A study of parasites and *MHCIIα* in Arctic charr(*Salvelinus alpinus*)

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Líf-& Umhverfisvísindadeild
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40 eininga ritgerð sem er hluti af
Baccalaureus Scientiarum gráðu í Líffræði
LÍF015M og LÍF030M

Leiðbeinandi
Arnar Pálsson

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A toda mi familia y amigos, los cuales siempre me han apoyado. En especial a mi abuelita Leri y a Fran porque cuidan de mí.

Útdráttur

Heimskautableikjan (*Salvelinus alpinus*)er áhugaverð tegund laxfiska útfrá landfræðilegum aðstæðum hennar og hugsanlegri samsvæða þróun. Í Þingvallavatni þrífast í samlyndi ólíkar afbrigði bleikju með ólíka hegðun og ólíkar fæðuvenjur. Þótt að þessar ólíku afbrigði hafi ólík einkenni svo sem stærð, lit og lögun, hafa þær svipaðan erfðafræðilegan bakgrunn. Þrátt fyrir það uppgötvaðist nýlega ónæmisfræðilegur munur dvergbleikja og murta í Þingvallavatni, nánar tiltekið í *MHCIIα* geninu. Fyrri rannsóknir benda flestar til þess að MHC arfblendningar séu hæfari þeir sem eru arfhreinir.

Í rannsókn okkar höfum við meðhöndlað 264 fiska úr hinum þrem ólíku afbrigði í leit að hugsanlegri tengingu á milli sníkjudýr hlaða og ólíkum líkamlegum (kyn, þyngd, aldur, staðsetning) eða erfðafræðilegum þáttum.

Við fundum ákveðin tengsl eða fylgni á milli nokkurra sníklanna sem rannsakaðir voru (*Diphyllobothrium sp., Dyplostomum sp., Eubothrium salvelini* og Nematodes) og ákveðinna líkamlegra breyta (þyngd og aldur) eins og við áttum von á en erfðafræðilegar niðurstöður voru ófyrirsjáanlegar. Það kann að skýrast af því að arfgerðir fengust úr fáum einstaklingum.

Niðurstaðan er sú að sníkjudýrasýking tengist mismunandi afbrigðum og líffræðilegum þáttum. Mögulegt er að breytileiki í *MHCIIα* tengist *Diphyllobothrium* sýkingum en frekari rannsókna er þörf til að kanna þá tilgátu til hlýtar.

Abstract

Arctic charr (*Salvelinus alpinus*) is a curious salmonid species due to its geographical situation and possibly sympatric evolution. In Lake Thingvallavatn different morphs with different behavior and feeding patterns cohabit. Though the different morphs differ in many characteristics such as size, color and form, they have similar genetic base. Nevertheless, recently were discovered the presence of immunological differences between dwarf and murta in Thingvallavatn, in the *MHCIIα* locus specifically. Previous studies indicate MHC heterozygotes have higher survival likelihood than homozygotes.

In our study we have processed 264 individuals among three of the different morphs searching for a possible connection between parasite load and different physical (sex, weight, age, location) or genetic (polymorphism in the *MHCIIα* region) factors.

We found some connections or correlations between some of the parasites studied (*Diphyllobothrium sp., Dyplostomum sp., Eubothrium salvelini* and Nematodes) and some physical variables (weight and age) as we expected but genetic results were unpredicted. This may because our genotyping sample is small.

In conclusion, parasite load is directly related with morph and some physical factors and aims to be directly related with *MHCIIα* polymorphism but we have not enough analyzed data to make our hypothesis consistent.

Table of contents

Figures

Tables

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And thanks to the rest of the people that works in the laboratory of the 3rd. floor in Askja and everyone that support and help me along this project, without them the experience would not have been the same.

1.Introduction

Salvelinus (known as charr) is a genus of salmonid fish with Holarctic distribution characterized as the freshwater fish most northerly founded among the seven genus (*Salmo*, *Oncorrynchus*, *Brachymystax*, *Hucho*, *Salvethymus*, *Acantholingua* and *Salvelinus)* belonging to Salmoninae subfamily of the Salmonidae family (Behnke 1980, Nelson 2006). There are forty-nine charr characterized species and among them it is common to find different morphs. The species are classified depending on the number of gill rakers and pyloric caeca whereas are described by a criteria based on: differences in vertebra numbers, size, color, age, feeding and morphological differences. (Savvaitova 1980, Behnke 1980). Due to all the high degree of variability that they present, Klemetsen talks about the "charr problem", which focuses on the existence of different polymorphisms in specific lakes at the same time (Klemetsen 2010).

Arctic Charr *(Salvelinius alpinus)* or the trout of the mountains as Linnaeus denominated it for first time in 1758, is the only species among the genus *Salvelinus* with a northern circumpolar distribution (Klemetsen et al. 2003). This species colonized Icelandic waters after the last glaciations; approximately 10000 years ago (Adalsteinsson 1992). It has been demonstrated that a marine ancestor of Arctic charr colonized Iceland in one single colonization event from a marine ancestor as all Icelandic populations form a monophyletic branch (Wilson et al. 2004) discussing the idea that different charr morphotypes evolved at different times in similar habitats of Iceland.

Thingvallavatn, Iceland's largest lake, is situated in a neovolcanic zone (Adalsteinsson 1992) (Fig.1.1). It was formed at the end of the last glaciation period (approximately 10000 years ago) by tectonic subsidence and glacial erosion. Since then, it suffered changes to its shape and size due to high volcanic and seismic activity in the neovolcanic zone. The lake covers an area of 83 km^2 and its depth varies from 34 to 114 meter (Adalsteinsson 1992). The catchment soil of the lake is composed by post-glacial lavas and receives springwater from the rifts at the north and east shores (Adalsteinsson et al. 1992, Saemudsson 1992; Snorrason et al. 1989).

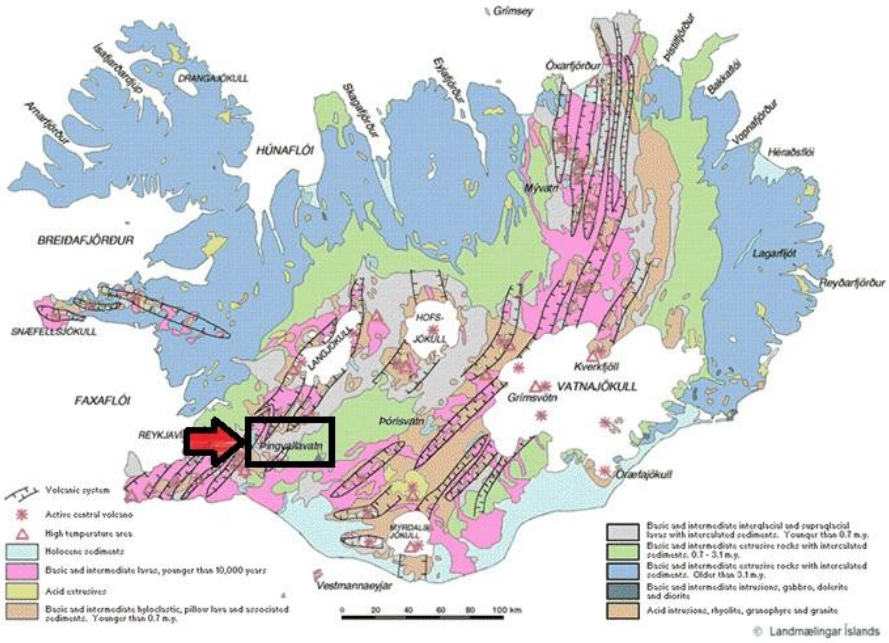


Fig.1.1 Map of Iceland showing the different mineralogy and age of the different layers which make up Iceland geology. The arrow marks the location of Thingvallavatn lake, which is located in the active volcanic belt. Source: Landmaelingar Íslands. 17.05.11 (http://www.lmi.is/)

Four different morphs of Artic charr co-exist in Lake Thingvallavatn: Small Benthic (often known as Dwarf) (SB), Large Benthic (LB), Planktivorous (PL) and Piscivorous (PI) (Fig.1.2). These morphs differ extensively in morphology and life-history characteristics. Lake Thingvallavatn has been isolated since Pleistocene epoch most likely preventing other invasion of artic charr stocks. Therefore it was believed for years that the species living in the lake had evolved in sympatry (Snorrason and Skúlason 2004), but recent studies based on morphs and patterns of genetic differentation propose a micro-allopatric scenario in which the small benthics would be evolved following an adaptative and repetitive evolution (Kapralova et al. 2011).

