Parasites and parallel divergence of individual MHC allelic richness in Icelandic threespine stickleback (*Gasterosteus aculeatus* L.): Contrasting habitats and population divergence

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90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

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General introduction

When individuals of a single species encounter different environments, local adaptation and thus phenotypic diversification among populations is expected. This intraspecific diversity, which is common among northern freshwater fish, can be observed even between populations that live in close proximity (sympatry) (Schluter, 2000a). Common ecological selection pressures often lead to parallel evolution as similar habitats favour the same phenotypic optima resulting in independent evolution of the same traits towards the same direction (Robinson & Schluter, 2000, Rundle et al., 2000, Schluter & Nagel, 1995). The identification of the ecological factors that facilitate this ecologically driven diversification has been the focus of many studies. The most common ecological factors associated with population divergence are intraspecific competition on the available food resources, exposure to different biophysical environmental cues and interaction with other species incorporated mainly in the form of predation (Schluter, 2000b, Vamosi & Schluter, 2002, reviewed by Reimchen, 1994).

Local adaptation is reflected in the external morphology of the fish as many morphological traits are associated with performance and fitness i.e. locomotion activity, foraging efficiency and protection against predators (McGuigan et al., 2003, Reimchen, 1994, Swain, 1992). However, in environments characterized by great niche availability utilization of vacant niches can result in increased intrapopulation phenotypic variation (Nosil & Reimchen, 2005, Wilson & Turelli, 1986). Differential exploitation and specialization to new alternative resources can also occur between sexes of the same population (Wootton, 1976). This intersexual competition for resources can lead to sexual dimorphism and its extent has been found to increase

with niche availability as sexes have the potential to explore a wider range of alternative niches (Nosil & Reimchen, 2005, Spoljaric & Reimchen, 2008).

An important ecological factor that can contribute to divergence among populations is the interaction between hosts and parasites. Although this mechanism was until recently largely neglected a growing number of studies suggest that parasites can also contribute to the divergence among their host populations by imposing strong selection pressures to their hosts (Buckling & Rainey, 2002, Summers et al., 2003). Parasites vary between habitats as they depend on environmental factors e.g. habitat type and water temperature but also on the density of intermediate and definite hosts (Halmetoja et al., 2000, Poulin & Morand, 2000). This variation is expected to lead to differential parasitism between populations foraging in different habitats causing divergence in the immune defence systems of their hosts which provides protection against pathogens (Kalbe et al., 2002, MacColl, 2009, Scharsack et al., 2007). A key part of the vertebrate adaptive immune system is the Major Histocompatibility Complex (MHC) and thus it is considered to be a perfect candidate for studying host-parasite interactions (Hedrick et al., 2001, Klein, 1979, Klein & O'Huigin, 1994, Westerdahl et al., 2005) as its high polymorphism is believed to be maintained by pathogen-mediated selection (Jeffery & Bangham, 2000).

A model species to study the evolutionary mechanisms underlying diversification and local adaptation is the threespine stickleback *(Gasterosteus aculeatus)*. Threespine stickleback have undergone multiple adaptive radiations since the retreat of the glaciers in the end of the Pleistocene period (10.000-15.000 years ago) (Schluter, 1993, Taylor & McPhail, 1999). It has a widespread distribution in the Northern hemisphere and is found in a wide range of heterogeneous environments

including both saltwater and freshwater habitats. As it faces diverse ecological opportunities within and among the new colonised freshwater habitats it is characterized by an extraordinary diversity in morphology, behaviour and life history (Bell & Foster, 1994). In Iceland, the unique geological conditions in lakes within the neovolcanic zones, with distinct habitat types formed by post glacial lava flows, appear to have promoted local adaptations resulting in two distinct benthic morphs, the lava and the mud (Kristjánsson et al., 2002, Ólafsdóttir & Snorrason, 2009, Ólafsdóttir et al., 2007).

In the first part of the thesis (manuscript I) I test the hypothesis that local adaptation to different parasitic fauna affects the genetic variation of the populations. By focusing on four lakes: two single morph lakes and two that are inhabited by the two distinct stickleback morphs –lava and mud, I first test if the level of parasitic infection varies consistently between the two sympatric morphs but also between sticklebacks found in the lakes with similar foraging environments experienced by the two morphs. Then if parasites contribute to the divergence of the two morphs I expect that selection pressures imposed by varying abundance of virulent parasite species will be reflected in the immune system of sticklebacks and variation in the number of MHC alleles between the two morphs will be observed. Thus I test if there are parallel differences in the MHC allelic richness between similar foraging habitats and if the MHC individual diversity has been affected by the diverse selection pressures imposed by contrasting parasite communities. Parallel differences in parasitic infection coupled with divergent MHC genotypes could indicate the role of parasites in the divergence of Icelandic stickleback morphs.

In the second part of the thesis (manuscript II) I relate intra-population morphological variation and sexual dimorphism to ecological opportunity (substrate availability and lake size) in Icelandic threespine stickleback in a larger geographical scale. First I examine if sexual dimorphism and population variation in morphology is related to substrate type or lake size. Secondly, I test if the extent of sexual dimorphism affects morphological variability within each sex.

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Manuscript I

Parasites and parallel divergence of individual MHC allelic richness between sympatric populations in Icelandic threespine stickleback morphs

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Abstract

Parasites are thought to be major ecological agents of selection. According to theory, differential parasitism between host populations has the potential to promote adaptation to local environments and thus rapid divergence due to host-parasite co-evolutionary interactions. Parasite mediated selection has been found to operate on the highly polymorphic genes of the major histocompatibility complex (MHC) which is part of the adaptive immune system of the jawed vertebrates. For the current study threespine sticklebacks (*Gasterosteus aculeatus*) sampled from four lakes located in South-Western Iceland, two of which are inhabited by two distinct stickleback morphs, apparently adapted to different benthic habitats, -lava and mud-, and two single morph lakes, were screened for parasites and genotyped for MHC class IIB diversity. The level of parasitic infection was found to differ consistently between the sympatric morphs but also between populations found in similar foraging habitats with fish from the lava/rocky habitats being more heavily infected. A parallel pattern was also found in individual MHC allelic variation with lava stickleback morphs exhibiting lower levels of variation compared to the mud morphs. The parallel divergence in MHC allele number between the sympatric morphs may be caused by different selection pressures imposed by varying abundance of parasite species causing different optima to be favoured in contrasting habitats. Consequently, parasite mediated selection could play a part in the divergence of the benthic lava-mud morphs in Icelandic lakes.

Introduction

Ecology has always been considered to play a significant role in the evolution of differences between populations and potentially in speciation (Bush, 1994, Darwin, 1859, Simpson, 1953). According to theory the process of adaptive radiation occurs when a population becomes the target of divergent selection as it encounters multiple discrete environments. This can lead to the formation of new species (Schluter, 2000a, Schluter, 2001, Simpson, 1953). During the last decade there has been growing interest in identifying which ecological factors facilitate this ecologically driven diversification. In many of the documented cases, local adaptation is attributed to intraspecific competition on the available food resources, exposure to different biophysical environmental conditions and interactions with other species mainly in the form of predation (Schluter, 2000b, Schluter, 2001, Vamosi & Schluter, 2002, reviewed by Reznick & Ghalambor, 2001). Recently a growing number of studies suggest that parasites can also be important agents of divergent selection (Buckling & Rainey, 2002, Scharsack et al., 2007, Summers et al., 2003).

Parasite communities can vary significantly between different habitats in terms of species diversity and abundance as they depend on environmental factors (e.g. substrate type, water temperature) and on the density of intermediate or definite hosts (Halmetoja et al., 2000, Poulin & Morand, 2000). This variation can lead to differential parasitism between host populations promoting adaptation to local environments and rapid divergence due to host-parasite co-evolutionary interactions (Lively, 1999, Summers et al., 2003). Moreover, parasites have been found to contribute to reproductive isolation between sympatric species by reinforcing assortative mating and thus have the potential to promote speciation (Haldane, 1949, Turelli et al., 2001, reviewed by Summers et al., 2003).

A perfect candidate for studying the effects of parasitism in the maintenance of genetic variation is the Major Histocompatibility Complex (MHC). MHC is a highly polymorphic multigene family part of the adaptive immune system of the gnathostome vertebrates against pathogens and thus an indicator of the host-parasites interactions (Hedrick et al., 2001, Klein, 1979, Klein & O'Huigin, 1994, Westerdahl et al., 2005). MHC variation also contributes to reproductive isolation and its role in mate choice and assortative mating has been demonstrated in many species, including threespine stickleback (Aeschlimann et al., 2003, Chaix et al., 2008, Milinski, 2006, Ober et al., 1997, Potts et al., 1991). Due to its role in fitness and mate choice it has been called a "magic trait" to speciation, a term firstly introduced by Gavrilets (2004).

MHC genes encode molecules found in the surface of the cells that bind and present self and non-self antigens to T-cells triggering an immune response against invading pathogens (Klein, 1986, Potts & Wakeland, 1990). The MHC region is divided in two subgroups, termed class I and II. Their main difference lies in the nature of the peptides they recognise. Class I molecules present endogenus peptides originated from viral or intracellular bacteria in contrast with class II molecules that recognise exogenous peptides derived from extracellular pathogens such as parasites (Jensen, 2007, Trowsdale, 1993). Both groups exhibit high levels of polymorphism which comprises high number of alleles and duplicated loci (Klein, 1979, Klein et al., 1998). This polymorphism is believed to be maintained by pathogen-mediated selection (Jeffery & Bangham, 2000). Three different models have been proposed to explain its high diversity: i) heterozygote advantage, ii) negative frequency-dependent selection and iii) fluctuating selection. All three mechanisms have been found to operate in wild populations and differentiation between them has been found to be

extremely difficult as in many cases they may interact (reviewed by Spurgin & Richardson, 2010).

The threespine stickleback (*Gasterosteus aculeatus*) is a model organism in evolutionary biology as it has undergone rapid adaptive radiation since the end of the Pleistocene period (10.000- 15.000 years ago) (Schluter, 1993, Taylor & McPhail, 1999). The retreat of the Pleistocene glaciers resulted in the formation of novel freshwater systems all across the northern hemisphere that were in turn colonised by marine stickleback populations (Bell & Foster, 1994, McPhail, 1994). This was frequently followed by rapid morphological divergence as colonising stickleback adapted to various habitats, and gave rise to the formation of different morphs that now vary in the level of genetic divergence and strength of reproductive isolation (reviewed by McKinnon & Rundle, 2002). Due to their widespread distribution, sticklebacks also encounter a wide range of parasitic fauna and they serve as definite or intermediate hosts to a great number of parasitic taxa (reviewed by Barber, 2007). The role of the parasites as agents of selection facilitating ecological and genetic divergence among stickleback morphs has only recently started to be explored. MacColl (2009) described differences in the level of parasitism between the benthic and limnetic stickleback "species pairs" found in sympatry in Paxton and Priest lakes, British Columbia, Canada. He demonstrated that the prevalence and abundance of parasites varied substantially between the host populations and that this variation was consistent across the two lakes studied, suggesting that the stickleback "species pairs" are under different selection pressures. The impact of these different selection pressures on the immune system of the fish was investigated further by Matthews et al. (2010) who showed that the two species pairs differ consistently in their MHC composition but also in their individual MHC allelic richness suggesting that different

selection pressures imposed by different parasite communities cause different optima to be favoured in contrasting habitats. Accordingly, studies on lake and river stickleback populations that live in sympatry in Northern Germany and are genetically distinct due to habitat specialization (Reusch et al., 2001a) have revealed a significant correlation between parasite diversity and MHC polymorphism among the different morphs, with river sticklebacks exhibiting lower number of different MHC classIIB alleles at the population level (Wegner et al., 2003) but also per individual fish (Milinski, 2006) while at the same time they are exposed to a less diverse range of parasites than the lake sticklebacks and they are more susceptible to infections when they come in contact with lake parasites (Scharsack et al., 2007).

Two different stickleback morphs have been described in Icelandic neovolcanic lakes, a lava morph which inhabits a complex structured benthic habitat made up of lava flows that created underwater rifts and hard rocks with holes and crevices- and a mud morph which is found in typical, vegetated soft mud habitats (Kristjánsson et al., 2002). Morphological and genetically divergent lava and mud morphs have been described in three Icelandic lakes: Thingvallavatn, Hredavatn and Myvatn (Kristjánsson et al., 2002, Ólafsdóttir & Snorrason, 2009, Ólafsdóttir et al., 2007). A detailed study on geographical and temporal variation in morphology and genetic composition in Thingvallavatn shows that the stickleback population within the lake is a complex composition of spawning groups, morphologically and genetically divergent to varying extent (Ólafsdóttir & Snorrason, 2009). Although strong assortative mating has been found among the two morphs in Thingvallavatn, (Ólafsdóttir et al., 2006) the authors speculate that the population structure within the lake may be temporally unstable and highly ecologically dependent. One of the ecological factors differing among the lava and mud habitat in Thingvallavatn is the

invertebrate fauna (Lindegaard, 1992) and thus the prevalence and abundance of intermediate hosts of certain parasites is expected to be different. This has important implications for adaptive divergence among the lava and mud morph in the lake. Firstly, parasite prevalence can create a strong and immediate divergent selection among spawning individuals in the two habitats. This is of augmented importance, as the MHC genotype may also influence mate choice. Second, differing parasite communities can affect the quality of the habitats at spawning sites by causing competition based structuring of spawning individuals prior to any selective forces acting on the populations.

In the present study stickleback populations from four lakes, including both lava and mud morphs from Thingvallavatn and Hredavatn and two single morph lakes (Bretavatn and Baularvallavatn) were screened for parasites and genotyped for MHC diversity. First, we want to ascertain if the level of parasitic infection varies consistently between the two sympatric morphs, but also between sticklebacks found in other lakes with similar foraging environments experienced by the two morphs. Second, as resistance against parasites is influenced by the MHC genes, we examine if MHC diversity has been affected by divergent selection in the two habitats. We focused on the MHC class IIB genes as they have been associated with resistance against parasites (Wegner et al., 2003) and mate choice decisions (Aeschlimann et al., 2003, Reusch et al., 2001b). If parasites contribute to the divergence of the two morphs then we expect that selection pressure imposed by varying abundance of virulent parasite species will be reflected in the immune system of sticklebacks and variation in the number of MHC alleles between the two morphs will be expected.

