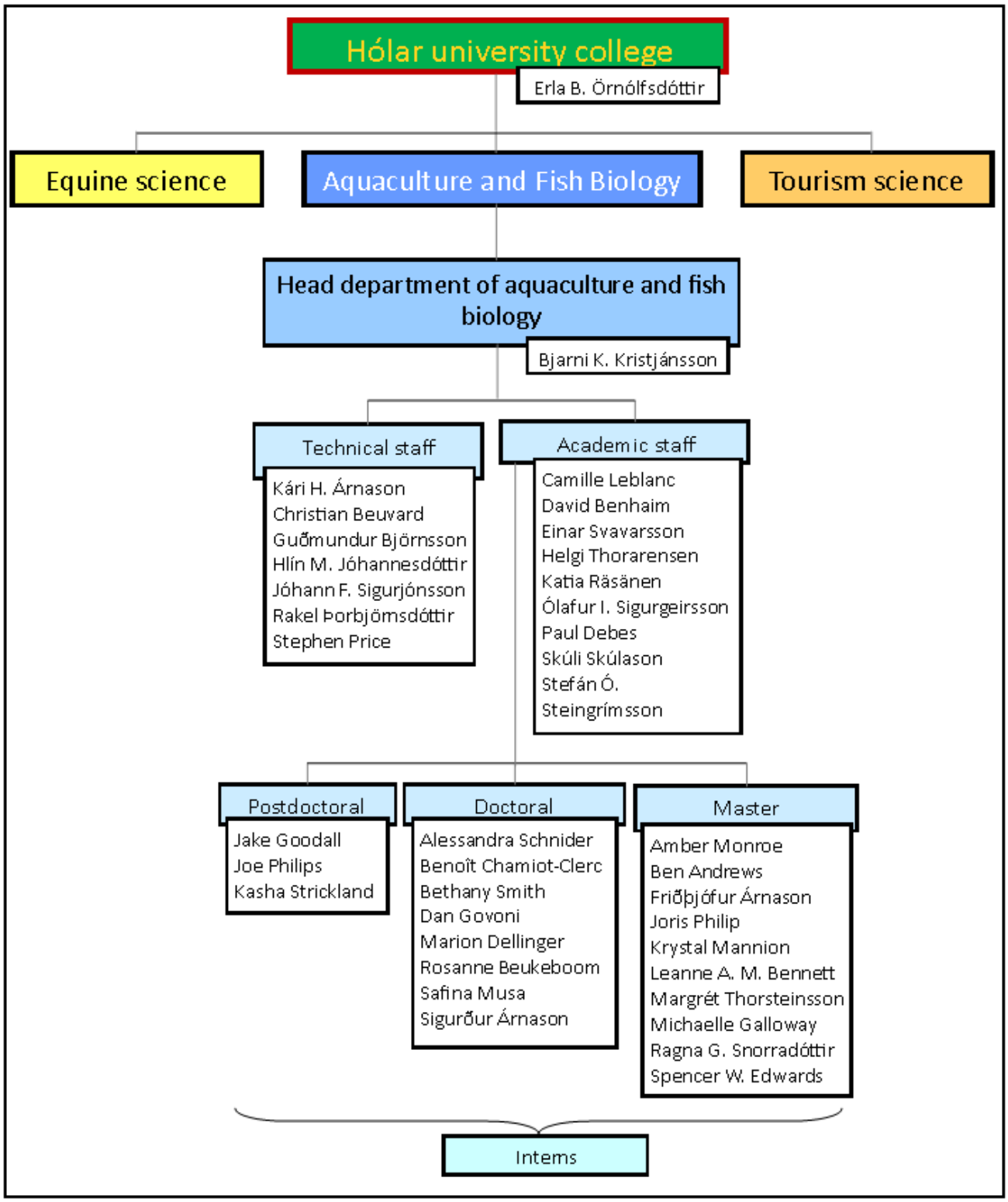
APPENDIX

Appendix 1 : Organization chart of Hólar University focus on fish biology department



SAMPLING and TREATMENT PROTOCOL of INVERTEBRATES COMMUNITIES in MESOCOSMS

Experiment in 3 phases in 40 mesocosm of 1000L:

