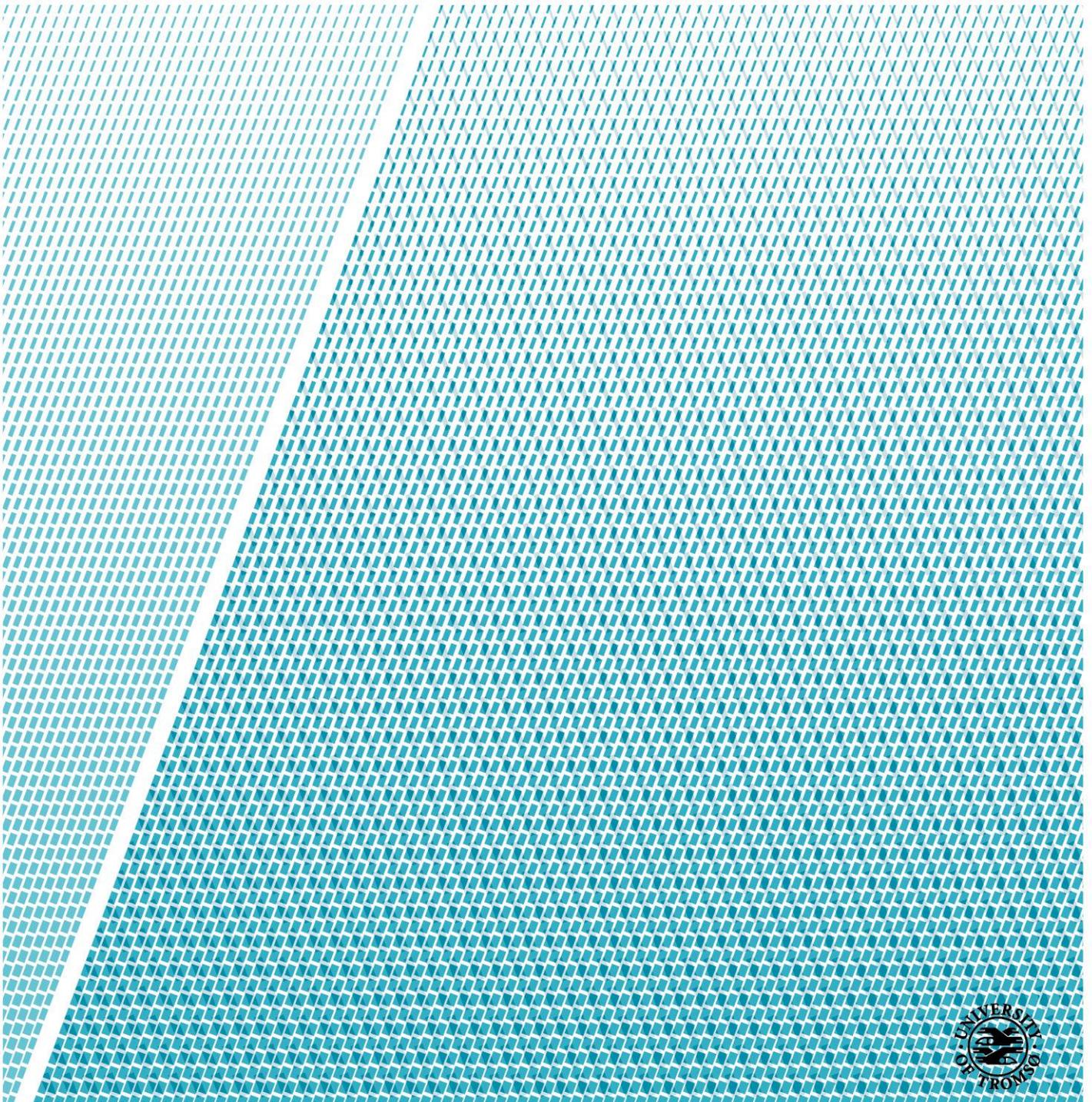


Comparison of eyfluke (*Diplostomum* spp.) infections of polymorphic whitefish (*Coregonus lavaretus* L.) populations in two sub-Arctic Norwegian lakes

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Abstract

Parasites might be small, but they are very important elements in the ecosystems. Parasites may have a strong negative influence on their host, and some species might even manipulate their hosts. Parasites commonly have complex life cycles and may use multiple different hosts. This study has been conducted in two sub-arctic lakes in Northern Norway with polymorphic whitefish populations. Both lakes, Suohpatjávri and Stuorajávri consist of three whitefish (*Coregonus lavaretus* L.) morphs, the near-shore large sparsely-rakerd morph (LSR), the pelagic densely-rakerd morph (DR) and the deep-water small sparsely-rakerd morph (SSR). Comparisons of the infection of eyeflukes in two eye habitats, the retina and vitreous humor (VH), of all three morphs were conducted both within and between each of the two lakes. The eyefluke has a rather complex life cycle, with both egg, two free-living larval stages and three different stages involving different hosts (snail, fish and bird). The results showed that there is a difference in eyefluke infection between the three whitefish morphs within both lakes, with a few exceptions. Further, the results showed a difference in eyefluke infection between the same morphs between the two lakes Suohpatjávri and Stuorajávri. Suohpatjarvi generally was more infected with eyeflukes than Stuorajávri for both eye habitats. I suspect that the large shallow areas in Suohpatjávri may favor the abundance of the intermediate host snails quite well, since the LSR morph was more infected with eyeflukes than the other two morphs. There was a difference in the eyefluke infection between the two eye habitats the retina and the VH. In fact, the overall highest infection of eyeflukes in the VH was found in whitefish from Suohpatjávri, while Stuorajávri had the overall highest infection of eyeflukes for the retina. The eyefluke infection did not differ as much between the DR and SSR morphs which could mean that either the cercariae find their way down to the profundal zone, or the SSR morph is migrating between the profundal and the upper-water zone. What is causing these differences is hard to say, there are many factors that may impact the abundance of eyeflukes, but the most important ones are suggested to be processes involving the different hosts. Especially the first intermediate host, the snail might have the greatest impact on the eyefluke infection, considering they are only found in more shallow parts of the lake.

Innhold

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1. Introduction

A long time has passed since Carl Linnaeus made his classification system for species, thinking of parasites as confused earth worms at first (Dobson, Lafferty, Kuris, Hechinger & Jetz, 2008). Thankfully our knowledge about parasites today has improved from back then (Dobson et al., 2008). We know more about them, but we still do not think of them as important pieces in the puzzle of biodiversity (Dobson et al., 2008). Dobson stated that about 75 percent of links in food webs involves parasites (Dobson et al., 2008). An example of this is the food web survey in Lake Takvatn in Troms County, Northern Norway (Amundsen et al., 2009). By adding parasites to an already complex food web, the number of links was doubled from 198 to 432 (Amundsen et al., 2009). This underlines the fact that parasites might be small, but their importance to the community is great. Digenean trematodes are reported to have a significant role in lakes and other waterbodies (Choudhury et al., 2016; Faltýnkóva, Sures & Kostadinova, 2016; Scholtz et al., 2016). Trematodes are also known to have high species diversity in sub-Arctic lakes, mainly dominated by salmonids (Soldanóxva et al., 2014). The Digenean trematode in question – the *Diplostomum* spp. has a complex lifecycle and a trophically transmitted parasite (Chappell, 1995; Kuris, 2003). The eyefluke has a complex life cycle with free-living stages (egg, miracidium and cercariae), first and second intermediate hosts (snails and fish) and a final host (bird). The complexity of the lifecycle may in turn may contribute to increasing the number of links and nodes between individuals within a foodweb (Amundsen et al., 2009). Here I will take a closer look at the distribution of eyeflukes in two polymorphic whitefish populations in two subarctic lakes in Northern Norway.

The main goal of any parasite, is to reproduce and complete its lifecycle. In order to do so the parasite needs to find a suitable host that may help the parasite reach the next level in its lifecycle. The definitive host is where the parasite becomes a sexually mature adult. Some parasites have direct lifecycles i.e. they only need one host to complete their lifecycle (Chappell, 1995; Goater, Goater and Esch, 2014). Examples of that are the monogeneans, as well as some nematodes and arthropods (Goater et al., 2014). Most animal parasites however have an indirect lifecycle, including one or several intermediate hosts where the parasites undergo developmental and morphological changes in each host (Goater et al., 2014). Intermediate hosts may serve as a prey for the final host i.e. trophic transmission (Goater et al., 2014). Complex lifecycles are fairly common and may include several different hosts,

free-living stages and larval stages, but the degree of complexity varies between parasite species (Chappell, 1995; Goater et al., 2014).

Parasites with a complex lifecycle are dependent on their hosts to reach the next level in the lifecycle, and to make sure the host is cooperative the parasite may have a trick to ensure a successful transmission. In order to complete their lifecycle, some parasites may manipulate their hosts (Kearn, 1998; Milinski, 1990). To get on with their next step in the life cycle, they may put their host in vulnerable positions and thereby making it easier for a potential predator to get a hold of their prey (Milinski, 1990). This kind of behavior has been shown among several parasite species like *Dicrocoelium dendriticums*' manipulation of ants or *Schistocephalus solidus*' manipulation of three-spined sticklebacks (Milinski, 1990).

Parasites of the genus *Diplostomum* von Nordmann 1832 (or eyeflukes) belong to the phylum Platyhelminthes under the class Digenea in the family Diplostomidae (Goater et al., 2014). The eyefluke has a three-host life cycle, as well as two (three with the egg) free-living stages. The life cycle starts with the eggs going out with feces from the final host (in this case birds, most likely a gull of the Laridae family) into the water (Goater et al., 2014). The egg hatches, and the first larval stage the miracidium is released (Goater et al., 2014). The free-living miracidium penetrates the molluscan host – the snail (mostly found in the littoral zone) and asexual reproduction occurs (Chappell, 1995; Goater et al., 2014). When the miracidium is inside the snail cilia are shed and they travel to the species-specific sites inside their molluscan host. In the snail the diplostomum turn into sporocysts that further develops redia (Goater et al., 2014). The redia then develops into more daughter redias or into cercariae emerging from the mollusk into the water (Goater et al., 2014).

The cercariae are short lived when they are in the free water masses and are highly time constrained to find a suitable host (Dr. Miroslava Soldanova; Institute of Parasitology; Biology Centre; Czech Academy of Sciences (personal communication)). If they find a suitable host, they penetrate their host going in through the skin of the fish (Goater et al., 2014). They travel with the lymph system or muscles and body tissue to the eye (Höglund, 1991; Lyholt & Buchmann, 1996; Ratanarat-Brockelman, 1974). When the cercaria has reached the eye, it develops into a metacercaria (Goater et al., 2014). If the fish then gets eaten by the final host, usually a bird, the cycle is complete (Goater et al., 2014). Metacercaria of the eye fluke can be found in different parts of the eye of the fish – in the retina, vitreous

humor (here after referred to as VH), lens and aqueous (Chappell, 1995). The eyeflukes in the retina and the VH from one host species are regarded to be two different genetic species (Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)).