Benthics (dwarf (SB) and LB) have dark coloration, relatively few gill rackers, chubby bodies, blunt snouts, long pectoral fins and short lower jaw. In contrast, planktivorous charr (PL) have silver coloration, high number of gills rackers, fusiform bodies, pointed snouts, smaller pectoral fins and longer lower jaws (Sandlund et al. 1987, Frandsen et al. 1988; Snorrason et al. 1989, Malmquist et al. 1992) (Fig.1.2).

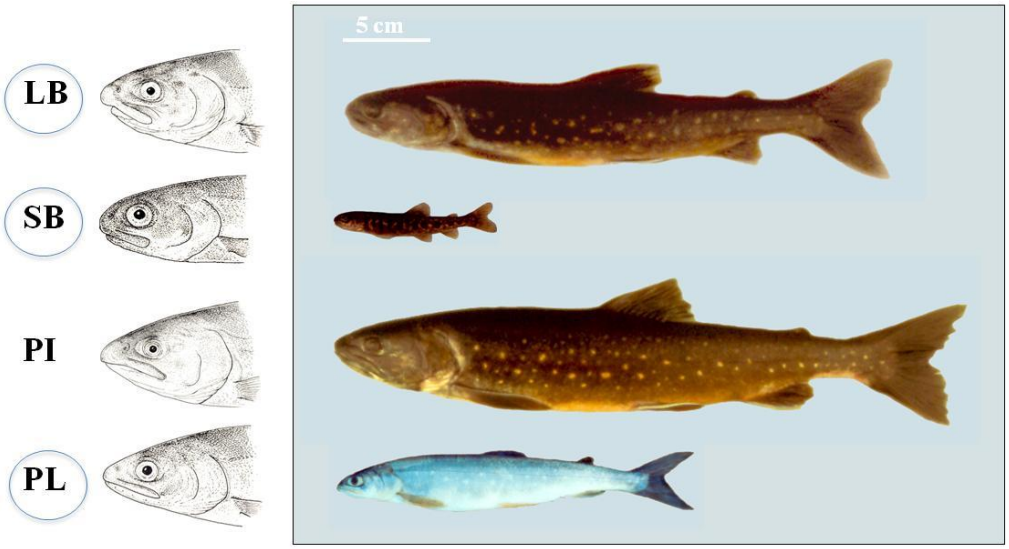


Fig.1.2 Picture of the different morphs of Arctic charr.. Rounded are the ones that we are going to look at in our study. Source: Kapralova "Biodiversity in Iceland 2010" presentation.

The morphs differ also to the life-history characteristics. Dwarfs (SB) mature at 2 years (males)-4 years (females) and approximately around 7.2 cm length (male). Planktivorous (PL) mature at 3-5 years and with a medium length of 15.2 cm while large benthics (LB) doing it among 5-10 years and a minimum length of 25.6 cm. (Skúlason et al. 1996). Large benthic, small benthic and pelagic charr utilize similar breeding stony littoral zone. Small benthic and pelagic charr have overlapping spawning time (October-November), whereas large benthic charr spawns July-August (Skúlason et al. 1989a).

Laboratory rearing experiments have shown a genetic component to differences in morphology, life-history characteristic and behavior (Skúlason 1989b, 1993, 1996, Eirikson et al. 1999). Previous study (Kapralova 2008) based on ten neutral microsatellite markers, have shown small but significant genetic differentiation between small benthic, large benthic and pelagic charr from Lake Thingvallavatn. That was demonstrated by analyzing the F_{st} (measure used in population genetics to find the differentiation between populations based on the genetic polymorphisms data and genetic distance (Gislason 1999; Excoffer et al. 2005)) between and within morphs. Kapralova (2008) got a statistically significant F_{st} values from 0.025 to 0.060 within Lake Thingvallavatn which are lower than the value (F_{st} = 0.234) corresponding for populations of Artic charr around Iceland. Which means that it has to exists some restriction's gene flow to maintain the phenotypic divergence among morphs (Kapralova 2008).

Because of the different habitat of each charr morph, they have different feeding preferences and therefore, each morph is affected by different parasites (Klemetsen and Grotnes 1980; Hindar and Jonsson 1982). The main food item of the benthic morphs is *Lymaea peregrea.* This gastropod is the first host of *Dyplostomum sp.* (fluke). The benthic morph is the second host of this parasite. *Dyplostomum sp* mechanism of infection is penetrating the skin of the fish and migrating to the eye (Frandsen et al. 1988). If a high infection persists, the fish could become blind. The pelagic (PL) are feeding on copepods and zooplankton. They are the second hosts of Cestodes as *Eubothrium salvelini or*

Diphyllobothrium sp. and Nematodes. (Frandsen et al. 1988; Knudsen et al. 2008)(Fig.1.3). It appears that seasonal dynamics often affect parasite infection. Several factors are involved in this process, for example external factors such as life cycle of the parasites, some temporary breakdowns in food/habitat segregation orecological factors (Sandlund et al. 1987, 1988; Malmquist 1988; Robertsen 2007) and internal factors of the host such as its age and sex (Malmquist 1988; Frandsen et al. 1988).

The parasite load is expected to be directly involved and intimately linked to the immune system of these salmonids, which functions as a defense against the attack of these external agents on the host organism (Koppang 2003; Conejeros 2008). In our case with the two small morphs of *Salvelinus alpinus*, in Lake Thingvallavatn.

Fig.1.3 Pictures of the different kind of parasites that we studied. Source: Pictures from poster of Kristmundsson and Ritcher, Keldur laboratories. Háskoli Íslands. Reproduced with permission.

Investigations have revealed that the immune system in fish is less differentiated that in other bigger organism like mammals. Various antigens can activate the adaptative immune system originating a specific antibody response (Koppang 2003).

The major histocompatibility (MHC) genes encode molecules that recognize fragments of pathogens (normally surface proteins) and then, present them to the T-lymphocytes to initiate an immune response (Steinmetz and Hood 1983; Klein 1986; Edwards and Hedrick 1998; Landry and Bernatchez 2001, Meyer and Thomson 2001). MHC belongs to a multigene family with two main subfamilies (class I and class II). Class I is associated with intracellular pathogens while class II is related with extracellular pathogens (Jensen 2007). Allele composition of this complex in *S. Salar* is affected by natural selection, selective pressure and environmental factors as has been documented in many studies (Landry and Bernatchez 2001; Koppang 2003; Bryja et al. 2006; Eyto et al. 2007).

Some alleles of MHC are more effective against the recognition of some specific pathogens than others so that provide better resistance to the individuals that carry them and higher survival likelihood (Dawkins et al. 1999; Lohm et al. 2002; Messaoudi et al. 2002). It has been shown that a high diversity of the MHC locus leads to an increment of survival probability against presence of new infections agents (Lamont 1998; Bernatchez and Landry 2003; Bonneaud et al. 2006) and that low divergence in that ones usually represent a strong selection to confront particular pathogens from a local area (Conejeros et al. 2008). In other words, it is predicted that individuals heterozygous for MHC will have a better response than homozygous individuals because the possession of two different alleles leads to an amplitude of recognition of more pathogen peptides (visit the discussion) (Penn at al. 2002, Kekäläinen 2009).