Material and methods

Sampling of fish and parasite screening

Threespine sticklebacks (*Gasterosteus aculeatus*) were sampled during summer 2009 (June and July) from four different lakes located in South-Western Iceland (Figure 1). Two of these lakes (Thingvallavatn, 64°11′N, 21°09′W and Hredavatn, 64°45′N, 21°36′W) are inhabited by two distinct stickleback morphs found in different benthic habitats, -lava and mud-, while the other two are single morph lakes (Bretavatn, 64°42′N, 22°4′W and Baularvallavatn, 64°55′N, 22°53′W) resulting in the sampling of six different populations. In Thingvallavatn and Hredavatn samples were collected from both mud and lava habitats while in the single morph lakes –Bretavatn and Baularvallavatn– samples were collected from a mud and a rocky-mud habitat respectively. Fish were caught in unbaited benthic minnow traps, lifted the day after setting and fishes collected were anaesthetized using CO_2 solution, kept in ice and transferred immediately to the laboratory for parasite screening. Standard length (± 0.005 mm) and mass (± 0.005 g) of each fish were recorded before dissection. To assess parasite load, fish were dissected and all the internal organs including the body cavity were examined under a dissecting microscope. Both eyes were removed and completely dissected. In case of infection, parasites were counted and identified to genus and in some cases to species level. However it was not possible to identify an encysted larval nematode found only in samples from Bretavatn. Gender of each fish was also determined by visual examination of the gonads. In total, five hundred and forty nine adult fish were scanned (Table 1). Forty eight fish (twenty four females and twenty four males) randomly chosen from each population were fin clipped. Fin clippings were preserved in 70% ethanol for genetic analysis.

MHC diversity

Total genomic DNA was isolated from fin clippings using NucleoSpin®Tissue Kit (Macherey-Nagel, Germany) following the manufacturer's standard protocol. In order to determine MHC diversity Single-Stranded Conformation Polymorphism (SSCP) analysis was performed. SSCP is a sensitive method used to detect even small differences between sequences (Orita et al., 1989, Sunnucks et al., 2000). Prior to the analysis a 247 bp fragment of the highly variable exon 2 of MHC class IIB genes which is the region that codes for the peptide-binding groove of the MHC class II β-chain (Brown et al., 1993) was amplified with polymerase chain reaction (PCR) using the primers GaIIEx2startF: 5'-GTC TTT AAC TCC ACG GAG CTG AAG G-3' for forward and GAIIExon2R_RSCA: 5'- ACT CAC CGG ACT TAG TCA G-3' for reverse amplification (Lenz et al., 2009). These primers were designed to amplify exon 2 in all known MHC class IIB loci which are estimated to be between two and four (Reusch & Langefors, 2005), and cover 88% of the exon (Lenz et al., 2009). PCR reactions consisted of 2 μl of DNA, 1 U of Taq polymerase, 5mM $MgCl_2$, 0.5 μM of each primer, 50 μM of each dNTP, and 1X PCR amplification buffer in 20 μl total volume. The cycling scheme followed consisted of an initial denaturing step of 95 ^{o}C for 5 min, 25 cycles of 94 ^{o}C for 60 s, 58 ^{o}C for 60 s and 72 ^{o}C for 60 s, with an extension step of 72 ^{o}C for 3 min. The PCR product was diluted 5x and used as template in a second PCR that contained the same reaction mix as described above following the same thermal profile but with a reduction of PCR cycles to 6. The low number of PCR cycles and the second reconditioning PCR step were performed in order to reduce the formation of artefacts (protocol developed by Lenz & Becker, 2008). Five microliters of the amplification product were mixed with equal volume of formamide mix containing 10mM NaOH and few grains of bromophenol blue, heated

to 95°C for 5 minutes (denaturation) and immediately transferred to ice-cold water for 4 minutes to hinder re-annealing. Eight microliters were loaded on SSCP GMA precast gels (Elchrom Scientific) and run in 0.75x TAE buffer at a constant temperature of 9 °C for 12 h at 6 V/cm. The electrophoresis was conducted on the SEA2000® electrophoresis apparatus (Elchrom Scientific). Gels were stained with SYBR Gold (Invitrogent) and gels were viewed and photographed on a transilluminator under UV-light (254 nm). To verify that the expected fragments were amplified, the PCR products of three random samples, representing different populations, were cloned and sequenced using the TOPO TA Cloning® Kit (Invitrogen, Germany) following the manufacturer's protocol.

Data analysis

Statistical analyses were performed in SPSS (release 15.0.0) and SYSTAT (release 12.02.00). Bonferroni corrections (Miller, 1981) were applied to Mann-Whitney U tests, chi-square tests and correlations tests when multiple comparisons were made. The p-value was adjusted to be compared directly to a significance level of 0.05.

Parasite load

After recording the abundance of each parasite species the prevalence, mean intensity and mean abundance were calculated for each population following the definitions of Bush et al. (1997). Further analyses were performed using only parasite species which occurred in all lakes and populations. Non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were used for detecting differences in the level of total parasite abundance and individual parasite load (number of species per individual) as test of normality and homogeneity of variance failed. Associations between parasite

prevalence and habitat were tested with a chi square test. Nonparametric Spearman's rank correlation coefficient (rho) was used to test if the abundance of parasites and intensity of infection was correlated with standard length (SL). Differences in the mean abundance and mean intensity of parasite infection between populations were tested using analysis of covariance (ANCOVA) in ranked transformed data (Conover & Iman, 1982) with fish SL as the covariate. Principal component analysis (PCA) was performed in log-transformed data to reduce the dimensionality of the multiple parasite data sets. The effect of lake, habitat (nested within lake), standard length and sex on the first two factors extracted from the PCA was determined by performing analysis of variance (ANOVA).

MHC data

Gels were photographed and Photoshop elements 4.0 (Adobe Systems Inc.) was used to process the bands obtained by the SSCP. Due to uncertainties during the determination of the exact size of the bands (extensive skewness) and thus to the identification of similar SSCP patterns between individuals our analysis was focused on variation in the number of MHC alleles found in each individual. As transformations failed to bring the distribution of the data close to normal, we used nonparametric tests (Kruskal-Wallis and Mann-Whitney U) to test if the average number of MHC alleles detected per individuals differed between populations and sexes. Correlation among MHC diversity and number of parasite species at the individual level as well as abundance of *D. baeri* and *S. solidus* (log-transformed) were tested by Spearman's rank correlation coefficient (rho) while differences in the infection level between individuals possessing different number of alleles were tested by Mann-Whitney U test.

Results

Parasite Load

Eight different parasite species from 3 taxonomic groups were recorded in total in the six populations studied: Cestoda (*Schistocephalus solidus*, *Proteocephalus* sp., *Diphyllobothrium dendtriticum*, *Diphyllobothrium ditremum*), Trematoda: Digenea (*Diplostomum baeri*, *Diplostomum spathaceum*, *Apatemon* sp.) and a larval nematode (unidentified) that was only found in Bretavatn. Table 1 shows the prevalence and mean intensity of the parasite species identified in each population. *Diphyllobothrium dendtriticum* and *Diphyllobothrium ditremum* are not included in the table as they represent isolated findings that occurred only in one sample from Bretavatn and in two samples -one from Bretavatn and one from Hredavatn lava- respectively.

Parasite communities did not differ between sampling sites, excluding the single occurrence of the unidentified nematode in Bretavatn (Table 1).

- Differences between habitats

Fish from the three habitats examined (mud, rocky-mud, and lava) differed significantly in the total abundance of parasites (all species summed, $H_{(2)} = 272.02$, $p < 0.001$, Kruskal-Wallis test) with sticklebacks from the lava habitat having more parasites than the other two habitats. The intermediate levels of total abundance exhibited by the fish from the rocky-mud habitat also differed significantly from the mud ($U = 2522$, $p < 0.001$, Mann-Whitney test) and lava habitat ($U = 5460$, $p < 0.001$, Figure 2). Moreover, sticklebacks from the mud habitat were infected with a lower number of parasite species per individual compared to fish from lava in both Hredavatn ($U = 2754.5$, $p = 0.012$) and Thingvallavatn ($U=2330.5$, $p < 0.001$). Consistently, fish from the rocky-mud habitat from Baularvallavatn had higher

individual parasite load ($U = 2608.5$, $p < 0.001$) in relation to fish from the mud habitat from Bretavatn.

Comparisons between Bretavatn (mud habitat) and Baularvallavatn (rocky-mud habitat) showed that there were significant differences between the prevalence of most of the parasite species with sticklebacks from Baularvallavatn exhibiting higher levels of prevalence except in the case of *D. spathaceum* (*S. Solidus*: $x^2_{(1)} = 33.37$, *D. baeri*: $x^2_{(1)} = 82.27$, *Apatemon* sp.: $x^2_{(1)} = 41.13$ and *D. spathaceum*: $x^2_{(1)} = 12.865$, $p < 0.001$ in all cases, Figure 3a). In the sympatric populations a significant association between the type of habitat and prevalence of *D. baeri* was found across both lakes examined, with lava morphs exhibiting higher levels of prevalence (in Thingvallavatn: $x^2_{(1)} = 14.79$, $p < 0.001$ and in Hredavatn: $x^2_{(1)} = 24.78$, $p < 0.001$). In Thingvallavatn additional associations between type of habitat and prevalence of other parasites were also detected (*S. solidus*: $x^2_{(1)} = 15.35$, $p < 0.001$, *Apatemon* sp: $x^2_{(1)} = 13.12$, $p < 0.001$) with parasite prevalence being always higher in the sticklebacks from the lava habitat (Figure 3b). Total abundance and intensity of infection (all parasites summed) were positively correlated with standard length (SL) in all populations (Rho between 0.207 and 0.588 and $p < 0.008$ in all cases). The patterns of mean abundance (MA) of each parasite when controlling for SL were similar to the patterns of prevalence with the difference that the MA of *Apatemon* sp. was not significantly different between the lava and mud habitat in Thingvallavatn (Table 2). Consistent differences between the sympatric populations were also found in the intensity of infection (controlling for SL) of the trematode *D. baeri* with lava morphs being more heavily infected than the mud morph (in Thingvallavatn: $F_{(1,159)} = 427.714$, $p < 0.001$ and in Hredavatn: $F_{(1,110)} = 61.298$, $p < 0.001$, Figure 4b). Significant differences in the intensity of *D. baeri* were also found between lakes Bretavatn and Baularvallavatn with sticklebacks from

Baularvallavatn harbouring more parasites (*D. baeri*: $F_{(1,143)} = 146.783$, $p < 0.001$, Figure 4a).

The first two factors extracted from the principal component analysis accounted for 52.55 % of the total variance (the first factor F1: 30.20% and the second factor F2: 22.35%). Figure 5a represents the loadings of individual parasite species on the two PCA axes while a plot of the mean scores of the first two factors extracted from the PCA is given in Figure 5b. Scores on the first axis were mainly associated with abundance of *D. baeri* and on the second axis with abundance of *S. solidus*. The nested ANOVA showed that the variation found in the principal component scores was significantly explained by SL, lake of origin and habitat type (nested within lake) in both factors extracted while sex had a significant effect only on the second factor (Table 3) as males were more heavily infected by the cestode *S. solidus* than females (mean abundance for males: 3.77± 0.28 SE, females: 1.97 ± 0.19 SE).

MHC data

Due to uncertainties during the determination of the exact size of the bands (extensive skewness) and thus to the identification of similar SSCP patterns between individuals our analysis was focused on variation in the number of MHC alleles found in each individual. The number of MHC-classIIB alleles detected per fish varied remarkably between individuals ranging from 2 to 7 different alleles (Figure 6).

- Differences between habitats

The number of MHC classIIB alleles per individual differed significantly between the populations studied ($H_{(5)}= 103.342$, $p < 0.001$) but also between habitat types ($H_{(2)} = 31.972$, $p < 0.001$). Fish from mud habitats had more alleles compared to the fish

from lava and rocky-mud habitat (U = 3874.5 and U = 1605.5 respectively, $p < 0.001$ in both cases) while no significant difference was detected between the last two habitats (U = 1604, $p = 0.252$). This between-habitat difference remained when the sympatric populations from Thingvallavatn and Hredavatn were examined separately (Hredavatn: U = 370.5, $p < 0.001$, Thingvallavatn: U = 356.5, $p < 0.001$) but not when fish from Bretavatn were compared to fish from the rocky-mud habitat (U = 1003, $p = 1.00$, Figure 7).

- Differences between sexes

Individual MHC richness did not vary significantly between sexes in any of the populations studied except the mud population from Thingvallavatn where females were found to possess fewer alleles than males (U = 139, sig = 0.019). However this difference was no longer significant after Bonferroni correction ($p = 0.114$).

- Parasites and individual MHC diversity

The number of different MHC alleles found within an individual did not correlate significantly with the parasite species found per fish (parasite diversity) in any of the populations examined ($p > 0.348$ in all cases). However, sticklebacks from the lava habitat tended to have the lowest number of parasite species when they possessed three alleles in contrast to sticklebacks from the mud habitat which showed a minimum around five alleles. In fish from the rocky-mud habitat an intermediate number of MHC alleles in relation to the other two habitats was observed (Figure 8). In addition, no significant correlation was observed between individual MHC variation and the abundance of *D. baeri* and *S. solidus* ($p > 0.197$) per individual except in the case of Baularvallavatn where there was a marginally significant

positive correlation between number of alleles and number of *D. baeri* parasites per individual (rho= 0.291, p = 0.05) and the case of Thingvallavatn lava where the correlation was negative (rho = -0.322, p = 0.031) (but not after Bonferroni corrections: Baularvallavatn: p = 0.6 and Thingvallavatn lava: p = 0.372). However, individuals with three alleles from Bretavatn, Baularvallavatn and the lava habitats seemed to be less heavily infected by the *S. solidus* and the same trend was observed among the mud morph individuals that had five or six alleles (Figure 9). Moreover in Thingvallavatn lava individuals possessing three alleles were significantly less infected by the cestode compared to individuals with four alleles (U= 29.5, p = 0.048). Concerning the abundance of *D. baeri,* sticklebacks from Thingvallavatn and Hredavatn with around five alleles suffered less regardless of their habitat of origin, while fish from Bretavatn and Baularvallavatn with three alleles seemed to be less susceptible to *D. baeri* infections (Figure 10).