According to earlier studies it seems that the metacercaria may affect the fishes' ability to detect both potential prey and predators (Chappell, 1995; Milinski, 1990). In a study on three-spined sticklebacks, Owen, Barber & Hart (1993) wanted to figure out if a low infection of eye flukes could have an impact on the fishes' ability to find prey. They conducted an experimental study in tanks, feeding the fish different prey from tubes (Owen et al., 1993). The fish was offered isopods of different sizes and their prey choice was recorded, they had uninfected fish as a control element (Owen et al., 1993). They found that the fish was affected by the eyefluke infection, even if there were only a few eyeflukes present, as they affected both the reactive distance to spot the prey and the prey selection of the fish (Owen et al., 1993). According to Bowmaker & Loew (2008) the eyesight is one of the most important senses for fish and is crucial for the fishes' ability to detect potential danger from predators as well as potential prey.

Speciation of whitefish (*Coregonus lavaretus* L.) is widely discussed amongst scientists, and Gunnar Svärdsöon referred to it as "The coregonid problem" (Svärdsöon, 1957). In lakes in northern Fennoscandia there can be three whitefish morphs existing in sympatry in a single lake. The most common type of whitefish morph is a large sized densely rakered morph (here after referred to as LSR), with gill raker number of varying between 20 to 30 approximately (Siwertsson et al., 2010). The LSR morph is known as a generalist because they utilize different lake zones and prey resources (Amundsen, Knudsen, Klemetsen & Kristoffersen, 2004b; Harrod, Mallela & Kahilainen, 2010). Another morph is the densely rakered morph (here after referred to as DR), with gill raker numbers varying between 30 and 40 approximately (Siwertsson et al., 2010). The DR morph utilizes the pelagic zone, with zooplankton as their main diet (Amundsen, 1988; Amundsen et al., 2004a, 2004b). The third morph, the small sparsley rakered morph (here after referred to as SSR), utilizes the profundal zone feeding on benthic macroinvertebrates (Amundsen, 1988). The gill raker number of the SSR morph varies from 15 to 20 in number (Siwertsson et al., 2010).

This survey was conducted in two lakes in Finnmark county, Northern Norway known to be polymorphic from previous studies (Siwertsson et al., 2010). In both Stuorajávri and Suohpatjávri, there are three morphs of whitefish (Häkli, Østbye, Kahilainen, Amundsen & Præbel, 2018), and both lakes are approximately 30 meters deep (Amundsen, 1988). The eye fluke has been found in both lakes. It has been conducted studies in both lakes before looking at parasite distribution – in general, fish diet and niche use but no one has looked at the distribution of eye flukes among the sympatric whitefish morphs in the two lakes (Amundsen, 1988; Amundsen et al., 2004b; Knudsen, Amundsen & Klemetsen, 2003). Nor has the eyefluke infection been focused much on in sympatric whitefish morphs in other lakes. The main focus has been on other salmonid species like brown trout (*Salmo trutta*), arctic charr (*Salvelinus alpinus*), three-spined sticklebacks (*Gasterosteus aculeatus*) and rainbow trout (*Oncorhynchus mykiss*) (Amundsen, 1988; Blasco-Costa et al., 2014; Chappell, 1995; Siwertsson et al., 2010). As mentioned above, the *Diplostomum* spp. may be found in the in more than one habitat within the eye. Here, we focused on two habitats; the retina and the VH. In a study from Iceland on eyeflukes among arctic charr, three-spined sticklebacks and brown trout published in 2014 (Blasco-Costa et al., 2014) they found that the eyeflukes inhabiting the microhabitats of the retina and VH were genetically different, and it is expected that such genetic differences are present here as well, due to their morphological differences.

To the best of my knowledge, there is no published work that to this day has focused on the differences in number of eyeflukes between the two habitats in the eye; the retina and VH in European whitefish. It has, however been published a few articles on what may affect the distribution of eyeflukes, and what causes such differences is widely discussed (Hechinger & Lafferty, 2005; Karvonen, Seppälä & Valtonen, 2004). Density and diversity in its potential final host the bird, may have an impact on the number of cercaria for the eye fluke in the fish (Hechinger & Lafferty, 2005). A report from 1996 about the bird community around Stuorajvri states that there are many bird species in the area around the lake basin (Strann & Nilsen, 1996). The combination of number of birds present in and around the lake basin and the size of the lake basin may have an impact in the number of eye flukes present in the fish (Hechinger & Lafferty, 2005). Stuorajávri is quite big with its 24 km² in comparison with Lake Suohpatjávri with its 2 km² range (Amundsen, 1988). Lake size may impact the habitat distribution for fish and morphs (Siwertsson et al., 2010). Suohpatjávri has relatively larger shallow areas where the first intermediate host the snail occurs i.e. a crucial part of the

lifecycle of the eyeflukes. In the beginning of the 1980's and again in the beginning of the 2000's, a large amount of fish was taken out in Stuorajávri (Amundsen, Kristoffersen, Knudsen & Klemetsen, 2002). This large outtake may have had an impact on number of eye flukes present. In addition to the factors mentioned over, factors like pH and temperature could have some effect on the free-living parasite stages (i.e. larval stages) as well (Marcogliese & Cone, 1996).

In this study, the main goal is to examine the distribution of eye flukes among the three different sympatric morphs of whitefish in each lake, and the differences between the two lakes – Stuorajávri and Suohpatjávri. On the basis of the information above, I hypothesized that there would be differences in eyefluke infection between the three sympatric whitefish morphs within both lakes. More specifically, I predicted that the LSR-morph would have the highest infection of eyeflukes, and the deep-water SSR morphs the lowest infection. I secondly hypothesized that there would be differences in the eyefluke infection for equivalent morph between Stuorajávri and Suohpatjávri. More specifically, I predicted that the infection would be highest in Suohpatjávri as the first intermediate host, snails, are found in most part of the lake. Thirdly, I predicted that there would be a consistent difference in eyefluke infection between the two habitats within the eye; both between the morphs and between the lakes, more specifically between the VH and the retina.

2 Material and methods

2.1 Study area

The study lakes Stuorajávri (69°08′ N; 22°47′ E) and Suohpatjávri (68°56′ N; 23°05′ E) are both situated in Kautokeino municipality in Finnmark county. Stuorajávri is 25 km² large and situated 374 meters above sea level. Suohpatjávri is 2 km² large and situated 323 meters above sea level (Amundsen, 1988). Both lakes are dimictic, oligotrophic lakes as well as a bit humic, and maximum depth in both is approximately 30 meters (Amundsen, 1988). The fish community in the lakes consist of six species; whitefish (*Coregonus lavaretus*) (most dominant), pike (*Esox lucius*), perch (*Perca fluviatilis*), burbot (*Lota lota*), arctic charr (*Salvelinus alpinus*), minnow (*Phoxinus phoxinus*) and brown trout (*Salmo trutta*) (Knudsen et al, 2003).

2.2 Background

In the beginning of the 1980's Kautokeino municipality and UiT took out 96 tons of whitefish from Lake Stuorajávri in an effort to make a future form commercial fishing in in the lake (Amundsen, 1988; Amundsen et al., 2002; Amundsen and Kristoffersen, 1998; Kristoffersen, Amundsen & Knudsen, 2004). The whitefish was highly infected by *Triaenophorus crassus* before the intensive fishing started (Amundsen, 1988; Amundsen et al., 2002; Amundsen and Kristoffersen, 1998; Kristoffersen et al., 2004). A new attempt to take out fish was done in 2002 and 2003 (Amundsen et al., 2002; Kristoffersen et al., 2004). After the intensive fishing the infection by *Triaenophorus crassus* in whitefish drastically declined, but now it is back to the same condition as in the 1980's (Amundsen, 1988; Amundsen et al., 2002; Amundsen and Kristoffersen, 1998; Kristoffersen et al., 2004). There is no record of such activity from Suohpatjávri.

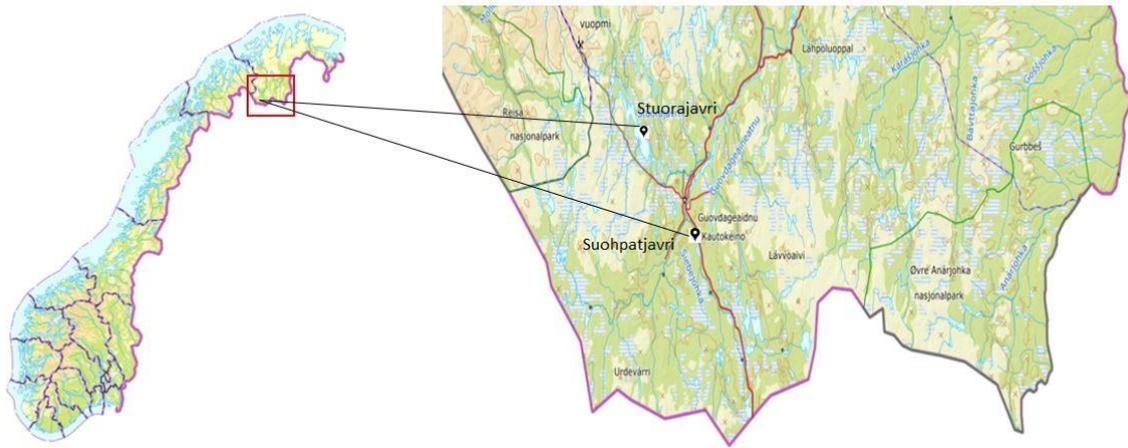


Figure 1. Map over study area in Finnmark, Northern Norway. Showing the location of Suohpatjávri and Stuorajávri (Norgeskart, 2018). Map Marker by P.J. Onori from the Noun Project.

2.3 Fish sampling

The fieldwork in Suohpatjávri was conducted in the autumn of 2016, in two separate periods from September 17th to 18th, and from October 16th to 19th. The fieldwork in Stuorajávri was executed from October 12th to 15th 2016. Multi-mesh floating and bottom survey gillnets were used to conduct the survey of the lakes in order to get fish samples from all three morphs. We standardized the fish catch to an intermediate size for all morphs in both lakes. Thus, by not sampling the bigger fish nor the smaller ones intentionally the catch is not what one may call representative with regards age and size of the fish. The gillnets fished for approximately 24 hours. The fish were sorted into plastic bags marked with the habitats for their capture as we picked up the gillnets (N=161).

The fish was brought back to an improvised field laboratory and put on ice until processed. For each fish, the length (mm) and weight (grams) was measured for each fish, sex and maturity level were noted, otoliths and gills were retrieved and stored on 96% ethanol for later analyses, and the eyes were extracted and examined for *Diplostomum spp.* eyeflukes.

2.3.1 Morph determination

The gills were used for morph determination. The number of gill rakers were counted under a stereo microscope. What kind of morph can be determined by the number, length and space

between the gillrakers, habitat and head morphology (Amundsen et al., 2004b; Svärdsön, 1957; Kahilainen & Østbye, 2006; Siwertsson et al., 2010). There are three morphs in both lakes; the SSR morph which is the small sparsely rakered morph, varies in gill raker number between 15 and 20 (Siwertsson et al., 2010). The LSR morph with slightly larger gill rakers ranging in number from 20 to 30 (Siwertsson et al., 2010). The DR morph with large and dense gill rakers ranging in number from 30-40 (Siwertsson et al., 2010).