- Setting up : without fish to allow ecosystem construction / runs on 4 weeks

- phase 1 : adult phase / runs on 3 weeks

- phase 2 : juvenile phase / runs on 8 weeks

*Note : 2 possibility was proposed for some phases : (*initial proposition) and finale/apply proposition*

Sampling of invertebrates : water column / benthic / epibenthic

1. SAMPLING ZOOPLANKTON in water column

sampling at the beginning and the end of phases 1 and 2 of the experiment. Use a pipe of 1.5m x 4.5cm of diameter, and that can close by a tennis ball link with a string. Method :

- Gently tap the side of the mesocosm to snatch the invertebrates fixe on the intern side and put them in suspension in the water
- Insert the pipe open into the water column and avoid any sudden gesture to avoid disturbing the ecosystem
- Close the end of the pipe thanks to the tennis ball by pulling the string
- Retire slowly the pipe of the mesocosm and pour water into a bucket
- Repeat the previous steps at different locations into the mesocosms until get 10L
- Filtering water through a mesh of 63 µm, until have a concentrate solution of 30-50ml
- Keep the sample in vial and add some ethanol 95%
- Repeat the previous steps for the 40 mesocosms
- Identification and counting all invertebrates until the lowest taxonomic level, and have some morphology measurement (length, biomass) of approximately 30 individuals.

2. SAMPLING EPIBENTHIC INVERTEBRATES

sampling at the beginning and the end of phases 1 and 2 of the experiment. Use a epibenthic trap for invertebrates, composed of a plexiglas plate with a hole (ø 80mm) in the centre (140mm*145mm*4mm), 4 legs (100mm long), a transparent plastic bucket fixed upside down on the plexiglas plate by thick rubber band with a hole (ø 8mm) in the centre of the lid and a funnel slide in with the narrow opening (ø 8mm) towards the bucket and the wide opening (ø 75mm) towards the ground fixed by rubber band. The funnel is fixed at the plate and the bucket thanks to the rubber band. (*Strings are attached to each leg to suspend the trap in the water and avoid putting it on the ground and regulate the position and the height of the trap in the mesocosm) or a string is attached on each side of the plate to bring down the trap to the bottom of the mesocosm and to be able to raise it afterwards. The length between the ground and opening funnel must be around 30mm. 2 traps per mesocosm have been put

- Fill up the trap with water/filtered water from mesocosm
- Put the trap delicately on the water by letting the trap submerging “upside down”, with funnel facing the water surface and after it’s full, turned the trap underwater with funnel

facing the sediment to avoid that the sieved water in the container from seeping out when the trap was deployed

- (*Attach the string linking to the legs on each side of the mesocosm and regulate the length to adjust the position and the height of the trap) or attach the trap at a string to put delicately the trap on the ground of the mesocosm
- Let the trap into the mesocosm 12 hours before retiring it
- Retire the tap slowly to avoid disturbing the ecosystem
- Filtering through a mesh of 63 µm, until have a concentrate solution
- Keep the sample in vial and add some ethanol 95%
- Repeat the previous steps for the 40 mesocosms
- Identification and counting all invertebrates until lowest taxonomic level, and have some morphology measurement (length, biomass) of approximately 30 individuals.

3. SAMPLING BENTHIC INVERTEBRATES

sampling at the beginning and the end of phases 1 and 2 of the experiment. Use a trap put in place before the sediment. the trap is a little pipe of 2.5-3 cm and 4.5 cm of diameter (37.8-47.7 cm3 of sediment per trap), with magnetic cercle at the top, that allow to retire the trap thanks to a magnet without disturb the ecosystem

- Put the trap in the bottom of the mesocosm and fixe it ((*9) or 6 per mesocosm) during the setting up phase
- Fill the mesocosms with sediments on 2-3 cm during the setting up phase
- Retire slowly (*3traps) or 2 traps per sampling thanks to a magnet (get 63.6-95.4 cm3 of sediments)
- Filtering all replicates together through a mesh of 63 µm, until have a concentrate solution
- Keep the sample in vial and add some ethanol 95%
- Repeat the previous steps for the 40 mesocosms
- Identification and counting all invertebrates until lowest taxonomic level, and have some morphology measurement (length, width, biomass) of approximately 30 individuals.