Discussion

The results of the present study demonstrate that there are clear differences in the prevalence, intensity of infection and abundance of parasites between different host populations and these can be related to substrate type. In general, fish from the lava and rocky-mud habitats were more heavily infected compared to fish from the mud habitat and also harboured more parasite species per individual. We expected that selection pressure by varying abundance of parasite species on fish specialized in different foraging habitats would result in different levels of individual MHC variation. Our data seem to support this hypothesis as we found a parallel divergence in the individual number of MHC class IIB alleles between the sympatric morphs, with lava morph sticklebacks being always associated with low individual allelic richness compared to the mud morph sticklebacks. Low MHC variation was also observed in the stickleback populations originated from the two single morph lakes with rocky-mud and mud habitat respectively. This low MHC individual variation, might be indicative of loss of genetic variation and thus reduced fitness and higher susceptibility to parasites, but could also indicate adaptive divergence in allele number as the optimal number of MHC alleles seemed to differ between populations depending on habitat specialization.

Alternatively, genetic drift could result in the observed pattern of MHC allelic diversity between the lava and the mud morphs. Low levels of MHC polymorphism have been associated with reduced genome wide variation (Bollmer et al., 2007, Hedrick, 2002, Miller et al., 2008). Such a loss of individual MHC variation due to genetic drift in populations with reduced effective population size (Ne) can affect significantly the fitness of a population by increasing the susceptibility to parasitic infections (Pearman & Garner, 2005, Radwan et al., 2010, Sommer, 2005). Previous

microsatellite data from Hredavatn and Thingvallavatn showed that lava sticklebacks from Thingvallavatn exhibited lower genetic variation at a population level compared to the mud morphs but not in Hredavatn. However, the divergence at microsatellite loci per individual did not differ between lava and mud stickleback morphs in both lakes (Thingvallavatn lava: 1.57, mud: 1.72 and Hredavatn lava: 1.51, mud: 1.5, G.Á. Ólafsdóttir, unpublished data). Therefore even if a correlation between MHC and genome wide variability cannot be fully ruled out; genetic drift does not seem to be sufficient to explain the overall pattern observed in our data indicating that selection has influenced the divergence in the individual MHC allele number found between morphs.

Although the general pattern observed shows higher parasite prevalence in the lava/rocky sites than in mud sites, the main consistent difference concerns the burden of the trematode *Diplostomum baeri*. Lava morphs exhibited significantly higher levels of infection than the mud morph sticklebacks and the same pattern was observed in the two single morph lakes with sticklebacks sampled from the littoral rocky-mud zone in Baularvallavatn harbouring more parasites compared to fish sampled from the mud habitat in Bretavatn. The abundance of *D. baeri* separates the sympatric morphs but also the other two lakes as revealed by the multivariate analysis. Moreover, the abundance of *S. solidus* separates also the mud and the lava habitats in Thingvallavatn as well as Baularvallavatn (rocky-mud) from Bretavatn (mud) as fish from the lava and rocky-mud habitats were more heavily infected with the cestode. Sex had also a significant effect, with male sticklebacks being more heavily infected with *S. solidus* than females, which is in accordance with a previous study concerning the trophic morphology of the fish in Thingvallavatn and Hredavatn (Kristjánsson, 2001). Males were found to have more limnetic characteristics (i.e.

longer jaw and gill rakers) than females, suggesting that they may consume more pelagic diet including copepods, the first intermediate host of *S. solidus*.

All these results suggest an association between infection patterns and foraging habitat, as ecological differences such as the abundance of intermediate hosts can affect the transmission rates of specific parasites (Halmetoja et al., 2000). The first intermediate host of *Displostomum* sp. is probably the aquatic snail *Radix peregra*, (previously known as *Lymnaea peregra*) as it is the only gastropod found at least in Thingvallavatn (Frandsen et al., 1989). Sticklebacks are infected when they come in contact with the *Diplostomum* sp. cercariae previously released from the infected snails (family Lymnaeidae). The cercariae penetrate the skin of the fish and migrate to the eye where they accumulate over repeated infections (Chappell, 1995, Karvonen et al., 2003, McKeown & Irwin, 1997, Whyte et al., 1991). The density of *Radix peregra* is higher in the shallow stony littoral zone (Snorrason, 1982) including the lava zone which explains the higher infection rate of the lava fish by *Diplostomum baeri*. Other differences observed mainly in the abundance and intensity of infection of *S. solidus* could probably be attributed to trophic specialization of the hosts. The first intermediate host of *S. solidus* is a wide range of Cyclopoid copepods (Smyth, 1946). Previous studies concerning the diet of the lava and mud sticklebacks in Thingvallavatn and Hredavatn did not reveal any difference in the occurrence of the Cyclopoid copepods between the two morphs (Kristjánsson, 2001), however these data might not reflect the actual diet preferences as the samples were taken during a single summer.

These differences found in the burdens of parasites could possible explain the parallel divergence in individual MHC variation observed between the sympatric morphs as fish from the lava and mud habitats showed different adaptive trends

concerning the optimum number of MHC alleles, however this was not consistent in the lava morph when *D. baeri* and *S. solidus* were examined separately (Figures 8 & 9). Mud morph individuals showed to harbour less parasite species when they possessed around five alleles (Figure 8), a pattern consistent for both *S. solidus* (Figure 9) and *D. baeri* (Figure 10). Conversely, individuals from the lava habitat showed a trend of harbouring less parasite species in general when they possessed around three alleles (Figure 8) and this was consistent for the cestode *S. solidus* (Figure 9). However, lava individuals with three alleles were more heavily infected by the trematode *D. baeri* than individuals with more alleles (Figure 10). This relationship was more pronounced in the lava population from Thingvallavatn where a significant negative correlation was observed between the number of alleles and abundance of *D. baeri* indicating that the infection level by the trematode is reduced when the number of alleles increases while on the contrary fish were significantly less infected by the *S. solidus* when they possessed three rather than four alleles. It is known that the lava morph moves to the lava littoral habitat only during the breeding season in order to spawn, in contrast with the mud morphs, while it is still unknown where they settle during the winter (Ólafsdóttir & Snorrason, 2009). It seems possible that in the environment where they live until they reach sexual maturity they are exposed to more limited range of parasites and only during the breeding period they move to the lava habitat where they come in contact with the high abundances of the *D. baeri*. Moreover parasites vary in their virulence and thus the selective impact of each parasite might differ in the formation of the individual MHC allelic variation. *Proteocephalus* sp. have not been associated with any harmful pathological effects on fish (Willemse, 1969). On the contrary, *Diplostomum* sp. have been associated with indirect host mortality as they affect the vision of their hosts making them more

vulnerable to predation and less effective in food acquisition (McKeown & Irwin, 1997, Owen et al., 1993) while *S. solidus* can have severe impact on the survival of its host and at the same time has detrimental effects on the growth and sexual development of sticklebacks (reviewed by Barber et al., 2008). It has been found that infected sticklebacks suffer from reduced performance during the breeding period and their chances to be preferred as mates are lower compared to uninfected fish (Barber et al., 1998). Thus, a possible explanation for the observed low MHC variation of the lava populations could be that the selective impact from *S. solidus* is higher compared to the *D. baeri* to which they are less exposed. It seems that the fitness cost imposed by the *S. solidus* is higher than that imposed by the *D. baeri* lowering the population mean as in that case it could be better to have multiple copies of fewer specific MHC alleles targeting the limited number of virulent parasites that they interact with (Matthews et al., 2010) in contrast with the mud morph sticklebacks that are exposed to the same parasitic fauna all year round. Moreover, our results seem to be in line with a recent study on limnetic and benthic stickleback species pairs from two lakes in British Columbia which showed that there is a consistent divergence in the individual MHC allelic richness between the two populations with limnetics having fewer alleles than the benthics (Matthews et al., 2010). Limnetic sticklebacks have also been found to generally suffer from higher parasite burdens than the benthics and more specifically being infected with higher abundances of *Scistocephalus solidus* and *Diplostomum scudderi* (MacColl, 2009). However, the benthic-limnetic system seems more complex than the mud-lava due to the existence of contrasting parasite communities which defer in their prevalence between the two species-pairs (MacColl, 2009).

Lack of information concerning the ecology of Bretavatn and Baularvallavatn and the genetic structure of their populations makes it more difficult to explain what causes the low MHC variation observed in these lakes. The littoral rocky-mud habitat in Baularvallavatn has a lot of similarities with the lava habitat concerning the prevalence and abundance of specific parasites (Figure 3 & 4). In addition, individuals possessing three or four alleles do not only seem to be infected with fewer parasites species but also seem less susceptible to the *S. solidus* and *D. baeri* parasites (Figure 9 & 10). Thus the low variation observed in the individual number of MHC alleles at the rocky-mud habitat could be associated with foraging habitat and selection pressures imposed by the parasites similar to the lakes with parapatric morphs.

In contrast with Baularvallavatn, which seems to follow a parallel pattern with the lava habitats, the observed MHC variation in Bretavatn was lower compared to the mud morphs despite the similarities in the habitat type. Strong genetic drift or insufficient selection for higher allele number can not be ruled out. However, sexually mature sticklebacks from the population in Bretavatn were shorter than the rest of the populations examined (Table 1) while at the same time they displayed more intense secondary sexual characters (i.e. blue eyes, bright red throat and blue-green body). Small size at maturity could indicate selection for early reproduction due to predation risk (McPhail, 1977) or resource availability (Schluter, 1995). Thus low MHC variability is possibly the result of reduced investment in the immune system compared to reproduction strategies such as the development of secondary sexual characters. However, the slightly different parasite communities observed in this lake (due to the presence of the unidentified nematode) and the observed adaptive trend of individuals with three alleles to be less intensively infected by the parasites *S. solidus* and *D. baeri* (Figure 9(a) & 10(a)) suggests that selection imposed by the parasites

has played also a role in shaping the individual MHC variation observed. Therefore, the mud population in Bretavatn might not represent the ecological variation of the mud sites in the two lakes with parapatric morphs.

In summary, our results demonstrate that there were consistent differences in the parasitic burdens between lava and mud morph sticklebacks in two lakes. A similar pattern was also observed between populations sampled from a mud and a rocky-mud habitat from two single morph lakes. Lava sticklebacks as well as fish from the rocky-mud habitat harboured more parasite species per individual compared to fish from the mud habitat while at the same time lava morphs exhibited reduced individual MHC diversity compared to their sympatric mud morph sticklebacks. The observed low MHC variability could partly be attributed to the effect of strong genetic drift however this does not seem sufficient to explain the observed patterns in the individual MHC classIIB allelic richness. Moreover, in all populations individuals with allele number close to the observed mean showed an adaptive trend by harbouring less parasite species and being less infected by the cestode *S. solidus* and/or by the trematode *D. baeri* that represent the most abundant species that were found to defer between the populations studied. Thus, the differences in the MHC allele number observed between the populations and the parallel divergence between the sympatric morphs may possible have been caused by different selection pressures imposed by varying abundance of parasite species found in contrasting habitats.

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Tables

Table 1. Prevalence (P %) and mean intensity (MI) of parasitic infection in threespine sticklebacks from four lakes. Lakes Thingvallavatn and Hredavatn are inhabited by two distinct morphs –lava and mud-. The last two rows show the mean standard length and mass of the samples (± S.E.).

		Site											
		Bretavatn (n = 100)		Baularvallavatn (n = 104)		Thingvallavatn				Hredavatn			
	Habitat	Mud		Rocky-mud		Mud (n = 77)		Lava (n = 97)		Mud (n = 91)		Lava (n = 80)	
Class	Parasite species	*P* (%)	*MI*	*P* (%)	*MI*	*P* (%)	*MI*	*P* (%)	*MI*	*P* (%)	*MI*	*P* (%)	*MI*
Cestoda	*Schistocephalus solidus*	38	3.47	77.9	4.17	35.1	2.41	64.9	4.98	80.2	6.08	78.8	5.03
Trematoda/Digenea	*Diplostomum baeri*	43	3.91	100	23.87	85.7	9.21	100	119.82	53.8	4.73	88.8	31.48
Trematoda/Digenea	*Diplostomum spathaceum*	25	1.24	6.7	1.86	2.6	1	13.4	1.15	9.9	1.33	10	1.56
Trematoda/Digenea	*Apatemon sp.*	20	1.5	64.4	2.85	67.5	3.33	89.7	3.16	46.2	3.21	50	2.6
Cestoda	*Proteocephalus sp*	12	1.33	25	2.12	31.2	2.38	24.7	2.38	9.9	2.11	15	3.33
Nematode	Unidentified	27	1.26	-	-	-	-	-	-	-	-	-	-
TOTAL		79	5.23	100	29.62	90.9	12.93	100	126.64	96.7	9.57	98.8	34.33
Mean SL ± S.E. (mm)		37.53 ± 0.48		41.72 ± 0.55		40.91 ± 0.65		41.10 ± 0.41		43.08 ± 0.43		40.94 ± 0.37	
Mean mass ± S.E. (g)		0.72 ± 0.04		0.85 ± 0.04		1.18 ± 0.07		1.12 ± 0.04		1.16 ± 0.05		0.89 ± 0.03	

Table 2. Mean abundance (MA) of parasites in sticklebacks from four lakes (*** $p < 0.001$ – Bonferroni adjusted for 15 tests, comparisons between sympatric morphs (lava-mud) found in Thingvallavatn and Hredavatn and between mud morph sticklebacks from Bretavatn and Baularvallavatn. ANCOVA was performed in ranked data and standard length was included as a covariable).