2.3.2 Age determination

The sagittal otoliths were used to determine the age of the fish using a stereo microscope. The otoliths could not be localized for all fish individuals, an overview of the age and number of individuals can be located in the appendix (Appendix x-x). Paper was used in order to clean the otoliths properly, and glycerin was added to make the otolith reading easier. The dark circles (winter zones) were counted to determine the age of the fish (Holden & Raitt, 1974).

2.3.3 Eye sampling

The eye flukes can be found in different parts of the eye; the lens, retina and vitreous humor (here after referred to as VH) (Chappell, 1995). Because eyeflukes in the lens was only found in a couple of fish, we chose to focus on the eyeflukes in the VH and retina, which most likely are two different species of *Diplostomum spp.* (Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)). The eyeflukes in the retina are small and move fast, whereas the eyeflukes in the VH are bigger and move slower.

The eye was taken out of the eye socket very carefully to ensure that the eye was not damaged. The eye was opened carefully in order to keep the VH and retina as intact as possible. The VH and lens were taken out of the eye and put in a petri dish, then they were separated from each other, and the lens was put in its own petri dish. The retina was ripped apart into really small pieces, using fine forceps to make sure that we could find all the eye flukes. The retina mass was put into a glass beaker in order to make it easier to pour in to a smaller petri dish, not too much liquid at a time. A stereo microscope with portable light or under light was used to disclose and count the eyeflukes. The eyeflukes were sampled with a glass pipette into a new petri dish before they were counted. Up to 30 individuals were preserved in a plastic test tubes with 96% ethanol for future genetic testing. The same procedure was followed for the eyeflukes in the VH, except the eyeflukes did not get put into a separate petri dish for counting (the mass of the VH is clear, which makes the eyeflukes a lot easier to see).

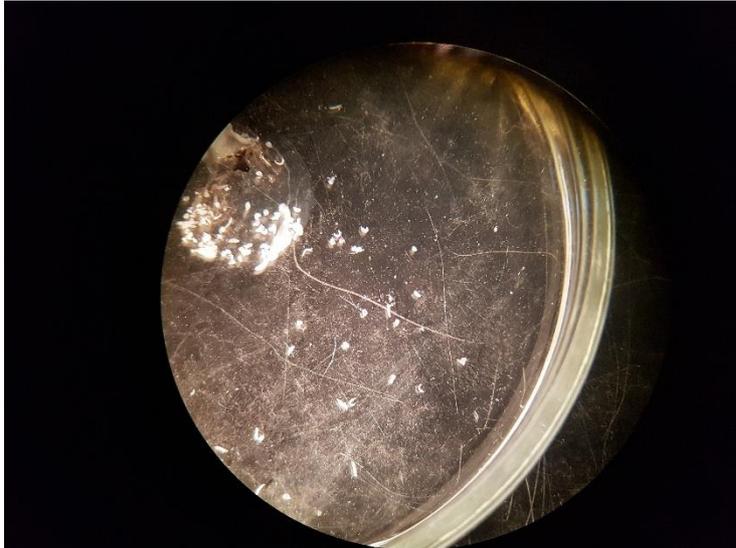


Figure 2. Photo showing what an ensemble of eyeflukes in the vitreous humor from whitefish may look like (Photo: private).

Originally, we started out with a plan to count the flukes in both of the eyes, but this is a time-consuming process and comparing the number of eyeflukes for both eyes from the same fish showed that they did not differ much with regards to the number of eyefluke. Similar infection between eyes has also been tested on arctic char, with the conclusion that the difference between the eyes was minimal (Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)). Some of the fish only one eye, and some eyes were broken. Thus, it was decided to count the flukes in one eye only per fish, and to switch between counting the left and right eye. For those individuals we already counted both eyes, the mean number of eyeflukes of both the retina and VH was calculated and used, hence the half values in Table 2.

2.4. An overview of the material

An overview of the total catch from both lakes for each morph as well as the total amount of each morph regardless of lake (Table 1). Table 2 gives an overview of total number of eyeflukes for each habitat in each morph within each lake, as well as the total amount for each lake. The comma for two of the morphs and two of the habitats in Suohpatjávri are there because both eyes were counted and to make up for this a mean was calculated, thus the comma (Table 2). There is an overview of mean length and age for each morph in both Suohpatjávri and Stuorajávri (Table 3). An overview of age for each morph in each lake is found in the appendix (Appendix 1-6).

Table 1. An overview of the number of each morph sampled in Stuorajávri & Suohpatjávri as well as the total number of each morph (LSR, DR and SSR) and fish.

Lake	LSR	DR	SSR	Total number of fish
Stuorajávri	32	31	29	92
Suohpatjávri	39	25	5	69
Total	71	56	34	161

Table 2. An overview of the total number of eyeflukes in Stuorajávri & Souphatjavri in both the retina and the VH eye habitats.

	Total retina	Total VH
Stuorajávri		
LSR	1724	770
DR	657	269
SSR	2778	528
Stuorajávri total	5159	1567
Suohpatjávri		
LSR	1434,5	7869,5
DR	173,5	1575,5
SSR	44	331
Suohpatjávri total	1652	9776
Total both	6811	11343

Table 3. An overview of mean age, mean length and number of each morph (LSR, DR and SSR) in Suohpatjávri (SU) Stuorajávri (ST).

	Mean age	Mean Length (mm)	Number of Morph
SU LSR	4	225	39
SU DR	4	204	25
SU SSR	3,8	222,8	5
ST LSR	7,7	349,8	32
ST DR	7,7	318,5	31
ST SSR	6,6	242,8	29

2.5 Statistical analysis

Quantitative descriptors and descriptive statistics

The prevalence, mean abundance and mean intensity have all been calculated in accordance with Bush, Lafferty, Lotz & Shostak, (1997).

Prevalence

Prevalence (P) is the number of infected hosts of a particular species (n), in this case the *Diplostomum* species in either the retina or the VH, divided by the total number of hosts that were examined (A) expressed as a percentage, i.e.:

$$P = n / A * 100$$

Mean abundance

Mean abundance (MA) is the total number of parasites (N) divided by the number of examined hosts (B):

$$MA = N / B$$

Mean intensity

Mean intensity (MI) is the average of parasites among the infected hosts in the population, estimated as the total number of parasites (N) divided by the total number of infected hosts (C):

$$MI = N / C$$

Data analyses

Mann Whitney U test

Parasites are rarely evenly or normally distributed but are typically aggregated distributed with some individual hosts that have very high infections while most of the others have low infections resulting in a highly skewed distribution. Non-parametric tests are used to do comparisons of data that are not normally distribution, and parasites fall within that group (Wilson & Grenfell, 1997). Hence, nonparametric Mann Whitney U-test (Wilcox test in R) testing the median (Nachar, 2008) was used for testing differences in eyefluke infection between independent groups, i.e. between the morphs within each lake, and between the total number of flukes in both the retina and the VH, as well as comparisons between equivalent morphs in the two lakes for the number of flukes in both the retina and VH.

Correlation between the retina and VH

The box plot function in Excel was used to see the correlation between the two eye habitats for each individual for each of the morphs in each lake.

Correlation test between number of flukes and age/length of the fish

Linear regressions were conducted between each habitat for each morph and age/length of the fish. When conducting several analyses on the same variable the risk of a type-1 error increases. A type-1 error is when you may reject a null-hypothesis that is true (Schmuller, 2017). Thus, a Bonferroni Adjustment was conducted on the regressions that indicated a significant p-value of 0,05 (Rice, 1989). As the threshold for significance level in R is at 0,05, the threshold was divided on the number of tests conducted – in this case 24 – 12 for age and 12 for length. 12 tests for the LSR, DR and SSR morph in Stuorajávri for both the retina and the VH and 12 for the LSR, DR and SSR morphs in Suohpatjávri for both the retina and the VH; resulting in Bonferroni adjustment of 0,0020833333.

All statistical tests conducted and illustrative graphs in this study were created using RStudio (Version 1.0.136) and Microsoft Excel (2016).

3. Results

3.1 Linear regression

3.1.1 Length

The number of eye-flukes in the retina was not related to the length of the LSR-morph (linear regression, $p=0,07859$), of the DR-morph ($p=0,9658$), nor of the SSR-morph ($p=0,5072$) in Suohpatjávri. In Stuorajávri and the number of eye-flukes in the retina were not related to the length of the LSR morph (linear regression, $p=0,518$) nor the DR-morph ($p=0,8057$). For the SSR-morph there was a relationship between the number of eye-flukes in the retina and the fish length (linear regression, $p=0,006181$), but not after the Bonferroni adjustment (Table 4).

In Suohpatjávri the number of eyeflukes in the VH for the DR and SSR morphs was not related to the length of the fish (linear regression $p=0,6699$ and $p=0,3953$). The LSR morph showed a significant relationship between number of eyeflukes in the VH and length (linear regression $p=0,01693$) but not after the Bonferroni adjustment. In Stuorajávri, neither the LSR morph or the DR morph showed a significant relationship between number of eyeflukes and fish length ($p=0,6884$ and $0,1899$). The SSR morph showed a significant relationship between number of eyeflukes and fish length ($p=0,01477$) but not after the Bonferroni adjustment (Table 4).

3.1.2 Age

The number of eye-flukes in the retina were significantly related (linear regression, $p<0,001$) to the age of the fish for the LSR morph in Suohpatjávri, and still significant after the Bonferroni adjustment. For the DR morph and the SSR morph, the number of eye-flukes in the retina were not related to the age (linear regressions, $p=0,06177$, $p=0,1873$, respectively). In Stuorajávri, there were no relationship between number of the eye-flukes and age nor in the LSR-morph, the DR morph or the SSR morph (linear regressions, $p=0,3518$, $p=0,4978$, $p=0,1282$, respectively, see Table 4).

The number of eyeflukes in the VH in Suohpatjávri was significantly related to age for the LSR morph ($p<0,001$) even after Bonferroni adjustment. The age of the fish was not related to number of eyeflukes for either the DR or SSR morphs ($p=0,09199$ and $p=0,09015$). In

Stuorajávri the number of eyeflukes for all three morphs – LSR, DR and SSR was not related to the age of the fish (respectively $p=0,7173$, $p=0,1594$ and $p=0,367$) (Table 4).

The LSR morph in Stuorajávri shows low abundance for both habitat for the youngest and the eldest individuals. The abundance for both habitats is at its highest for individuals between the age of 6 and 8 years old. The abundance is higher for the retina than the VH (Appendix figure 1). Among the DR morph in Stuorajávri, the individuals with age 5 and 7 have a higher abundance in the retina than the rest. The abundance in the VH is at its highest at age 8. The DR morph has lower abundance than the LSR morph in both habitats (Appendix figure 2). For the SSR morph, the abundance is increasing with age, except for a small drop at age 6 and age 9, in both habitats. Abundance is at its highest among individuals at age 7 and 8. The abundance for retina is higher than for the VH among the SSR morphs as well (Appendix figure 3).