(* = setting up phase)

Note : for the experiment we need 6 traps per mesocosms (2 traps for at the beginning and the end of phase 1 and 2 so 3 sampling), so, a total of 240 traps for the entire duration of the experiment is necessary(6*40 mesocosms)

Sampling of adult fish and further stomach analyse after mesocosm experiment

sampling after the first phase

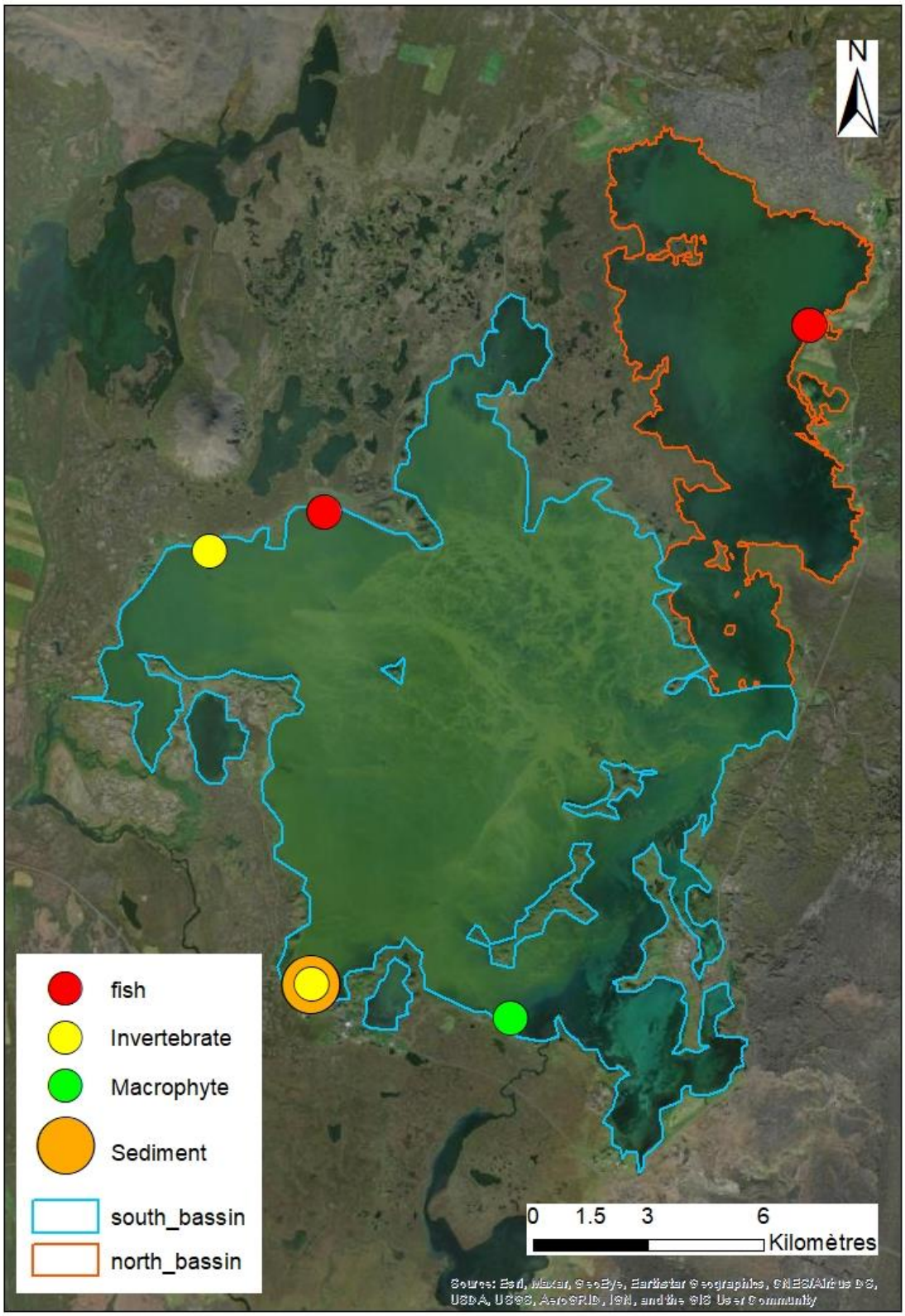
- Take out all fish with a net
- Fish euthanasia by putting them in Phenoxy solution
- Measure of fish (length, weight) and take a picture before putting them into ethanol 95% to conserve them
- Dissection of adults in petri dishes with ethanol 95%, after opening the belly and stomach to spread the food contents in the dish

- Identification of diet items (mostly from the head) to the lowest taxonomic level and counted. The elements can be preserved in a vial into 95% ethanol.