	Site								
	Bretavatn	Baularvallavatn		Thingvallavatn			Hredavatn		
Habitat / Parasite species	mud	rocky-mud	*F-value*	mud	lava	*F-value*	mud	lava	*F-value*
	MA	MA	*(1,200)*	MA	MA	*(1,170)*	MA	MA	*(1,164)*
S.solidus	1.32	3.25***	21.380	0.84	3.24***	21.528	4.88	3.96	0.019
Proteocephalus sp.	0.16	0.53	3.977	0.74	0.59	1.197	0.21	0.5	0.479
Nematode	0.34	-	-	-	-	-	-	-	-
D. baeri	1.68	23.87***	368.753	7.90	119.82***	509.851	2.55	27.94***	102.182
Apatemon sp.	0.30	1.84***	41.489	2.25	2.84	5.623	1.48	1.3	0.759
D. spathaceum	0.31***	0.13	13.273	0.03	0.15	6.289	0.13	0.18	0.031

Table 3. Results from nested ANOVA showing effects of standard length (SL), sex, lake and habitat type (nested within lake) on the principal component scores of the first two factors extracted.

	Source	d.f.	SS	MS	*F*	*P*
F1	SL	1	74.957	74.957	187.0142	< 0.001***
	Sex	1	0.034	0.034	0.0848	0.771
	Lake	3	169.179	56.393	141.6986	< 0.001***
	Lake:Habitat	2	83.713	41.857	104.4303	< 0.001***
	Residuals	535	214.432	0.401		
F2	SL	1	17.89	17.892	23.446	<0.001***
	Sex	1	63.30	63.303	82.951	< 0.001***
	Lake	3	24.82	8.274	10.842	< 0.001***
	Lake:Habitat	2	30.29	15.147	19.849	< 0.001***
	Residuals	537	408.28	0.763		

Figures

Figure 1. Sampling locations within Iceland. Grey points indicate lakes inhabited by both stickleback morphs, -lava and mud- while black points indicate single morph lakes where fish were sampled from a mud (Bretavatn) and a rocky-mud habitat (Baularvallavatn).

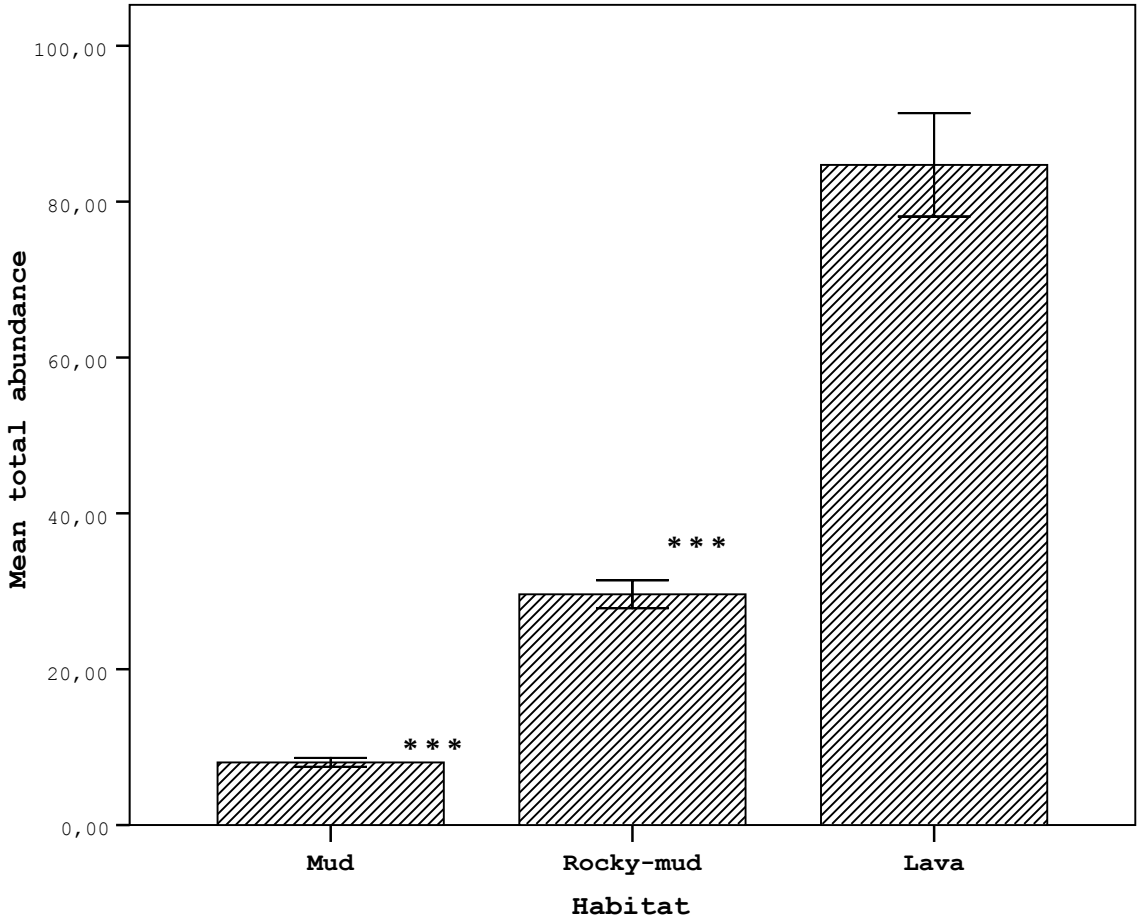

Figure 2. Mean total abundance (± 1 S.E.) of parasites (all species summed) differed significantly between sticklebacks from different habitat types ($p < 0.001$, Kruskal-Wallis test) with sticklebacks from rocky-mud habitat exhibiting intermediate levels that differed significantly from both the mud and the lava habitat (***$p < 0.001$ in both cases, Mann-Whitney test).

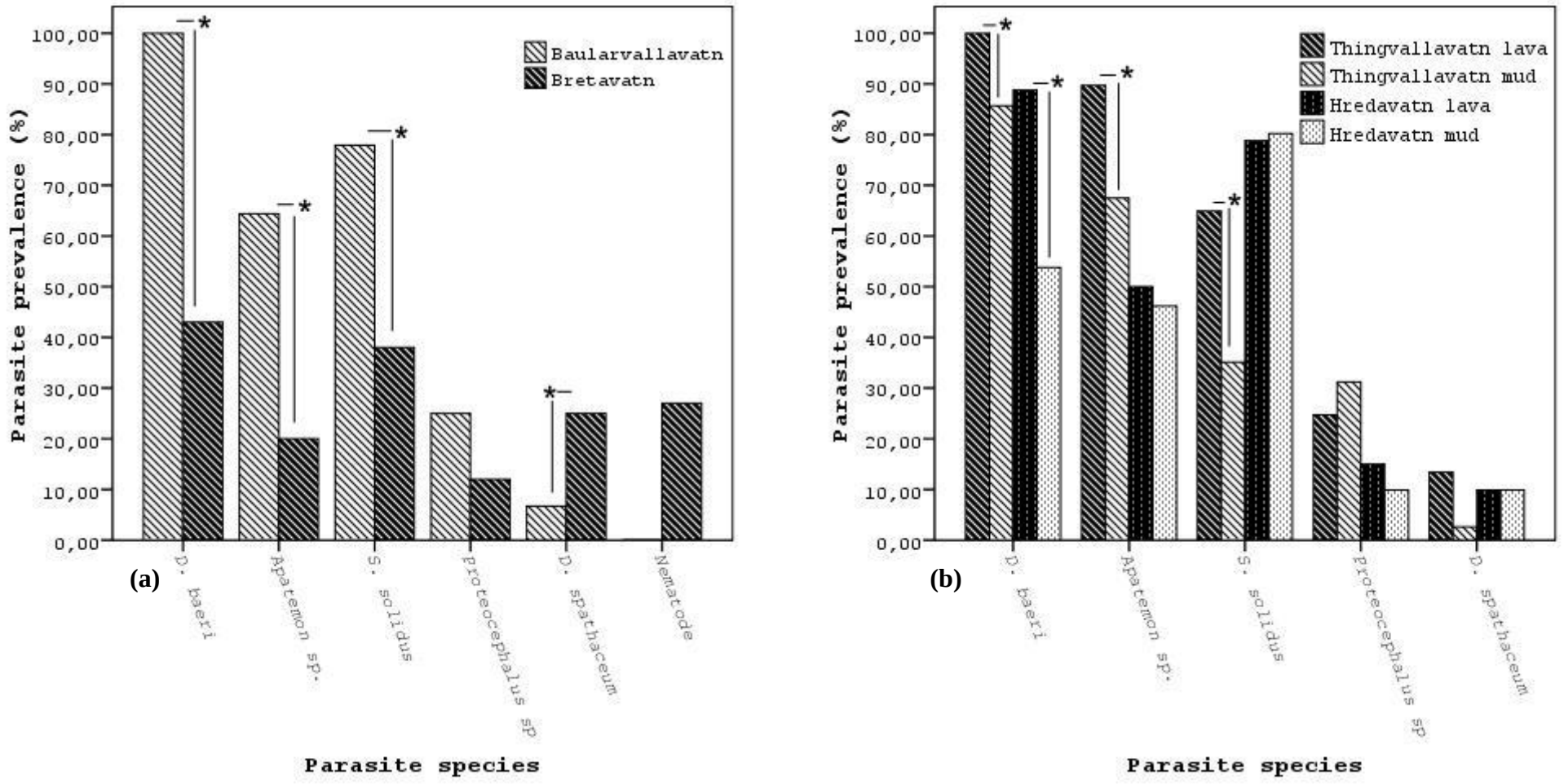

Figure 3. Prevalence of parasitic infection (a) of sticklebacks from Bretavatn (mud habitat) and Baularvallavatn (rocky-mud habitat) and (b) of sympatric morphs –lava and mud- in Thingvallavatn and Hredavatn. Prevalence of *D. baeri*, *Apatemon* sp and *S. solidus* was significantly higher in sticklebacks from Baularvallavatn in contrast to the prevalence of *D. spathaceum*. An unidentified nematode was present only in Bretavatn. Differences in the prevalence of *D. baeri* infection between sympatric lava and mud morphs were consistent in both lakes, while additional associations between habitat and prevalence of other parasite species were detected only in Thingvallavatn (*$p < 0.001$ - Bonferroni adjusted for 15 tests, x^2-test).

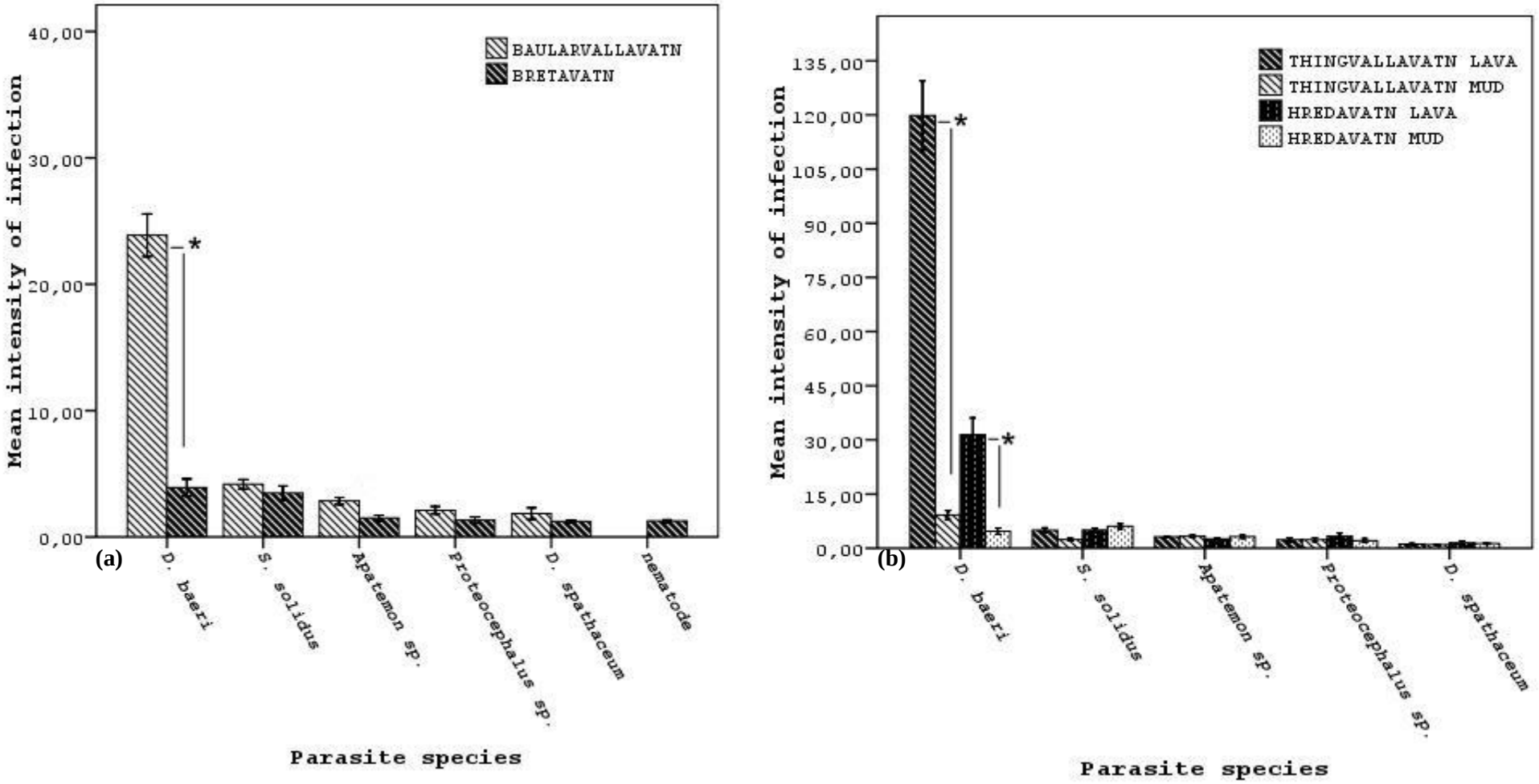

Figure 4. Mean intensity of parasite infection (± 1 S.E.) (a) of sticklebacks from Bretavatn (mud habitat) and Baularvallavatn (rocky-mud habitat) and (b) of sympatric morphs –lava and mud- in Thingvallavatn and Hredavatn. Mean intensity of *D. baeri* infection was higher in sticklebacks from Baularvallavatn compared to the ones from Bretavatn and in lava stickleback morph compared to mud morph in the sympatric populations (* $p < 0.001$, Bonferroni adjusted for 15 tests, ANCOVA in ranked data with standard length as a covariate.