In lake Suohpatjávri the LSR morph has a high abundance for retina, while the abundance for VH is lower. Abundance is at its highest at age 4, and lowest at age 6 and 9 (Appendix figure 4). The abundance for the DR morph is at its highest at ages 3 and 4, and lowest at 6 years old. The abundance for the VH is higher for ages 3 and 4 than 5 and 6, but it is minimal (Appendix figure 5). The SSR morph has the lowest abundance for age 3, and slightly higher for age (Appendix figure 6).

Table 4. The relationships (linear regression) between the number of eyeflukes and the length and the age of the three different whitefish morphs (LSR, DR, SSR) in Souphatjavri and Stourajavri for both eye habitats – the retina (R) and vitreous humor (VH) with significant p-values and Bonferroni adjustment marked in bold.

Parameters	Habitat in eye	Lake	Morph	R2	P-value	Bonferroni adjustment P
Length	R	Suohpatjávri	LSR	0.05963	0.07859	not significant
Length	R	Suohpatjávri	DR	-0.04339	0.9658	not significant
Length	R	Suohpatjávri	SSR	-0.1223	0.5072	not significant
Length	R	Stuorajávri	LSR	-0.0188	0.518	not significant
Length	R	Stuorajávri	DR	-0.03229	0.8057	not significant
Length	R	Stuorajávri	SSR	0.2184	0.006181	not significant
Length	VH	Suohpatjávri	LSR	0.1281	0.01693	not significant
Length	VH	Suohpatjávri	DR	-0.03508	0.6699	not significant
Length	VH	Suohpatjávri	SSR	-0.005199	0.3953	not significant

Length	VH	Stuorajávri	LSR	-0.0277	0.6884	not significant
Length	VH	Stuorajávri	DR	0.02603	0.1899	not significant
Length	VH	Stuorajávri	SSR	0.1712	0.01477	not significant
Age	R	Suohpatjávri	LSR	0.5352	0.0001893	SIGNIFICANT
Age	R	Suohpatjávri	DR	0.1965	0.06177	not significant
Age	R	Suohpatjávri	SSR	0.3217	0.1873	not significant
Age	R	Stuorajávri	LSR	0.03401	0.3518	not significant
Age	R	Stuorajávri	DR	-0.01363	0.4978	not significant
Age	R	Stuorajávri	SSR	0.1598	0.1282	not significant
Age	VH	Suohpatjávri	LSR	0.4643	0.0009801	SIGNIFICANT
Age	VH	Suohpatjávri	DR	0.1604	0.09199	not significant
Age	VH	Suohpatjávri	SSR	0.5602	0.09015	not significant
Age	VH	Stuorajávri	LSR	-0.08379	0.7173	not significant
Age	VH	Stuorajávri	DR	0.1601	0.1594	not significant
Age	VH	Stuorajávri	SSR	0.03137	0.367	not significant

3.2 Prevalence

All of the whitefish from both Souhpatjavri and Storajavri are infected with eye flukes. In Stourajvari there is a 100% prevalence among all morphs, and in both of the habitats of the eye, in the retina and vitreous humor (VH). In Suohpatjávri there is a 100 % prevalence for eye-flukes in the retina, for all three morphs. In the VH the LSR morph and the SSR morph has a 100% prevalence, while the DR morph has a prevalence of 88% (Figure 3).

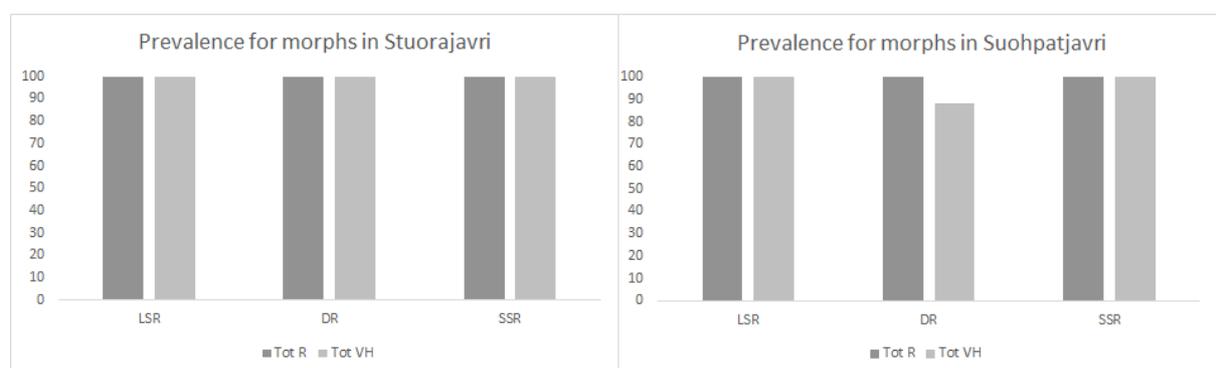


Figure 3. Prevalence of eyeflukes for the three different morphs (LSR, DR, SSR) in both eye habitats, the retina (Tot R) and VH (Tot VH) from both Stuorajávri and Suohpatjávri.

3.3 An overview of the total infection – Mann Whitney U-test (MWU) and abundance

Retina

In Stuurajávri, the LSR morph had an abundance of 86,8 eyeflukes in the retina and the DR-morph 21,2 eyeflukes, thus the infection in the retina was significantly different between the LSR-morph and the DR-morph (MWU, $p < 0,001$). The DR-morph had the lowest infection among the morphs. There were also significant differences in the eyeflukes in the retina between the DR- and the SSR-morph with 59.5 flukes (MWU, $p < 0,001$) and between the LSR- and the SSR-morph ($p < 0,05$) (Table 2). In Suohpatjávri the abundance of eyeflukes in the retina was significantly different between the LSR morph (abundance of 36,7 flukes) and DR morph (abundance of 6,9 eyeflukes) (MWU, $p < 0,001$) and between the LSR and SSR morph (abundance of 8.8 eyeflukes) (MWU, $p < 0,001$). However, there was no difference in the abundance of the DR and SSR morph ($p = 0,1149$) (Table 2). Stuurajávri appears to have higher abundance of eyeflukes in the retina than Suohpatjávri (Figure 4 and 5, for details see Appendix table 7).

VH

In Stuurajávri the infection in the VH was significantly different between the LSR (abundance of 16.5) and DR morph (abundance of 8.7) (MWU, $p < 0,001$), between the DR and SSR morph (mean abundance of 26,5) (MWU, $p < 0,001$) and for the LSR and SSR morph (MWU, $p < 0,05$). In Suohpatjávri the abundance in the VH was significantly different between the LSR (abundance of 201,8) and DR morph (abundance of 63) (MWU, $p < 0,001$) and the LSR and SSR (abundance of 66.2) morph (MWU, $p < 0,05$). However, the DR and SSR morph did not show any difference in abundance ($p = 0,8455$) (Table 2). Suohpatjávri seems to have a higher abundance of eyeflukes in the VH than Stuurajávri (Figure 4 and 5, for details see Appendix table 7).

Retina and VH within lakes

In Stuurajávri the abundance in the retina and VH showed significant differences between the two habitats for the LSR morph (MWU, $p < 0,05$) and the SSR morph (MWU, $p < 0,001$). However, the DR morph showed no significant differences between the two habitats (MWU, $p = 1$). In Suohpatjávri the abundance in the retina and VH exhibited significant difference for all three morphs, LSR (MWU, $p < 0,001$), DR (MWU, $p < 0,001$) and SSR (MWU, $p < 0,05$) (Table 5).

Retina and VH between lakes

The retina exhibited a significant difference in abundance between the two lakes for the LSR morph (MWU, $p < 0,05$) and the SSR morph (MWU, $p < 0,001$). In contrast, the DR morph only exhibited a trend between the two habitats (MWU, $p = 0,05077$). The VH showed a significant difference in abundance between the two lakes for the LSR morph (MWU, $p < 0,001$), the DR morph (MWU, $p < 0,001$) and the SSR morph (MWU $p < 0,05$) (Table 5).

Table 5. Pairwise comparisons (MWU) of infection of eyeflukes among the whitefish morphs LSR, DR and SSR within lakes and between lakes – Stourajávri (SU) and Suohpatjávri (SU). Significant p-values are marked with bold, while the trend is marked with both bold and italic.

Lake	Habitat	Morph	P-value
Stourajvri ST	Retina	LSR/DR	1.98e-09
ST		DR/SSR	2.354e-10
ST		LSR/SSR	0.004131
ST	VH	LSR/DR	1.297e-07
ST		DR/SSR	1.028e-05
ST		LSR/SSR	0.1149
ST	Retina/VH	LSR	0.001849
ST		DR	1
ST		SSR	9.393e-09
Suohpatjávri SU	Retina	LSR/DR	8.27e-06
SU		DR/SSR	0.3002
SU		LSR/SSR	0.03333
SU	VH	LSR/DR	3.876e-05

SU		DR/SSR	0.8455
SU		LSR/SSR	0.03504
SU	Retina/VH	LSR	6.744e-09
SU		DR	1.344e-09
SU		SSR	0.007937
Stourajavri/Suohpatjávri	R/R	LSR	0.01494
ST/SU		DR	0.05077
ST/SU		SSR	0.0007234
ST/SU	VH/VH	LSR	2.346e-09
ST/SU		DR	1.403e-09
ST/SU		SSR	0.001095

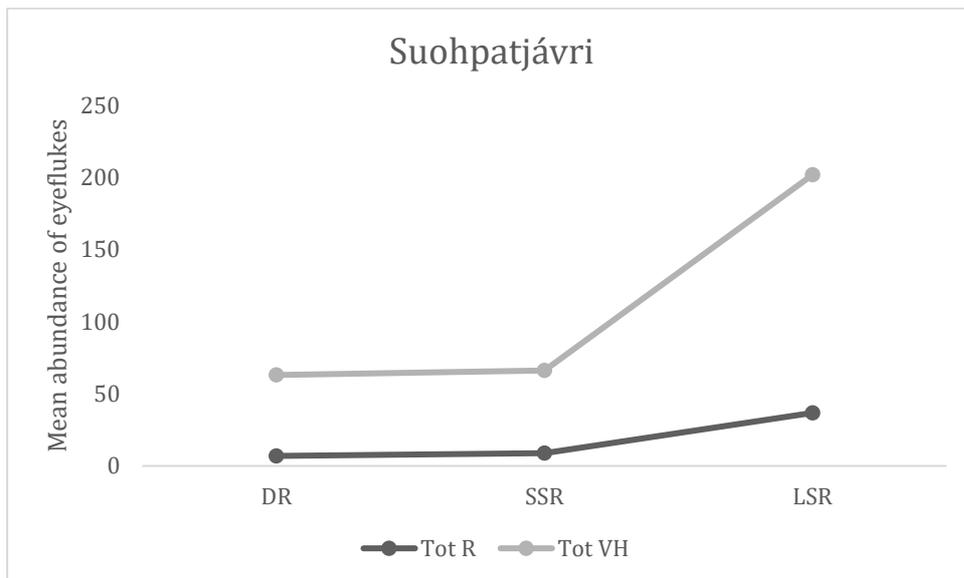


Figure 4. Abundance of eyeflukes in the retina (Tot R) and VH (Tot VH) in the three different morphs of whitefish (LSR, DR and SSR) in Suohpatjávri (for details see Appendix table 7).