1.1 AIMS

The aim of this study is to determine the parasite infection patterns in three different morphs of Arctic charr in Lake Thingvallavatn, with mainly focus on the two small morphs (murta and dwarf). These morphs differ extensively in their habitat and resources utilization, therefore we expect to observe large divergence in parasite load between small benthic, pelagic and large benthic (Frandsen et al. 1988).

We are also going to test if these phenotypic differences reflect genetic differences as results of recent studies shown. More specifically, we will investigate whether alleles MHC II alpha locus differ between morphs and if they are associated with a given parasite infection.

Thus, we are going to study a sample of the arctic charr population from Lake Thingvallavatn and investigate:

a) The prevalence of the different kind of parasites in each morph.
b) Which factors define the parasite load.
c) If any correlation exits between parasites infection.
d) If the genetic differences (genotypes) explain the different parasite load and infection.

These approaches are the *leitmotiv* which we try to answer in the results and clarify in the discussion.

2. Materials and methods.

2.1 Sampling

Artic charr were caught by gill netting in two different spawning locations (Mjóanes and Ólafsdráttur) by the shores of Thingvallatn lake (64º10'N, 21º10'W) on September 30 and October 12 of 2010 (Fig.2.1). The sampling yielded a unequal percentage of each sex (a total of 141 females and 123 males), morph (131 murtas, 113 dwarfs and 19 large benthics) and location (141 samples were collected in Ólafsdráttur and 123 in Mjóanes (Fig.2.1)), being a total of 264 fish processed. As can be appreciated the number of large benthics is lower than the others as the study is mainly focused in the other two morphs named before.

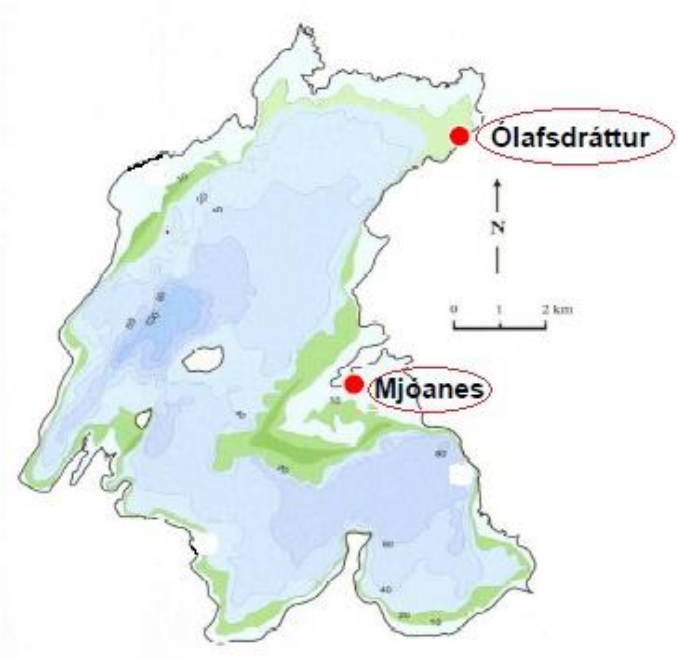


Fig.2.1 Thingvallavatn lake with the situation of the two shores were the specimens were caught (from Kapralova 2008).

The different specimens were classified, each put in an independent bag and gathered in larger bags based on morph criteria (according to Snorrason et al. 1989). Some were mated and some not. After that, they were frozen at -10ºC until the dissection process days later.

2.2 Processing & dissection

A random set of 10-20 individuals were selected for analysis each day. Each was thawed, photographed and their length (cm) and weighed (g) was measured. Their sex was determined and whether they had been mated. Mature sexed and their right pectoral fin was removed and frozen for the subsequent DNA extraction. The parasite extraction was divided in two sections: head and body. From the head we removed the eyes and otholites, stored them in eppendorf tubes and froze them for the posterior analysis. From the body, we cut and analyzed all the organs separately to count the amount of parasites and to categorize the different types (see sections below).

2.2.1 Age determination

The head of each sample was removed and the brain was smashed until we got the otholites (small bony accumulations located in the inner ear whose function is to serve as a compass to fish) which we stored away in eppendorf tubes at room temperature. We analyzed them to determine the age of the fishes.

The age was estimated following an observation criteria in function of the ossification growing marks presented by pair of otholites per individual. They were visualized under a microscope Leica KL200 LED with two auxiliary light arms at 2x times magnification. The ratio followed was one year per mark (Jonsson 1976).

2.2.2 Eye parasites

Two eyes were extracted per individual and at least one of them was processed. First, the eye was popped out and the content was poured on a flat slide with a cover slip over (Kristmundsson and Ritcher 2009). Sigrún Reynisdóttir (SR) processed them using a Leica 5x times microscope and dividing in 45 felts the slide. The estimation of the average number of parasites (*Dyplostomum sp*.) was done by analyzing one felt first and counting the number by view in all slide. The felt ratio followed in all the slide goes from 0-4; being 0 the total absence of parasites; 1 equivalent to 1 parasite per felt; 2 a lower total ratio represented by the presence from 1 to 3 individuals per felt; 3 a moderate total ratio equivalent a 4-10 individuals per felt and 4 total invasion which means more than 10 *Dyplostomum sp*. per felt (Koppang et al. 2003).

2.2.3 Intestine parasites

We opened the fish with cutting from the inferior part of the vent and separating the superior flesh to better see the insides. Then, we extracted the eggs or the milt, depending on sex. Normally there were no diseases or parasites in that area, but it is always good to have a look before ridding of it.

Carefully, we extracted the liver, stomach and intestine and analyzed them looking for parasites and marks of diseases. After finding them, we took and separated them for the posterior classification. We looked into the organs specified before looking for *Eubothrium salvelini*. For *Diphyllobothrium sp*. and Nematodes we looked around all the cavities as they were occasionally around there or stuck to the flesh (Frandsen et al. 1989; Kristmundsson and Ritcher 2009).

To quantify all the different parasites we used different scales but all of them following an observation criteria. The scale used for *Diphyllobothrium sp*. was from 0 to 3, being 0 the total absence of parasites; 1, from 1 to 3 per individual; 2, from 4 to 7 per individual and 3, more than 8 parasites per individual. For *Eubothrium salvelini*, we only annotated the presence (1) or the absence (0) of the parasite in the individual. In the case of Nematodes we annotated the number of them per individual.

The data were obtained by a single observer (Cristina Bajo Santos (CBS)) but the scale was set up jointly by CBS, Kalina H. Kapralova and SR.

2.3 Molecular work

DNA was extracted from a piece of tissue of the right fin stored before of 262 samples by a standard phenol chloroform procedure (Conejeros P. et al. 2008). This was done by SR and provided to CBS.

A polymerase chain reaction (PCR) was used to amplify our fragment following the protocol (for one sample (table 2.1)) with the forward primer (*MHCIIα*-f6: 5'- CCA GAG ACA ATA GGT AAG AGA GAG A-3') and the reverese (*MHCIIα*-r5: 5'-TGG GAA CAC ATT TAG CAT CA-3') and starting from a primer stock of 100ng/µl. The rest of reagents used can be seen in the table.

Table 2.1 PCR protocol.

DNA	10XBuffer	dNTPs	R primer	F primer	Taq	ddH20	Total Volume
2 µl	2 µl	2 µl	0,4 µl	0,4 µl	0,2 µl	13 µl	20 µl

The PCR program followed was CHANG, which is based on a denaturation temperature of 94ºC, anneling temperature of 53ºC and an extension temperature of 72ºC, repeating it 35 times and maintain it at 12ºC forever in the end. The follow graphic (Fig.2.2) explain it in detail.