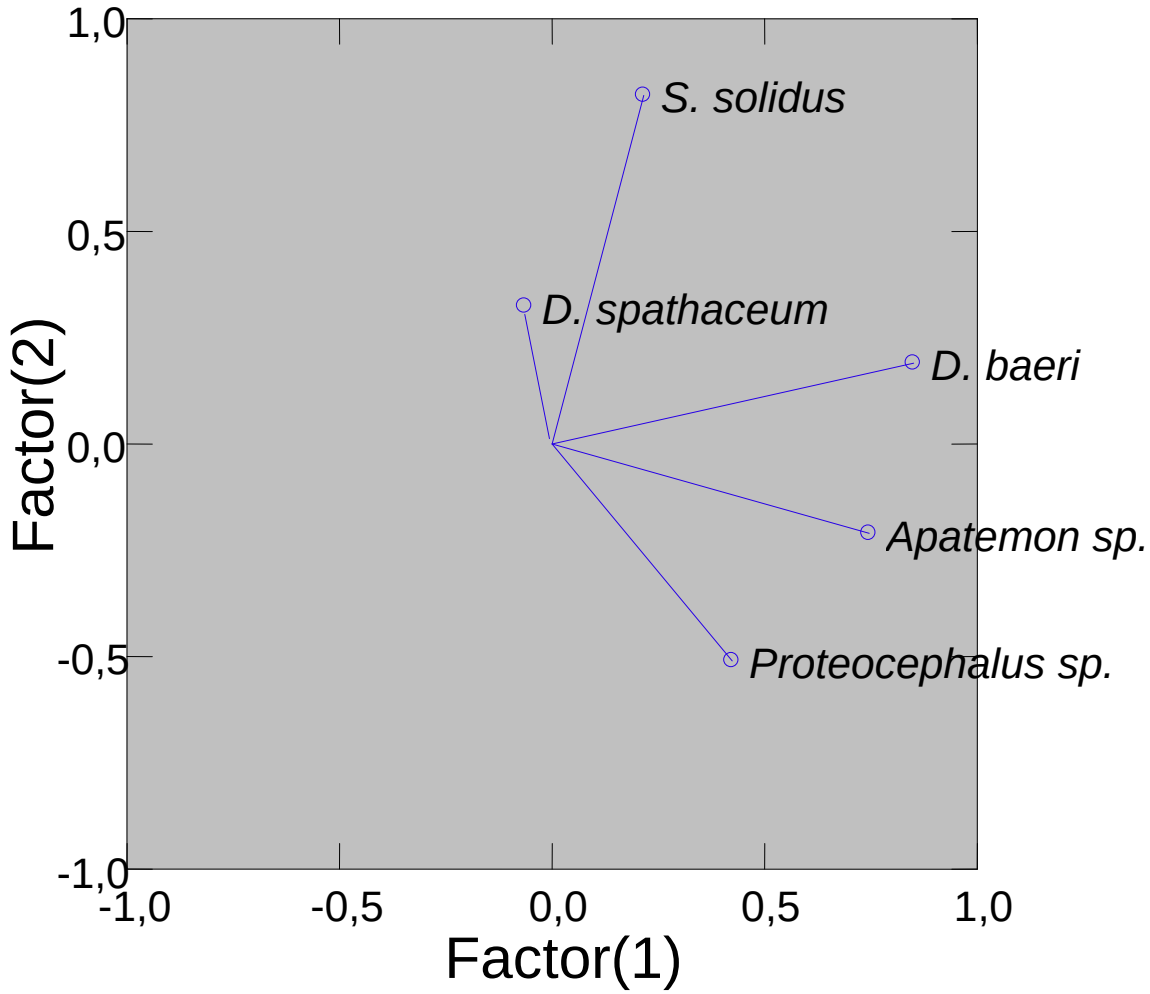

Figure 5a. Loadings of individual parasite species on the two PCA axes.

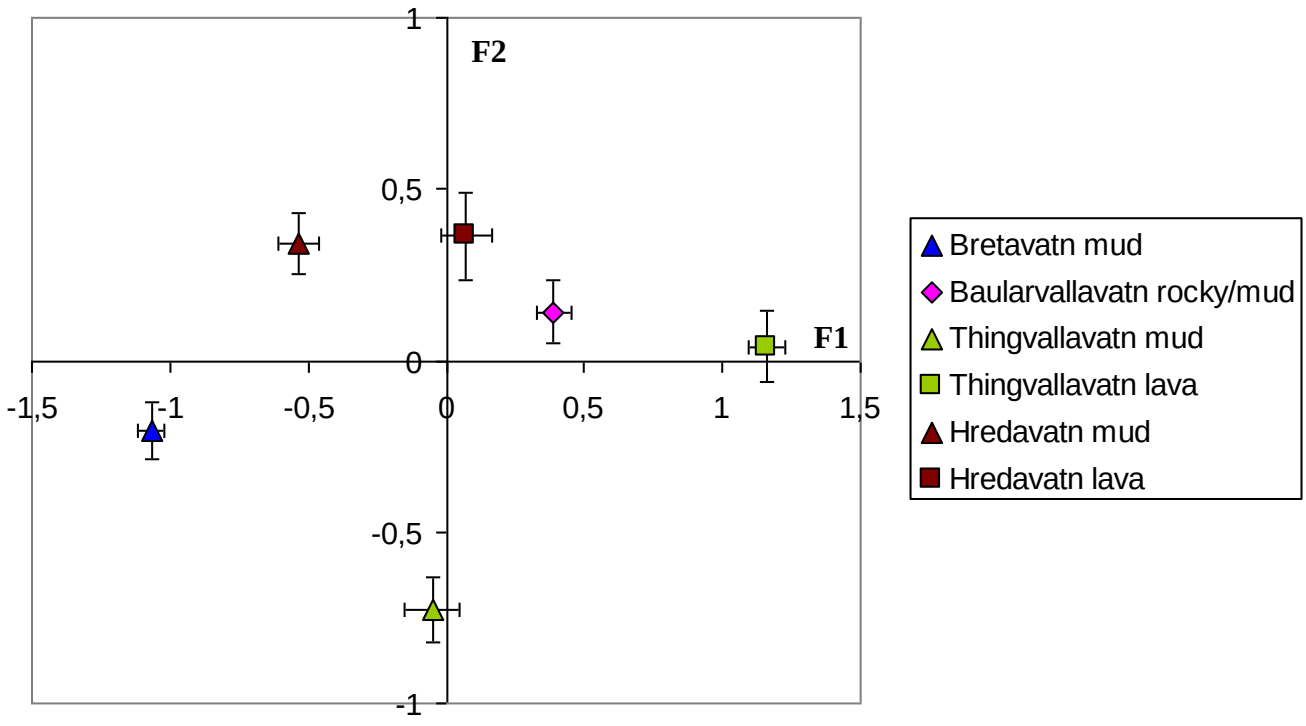

Figure 5b. Mean (± S.E. in both directions) of the scores on the first two factors extracted by the Principal component analysis grouped by population.

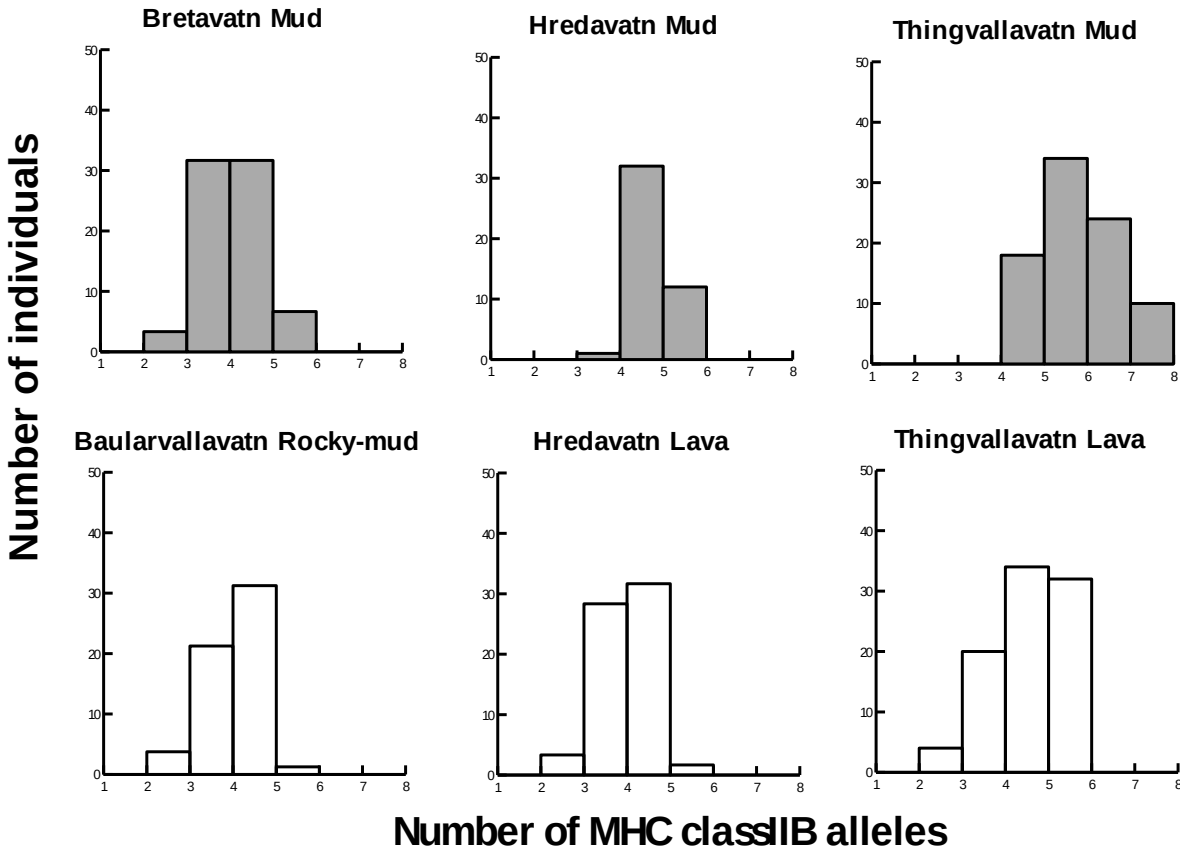

Figure 6. Frequency distributions of the number of MHC alleles detected by SSCP per individual.

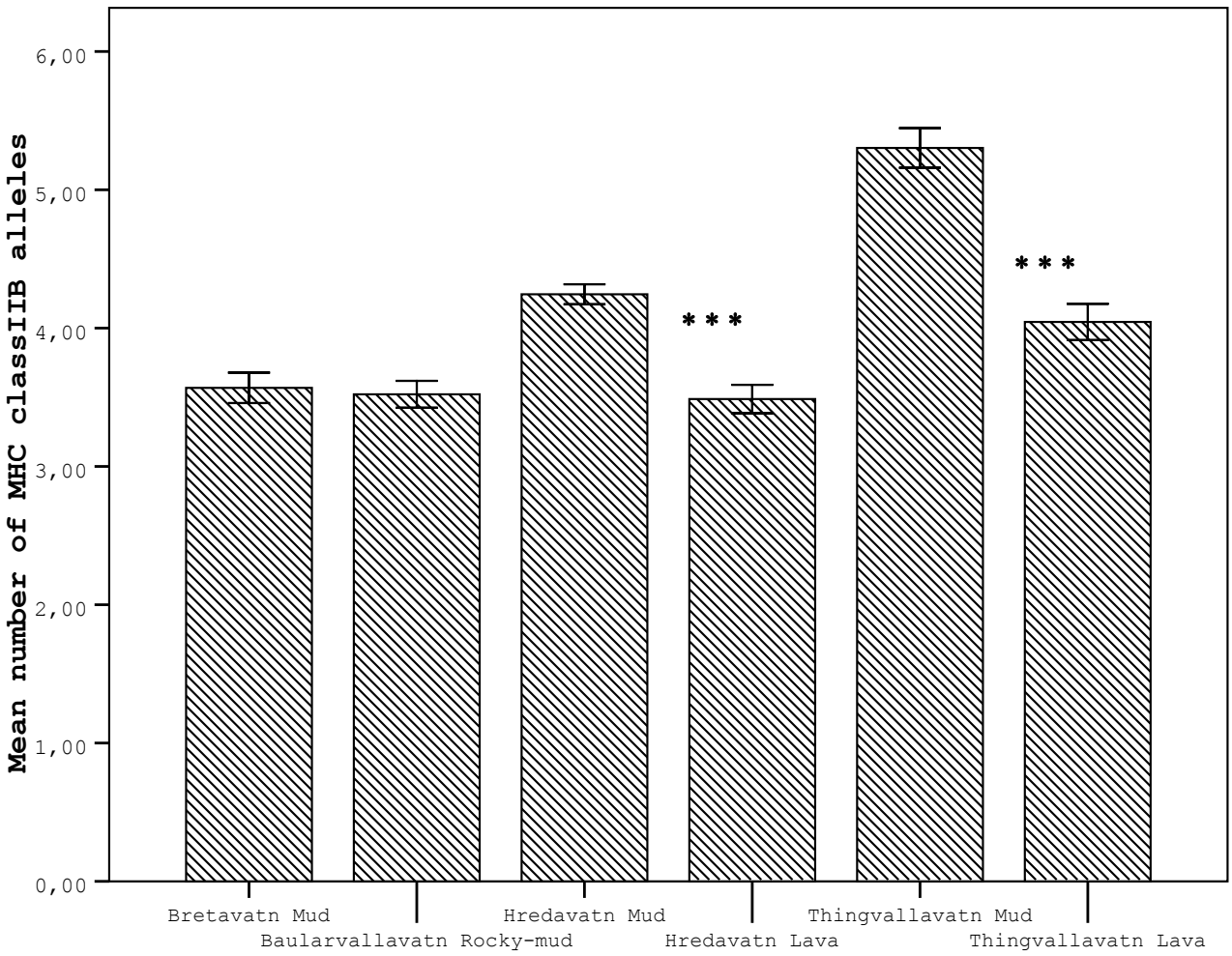

Figure 7. Mean number (± 1 S.E.) of MHC classIIB alleles in each population studied. Sympatric populations differed consistently in the number of MHC alleles with sticklebacks from the mud habitat having more alleles than sticklebacks from the lava habitat (*** $p < 0.001$, Mann-Whitney test).