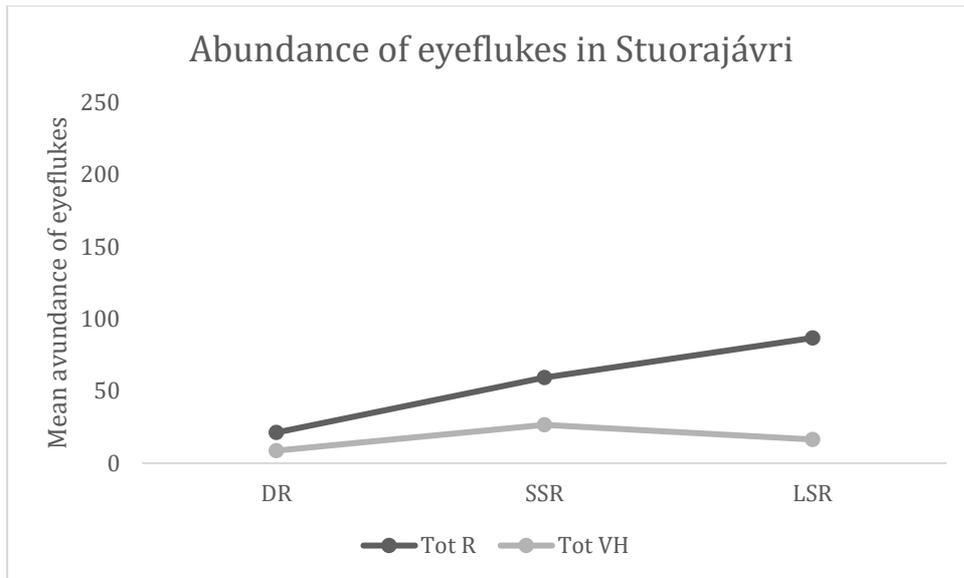


Figure 5. Abundance in the retina (Tot R) and VH (Tot VH) for the three whitefish morphs (LSR, DR and SSR) in Stuorajávri (for details see Appendix table 7).

3.4 Eyefluke distribution

The eyeflukes are unevenly distributed among the two habitats, and also between the morphs. Among the LSR morph in Suohpatjávri the majority of individuals appear to have relatively low infection in the retina and higher in the VH, with a few outliers and this also seems to be the case for the DR and SSR morphs (Figure 10, 11 and 12). In Stuorajávri, the pattern for the three morphs appears to be different, showing a higher infection in the retina than in the VH – the opposite of Suohpatjávri with the exception of the DR morph which seems to show the same pattern as the morphs in Suohpatjávri (Figure 13, 14 and 15). All boxplots exhibit a positive correlation between the two eye habitats (retina and VH) (Figure 10, 11, 12, 13, 14 and 15). Also, if the number of eyeflukes in the retina increases, as does the number of eyeflukes in the VH and the other way around (with a few exceptions) (Figure 10, 11, 12, 13, 14 and 15).

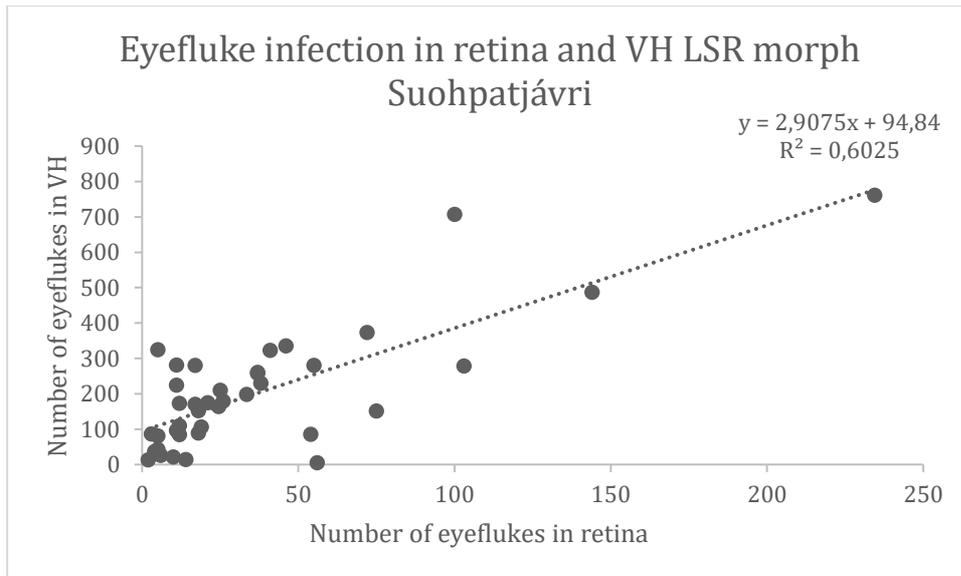


Figure 6. Eyefluke infection of both habitats (retina and VH) for each individual of the LSR morph in Suohpatjávri.

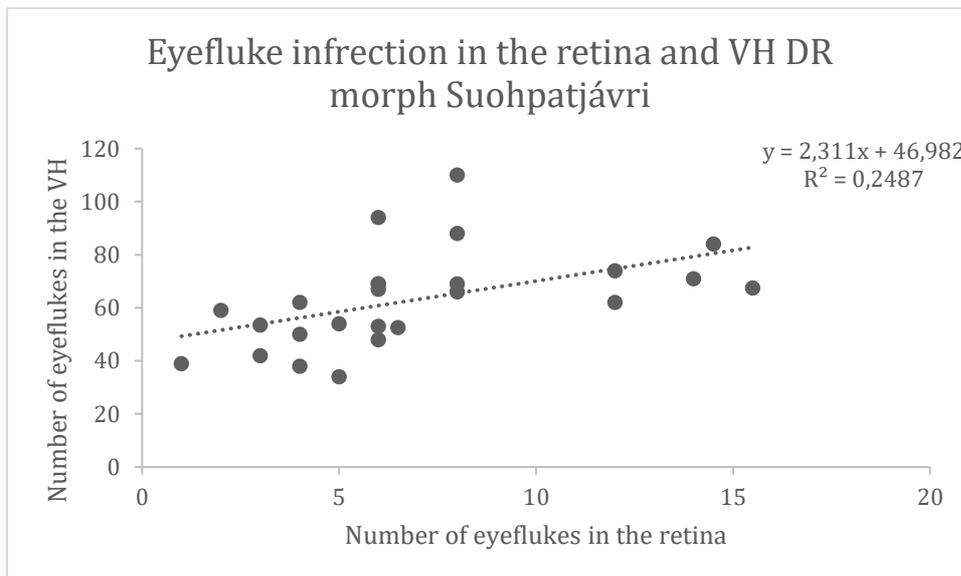


Figure 7. Eyefluke infection in both habitats (the retina and VH) for each individual of the DR morph in Suohpatjávri.

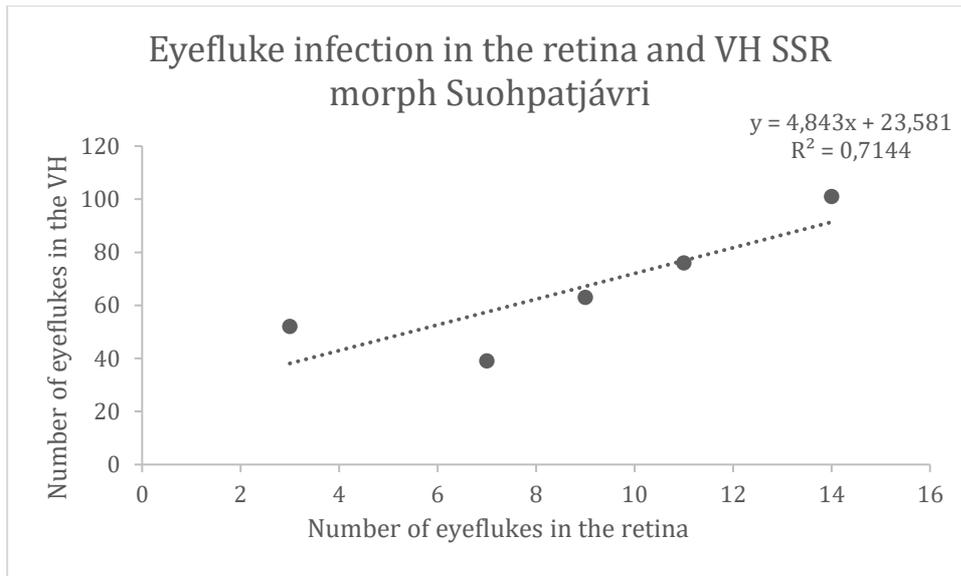


Figure 8. Eyefluke infection for both habitats (the retina and VH) for each individual of the SSR morph in Suohpatjávri.

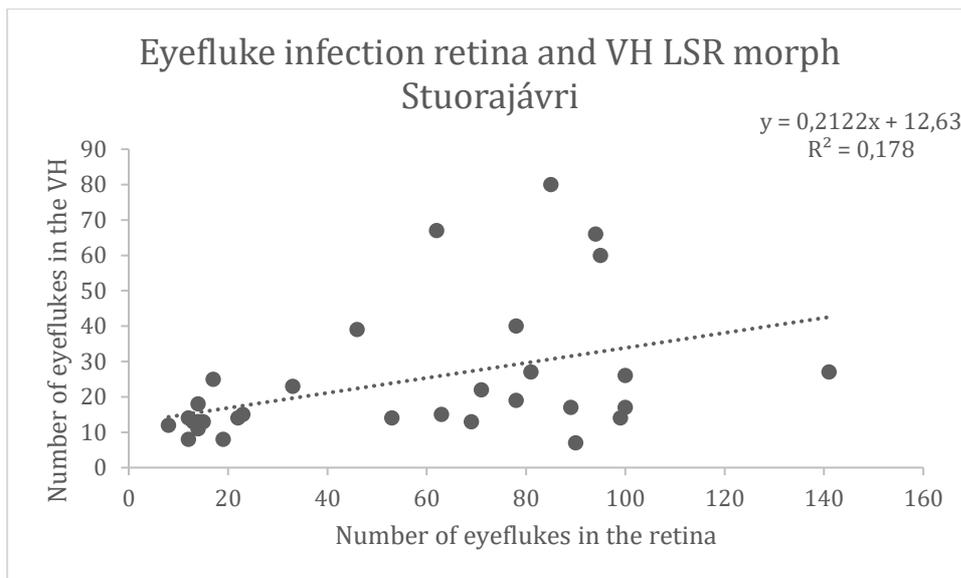


Figure 9. Eyefluke infection for both habitats (the retina and VH) for each individual of the LSR morph in Stuurajávri.