Appendix 3 : Summary table of different steps in the implementation of the experiment

Preparation of mesocosms : cutting and cleaning	Implementation of mesocosms on site and upgrade	Filling of mesocosms with water	Cleaning of marine sand and filling of mesocosms
			
Manufacturing of sediments and invertebrate traps	Setting of traps into mesocosms	Collect of sediment at Mývatn lake	Filling of mesocosms with sediment
			
Collecting of invertebrate communities at Mývatn lake	Collecting of macrophyte at Mývatn lake	Filling of mesocosms with invertebrate communities	Filling of mesocosms with macrophyte
			
Collecting of fish	/	Filling of mesocosms with adult fish (according to NF, WH, WL, CH, CL treatment)	Filling of mesocosms with juvenile fish (according to NF, juvenile treatment)
/	/	/	/
Water chemistry measurement at the beginning and ending of each phase	Collect of pelagic invertebrate at the beginning and ending of each phase	Collect of pelagic invertebrate at the beginning and ending of each phase	Collect of pelagic invertebrate at the beginning and ending of each phase
		/	/

Appendix 4 : Map of points and elements collected for the mesocosm experiment in Mývatn lake



Stickleback crossing (Mýstickle rearing experiment, Verið)

Checking and selecting gravid females (wet lab)

On a daily basis (once or twice a day), females are thoroughly checked and gravidness is assessed. From Schluter lab "A female ready to spawn can be identified by her abdomen shape at the cloaca. If ready to lay eggs, one can almost see the first egg; at this point her abdomen at the cloaca is sharply angled, almost like the corner of a box. The eggs should come after gently squeezing her body above and forward of the egg mass and, while maintaining pressure, sliding your fingers posteriorly. The eggs, when they appear, will stick to one another in a clump. If they dis-aggregate then the female was not ready and you should throw the eggs out". NOTE: ripe females relatively quickly release their eggs, so it is not a good idea to wait too long before crossing. Individuals that look close to spawning are separated out into a (properly labelled by origin and date) bucket.

Females that look likely to be ready the next day can also be separated (different bucket), to save sorting time the day after. Where possible, do not feed gravid females immediately before stripping.

Females that are ready to be stripped will have a nearly angular swollen cloaca and clearly visible eggs through the abdomen (see above). It is not always the case that fish that look likely to be easily strippable, actually are. Assessing when females are ready to be stripped will become easier with practice.

Sexually mature males are easy to recognize and will have the characteristic male coloration (obvious blue eyes and orange-red throat).


Adult male (and juveniles)

Adult female

At Verið, crosses are easiest done in the upstairs lab (which has camera set up and microscopes). When all fish have been checked, transport relevant fish up to the lab in small plastic buckets to attempt crossing. Make sure that everything is clean and label the buckets accordingly.

Crossing fish (upstairs lab, Verið)

Equipment needed:

- Small petri dishes
- Dissection kit that includes:
  - Sharp scissors
  - Various fine forceps
- Pasteur pipettes
- Phenoxyethanol
- Small containers for keeping live fish

- Dip net for handling fish
- 1.5ml eppendorf tubes
- 95% ethanol (for storing fin clips)
- 70% ethanol for cleaning equipment
- 2ml eppendorf tubes filled with 1ml Ginzenberg‘s solution
- Eppendorf tube rack
- Data collection sheets for keeping track of crosses. **Essential data to record**: Date of cross, origin of fish (M+F), ID of fish, weight of fish (pre and post stripping), crossing time, water change time, time eggs added to incubation tank, additional notes, operator
- Paper towels
- Electrician tape for labelling dishes
- Sharpies and pencils
- Digital scale
- Digital camera, stand and lights (see separate SOP for photographs)

Heavily gravid females should be crossed as soon as possible as it is possible for individuals to rapidly reabsorb/lose eggs.