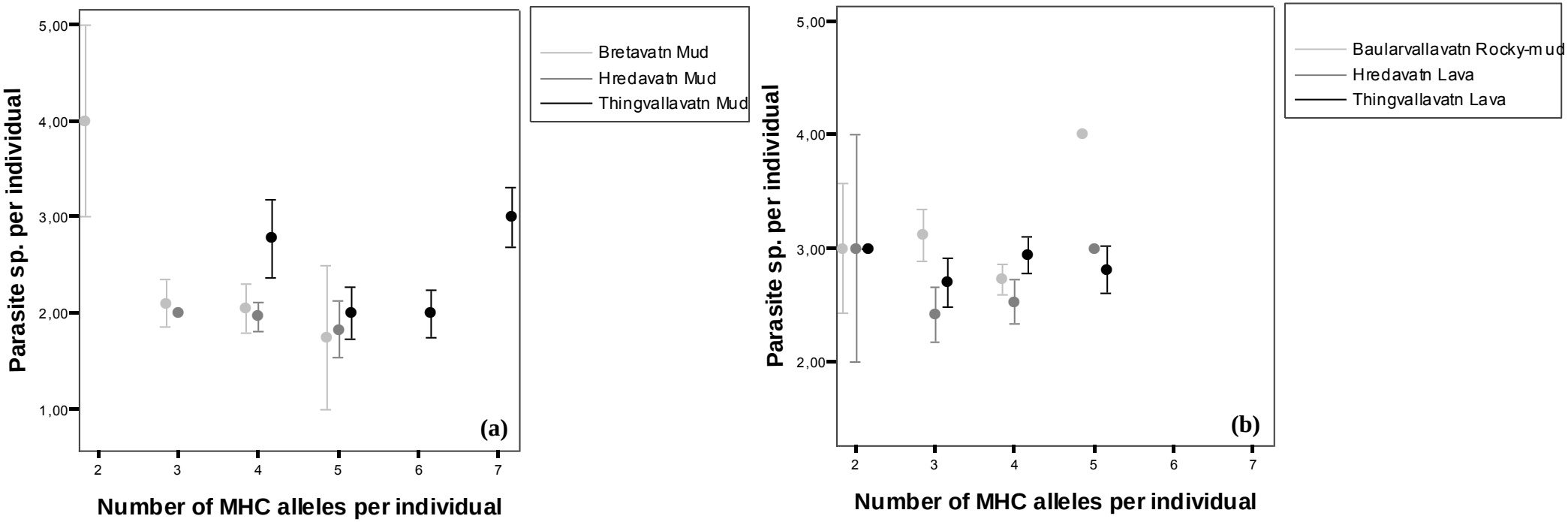

Figure 8. Number of MHC classIIB alleles vs. number of parasite species (mean ± 1 S.E.) present in individual fish. Fish tended to be infected with the lowest number of parasite species when possessing five (a) or three (b) alleles depending on the habitat of origin (mud and lava respectively) while an intermediate number in relation to these two habitats was detected in fish from the rocky-mud habitat (b).

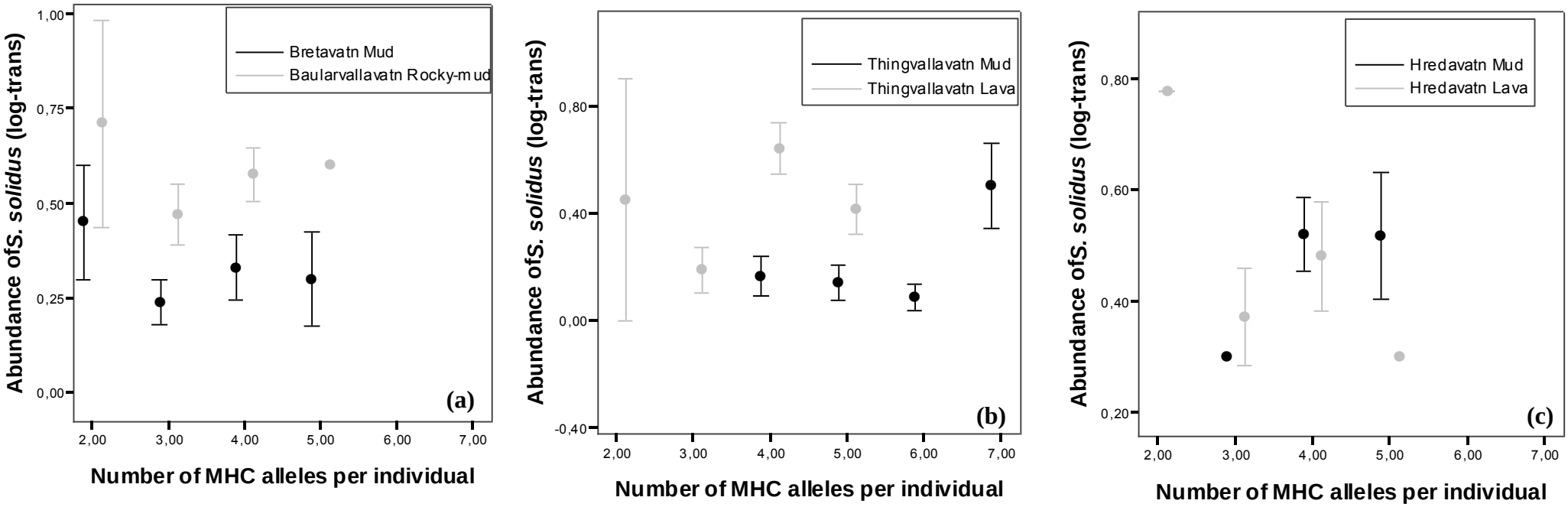

Figure 9. Number of MHC classIIB alleles vs. number of *S. solidus* (mean ± 1 S.E.) present in individual fish. Fish from Bretavatn, Baularvallavatn (a), but also from the lava populations (b and c) tended to be less heavily infected by the cestode *S. solidus* when they possessed three alleles (a) in contrast with mud morph individuals from Thingvallavant and Hredavatn (b and c) that were less heavily infected when they had more alleles (~5).

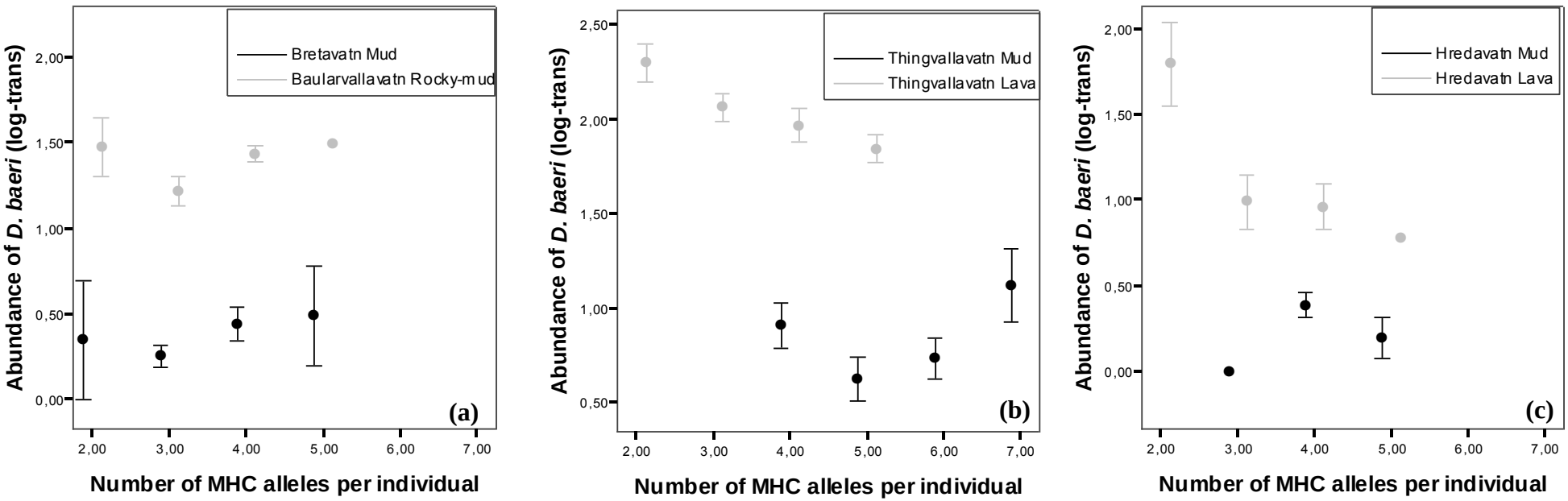

Figure 10. Number of MHC classIIB alleles vs. number of *D. baeri* (mean ± 1 S.E.) present in individual fish. Fish from Bretavatn, Baularvallavatn (a), tended to be less heavily infected by the trematode *D. baeri* when they possessed three alleles (a) in contrast with individuals from all the other populations (b and c) that were less heavily infected when they had more alleles (~5).

Manuscript II

Morphological variability and sexual dimorphism related to ecological opportunity in Icelandic threespine stickleback (*Gasterosteus aculeatus* L.)

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Abstract

Resource polymorphism and phenotypic differentiation is common in vertebrates as they adapt to local environments. Ecological specialization can promote morphological divergence both between populations and between the sexes. Ecological opportunity theory suggests that in heterogeneous environments with high intraspecific competition and low interspecific competition or predation pressure phenotypic variation increases as individuals that switch to under-exploited resources undergo weaker competition. The present study investigates sexual dimorphism and morphological variation in relation to substrate type and lake size among populations and the sexes in threespine stickleback (*Gasterosteus aculeatus*). Threespine stickleback were sampled from 34 sites in 22 lakes in Iceland inhabiting three main substrate types: mud, vegetation and rock. Lake size, generally representing increased niche availability, was positively correlated with phenotypic variance within populations and sexual dimorphism, most likely representing increased ecological opportunity. Finally, our result do not support that increased sexual dimorphism restricts phenotypic variation within each sex at the population level.

Introduction

Resource polymorphism and phenotypic differentiation due to adaptation to local environments is common in vertebrates (Robinson & Schluter, 2000, Skúlason & Smith, 1995, Smith & Skúlason, 1996). Selection acts on the morphology, behavior and/or physiology increasing the efficiency of local resource use and habitat use (Schluter, 2000). Different resource and habitat use may lead to population diversification which in turn may result in distinct morphs within species or even to speciation (Smith & Skúlason, 1996, Snorrason & Skúlason, 2004). Many ecological factors could promote divergence such as lake size, habitat complexity, food type, inter- and intraspecific competition and predation pressure (Robinson & Schluter, 2000, Schluter, 2000, Skúlason & Smith, 1995, Skúlason et al., 1999, Smith & Skúlason, 1996, Snorrason & Skúlason, 2004).

Exploitation of vacant niches according to the ecological opportunity theory is enhanced in heterogeneous environments with high intraspecific competition and low interspecific competition or predation pressure (Bolnick, 2001, Wilson & Turelli, 1986). Thus, phenotypic variation increases as individuals that switch to under-exploited resources experience weaker competition (Bolnick, 2001, Wilson & Turelli, 1986). This is empirically supported by a documented positive relationship between lake size, trophic niche availability (Barbour & Brown, 1974) and niche utilization of lake populations in threespine sticklebacks (Nosil & Reimchen, 2005).

Different age classes, sexes and individuals within a population can exploit different resources with resulting ecological specialization (Losos et al., 1998, Reimchen & Nosil, 2002, Reimchen & Nosil, 2004, Schluter, 1993 reviewed by Bolnick & Doebeli, 2003). Intersexual competition for resources can lead to sexual dimorphism while difference in specialization among individuals can lead to

population divergence. There is evidence for both being driven by similar processes (Bolnick & Doebeli, 2003, Reimchen & Nosil, 2004). Sexual dimorphism is common in nature. Generally, two mechanisms underlie sexual differences that are not directly related to reproduction, sexual selection (Darwin, 1879) and ecological niche partitioning (Shine, 1989, Slatkin, 1984). Sexual selection can lead to sexual dimorphism when the preference for a specific trait results in advanced fitness to males and females. For example, in sticklebacks it is common for females to be larger than males. This is generally believed to be sexually selected by male preference for larger females (Kraak & Bakker, 1998, Rowland, 1994).

Habitat heterogeneity may contribute to the evolution of sexual dimorphism. For example, in threespine stickleback (*Gasterosteus aculeatus*) there is evidence for differential habitat use by males and females often with males resembling benthic morph and females limnetic (Reimchen, 1980, Reimchen, 1984, Reimchen & Nosil, 2004, Reimchen & Nosil, 2006). It has been argued that increased sexual dimorphism within a population inhibits population divergence as available niches are already exploited by the sexes, eliminating disruptive selection within each sex (Bolnick & Doebeli, 2003). However, population divergence and trophic sexual dimorphism are not mutually exclusive for example both are found to exist in intralacustrine benthic and limnetic populations of threespine stickleback (McPhail, 1992, Spoljaric & Reimchen, 2008).