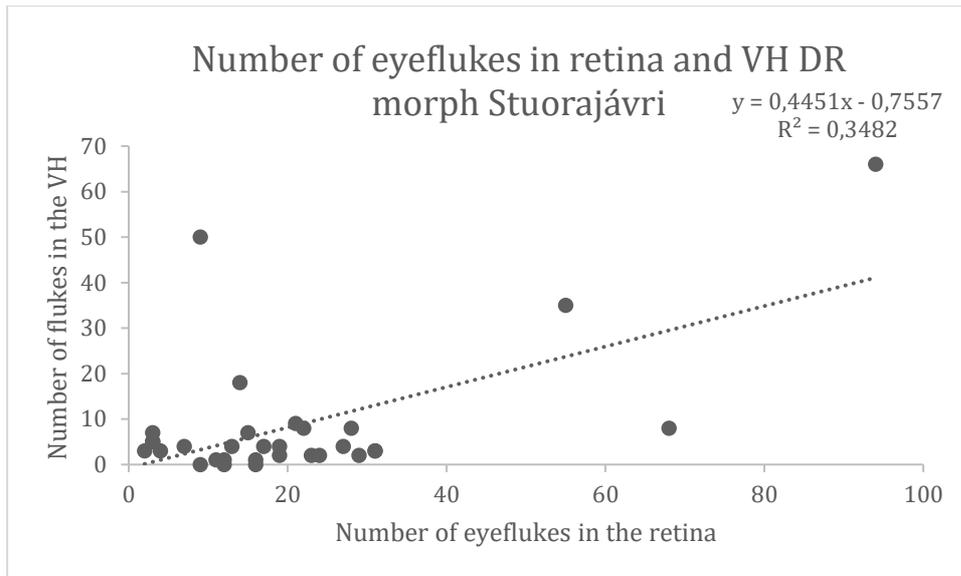


Figure 10. Eyefluke infection of both habitats (the retina and VH) for each individual of the DR morph in Stuurajávri.

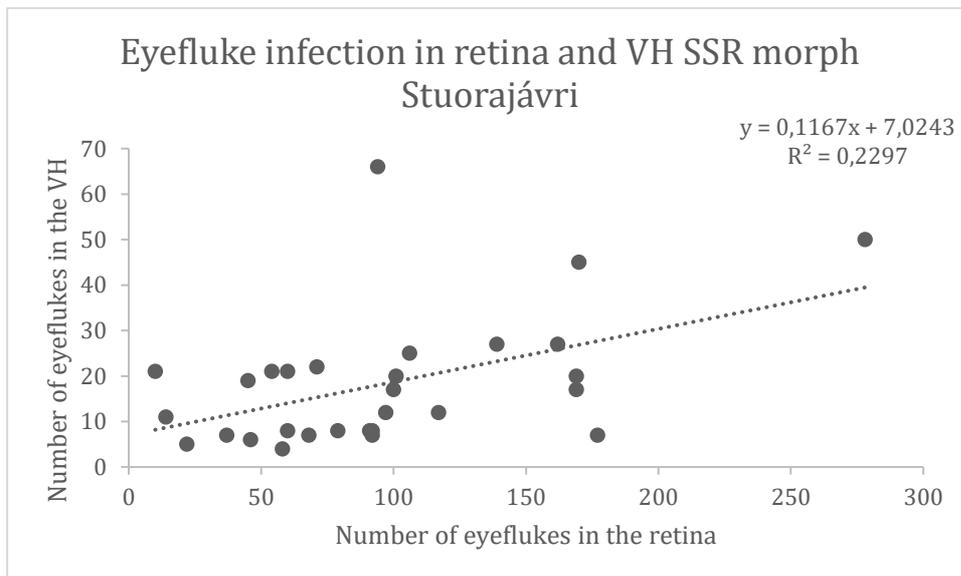


Figure 11. Eyefluke infection of both habitats (the retina and VH) for each individual of the SSR morph in Stuurajávri.

4. Discussion

To the best of my knowledge this study is the first to look into the distribution differences of eyeflukes in the vitreous humor (VH) and the retina among polymorphic whitefish populations within and between two lakes in Northern Norway. Generally, the whitefish in Suohpatjávri had higher infection of eye-flukes than in Stuorajávri. As expected within both lakes, the morphs exhibited different parasite infection patterns of eyeflukes. Furthermore, the result also suggested that there are different eyefluke distributions between the two habitats in the eye – the retina and the VH. Unexpectedly, there were differences in parasite infection between the two eye habitats between the lakes, as Suohpatjávri had more flukes in the retina than in the VH, while in Stuorajávri it was the other way around.

There were two habitats within the eye that had infection; the retina and the VH, which has been found to be infected in other studies (Blasco-Costa et al., 2014). In many studies there has been more focus on the VH – usually together with the lens rather than the retina (Blasco-Costa et al., 2014). From personal experience, the flukes in the retina are relatively smaller and move differently in contrast with the flukes in the VH which are bigger and move quite slow. Such differences are also noted from other studies (Blasco-Costa et al., 2014).

Therefore, I presume that there are two separate species of eyeflukes infecting each habitat, as there are shown to be two genetically different eyefluke species in other studies (Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)). Further DNA analysis will fully shed light on this topic but has not been done to this date. Furthermore, there has been several species of eyeflukes documented among northern salmonid species (Blasco-Costa, et al., 2014). The eyeflukes from whitefish could be same generalist species as earlier found in arctic charr and trout (Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)).

The prevalence of eyeflukes for both Stuorajávri and Suohpatjávri was a 100% for all three morphs for infection of the species in the retina. The prevalence was also high (100%) for the species in the VH except for the DR morph in Suohpatjávri which showed a prevalence of 88%. In comparison a study from Finland showed prevalence values of 72,7% (Karvonen et al., 2004) which makes the prevalence for both Stuorajávri and Suohpatjávri high. The infection pressure for all whitefish morphs therefore seems to be high, and high prevalence of eyeflukes is commonly seen among many fish species (Marcogliese et al., 2001). As

expected, overall it seems to be higher infection of eyeflukes in Suohpatjávri than in Stuorajávri. In Suohpatjávri the overall number of eyeflukes per fish was moderate and the highest total number within the VH was more than 700 individuals per eye, and the highest number of individuals in the retina was 234. While in Stuorajávri the highest number of eyeflukes in the retina was 278, and for the VH it was 80. In comparison, there is reported more than 4000 individuals of eyeflukes per eye in other studies (Bouillon, 1984; Shostak, Tompkins & Dick, 1987). Altogether, this study shows that there is a high infection pressure of the eyeflukes in the retina and VH for all three morphs of whitefish in both lakes.

There seems to be a low effect of the age and length of the different whitefish morphs fish on the eyefluke infection, in contrast with what is shown in other studies (Padrós, Knudsen & Blasco-Costa, 2018). Only two out of the total twelve linear regressions were significant (from Stuorajávri only). This low impact on age and size on eyefluke infection is probably an effect of standardizing the fish catch to an intermediate size for all morphs in both lakes. Thus, by not sampling the bigger fish nor the smaller ones intentionally during the net fishing, the relationship between fish age and length and eyeflukes is not significant in most cases in this study. The relationship between length/age of the fish and the number of eyeflukes has not been a main focus as in previously published articles on this topic (Padrós et al., 2018).

The habitat choice of the fish may have an impact on the infection pressure. Since the snail which is the first intermediate host breeds in the littoral zone (Chappell, 1995), I assumed that the LSR morph utilizing the littoral zone would have a higher infection of eyeflukes than the other two morphs. The DR morph would have the second highest eyefluke infection, I assumed that there would be a high density of cercarias in the littoral zone causing the cercariae to make their way out into the pelagic in the upper water level, known to be the home of the DR morph and thereby infecting the DR morph. The SSR morph residing in the profundal zone was suspected to be the one with the least infection of eyeflukes because of their deep-water habitat choice. This however did not turn out to be the case.

The infection differences between the morphs with regards to the retina eye habitat were all significant in Stuorajávri. In Suohpatjávri the LSR and DR morph and the LSR and SSR morphs were significantly different in regards to number of flukes in the retina. Several articles report that there is an association between habitat choice of the fish host and their parasite infection (Holmes, 1990; Knudsen et al., 1997). In an article from several lakes in

Europe from 2013 they found that in Lake Luzerne and Lake Brienze the profundal morph had no eyefluke infection at all, while fish living in more shallow parts of the lake were infected (Karvonen, Lundsgaard-Hansen, Jokela & Seehausen, 2013a). A similar infection pattern seems to be the case for the eyeflukes in the retina of the three morphs in Suohpatjávri. The LSR and DR morphs exhibited a higher eyefluke infection than the SSR morph (in this case the SSR morph was infected) though the overall eyefluke infection in the retina was low. Different whitefish morphs have very specific habitat preferences (Amundsen, 1988; Kahilainen, Lehtonen & Könönen, 2003; Præbel et al., 2013). Thereby, one would expect that the LSR morph should be the most infected morph due to its choice of habitat, since it is the same habitat (the littoral zone) as the snails, the first intermediate hosts reside in. Generally, the LSR-morph is the most infected morph of eyeflukes in the retina as predicted.

However, in the VH eye habitat there were no differences in infection between the LSR and SSR morphs in Stuurajávri. In Suohpatjávri the DR and SSR morph had no significant difference in eyefluke infection in the VH. The SSR morph resides in the deeper part of the water column, I therefore assume the SSR morph would be less exposed to swarming parasite larvae than the other morphs (LSR and DR) residing in the upper water column and closer to shore. A relatively similar infection pressure in the SSR morph compared with other morphs suggests that either the parasite larvae are transported down to deeper areas or the SSR morph may migrate between shallow and deeper areas. Thus, differences in habitat preference of each of the whitefish morphs and transmission possibilities from snails may explain differences in infection of eyeflukes as observed.

The eyefluke infection in Stuurajávri among the three morphs suggests that there are some differences between the two eyefluke species-groups. The comparisons between the two habitats in the eye, revealed a significant difference between the retina and the VH for two of the three morphs – the LSR and the SSR morph. The DR morph did not have significant difference of infection between the two eye habitats. In Suohpatjávri all three morphs exhibited significant infection difference between the two eye habitats. Other studies published about differences between habitats within the eye mostly focused on the differences between the VH and the lens and found that there were significant differences between the two habitats (Locke, McLaughlin & Marcogliese, 2010). To be able to say why there are such differences between the fish morphs and eye habitats is hard without further and more in-

depth research with the main focus on this particular predicament. The genetic analysis may help with shedding some light as to why there are such differences. Several studies have showed that habitat and diet may have some influence on the degree of parasite infection of sympatric fish morphs (Holmes, 1990; Knudsen et.al 1997).

As expected, the results showed clear differences in eyefluke infection between most of the morphs both within and between the lakes. As expected, the number of eyeflukes in each eye habitat would differ both within and between the lakes. What was unexpected was that one of the lakes, Suohpatjávri had a generally higher infection in the VH than in the retina, while Stuorajávri had a generally higher infection in the retina than in the VH. What may be the cause for those differences is hard to say, but below I shed some light on possible causes that have been reported in literature. The comparisons for the VH were all significant. The findings above support the results for the abundance analysis, showing that Suohpatjávri has more flukes in the VH than in the retina, and Stuorajávri the other way around. A study mentioned earlier (Karvonen et al., 2013a) found not only differences between morphs within the same lake, but they found different patterns in the eyefluke infection between the lakes as well, the profundal morph had no infection in two of the lakes – Lake Luzerne and Lake Brienze (Karvonen et.al. 2013a).