1. Lay out everything that you need and ensure that you have set up a good working area to ensure efficient flow

2. Select a male and add to a strong phenoxyethanol solution

3. After a few seconds, the male should be anesthetised (confirm that male does not respond to stimulus by pinching him with a forcep) NB: Important that the male is unresponsive as you are effectively doing a surgery (cutting out testes) on a live fish

4. Weigh the male, record weight

5. Take a photograph of the male including it’s ID label (**see separate photography protocol**)

6. Dissect out the male gonads and add to the 2ml Eppendorf tube with Ginzenberg‘s. Confirm male death by decapitation using scissors
    1. To dissect gonads, securely hold anaethatised fish and with dominant hand use dissection scissors to make an incision using the cloaca as a starting point
    2. Carefully cut upwards and then along the lateral line towards the pectoral fin, being careful not to cut any internal organs along the way (stop a few mm short of fin)
    3. Once the incsion has been made, use fine curved forceps to identify testes. These are usually dark grey and speckled (see pic). Pull testes away from each other
    4. Gently hold and pull testes away from other internal structures and remove by cutting with dissection scissors
    5. Add testes to petri dish with water

7. Using fine forceps, crush the testes to release sperm. The sperm solution is usually clearly visible as whitish fluidin 95% ethanol. Leave the sample out overnight. Then remove all the ethanol and replace it with fresh 95% and store the sample at -20°C
8. Check again to ensure that the male is dead and store the male individually in a small plastic bag with an ID label. Freeze at -20C. (These are backups in case something goes wrong with any samples. They can be discarded once we know all is ok)

9. Select a female and remove from bucket with dip net or with hand (wear nitrile gloves and always wet your hands before touching fish)

10. Weigh female (pre-stripping weight) on a digital balance. **Be sure to calibrate scale**

11. Secure the female fish in one hand with the cloaca oriented towards the petri dish (approx 5cm away). Exact technique is slightly specific to the handler here. **The petri dish must be clean and dry and contain no water**. **Any water present will make the eggs start to water harden, making fertilisation more difficult**

12. With the other hand, starting near the upper to mid abdomen squeeze and push the egg mass towards the cloaca. This step requires some judgement, it is a good idea to start gently and gradually increase pressure if you think necessary. A 'ripe' female does not require much squeezing pressure to be stripped of eggs.
    Squeeze out as many eggs as possible directly into the Ginzenberg's solution in the 2ml Eppendorf tube. **Once the flow of eggs has obviously neared the end, stop as you can else harm the female (bleeding of cloaca)**
    If half sibling crosses are needed into the petri dish. Split the clutch into two using a wet brush and carefully add the egg mass to the respective Eppendorf tube containing sperm from one male.

Stripping eggs

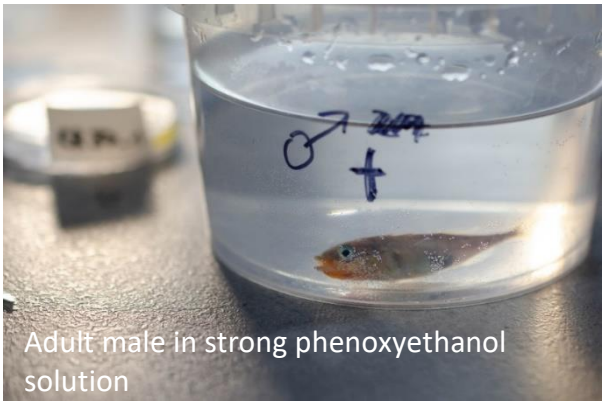
Stripping eggs

13. Weigh female again (make sure to tare the scale before each weighing) to get post-stripping weight (this gives you more accurate female body mass and allows calculating clutch mass= female post-stripping mass - female pre-stripping mass)

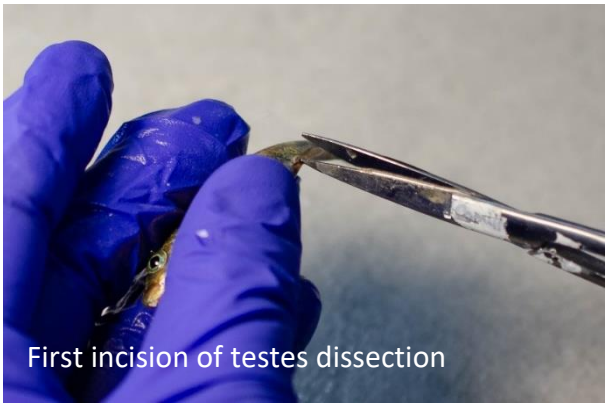
14. Put female into a small cup with water to give her a moment to recover

15. After 10min pour the egg-sperm solution into a small petri dish and add safe tap water (cold tap water is safe to use in Iceland) to the petri dish until eggs are covered

16. Gently shake petri dish in an orbital motion to ensure good contact with sperm and eggs. Important: record fertilisation time on data sheet. Let eggs sit for 20min (assure they are fully covered with water).