Threespine stickleback have colonized a great variety of freshwater habitats (small ponds, larger lakes, rivers) since the end of the Pleistocene period (10.000-15.000 years ago) and have undergone multiple adaptive radiations (Schluter, 1993, Taylor & McPhail, 1999) showing extraordinary diversity in morphology, behavior and life history (Bell & Foster, 1994, Kristjánsson et al., 2002a, Lavin & McPhail,

1985, McKinnon & Rundle, 2002, Wootton, 1984). In Iceland, stickleback exhibit high variation in morphology and behavior between sympatric populations and also between allopatric populations associated with different types of habitat. Population divergence in morphology has been detected between and within the lakes and also between the sexes (Kristjánsson et al., 2002a, Ólafsdóttir et al., 2006, Ólafsdóttir & Snorrason, 2009). Behavioral differences have been revealed in feeding and antipredator behavior while assortative mating between different morphs has been found within a lake (Doucette, 2001, Doucette et al., 2004, Ólafsdóttir et al., 2007).

In the present study we relate intra-population morphological variation and sexual dimorphism to ecological opportunity (substrate availability and lake size) in Icelandic threespine stickleback. First, we test if sexual dimorphism and population variation in morphology is related to substrate type and/or lake size. We expect that both or either sexual dimorphism and population variation to increase with lake size as in bigger lakes more niches are available to be exploited (Barbour & Brown, 1974, Nosil & Reimchen, 2005). Second, we test if there is a relationship between the degree of sexual dimorphism and population diversity in morphology within each sex. A negative correlation would support that the alternate utilization of vacant niches by males or females may inhibit population divergence (Bolnick & Doebeli, 2003).

Material and methods

Collection and handling of samples

Threespine stickleback were sampled from 22 lakes (a total of 34 sites) across Southwest, West and North of Iceland (Figure 1). The lakes differ in size, number and type of habitats. Fish were collected from all of three substrate types found in each lake: soft mud, rocky and vegetated, using benthic minnow traps (mesh size 3.2 mm) during June-July of 2008. All fish were anaesthetized using CO_2 solution, and then preserved in buffered 10% formalin before being stored in 70% ethanol. The fish were stained with Alizarin Red 1% KOH (e.g. Bell, 1982) to facilitate measuring of morphometric characters. Standard length, lateral plate length (the plate under the first dorsal spine), head length, body depth, upper-jaw length, snout length, eye diameter, pectoral fin length, first dorsal-spine length, pelvic-spine length, and pelvic-girdle length were measured from the left side of each fish with a digital caliper to the nearest 0.005 mm (Figure 2). From each population fish larger than 27 mm were randomly chosen. The left side of all the mature fish was scanned (HP Photosmart 6200) (e.g. Kalous et al., 2010). To visualize morphological differences in body shape, landmark-based geometric morphometrics was used covering the body shape with 22 landmarks on fixed morphological features and one sliding semi-landmark (Figure 3) using tpsDig program (Rohlf, 2008). Sliding semi-landmarks are defined relatively to other landmarks (e.g. between A and B landmark) and by sliding to the left or to the right on a curve that connects the other landmarks reducing the amount of shape change between each specimen and the centroid size of all specimens (Adams et al., 2004, Rohlf, 2010). Relative warp analysis was applied with tpsRelw software (Rohlf, 2010) to analyse for differences in morphology. The analysis aligns, rotates and scales the landmarks to a centroid size. Then the programme calculates

partial warp scores that represent the amount of bending energy needed for scaling all the coordinates of all fish to fit on the centroid size. The weight matrix of partial warp scores was saved and then used for further statistical analyses.

The sex of mature fish was determined by visual examination of the gonads. A total of 1012 fish were used in this study, 988 female and 624 males. For sexual dimorphism 33 populations out of 34 were used as the population from the mud habitat in Torfavatn consisted only of females while in sex-specific analyses 34 and 33 populations were used in female and male analyses respectively.

Statistical analysis

Statistical analyses were performed using SPSS (release 15.0.0). In order to correct for size all trait measurements were size-standardized by calculating the residual values from a regression of each of the 11 traits against the standard length. First, heterogeneity of slopes among populations was checked by analysis of covariate (ANCOVA) with standard length as a covariate. When the slopes were heterogeneous size-standardization was calculated using one regression slope by combining all populations while when the slopes were the same indicating similar allometry among populations, separate within-population slopes were used (Reist, 1985).

Bonferroni corrections (Miller, 1981) were applied when multiple comparisons were made to reduce Type I error. The p-value was adjusted to be compared directly to a significance level of 0.05.

- Association between morphological variability, substrate type and lake size

Separate analysis was undertaken for male and female stickleback in order to eliminate the effect of sexual dimorphism from population comparisons. The

allometric slopes of body depth (only in females), 1st dorsal spine, pectoral fin, jaw and snout length (only in males) did not differ among the populations and thus residuals were calculated by separate within-population regressions. Only the size-free residuals of the trait values were used for further analysis. In order to reduce the dimensionality of the morphological data set principal component analysis (PCA) of these residuals was conducted for male and female stickleback separately. Variance of PCA scores was calculated within each population for each axis. Residual variance extracted from a regression of population variances against population means was used as a measure of variability instead of coefficient of variation, as residual variance controls for associations between means and variances more precisely than the coefficient of variation (Nosil & Reimchen, 2005). Possible associations between residual variance and substrate type were examined by One-way ANOVA with substrate type as factor. Correlation between residual variance and lake size was tested by using non parametric Spearman's correlation as the residual variances calculated for each population were not normally distributed. PCA was also performed on the weight matrix of the partial warp scores obtained from sexually mature male and female stickleback (analysed separately) and the same analyses as described above applied to the PC scores.

- Sexual dimorphism, substrate type and lake size

The same procedure described above was performed in order to correct all traits for size but including both sexes in the analysis. The allometric slopes of all traits differed between populations and thus residual values were obtained by combing all fish from all populations as described above. For continued analysis the residuals of the trait values were used. Independent sample t-tests were used on each trait to

investigate differences between the sexes within each population. DFA analysis with sex as discriminant factor was performed on the measured traits combined and also on the weight matrix of partial wrap scores to check whether sex allows the two groups to be distinguished based on patterns in overall shape variation.

To test differences in the degree of sexual dimorphism among substrates, a dimorphic index was calculated based upon the DF scores (mean males minus mean females) of the DFA analysis performed on measured traits and from the weight matrix of partial warp scores (see above). Linear regression was used to investigate the relationship between lake size (log- transformed) and the dimorphic indices, while cubic regression were also used to check for departures from linearity. Finally, associations between the dimorphic indices and substrate type were checked by One-way ANOVA with substrate as factor followed by post-hoc tests.

- Correlation of sexual dimorphism and morphological variation within populations

Correlation between the PC axis residual variance (see above) and the sexual dimorphism index (calculated from the DF1 of measured traits) was tested with non parametric Spearman's correlation. The same analysis was performed using the sexual dimorphism index and residual variance calculated from the weight matrix of partial warp scores (see above).

Results

Association between morphological variability, substrate and lake size

The variation in morphology was reduced by principal component analysis for each sex separately. In male stickleback three factors were extracted accounting for 45% of the total variance. The first axis (PC1) explained 21% of the variance and was mainly associated with body depth and lateral plate length. The second axis (PC2) explained 11% of the variance and was correlated with traits associated with antipredator structures: pelvic spine, pelvic girdle and 1st dorsal spine length while the third axis (PC3) accounted for 11% of the total variance and was correlated with head morphology: snout, jaw and head length. No significant associations were observed between residual variance and substrate type (PC1: $F_{(2,30)} = 1.428$ and $p = 0.256$; PC2: $F_{(2,30)} = 0.700$ and $p = 0.505$; PC3: $F_{(2,30)} = 2.161$ and $p = 0.133$).

The residual variance (see materials and methods) of the PC1 and PC3 scores, calculated for each population, was positively correlated with lake size (Spearman's correlation coefficient: PC1: rho = 0.412 and p = 0.017; PC3: rho = 0.405 and p = 0.019, however after Bonferroni corrections the relationship was marginally significant: PC1: p = 0.051 and PC3: p = 0.057, Figure 4).

The principal component analysis performed on female stickleback gave two axis explaining 41% of the total variance. The first axis (PC1) accounted for 28% of the total variance and was positively correlated with snout, jaw and head length (head structure) while the second axis (PC2) explained 13% of the variance and was associated with pelvic spine, 1st dorsal spine and pelvic girdle length (antipredator structure). No significant associations were observed between residual variance and substrate type (PC1: $F_{(2,31)} = 0.679$, $p = 0.514$; PC2: $F_{(2,31)} = 0.407$, $p = 0.669$).

A significant positive correlation was observed between the residual variance of the second axis scores and lake size (Spearman's correlation coefficient: PC2: rho = 0.410 and p = 0.032, Bonferroni corrected) (Figure 5).

The same analyses performed on the PC scores obtained from the PCA performed on the weight matrix of the partial warp scores of male and female stickleback (analysed separately) did not show any significant relationship between shape variance and lake size (all $p > 0.557$) or substrate type (all $p > 0.133$) in either sex. A summary of all the tested associations between morphological variability, substrate type and lake size are given in Table 3.

Sexual dimorphism, substrate type and lake size

Most of the traits measured were found to be proportionally larger in male than female stickleback only the length of pelvic girdle was, in most populations, larger in females. Males tended to have longer heads (in 33 of 33 populations, 82 % (before bonferroni corrections) or 45% (after bonferroni corrections) differed significantly), longer jaws (in 31 of 33 populations, 67/39% significantly), snouts (in 30 of 33 populations, 67/33% significantly), first dorsal spines (in 26 of 33 populations, 54/23% significantly), deeper bodies (in 28 of the 33 populations, 46/7% significantly), bigger eyes (diameter, in 27 of 33 populations, 44/26% significantly), longer lateral plates (in 26 of 33 populations, 38/15% significantly), longer pelvic fins (in 17 of 33 populations, 41/6% significantly) and pelvic spines (in 27 of 33 populations, 44/19 % significantly) while females tended to have larger pelvic girdles (in 24 of 33 populations, 42/4% significantly) and larger standard length (in 28 of 33 populations, 68/32% significantly).

The discriminant function analysis performed on the measured traits correctly classified 77.5% (Jackknife procedure) of the individuals according to their sex. The DF1 was positively associated with the head, jaw and snout length while pectoral girdle length and standard length were negatively associated with the axis (Table 2). The discriminant function analysis performed on the weight matrix of partial warp scores correctly classified 85.2% (Jackknife procedure) of the individuals according to their sex. Male stickleback were mainly associated with positive DF1 scores while females with negative (Figure 6).

The degree of sexual dimorphism calculated for each population from the DF scores performed on the trait values (see materials and methods) was not associated with substrate type (mud, vegetation or rock) ($F_{(2,30)} = 3{,}025$, $p = 0.064$). However, the degree of sexual dimorphism on the overall shape morphology (DF1 scores from the Discriminant analysis on the weight matrix of the partial warp scores) was significantly associated with substrate type ($F_{(2,\ 28)} = 5.25$, $p = 0.012$). Post hoc tests with Bonferroni corrections revealed higher sexual dimorphism in vegetated habitats (both $p < 0.015$, degree of sexual dimorphism in overall shape: vegetation: 2.35; mud: 1.83; rock: 1.72).

The dimorphic index based on the traits values was not significantly correlated with the size of the lake (linear regression, $r^2 = 0.003$, $p = 0.750$). However, cubic regression analysis was found to explain significantly the relationship between the degree of sexual dimorphism and size of the lake ($r^2 = 0.317$, $F_{(3,29)} = 4.490$, $p = 0.01$, Figure 7). However, no significant associations were observed between the magnitude of the overall shape sexual dimorphism and lake size (linear regression, $r^2 = 0.025$, $p = 0.394$; cubic: $r^2 = 0.057$, $p = 0.659$). A summary of all the tested associations between sexual dimorphism, substrate type and lake size are given in Table 3.

Correlation of sexual dimorphism and morphological variation within populations

The only significant correlation between sexual dimorphism and morphological variance was found between the residual variance of male population PC3 (representing variation in head morphology) and the sexual dimorphic index of DF1 of the measured traits (Spearman's correlation coefficient: rho = 0.384, p = 0.027, Table 3). No association was observed between sexual dimorphism and morphological variance in female populations. Finally, no significant associations were observed between the magnitude of the overall shape sexual dimorphism index and residual variance of PC scores in either sex (all $p > 0.600$, Table 3).

Discussion

Under the ecological opportunity hypothesis increased phenotypic variance is expected in heterogeneous environments as exploitation of new resources is available (Simpson, 1953). New morphologies, that were previously selected against or not influenced by selection, could now be expressed resulting in increased phenotypic variance within the population (Roughgarden, 1972, Van Valen, 1965, reviewed by Schluter, 2000). In the current study, we found a positive correlation between lake size and variability of the PC1 and PC3 scores, representing body depth and traits in the head region respectively, in male stickleback populations. A positive relationship was also observed in female stickleback in PC2 scores (linked to defence structures: pelvic girdle, pelvic spine and 1st dorsal spine length). The increased variance in body depth and trophic related structures found in the head are in accordance with the greater niche availability hypothesis but the increased variance in defence structure traits contradicts with previous findings (Nosil & Reimchen, 2005), where variation in dorsal spine length and number of lateral plates was negatively correlated with lake size. The authors suggested that the decrease in phenotypic variance for the defence traits in larger lakes results from increased predation pressures that favour well developed defence traits. Lack of information concerning the predation regime in the lakes used in the present study makes it difficult to explain what causes the increased variance in the defence structure traits observed in larger lakes.

Previous studies (Nosil & Reimchen, 2005, Spoljaric & Reimchen, 2008) have demonstrated that sexual dimorphism is more expressed in large lakes than small ponds as in large lakes male and female stickleback have the opportunity to explore a wider range of different niches. In our study, a positive correlation between size of the lake and degree of sexual dimorphism was also present. However, a significant

departure from linearity was observed indicating that in our case the relationship is more complex perhaps because of a confounding effect of variable number of substrate types within each lake. Moreover, the degree of sexual dimorphism in the overall shape morphology based on the geometric morphometric analysis was found to be associated with substrate type suggesting that different ecological factors may enhance or hinder the expression of sexual dimorphism.