Lakes are different in ecological and abiotic factors. Both biotic and abiotic factors may have impact on the different life-stages of the parasite (Bagge, Poulin & Valtonen, 2003; Hechinger & Lafferty 2005; Höglund, 1995; Karvonen et al., 2013b; Lyholt & Buchmann, 1996; Marcogliese & Cone, 1996). In an article about eel off the coast of Canada they found that pH has an effect on the number of eyeflukes (Marcogliese & Cone, 1996). The pH had an impact on the free-living larval stage of the eyeflukes, the article found that the lower the pH the less parasites in the host. This has an enormous effect on the occurrence of trematodes since their first intermediate host – the snail struggles in acidic conditions (see Marcogliese & Cone, 1996 and references therein). Given that both of the lakes in this study are situated in the same watercourse, I assume the difference in pH is of relatively low, and thereby of low importance in regard to across lake differences in the distribution and number of eyeflukes.

One factor that may impact the distribution and number of eyeflukes is the temperature – the cercariae only start hatching from the snail at a certain temperature i.e. the length of the period that the snail can shed larvae and the life expectancy of the free-living larvae.

Karvonen with others found that although the size of the fish having an impact on the infection, the water temperature has a greater impact (Karvonen et al, 2013b). Other articles states that in order for the cercariae to hatch from the snail, the water temperature has to be between 3 to 10 degrees Celsius (Karvonen et.al, 2013b; Lyholt & Buchman, 1996). Again, the two lakes are a part of the same watercourse so the difference in temperature may not be that great, but it could favor a higher temperature in more shallow and smaller lakes (taking less time to warm up than bigger lakes (Wetzel, 2001) which in this case would favour a higher infection in Suohpatjávri than in Stuorajávri.

Another factor that may impact the infection level of eyeflukes is the abundance of the first intermediate host, the snail (Hechinger & Lafferty, 2005; Karvonen, Savolainen, Seppälä & Valtonen, 2006). The snail abundance may not be the same in both lakes. Since Stuorajávri is a lot bigger than Suohpatjávri i.e. more wind exposed which provides more spreading for the cercariae and more deep areas, the littoral zone is the key here. Suohpatjávri has a relatively larger littoral zone because of its smaller size i.e. more shallow areas than Stuorajávri. Since the snails mainly reside in the littoral zone, Suohpatjávri might have a greater abundance of snails and thereby “shed more parasite larvae”. A study looking at the relationship between prevalence of eyeflukes and the snail found that the eyeflukes were more common in smaller sized lakes, and rare in bigger lakes (Voutilainen, van Ooik, Puurtinen, Kortet & Taskinen, 2008). Hence, this might be one of the key factors contributing to the differences between the two lakes and thus very important.

The availability of the second intermediate host, the whitefish may play a significant role, as well as the density of each of the three morphs. The cercariae is short lived and can only live a few days in the free water masses before finding a host, otherwise it dies (Karvonen et.al., 2006). In a study from Sweden they found that density of cercariae was more important for the transmission than fish density (Höglund, 1995). A study from Finland and Russia found that rather than the density of fish hosts playing a big role in parasite infection, and the density of hosts had a greater impact (Bagge et al., 2003). In Stuorajávri and Suohpatjávri the LSR and DR morphs have quite high density, while the SSR morph is not as common, especially in Suohpatjávri. A total number of 161 fish from the two lakes were examined, but there were only caught five individuals of the SSR morph in Suohpatjávri. Five individuals do not say a whole lot about the situation among the SSR morph, but it is still something to report as all five individuals were infected with eyeflukes both in the retina and the VH.

As mentioned over, the cercariae is short lived and is dependent on finding its new host within a few days. The egg or miracidium might get snapped up from an unwanted predator at any time like fish larvae or some kind of invertebrate, putting its lifecycle to an end. As mentioned earlier in the discussion, the diplostomum found in the eye of the whitefish in both lakes is most likely the same genetic species that has been found in arctic charr, trout and three-spined sticklebacks in Iceland (Blasco-Costa et.al., 2014; Dr. Isabel Blasco-Costa; Department of Genetics and Evolution, Geneva (personal communication)). Other fish species than whitefish are also present in both lakes, and several studies report of eyeflukes in the eyes of perch as well (Höglund & Thulin, 1990; Höglund & Thulin 1982). Given this information, it is possible that the perch in both lakes is infected with the same generalist eyefluke species as well, thereby sharing the infection rate with the whitefish in both lakes, but to a larger extent in Suohpatjávri, this needs to be looked further into. The density of perch is greater in Suohpatjávri than in Stuorajávri (Knudsen et al., 2003). Pike, which is known to be a predator is present in both lakes (Amundsen, 1988). The density of pike in Stuorajávri is higher than in Suohpatjávri (Amundsen, et al., 2002). With the pike present in both lakes, one may assume that the presence of the predator has an impact on the density of potential fish hosts with the lake. In turn, the predation on potential hosts from pike might contribute to a less dense host population and which may result in a lower infection of eyeflukes. Thus, availability of the second intermediate host, the whitefish, is very important.

Human impact may have a certain effect on fish density, in the early start of the 2000's, an effort was made to take out a lot of fish from Stuorajávri in order to see if this had any effect on the infection of *Triaenophorus crassus* in whitefish which has been known to be a severe problem in the lake (Amundsen, 1988; Amundsen et al., 2002, Kristoffersen et al., 2004). Several tons of fish were removed over a period of two years (Amundsen, 1988, Amundsen et al., 2002; Kristoffersen et al., 2004). This could have had an impact on the distribution of eyeflukes by changing the transmission rates through the life cycle of the parasite, taking out such a vast number of individuals serving as potential host. Fish removal was conducted two time, the first time in the 1980's and the last time in year 2000 Since this is more or less 40 and 15 years ago now, it is less likely that it still has an effect. Again, this might be a slow process.

Last but not least, the abundance of birds might have an impact on the infection pressure of eyeflukes. Several articles have reported a connection between bird abundance and abundance

of eyeflukes (see Hechinger & Lafferty, 2005 and references therein). There is one report on bird life around Stuurajávri, reporting finding of quite a few species (Strann & Nilsen, 1996). There are no reports on the bird life around Suohpatjávri, but it is reasonable to assume that birds are abundant given the number of eyeflukes found in the eye of the fish. As mentioned over, the main goal of any parasite is to fulfill its life cycle, and in this case the life cycle is complex (Chappell, 1995; Goater et.al, 2014). The more complex the life cycle gets, the harder it gets for the parasite to complete it. Several articles state that the parasite has the ability to manipulate the host in ways that benefits the parasite and increases the likelihood of the parasite fulfilling its life cycle (Milinski, 1990). Articles published on the subject state that the eyefluke compromises the eyesight of the fish reducing its ability to detect both potential prey and predators (Crowden & Broom, 1980; Milinski, 1990; Owen et.al., 1993). The reduced predator detection of infected fish makes the fish more vulnerable, and they are more at risk for becoming a prey, for a fish-eating bird for instance. By getting eaten by a bird, the lifecycle is completed and the cycle starts all over again. The new egg produced is passed through the feces of the bird and from there it might go into a lake, ensuring the lifecycle continues or it might land outside of the lake, putting the lifecycle to an abrupt end. Thus, the spreading of the eggs might be of great importance.

In conclusion, my hypothesis that there would differences in eyefluke infection between the three sympatric whitefish morphs within both lakes is supported for all comparisons for both eye habitats with a few exceptions. I secondly hypothesized that there are differences in the eyefluke infection for the equivalent morph between Stuurajávri and Suohpatjávri, and all comparisons exhibited a significant difference except for the comparison for the retina between the DR morph in both lakes. More specifically, I suggested that the infection would be higher in Suohpatjávri mainly because the first intermediate host, snails, are found in most part of the lake because of its small size and not being too deep, which my results support. Thirdly, I predicted that there will be a difference in eyefluke infection between the two habitats within the eye; more specifically between the VH and the retina. For Stuurajávri two of three comparisons of eyefluke density between the VH and retina were significantly different for LSR and SSR morphs, however not for the DR morph. In Suohpatjávri however all comparisons were significant and overall the prediction is supported. There are many potential factors that may cause such differences in eyefluke infection across lakes and between morphs. It is hard to say which factor has more impact than the other, but I assume

that the most crucial thing to ensure parasite survival is the abundance of hosts, more specifically the density of the first intermediate host, the snail. The second intermediate host, the fish might be very important, as well as the habitat choice of the fish which might be crucial for the infection pattern. Last but not least the final host, density of birds in the area are important factors. When the results of the genetic testing are ready, it may be able to shed some light as to whether or not the eyeflukes in the retina and the VH are two separate species like assumed. In the future, a more in-depth research within and between both lakes is highly recommended, to try and find out why there are such differences.

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Appendix

Appendix table 1. An overview of the age and the number of individuals with that age for the LSR morph in Stuorajávri.

LSR Stuorajávri	
Age	Number of individuals
5	1
6	2
7	10
8	12
9	4
10	1
11	1

Appendix table 2. An overview of the age and the number of individuals with that age for the DR morph in Stuorajávri.

DR Stuorajávri	
Age	Number of individuals
5/5+	3
6	6

7	7
8	8
9	2
10	1
11	2
15	1

Appendix table 3. An overview of the age and the number of individuals with that age for the SSR morph in Stuorajávri.

SSR Stuorajávri	
Age	Number of individuals
3	1
4	3
5	5
6	3
7	7
8	5
9	5

Appendix table 4. An overview of the age and the number of individuals with that age for the LSR morph in Suohpatjávri.

LSR Suohpatjávri	
Age	Number of individuals
2	6
3	11
4	13
5	5
6	3
7	2
8	1

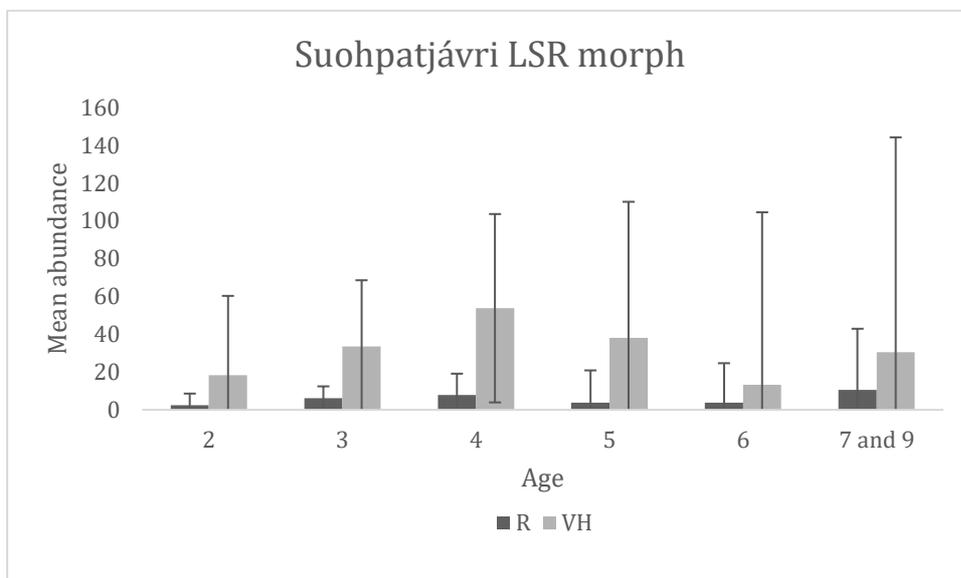
Appendix table 5. An overview of the age and the number of individuals with that age for the DR morph in Suohpatjávri.