Adult male in strong phenoxyethanol solution

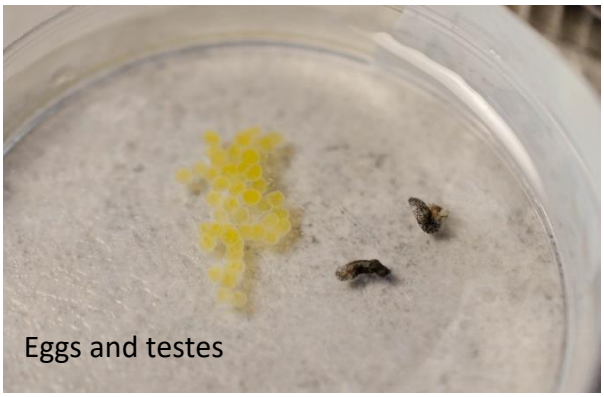


First incision of testes dissection

Testes dissection

Testes dissection (testes visible)

Releasing sperm from testes



Eggs and testes

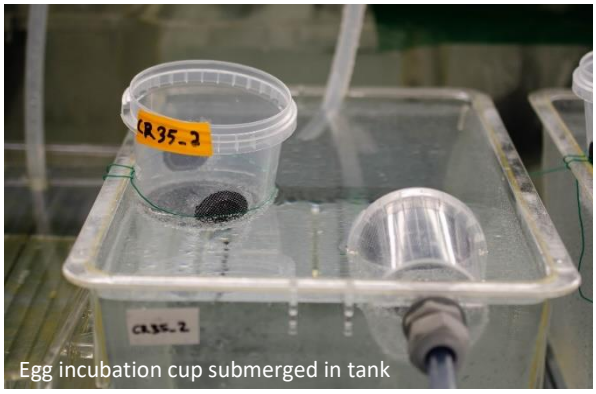
Gently swirling to aid fertilization

Additional help is beneficial to help record fertilization time etc.

17. 30mins after mixing the eggs and sperm, remove water and gonads from the petri dish with a 3ml single use plastic pipette. Add fresh water (cold tap water) so that the eggs are well submerged. Record water change time.

18. After another 30mins, photograph the eggs **(Dropbox -> stickleback data -> 1Readmefiles -> Rearing experiment 2020 -> FishAndEggsPhotographing_JUN2020.doc)**
19. The fertilised eggs can be placed to a clearly labelled embryo rearing cup (with mesh in bottom and sides) in a relevant holding tank. (**See separate protocol for embryonic rearing)**


Egg incubation cup



Egg incubation cup submerged in tank

20. Clean all dissection equipment by wiping with paper (to remove any tissue) followed with 70% EtOH and start with the next cross

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2020-2021

The impacts of Threespine Stickleback on invertebrate communities, in mesocosm experiment, Iceland

My internship was part of a research on the impact of the threespine stickleback *(Gasterosteus aculeatus)* on its environment. This study is based on different types of stickleback existing within the same lake system: Mývatn. These different populations have developed different phenotypes depending on their habitat of origin. This study uses fish from a warm basin and a cold basin at different densities in a mesocosm experiment to reproduce the lake's ecosystem. This experiment tries to understand the short-term but also long-term effects of stickleback on their environment. My internship topic focused on the impact of stickleback on invertebrate communities. Despite a delay in the experiment, the results obtained showed that the seeding of mesocosms from an invertebrate communities taken from a lake is possible and very effective. However, there was a loss of species diversity completely different from the initial stand. Therefore, the study aiming to reproduce the Mývatn ecosystem is not fully respected, but still represents a good approach to the study of the stickleback on its environment or on invertebrate communities.

Key worlds : Threespine Stickleback, invertebrates, adaptation, phenotype, mesocosm

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