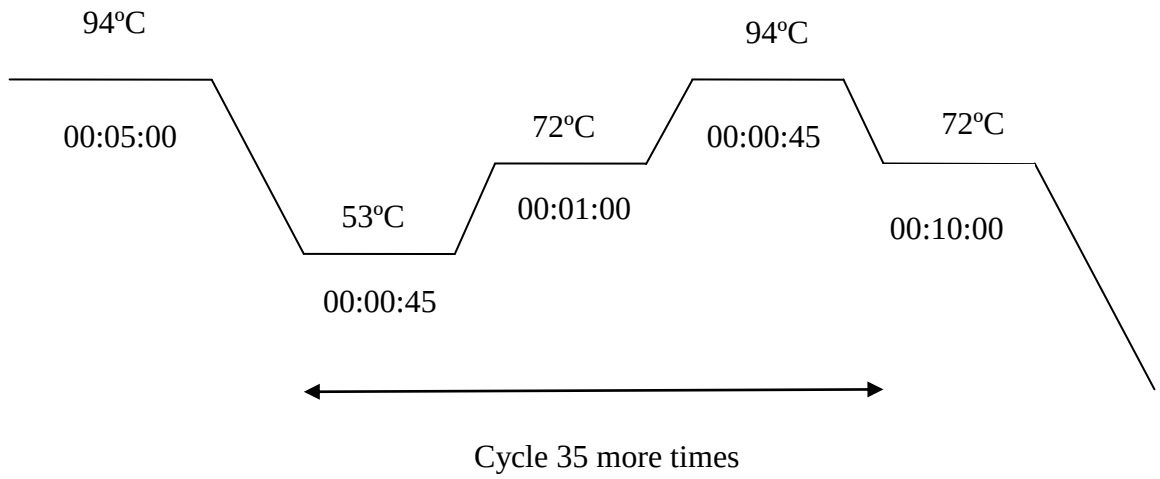


Fig.2.2 Graphic of the CHANG program used to amplify the *MHCIIα.*

The products were stored in freezer or run directly on a gel. They were separated by agarose electrophoresis at 1 % agarose + ethidium bromide gel. To check the presence and the correct size of the fragments we used a fluorescence imaging system.

After the electrophoresis check I purified the PCR product before beginning the sequencing with an Exo-SAP procedure following a protocol (table 2.2). We ran the mixture on a Exo-SAP program consist of 35 minutes at 38ºC and 20 more minutes at 80ºC in the PCR machine. With this, we eliminated the primers from the previous PCR reaction and removing all the ssDNA, which is required for DNA sequencing.

Table 2.2 Exo-SAP protocol.

PCR product	ddH_2O	(Exo1)Fosfatase buffer	Antatric phosphatease 0,2x5U/ µl ~1U	Exo1 0,1x20U/ µl~2U	Total Volume
5 µl	3,7 µl	1 µl	0,2 µl	0,1 µl	10 µl

After purifying the DNA fragment I proceed to sequencing. First I did the sequencing reaction adding termination dyes to be recognized by the sequencer. In that step we follow a sequencing protocol (Table 2.3) and the sequencing program in the PCR machine (Fig.2.3).

Table 2.3 Protocol sequencing program.

Exo-SAP product	5 µl
ddH2O	5,25 µl
VII 5xbuffer	2,76 µl
TRR BigDye	0,49 µl
R primer (mentioned before in the PCR protocol)	1,5 µl
T.V.	15 µl

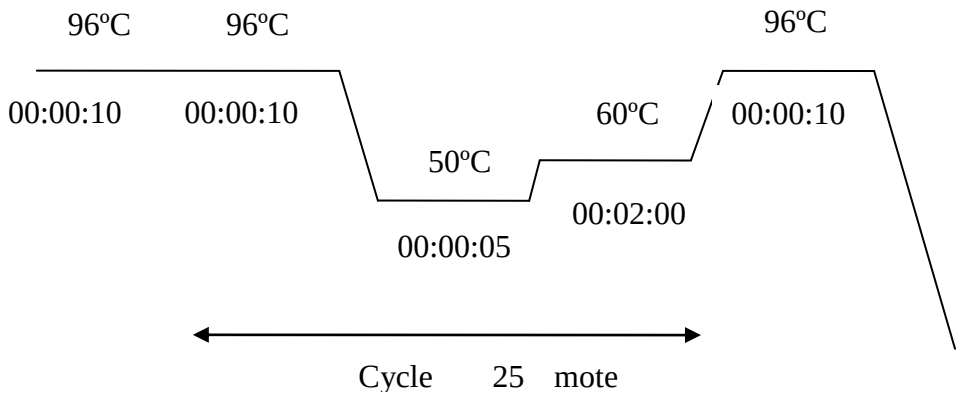


Fig.2.3 Graphic representation of the sequence program.

The DNA sequencing reaction was purified with standard ethanol precipitation. The products were run on an AB 3500xL Applied Biosystems Genetic Analyzer.

2.4 Data processing

I compiled all the data from the different procedures in an excel sheet (Appendix A).

The genotypic data obtained from the sequencer was translated from a base calling software called Sequencing Analysis v.5.4. (https://products.appliedbiosystems.com) and edited by Phred phrap and consed software elaborated by Phil Green laboratories (Gordon D. 2004). Phred reads the base calling sequence and assigns a value of each one of them. After that, Phrap assemble the sequence in the most suitable contings by a DNA shotgun technique. Finally, consed allow us to check the sequence, view the differences between them and modify the possible mistakes that could have in the assembling.

Later on we align the sequences using ClustalW software online version (http://www.genome.jp/tools/clustalw/) (Li KB 2003). For a double checking of the sequences and correcting the possible mistakes we use a GeneDoc 2.1 program (http://www.nrbsc.org/gfx/genedoc/) (Nicholas KB 1997). The information obtained after all this steps were collected in an Excel sheet for the posterior analysis (Appendix A).

2.4.1 Statistical analysis

After getting all the data mentioned before we proceed to find which of the previous factors are relevant and indicative of the parasite-morphology-genotypic relations that we tried to elucidate.

For that we did statistical analysis such as ANOVA (aov in R) or linear regressions (lm in R) to look if there is any significant relation or not between factors using the R-project.org platform (http://www.r-project.org/) (R development Core Team 2005). With the same platform we have done some histograms of the frequency of the different parasites and genotypes within morph for seeing clearly the prevalence of them. We also calculated averages, standard deviation and sample size. To test the correlation of parasites we used (corr.test in R) among them by type and sex separately.

Finally we sought to evaluate whether the *MHCIIα* polymorphism correlated with parasites. We only looked at murtas because only 24 murtas and 4 large benthics were genotyped. We ran a single model (Y = Genotype) for each of the parasites and also for weight and age.

$$Y = \text{genotype} \times \text{weight} + \text{error}$$

$$Y = \text{genotype} \times \text{age} + \text{error}$$

We ran also a more elaborated model to test for interaction effects on P1 prevalence.

3. Results

3.1 Factors that correlate with parasite load.

A lot of factors are involved in an aquatic ecosystem and the creatures that live in it are affected by them. Some of them will be more influential than others and these ones will define the characteristic of the species. In our case we are interested in finding out if there is any connection between the parasite load of arctic charr and one or more of the physical factors.

To investigate that, we studied a sample of 264 fishes annotating all the physical variables as weight, age and morph. After that we dissected, counted and classified the parasites making a table with all the information that it shows in Appendix A.

To check which factor was the most predominant we did statistical analysis like ANOVA. The results for three parasites (*Diphyllobothrium sp*., *Dyplostomum sp*. and Nematodes) are shown in table 3.1.

Table 3.1. P-values from ANOVA's among physical factors and parasites.

Parasites	Location	Sex	Weight	Age
Diphyllobothrium sp.	0,31	0,41	0,027*	0,52
Dyplostomum sp.	0,23	0,57	0,006*	0,90
Nematodes	0,98	0,0095**	0,39	0,08

*= <0, 05; ** =< 0, 01

In light of these results, we can see a significant relation between weight and two of the parasites (*Diphyllobothrium sp.* and *Dyplostomum sp.).*This means that a fat individual normally is being more parasites infected than a thin one. In summary, weight increases parasite load (data not shown).