DR Suohpatjávri	
Age	Number of individuals
3	8
4	9

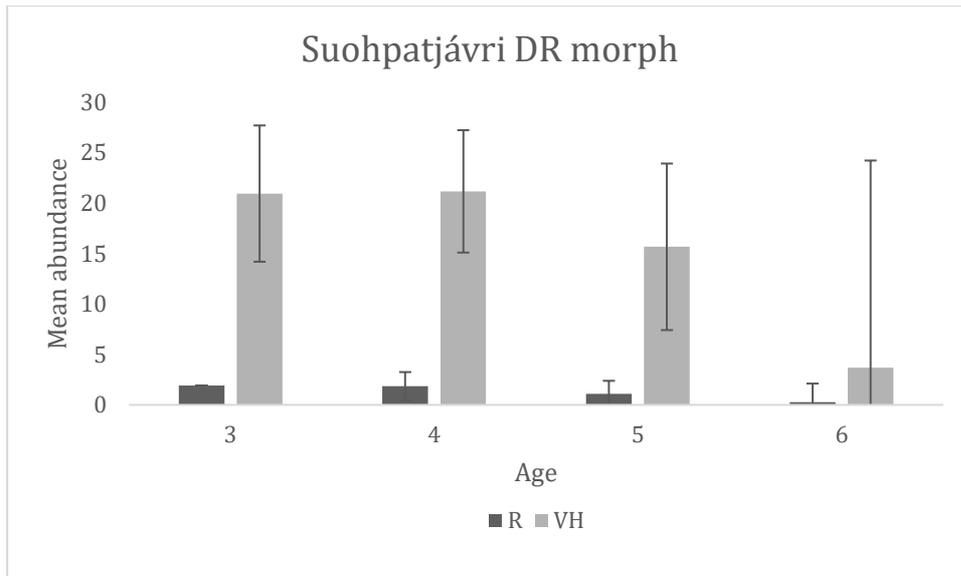
5	5
6	2

Appendix table 6. An overview of the age and the number of individuals with that age for the SSR morph in Suohpatjávri.

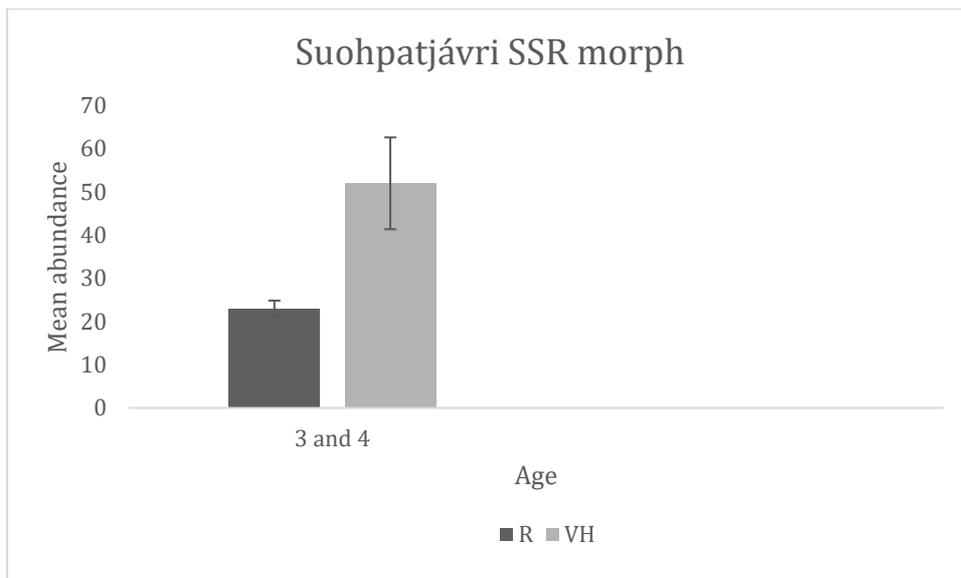
SSR Suohpatjávri	
Age	Number of individuals
3	1
4	4



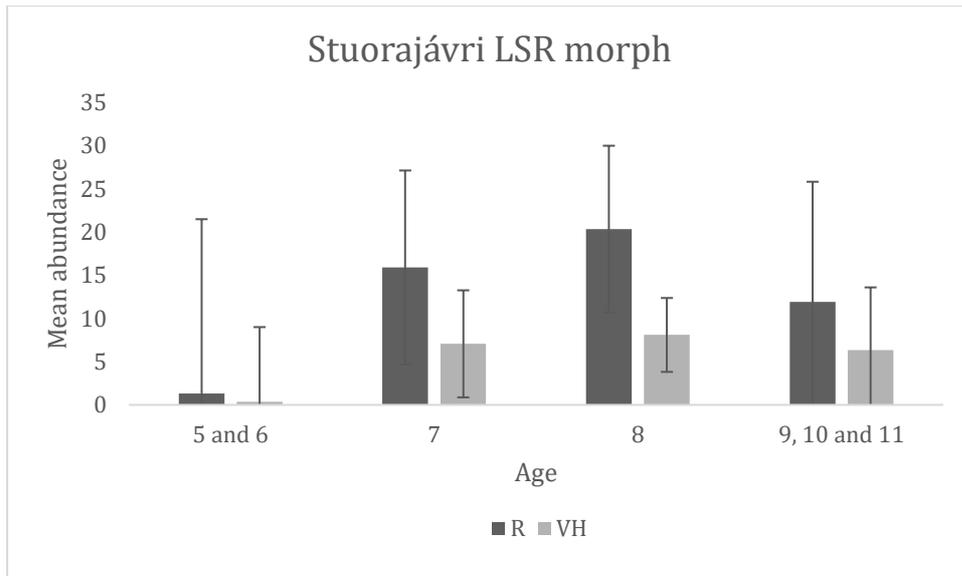
Appendix figure 1. Mean abundance plotted against age of the LSR morph in Suohpatjávri \pm SE.



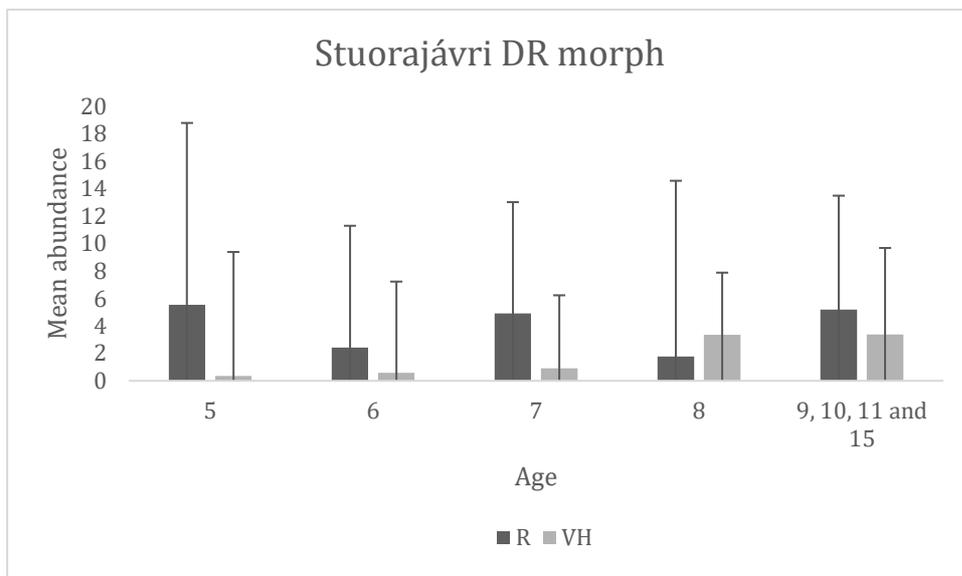
Appendix figure 2. Mean abundance plotted against age of the DR morph in Suohpatjávri \pm SE.



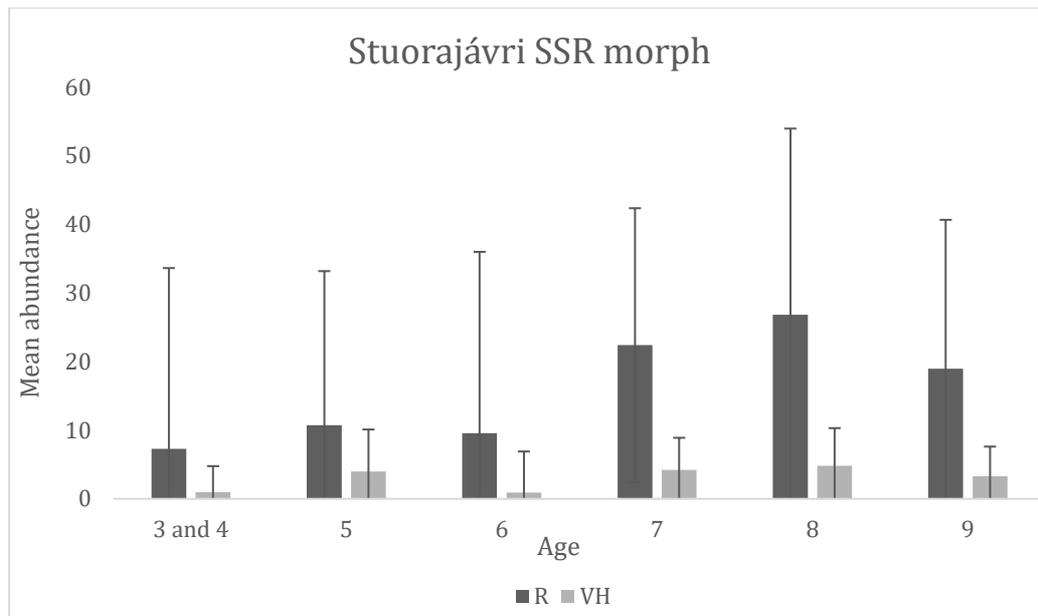
Appendix figure 3. Mean abundance plotted against age of the SSR morph in Suohpatjávri \pm SE.



Appendix figure 4. Mean abundance plotted against age of the LSR morph i Stuorajávri \pm SE.



Appendix figure 5. Mean abundance plotted against age of the DR morph in Stuorajávri \pm SE.



Appendix figure 6. Mean abundance plotted against age for the SSR morph i Stuorajávri ± SE.

Appendix table 7. The abundance of both eye habitats for each morph (LSR, DR and SSR) for Stuorajávri (ST) and Suohpatjávri (SU) including values for mean and standard error (\pm SE).

	LSR ST	DR ST	SSR ST	LSR SU	DR SU	SSR SU
Abundance retina	1724	657	2778	1142	137	44
Abundance VH	770	269	528	7690	1402	331
Mean retina	53,9	21,2	95,8	31,7	6,2	8,8
Mean VH	24,1	8,7	18,2	202,4	63,7	66,2
\pm SE retina	6,6	3,5	11,0	5,2	0,7	1,9
\pm SE VH	3,3	2,7	2,7	27,5	4,4	10,6