The statistics show also a significant relation between Nematodes infection and sex. Females have on average 1,06 parasites but males 0.6 (standard deviation (2,01 and 1,54 respectively), meaning a predilection for females than males (Table 3.2).

Table 3.2. Means and standardl deviation of Nematodes by sex.

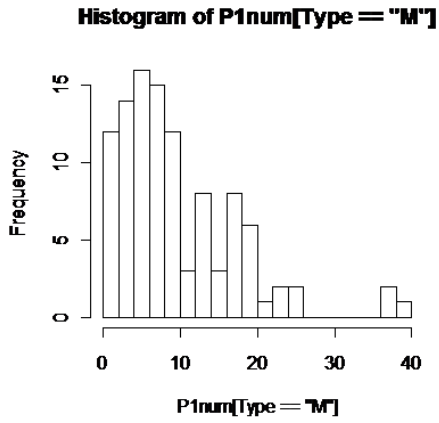
	Mean	Statistical D.
Female	1,06	2,01
Male	0,66	1,54

3.2 Prevalence of different kind of parasites by *Arctic charr* morphotype.

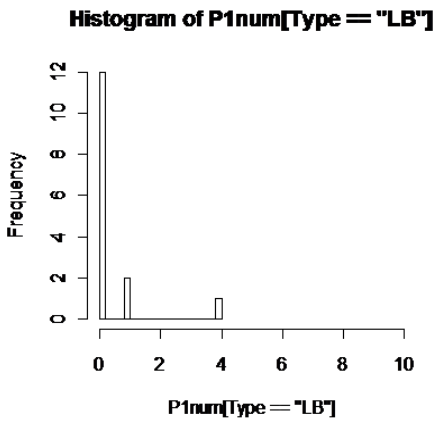
Our objective was study the different parasite load which affects arctic charr individuals of the community established in lake Thingvallavatn. We expected a different parasite load in each morph (murta (M), dwarf (D) and large benthics (LB)) due to all the ecological and behavior differences that conform.

To do that, we took the data from Appendix A and we drew some histograms and tables were we can see the prevalence and number of the different parasites in the different morphs (Fig. 3.1) and tables 3.3 and 3.4.

A



B



C

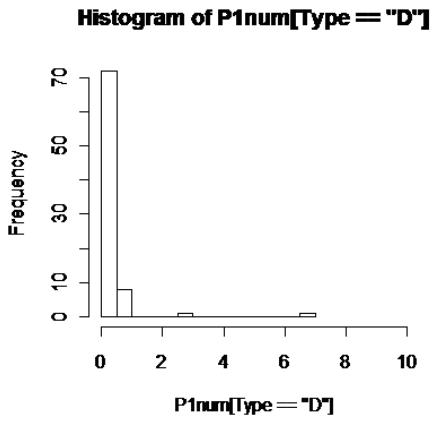


Fig 3.1 Parasite load in Arctic charr from Lake Thingvallavatn. A shows the amount and the frequency of *Diphyllobothrium sp.* (P1num) in murta, B shows the amount and the frequency of *Diphyllobothrium sp.* (P1num) in large benthic and C shows the amount and the frequency of *Diphyllobothrium sp.* (P1num) in dwarfs.

The histograms show a clear trend murta's are more infected by *Dyphillobothrium sp.* than dwarfs or large benthic. This makes sense, cause the main food of pelagic fishes are copepods and zooplankton, which are the second host of *Dyphillobothrium sp.*

It is clear that Nematodes are more common in murta's female individuals than in other morphs or in males(Table 6). This corroborates the results of the previous point of the results.

Table 3.3. Nematode load distribution between morph and sex.

Morph	Infected	No infected	No data
Dwarf	6	76	31
Murta	68	37	26
Large Benthic	2	13	4

A similar effect appears in the study of *Eubothrium salvelini* infection. Murta's is the most affected morph with a clear difference in comparison with the other morphs as it shown in table 3.4.

Table 3.4. Summary of the number of individuals infected by *Eubothrium salvelini* by morph.

Type of morph	Sex	Infected	No infected	No data.
Murta	Male	19	55	18
Murta	Female	36	18	3
Dwarf	Male	0	19	11
Dwarf	Female	1	59	23
Large Benthic	Male	1	14	3
Large Benthic	Female	0	0	1

In contrast, we can see (Fig.3.2) a clear increase of *Dyplotomum sp.* in dwarfs compared to murtas. It is visible a similar amount of parasite load in large benthics but the sample of LB consist of only 19 individuals, while the data acquired of dwarfs are based on 113 individuals.

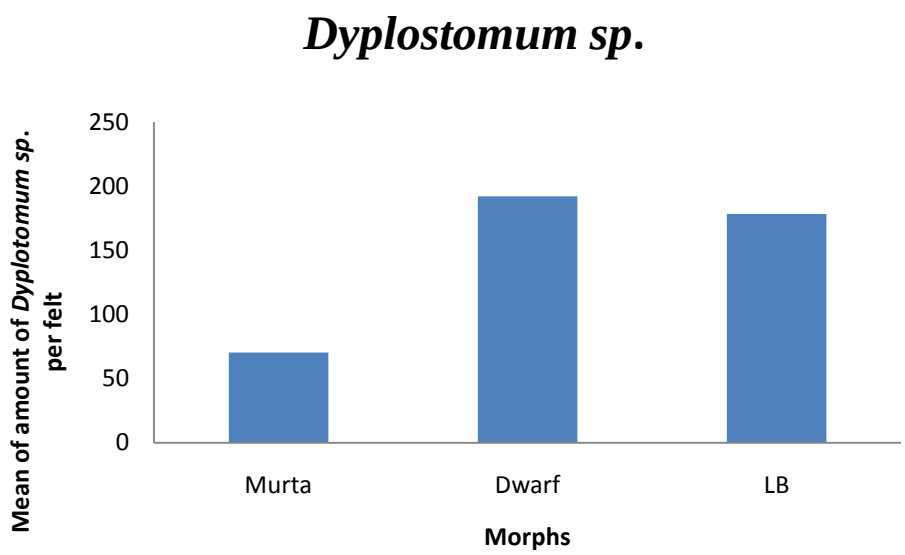


Fig 3.2. Representation of prevalence of *Dyplotomum sp.* by morph.

These results follow the hypothesis expressed in the introduction and discussed by Frandsen et al. 1988.

3.3 Correlation between parasites infection.

One individual that have a disease or that it is infected with some kind of parasite tends to be weak against parasite invasion and prone to catch other diseases than a healthy fish. Due to this, we expected that an individual highly infected show more than one kind of parasites.

For discovering if there exists a correlation between parasites, we did ANOVA's for *Diphyllobothrium sp*., *Dyplostomum sp*. and Nematodes and correlation test for *Eubothrium*. The results are shown in table 3.5.

Table 3.5 Table with Pearson's coefficient(pr) below the diagonal and p-values of correlations and ANOVA's among parasites above the diagonal.

	Dyplostomum sp.	*Diphyllobothrium sp.*	*Eubothrium*	Nematodes
Dyplostomum sp.	----------	0,84	0,65	0,082
Diphyllobothrium sp.	0,25	------	0.0006	0,86
Eubothrium	NA	NA	--------	0,06
Nematodes	-0,025	0,17	NA	---------

NA = Not Available.

The results show that there is not any significant relation among parasites except between *Eubothrium* and *Diphyllobothrium sp.* which appear clearly significant (p = 0.0006). We haven't got a clear explanation for it. The presence of one of them may influence the presence of the other but we don't know how. We can see also that there is a nominally significant (0.1- 0.05) tendency for *Eubothrium* and Nematodes infections to be related. Likewise, all the relations are direct except between Nematodes and *Dyplotomum sp*, which is reverse and nominally significant, meaning that the presence of one of them decrease the presence of the other. We did ANOVA's and linear regression models taking more than three parasites at a time but the results were not significant in any case.

3.4 Association between *MHCIIα* genotype and parasites.

The hypothesis was that a genetic difference in *MHCIIα* gene could explain the different level of infection among the different morphs and maybe at the same time could be modulated by other attributes.

To assess this, we genotyped samples of different morphs. Only 60 were successfully genotyped (Appendix A) and we made a statistical plot (Fig.3.3) with murtas (36 genotyped individuals) showing the different genotypes and the score of *Diphyllobothrium* infection.

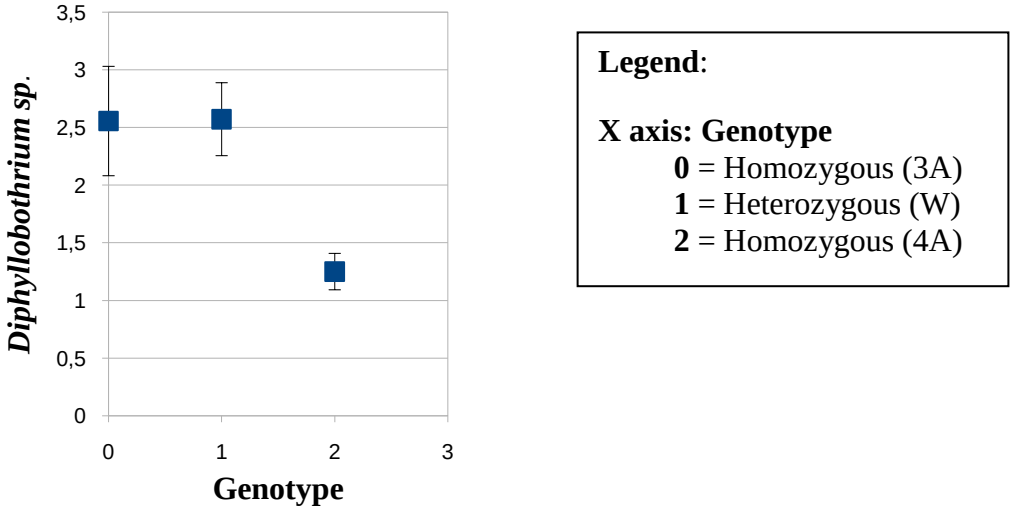


Fig 3.3 Association between average *Diphyllobothrium sp*. infection and *MHCIIα* genotype. The SD, variance is graphed on the averages.

Homozygous individuals (4A) are less infected with *Diphyllobothrium sp*. than the others genotypes (Fig.3.3 and Table 3.6). We checked also if some physical variables could interact with the genotype. Sex, location and type didn't give any significant results, but the age appears to interact with the genotype (Table 3.6). This could mean that the effects of *MHCIIα* genotype on *Diphyllobothrium sp*. parasite infection depend on the age of the fishes (small pelagic) but note that our sample is small and it is possible that those signals are due to chance. More individuals have to be genotyped to test this hypothesis further.

Table 3.6 Relation among genotype, parasite load and age.

Genotype	2	5,97	2,99	5,42	0,01	*
Age	1	0,09	0,92	0,17	0,69	·
G x A	2	4,34	2,17	3,94	0,03	*
Residuas	29	15,49	0,55			

*= p< 0.05; ·= p>0.05

4. Discussion

As my results show, the most decisive character related with the parasite load is the morphotype. Small pelagic morph (murta) presents higher rate of infection of *Diphylobothrium sp*; Nematodes and *Eubothrium salvelini*. This is easily explained with their food habits based on copepods and zooplankton, second hosts of the parasites mentioned before (Knudsen 2008). In the other hand, there is a clear enrichment of *Dyplostomum sp*. in dwarfs which is also related with the food habits because in this case, the main food item of the benthivorous charr is the gastropod *Lymaea peregrea*, (the first host of *Dyplotomum sp).* These results confirm our hypothesis that different morphs carried different level of parasite load as it is shown in previous studies on Lake Thingvallavatn (Frandsen et al. 1988).

The parasite load appears also affected by weight in the case of *Dyplostomum sp.* and *Diphyllobothrium sp*. The number of parasites increases with weight. We tested also the age as some studies (Frandsen et al. 1988) where were detected a direct relation between age and number of parasites, but our results did not show this. The Nematodes infection level has relation with sex. They are more common in females than in males which is corroborating by some studies (Fraser 2009).

Looking for some correlation pattern among different parasites we find positive one between *Eubothrium* and *Diphyllobothrium sp*. The two of them are endoparasites of the stomach and intestine area (*Eubothrium* inside and *Diphyllobothrium sp*. on it). Therefore they can live together in the same individual without directly competing with the other. At the same time, infected individuals may have more probability to get other infections than healthy individuals, consistent with our results and other studies (Frandsen et al. 1988; Kekäläinen 2009). We observed also a positive nominally significant relation between Nematodes and *Eubothrium* infections, may be for the same reason. Nematodes establish themselves around the cavities, more or less in the same area as *Diphyllobothrium sp*. meanwhile *Eubothrium* lives inside the stomach, so they can cohabit together seemingly without problem.

On aim of my study, was to test the possible relation between the different *MHCIIα* genotypes and parasite load in Arctic charr. We only could look at one morph (murtas) because of the genotyping success. We were expecting results had followed the previous studies of polymorphism in *MHCIIα* (Landry et al. 2001; Penn et al. 2002; Conejeros 2008; Kekäläinen 2009); showing that there was a significantly higher frequency of heterozygous and that these have a less rate of parasite infection. But we obtained that homozygous (4A) has lower infection of *Diphillobothrium sp*. than heterozygous. That's a bit controversial but our sample size is rather low, only 35 small pelagic individuals. We need to genotype more individuals to firmly test this hypothesis.

We also found a relation between age and genotype which could be explained with the knowledge that an older individual has more probability and time to get infected than a younger one (Frandsen et al. 1988). But as before, we can only speak about a tendency due to our sample size is small, but this is a beginning statement for future studies in the area.

In conclusion, parasite load is directly related with morph and some physical factors and aims to be directly related with *MHCIIα* polymorphism but we have not enough analyzed data to make our hypothesis consistent.

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List of webpages.

Several webpages and tools were used in this study.

Sequencing Analysis v.5.4: https://products.appliedbiosystems.com

ClustalW: http://www.genome.jp/tools/clustalw/

GeneDoc 2.1: http://www.nrbsc.org/gfx/genedoc/

R-project: http://www.r-project.org/

Appendix A

Parasite load & physical character of Arctic charr.

Legend.

Location : O = Ólasfdráttur
M=Mjóanes

Type: M = Murta
D = Dwarf
LB = Large Benthic

Age: Years in number.

Weight: In grams.

P1*: Diphyllobothrium sp*.
0 = No presence
1 = 1-3 per indv.
2 = 4-7 per indv.
3 = > 8 per indv.

Nematodes: Number per fish

Embor.*: Eubothrium salvelini*
0 = No present.
1 = Present.

Eyescore *: Dyplostomum sp.*
0 = Absence
1 = 1 ind/felt
2 = 1 < 3 ind/felt
3 = 4-10 ind/felt
4 = >10 ind/felt

Gtyp: Genotype
0 = Homozygous (3A)
1 =Heterozygous(3AW)
2 = Homozygous (4A)

Appendix A: Parasite load & physical character of Arctic charr.

Identifier	Location	Sex	Type	Age (years)	Weight	P1codeALL	Nematodes	Embor	eye_score	gtyp
2001	O	Male	M	4	49,39	1			2	1
2002	O	Male	M	5	67,47	2			3	1
2003	O	Female	M	6	128,14	3			2	
2004	O	Male	M	5	63,78	3			1	1
2005	O	Male	M	5	78	2			2	
2006	O	Male	M	6	89,91	2			2	0
2007	O	Male	M		78,6	3			2	1
2008	O	Male	M	7	52,69	3			2	0
2009	O	Male	M	5	68,61	3			3	1
2010	O	Male	M	5	39,85	1			3	2
2011	O	Male	M	6	65,88	3			3	1
2012	O	Male	M	6	70	0			1	
2013	O	Male	M	6	64,55	3			2	1
2014	O	Male	M	6	67,33	3			2	0
2015	O	Male	M	6	86,07	3			1	0
2016	O	Female	M	6	74,2	0			1	2
2017	O	Female	M	7	69,1	1			4	0
2018	O	Male	M	7	65,7	1			1	
2019	O	Female	D	7	27,07	0			1	
2020	O	Female	D	4	60,3	0			2	2
2021	O	Female	D	5	87,25	0			3	2
2022	O	Male	D	4	35,3	0			2	2
2023	O	Female	D	7	48,08	1			2	2
2024	O	Female	D	4	43,52	0			3	2
2025	O	Female	D	6	72,8	0			3	2
2026	O	Female	D	5	43,78	0			2	2
2027	O	Male	LB	7	357,93	0			3	2
2028	O	Male	LB	8	390,5	2			3	2
2029	O	Male	LB	8	247,5	1			3	2
2030	O	Female	LB	9	241,4	0			1	0
2031	O	Female	D	6	13,34	1			2	
2032	O	Female	D	5	40,87	0			1	
2033	O	Female	D	4	32,04	0			0	2
2034	O	Female	D	4	33,41	0			3	2
2035	O	Male	D	4	22,29	0			1	2
2036	O	Male	D	4	34,27	0			2	2
2037	O	Male	D	4	28,97	0			2	
2038	O	Male	D	5	51,2	0			3	2
2039	O	Male	D	5	51,36	0			3	2
2040	O	Female	D	4	25,27	0			1	
2041	O	Male	D	4	38,94	0			2	2
2042	O	Male	D	4	44,5	1			3	2
2043	O	Male	D	6	32,64	1			2	
2044	O	Male	D	4	35,79	1			3	
2045	O	Male	D	5	33,41	0			4	
2046	O	Female	D	7	64,9	0			3	2
2047	O	Female	D	9	125,76	0			3	2
2048	O	Female	D	6	58,69	0			3	2
2049	O	Female	D		21,97	0			3	
2050	O	Female	D	5	39,2	0			3	2
2051	O	Female	D	5	52,18	0			3	
2052	O	Female	D	5	68,47	0			3	2
2053	O	Female	D	6	36,6	0			2	
2054	M	Male	M	4	85,53	2			2	
2055	M	Male	M	4	82,44	3			2	
2056	M	Male	M	6	86,55	3			1	
2057	M	Male	M	6	92,35	3			2	1
2058	M	Male	M	5	69,22	3			1	
2059	M	Male	M	6	73,19	3			1	
2060	M	Male	M	5	62,3	0			2	
2061	M	Male	M	5	88,57	3			2	
2062	M	Male	M	6	69,07	1	0	Inf	1	2
2063	M	Male	M	5	81,26	3	0	Not	1	0
2064	M	Male	M	5	61,81	3	0	Not	2	1
2065	M	Male	M	6	69,85	2	0	Not	1	
2066	M	Male	M	6	78,67	3	0	Not	1	0
2067	M	Male	M	5	91,09	3	0	Not	1	
2068	M	Male	M	5	65,8	3	0	Inf	2	
2069	M	Male	M	6	77,9	2	0	Inf	1	

2070	M	Male	M	6	85,7	3	0	Inf	2	
2071	M	Male	M	5	79,72	1	0	Inf	1	1
2072	M	Male	M	5	99,24	3	0	Not	1	0
2073	M	Male	M	7	87,89	3	3	Inf	2	0
2074	M	Male	M	5	62,59	2	0	Not	2	
2075	M	Male	M	5	74,24	2	0	Not	3	0
2076	M	Male	M	5	63,6	2	0	Not	2	
2077	M	Male	M	6	80,95	3	0	Not	2	
2079	M	Male	M	6	79,24	3	0	Inf	1	1
2080	M	Male	M	7	72,68	3	3	Inf	2	
2081	M	Male	M	5	80,42	3	0	Inf	2	
2082	M	Female	M	5	89,27	3	0	Inf	1	2
2083	M	Female	M	6	72,95	3	0	Inf	1	
2084	M	Female	M	4	66,18	3	0	Inf	2	
2085	M	Female	M	6	85,47	3	0	Inf	1	0
2086	M	Female	M	5	83,56	3	0	Inf	1	1
2087	M	Female	M	5	87,77	3	0	Not	2	0
2088	M	Female	M	6	72,54	3	0	Not	2	0
2089	M	Female	M	6	91,19	2	0	Not	2	1
2090	M	Female	M	5	84,75	2	0	Inf	2	0
2091	M	Female	M	6	89,45	3	1	Not	1	1
2092	M	Female	M	7	71,72	3	0	Not	3	0
2093	M	Female	M	6	102,2	1	0	Not	1	0
2094	M	Female	M	6	77,03	3	8	Not	1	
2095	M	Female	M	7	62,05	2	2	Inf	2	0
2096	M	Female	M	7	70,25	3	0	Not	2	0
2097	M	Female	M	6	100,77	3	7	Inf	1	
2098	M	Female	M	7	71,05	1	1	Inf	1	
2099	M	Female	M	6	77,08	1	2	Not	2	
2100	M	Female	M	6	74,92	1	4	Inf	1	
2101	M	Female	M	7	70,34	0	0	Inf	1	
2102	M	Female	M		69,89	2	3	Inf	2	
2103	M	Female	M	5	101,94	2	1	Inf	1	
2104	O	Female	D	6	37,44	1	0	Not	3	
2105	O	Female	D	5	75,95	1	0	Not	2	
2106	O	Female	D	4	26,24	0	0	Not	4	
2107	O	Male	D	4	46,15	0	0	Not	1	
2108	O	Female	D	5	25,05	0	0	Not	3	
2109	O	Female	D	5	26,64	0	0	Not	2	
2110	O	Female	D	5	18,92	0	0	Not	1	
2111	O	Female	D	5	37,05	0	0	Not	2	
2112	O	Female	D	4	19,29	0	0	Not	1	
2113	O	Female	D	3	23,1	0	0	Not	1	
2114	O	Female	D	6	28,73	0	0	Not	1	
2115	O	Female	D	5	27,91	0	0	Not	1	
2116	O	Female	D	4	31,03	0	0	Not	2	
2117	O	Female	D	5	39,76	0	0	Not	2	
2118	O	Female	D	4	26,25	0	0	Not	3	
2119	O	Female	D	3	22,01	0	0	Not	1	
2120	O	Male	D	5	19,97	0	0	Not	1	
2121	O	Female	D	4	41,55	0	0	Not	1	
2122	O	Female	D	7	27,25	0	0	Not	1	
2123	O	Female	D	5	61,39	0	0	Not	3	
2124	O	Male	D	5	23,44	0	0	Not	2	
2125	O	Male	D	4	22,77	0	0	Not	1	
2126	O	Male	D	4	25,17	0	0	Inf	1	
2127	O	Male	D	5	22,7	0	0	Not	1	
2128	O	Male	D	3	20,62	0	0	Not	1	
2129	O	Female	D	5	60,7	0	0	Not	3	
2130	O	Female	D	5	29,65	0	0	Not	1	
2131	O	Male	D	4	25,62	0	0	Not	2	
2132	O	Male	D	8	31,35	0	0	Not	0	
2133	O	Male	D	4	29,21	0	0	Not	3	
2134	O	Male	D	3	24,06	0	0	Not	2	
2135	O	Male	D	4	22,01	0	0	Not	1	
2136	O	Female	D	5	47,16	0	0	Not	2	
2137	O	Female	D	5	23,09	0	0	Not	1	
2138	O	Female	D	6	26,85	0	0	Not	1	
2139	M	Male	M	5	60,56	1	0	Not	1	
2140	M	Male	M	5	67,7	3	4	Inf	1	

Appendix A(continued): Parasite load & physical character of Arctic charr.

2141	M	Male	M	5	70,09	1	0	Inf	1
2142	M	Male	M	5	102,72	3	10	Inf	1
2143	M	Male	M	5	78,82	2	0	Not	1
2144	M	Male	M	6	83,89	3	0	Inf	1
2145	M	Male	M	6	78,06	3	1	Inf	1
2146	M	Male	M	4	63,82	3	1	Inf	1
2147	M	Male	M	7	60,52	2	6	Not	2
2148	M	Male	M	6	105,27	3	0	Not	1
2149	M	Male	M	6	61,62	0	0	Inf	2
2150	M	Male	M	6	75,93	2	1	Inf	1
2151	M	Male	M	5	64,13	2	0	Inf	1
2152	M	Male	M	5	67,8	1	0	Inf	2
2153	M	Male	M	5	70,7	1	0	Not	1
2154	M	Male	M	7	80,2	1	0	Not	2
2155	M	Male	M		67,13	3	2	Not	1
2156	M	Male	M	5	69,65	3	2	Not	1
2157	M	Male	M	5	76,05	3	1	Inf	1
2158	M	Male	M	5	66,8	3	1	Not	1
2159	M	Male	M	5	74,6	3	0	Not	2
2160	M	Male	M	5	80,2	1	0	Inf	1
2161	M	Male	M	5	67,5	2	2	Inf	2
2162	M	Male	M	6	74,98	2	0	Inf	1
2163	M	Male	M	6	65,3	1	1	Inf	1
2164	M	Female	M	5	81,5	3	4	Inf	2
2165	M	Male	M	5	87,99	2	5	Inf	2
2166	M	Female	M	4	76,9	3	2	Not	2
2167	M	Male	M	6	71,55	3	0	Not	1
2168	M	Male	M	5	75,6	1	0	Inf	2
2169	M	Male	M	5	79,02	1	1	Not	2
2170	M	Male	M	6	82,14	2	2	Inf	1
2171	M	Male	M	5	84,4	3	1	Inf	1
2172	M	Male	M	5	81,9	2	3	Not	1
2173	O	Female	D	7	33,72	0	0	Not	1
2174	O	Female	D	6	39,12	0	0	Not	1
2175	O	Female	D	7	54,42	0	0	Not	1
2176	O	Female	D	5	29,11	0	0	Not	4
2177	O	Male	D	5	61,16	0	0	Not	2
2178	O	Male	D	5	35,5	1	0	Not	3
2179	O	Female	D	7	42,76	0	0	Not	1
2180	O	Female	D	4	29,55	0	0	Not	3
2181	O	Male	D	6	35,77	1	0	Not	2
2182	O	Male	D	4	24,05	0	0	Not	2
2183	O	Female	D	5	27,01	0	0	Not	1
2184	O	Male	D	6	24,56	0	0	Not	3
2185	O	Female	D	6	32,4	0	0	Not	3
2186	O	Female	D	5	37,22	0	0	Not	0
2187	O	Male	D	5	24,96	0	0	Not	3
2188	O	Female	D	9	29,14	1	0	Not	0
2189	O	Female	D	7	25,59	3	0	Inf	1
2190	O	Female	D	5	32,78	0	0	Not	2
2191	O	Female	D	3	33,23	0	0	Not	3
2192	O	Male	D	3	22,32	0	0	Not	1
2193	O	Female	D	6	65,44	0	0	Inf	3
2194	O	Female	D	5	58,53	0	0	Not	2
2195	O	Female	D	5	36,14	1	0	Not	4
2196	O	Female	D	6	20,53	0	0	Not	1
2197	O	Female	D	5	23,84	0	0	Not	1
2198	O	Female	D	5	34,35	0	0	Not	3
2199	O	Female	D	6	60,53	0	0	Inf	4
2200	O	Female	D	7	53,58	0	0	Not	3
2201	O	Female	D	6	45,54	0	0	Inf	2
2202	O	Female	D	6	82,94	0	0	Not	3
2203	O	Female	D	7	95,5	0	0	Not	3
2204	O	Female	D	8	23,31	0	0	Not	1
2205	O	Female	D	5	56,43	0	0	Not	4
2206	O	Female	D		32,87	1	0	Inf	1
2207	O	Female	D	5	45,59	0	0	Not	2
2208	O	Female	D	4	40,9	0	0	Not	2
2209	O	Female	D	5	25,37	0	0	Not	4
2210	O	Female	D	8	90,03	0	0	Not	3

Appendix A (continued): Parasite load & physical character of Arctic charr.

2211	M	Female	D	5	53,79	0	0 Not	2
2212	M	Female	M	7	92,1	3	3 Inf	1
2213	M	Female	M	9	90,09	3	2 Inf	1
2214	M	Female	M	7	96,73	3	4 Inf	1
2215	M	Female	M	6	69,31	3	2 Inf	2
2216	M	Female	M	7	75,55	3	2 Inf	2
2217	M	Female	M	6	76,94	3	2 Inf	2
2218	M	Female	M	6	81,88	2	3 Inf	1
2219	M	Female	M	5	78,3	3	4 Inf	1
2220	M	Female	M	5	78,02	1	5 Not	1
2221	M	Female	M	5	82,05	2	0 Inf	1
2222	M	Female	M	6	68,34	3	5 Not	1
2223	M	Female	M	7	73,83	3	0 Inf	1
2224	M	Female	M	6	71,63	3	8 Inf	2
2225	M	Female	M	5	82,32	2	5 Inf	2
2226	M	Female	M	8	64,69	2	7 Inf	2
2227	M	Female	M	6	94,4	3	2 Inf	1
2228	M	Female	M	6	84,76	2	3 Inf	1
2229	M	Female	M	6	70,81	0	0 Not	1
2230	M	Female	M	7	89,85	3	1 Inf	2
2231	M	Female	M	6	98,24	3	2 Inf	3
2232	M	Female	M	8	94,16	3	3 Inf	2
2233	M	Female	M	8	82,7	3	11 Inf	1
2234	M	Female	M	7	100,2	3	3 Inf	2
2235	M	Female	M	7	80	3	2 Inf	1
2236	M	Female	M	7	79,6	3	2 Inf	1
2237	M	Female	M	5	76	1	2 Inf	2
2238	M	Female	M	5	89,74	2	1 Not	1
2239	M	Female	M	6	89,2	3	0 Inf	2
2240	M	Female	M	7	84,89	3	0 Inf	1
2241	O	Female	M	7	96,49	3	2 Inf	1
2242	O	Male	LB	7	234,4	0	0 Not	3
2243	O	Male	LB	5	110,2	1	0 Inf	2
2244	O	Male	LB	8	220,5	2	2 Inf	3
2245	O	Male	LB	5	164,5	0	0 Not	2
2246	O	Male	LB	6	115,15	0	0 Not	1
2247	O	Male	LB	9	243,3	0	0 Not	3
2248	O	Male	LB	7	179,15	0	0 Not	3
2249	O	Male	LB	5	120,12	0	0 Not	3
2250	O	Male	LB	9	259,35	0	0 Not	3
2251	O	Male	LB	7	245,4	0	0 Not	3
2252	O	Male	LB	4	56,95	0	0 Not	1
2253	O	Male	LB	7	285,2	0	0 Not	3
2254	O	Male	LB	7	176,79	1	0 Not	1
2255	O	Male	LB	7	124,25	0	0 Not	1
2256	M	Male	LB	8	165,9	0	0 Not	1
2257	M	Female	D	7	28,08	0	0 Not	4
2258	M	Female	D	5	83,4	1	0 Not	2
2259	M	Female	D	4	28,2	0	0 Not	2
2260	M	Female	D	6	82,6	0	0 Not	3
2261	M	Female	D	4	25,15	0	0 Not	3
2262	M	Female	D	7	114,12	0	0 Not	3
2263	M	Female	D	7	71,5	0	1 Not	3
2264	M		D	4	17,36	1	0 Not	1