Sexual dimorphism in the body shape of threespine sticklebacks is widespread and usually parallel (Aguirre et al., 2008, Caldecutt & Adams, 1998, Kitano et al., 2007, Reimchen & Nosil, 2006, Spoljaric & Reimchen, 2008). In most studies males have been found to have larger heads, deeper body and smaller body size (Kitano et al., 2007, Reimchen & Nosil, 2006, Spoljaric & Reimchen, 2008). Many of the differences between the sexes can be attributed to disruptive selection as males and females utilize alternative resources (Reimchen, 1980, Reimchen et al., 2008) although sexual selection also plays a role (Darwin, 1879, O'Donald, 1967). The results from the current study are in line with previous reports. Males usually have deeper bodies, longer heads and jaws which reflect adaptations to a more benthic habitat and diet (Aguirre et al., 2008, Kitano et al., 2007, Walker, 1997) while females exhibit larger pelvic girdles which is consistent with the higher predation risks that they may encounter due to their more limnetic distribution (Reimchen, 1994, Reimchen et al., 2008). However, the snout, and eye diameter, that are usually found larger in males (Aguirre & Akinpelu, 2010, Kitano et al., 2007, Kristjánsson et al., 2002b) are indicative of a more limnetic diet (Caldecutt & Adams, 1998) suggesting that sexual selection plays an important role in the direction of trait divergence between the sexes. Most of the traits studied are used by males during the breeding period as part of their courting behaviour and thus are sexually selected (Foster, 1994,

Kitano et al., 2007) and the same applies for the traits found larger in females (body size, girdle) as they have associated with higher reproductive rates (Kitano et al., 2007, Kraak & Bakker, 1998).

Finally, we expected the phenotypic variation of traits that exhibited increased sexual dimorphism to be reduced within each sex because male and female stickleback would be substantially differentiated and occupied the available alternative niches (Bolnick & Doebeli, 2003). Natural selection would then be expected to stabilize the trait value at an optimum that confers the higher fitness advantage to each sex (Bolnick & Doebeli, 2003). However, the only significant association detected between the degree of sexual dimorphism and residual variance was positive and concerned the residual variance of PC3 that represents head morphology in male stickleback populations. This finding suggests that sexual dimorphism does not always result in reduced trait variance within each sex i.e. sexual dimorphism does not necessarily hinder the evolution of different morphs.

In conclusion, we find that stickleback populations in smaller lakes with low habitat heterogeneity exhibited lower trait variance compared to populations found in larger lakes. These results suggest that in lakes with higher niche availability stabilizing selection towards specific phenotypes is more relaxed and exploitation of diversified habitats is promoted resulting in increased phenotypic variance. Finally, sexual dimorphism in body shape was found to be widespread and higher in larger lakes probably reflecting the increased ecological opportunity of the sexes.

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Tables

Table 1. Description of the landmark positions used in our study.

Landmark number	Landmark position
1	Anterior extend of maxilla
2*	Lachrymal at nasal capsule
3	Dorsal extent of orbit
4	Posterior extent of supraoccipital
5	Anterior insertion of first dorsal spine
6	Anterior insertion of second dorsal spine
7	Anterior insertion of third dorsal spine
8	Posterior extent of caudal peduncle
9	Posterioventral extent of preopercular
10	Insertion point of pelvic spine into the pelvic girdle
11	Dorsal extent of the ascending branch of the pelvis
12	Posterior extent of ectocorocoid
13	Dorsal extent of ectocorocoid
14	Anterior extent of ectocorocoid
15	Posteriodorsal extent of operculum
16	Ventral extent of operculum
17	Anteriodorsal extent of operculum
18	Dorsal extent of preopercular
19	Posterioventral extent of preopercular
20	Anterioventral extent of preopercular
21	Centre of eye
22	Upper origin of pectoral fin
23	Lower origin of pectoral fin

*Sliding landmark between landmarks 1 and 3.

Table 2. Loadings on Discriminant function Axis 1 from analysis performed on 11 morphometric characters for male and female stickleback.

Variable	Loadings
Head length	0.682
Jaw length	0.577
Snout length	0.531
Standard length	-0.374
Body depth	0.286
Lateral plate length	0.245
Dorsal spine length (1^{st})	0.238
Eye diameter	0.238
Pelvic spine length	0.233
Pelvic girdle length	-0.176
Pectoral fin length	0.093

Table 3. Summary of the main results of the current study. Association between degree of sexual dimorphism (mean male minus mean female based upon the DF scores obtained from DFA on the morphological traits (a) and on the weight matrix of partial warp scores (b)) and lake size, substrate type and residual variance calculated for each population based upon the PC scores obtained from PCA on the morphological data (A) and on the weight matrix of partial warp scores (B), males and females analysed separately; and association between residual variance and lake size and substrate type. Bold letters indicate that the relationship was significant. Note: * and ** indicate that the tests were marginally significant after Bonferroni corrections (p = 0.051 and 0.057 respectively).

			Males (A)			Females (A)		Males (B)			Females (B)		
Sexual dimorphic index calculated from:	Lake size	Substrate type	Res. variance PC1	Res. variance PC2	Res. variance PC3	Res. variance PC1	Res. variance PC2	Res. variance PC1	Res. variance PC2	Res. variance PC3	Res. variance PC1	Res. variance PC2	Res. variance PC3
a) DF scores - traits measured	Linear regr.: $r^2 = 0.003$ **Cubic regr.: $r^2 = 0.317$**	$F_{(2,30)} = 3.025$	rho = 0.256	rho = 0.10	**rho = 0.384**	rho = -0.136	rho = -0.088	-	-	-	-	-	-
b) DF scores- morphometric analysis	Linear regr.: $r^2 = 0.025$ Cubic regr.: $r^2 = 0.057$	**$F_{(2,28)} = 5.25$**	-	-	-	-	-	rho = -0.017	rho = 0.600	rho = 0.017	rho = -0.098	rho = 0.109	rho = -0.069
Lake size	-	-	**rho = 0.412 ***	rho = 0.067	**rho = 0.405 ****	rho = 0.247	**rho = 0.41**	rho = -0.110	rho = -0.024	rho = 0.069	rho = -0.075	rho = -0.239	rho = -0.069
Substrate type	-	-	$F_{(2,30)} = 1.428$	$F_{(2,30)} = 0.70$	$F_{(2,30)} = 2.161$	$F_{(2,31)} = 0.679$	$F_{(2,28)} = 0.407$	$F_{(2,28)} = 1.739$	$F_{(2,28)} = 0.619$	$F_{(2,28)} = 2.364$	$F_{(2,31)} = 0.239$	$F_{(2,31)} = 2.081$	$F_{(2,31)} = 0.234$

Figures

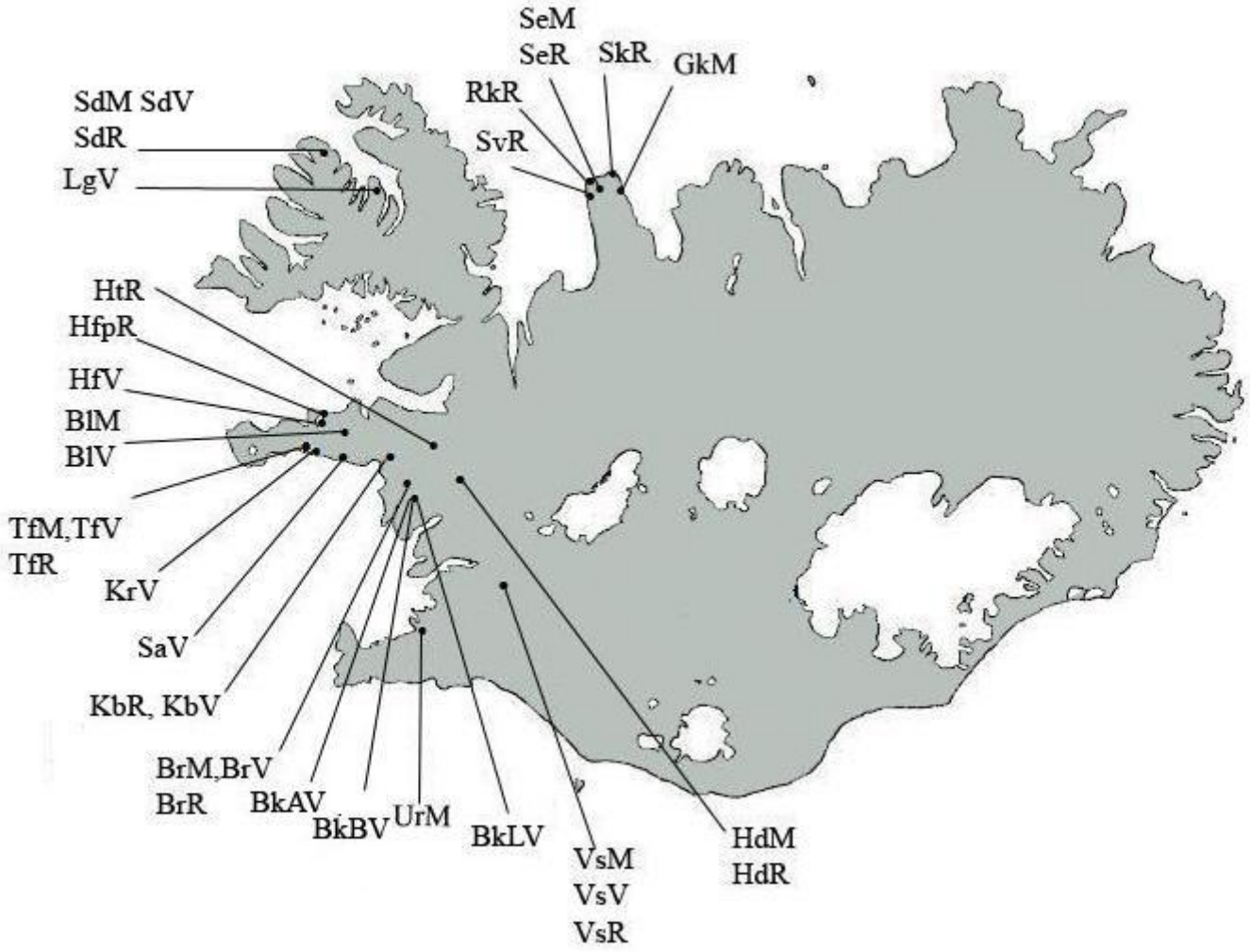

Figure 1. Sample localities of the stickleback populations used in this study. Type of substrate (Mud = M, Vegetation = V, Rock = R) and Lake abbreviations (Sd, Sydradalsvatn; Lg, Laugabolsvatn; Ht, Hitarvatn; Ffp; Hf, Hraunsfjordur; Hfp, Hraunsfjordur pond; Bl, Baularvallavatn; Tf, Torfavatn; Kr, Kirkjuholl; Sa, Sauratjorn; Kb, Kolbeinsstadatjarnir; Br, Bretavatn; Ur, Urridakotsvatn; BkL, Brokavatn lake; BkA Brokavatn pond a; BkB, Brokavatn pond b; Vs, Vatnsvik (in Thingvallavatn); Hd, Hredavatn; Gk, Galtakarsvotn; Sk, Skaletjorn; Se, Selvatn; Rk, Reikavatn; Sv, Sveinningstjorn.

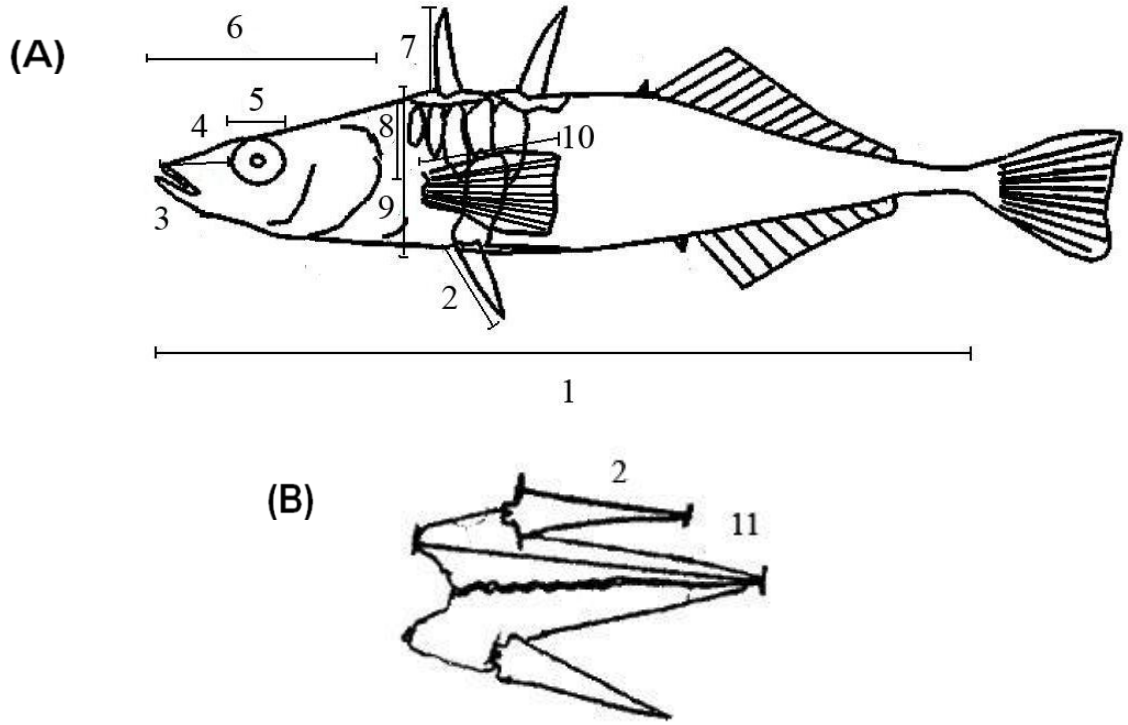

Figure 2. Schematic figure of morphological traits of the left side (A) of a threespine stickleback used in the current study. Standard length (1), pelvic-spine length (2), upper-jaw length (3), snout length (4), eye diameter (5), head length (6), first dorsal-spine length (7), lateral plate length (8), body depth (9) and pectoral fin length (10). Schematic figure of a ventral side (B) of pelvic-spine length (2) and pelvic-girdle length (11).

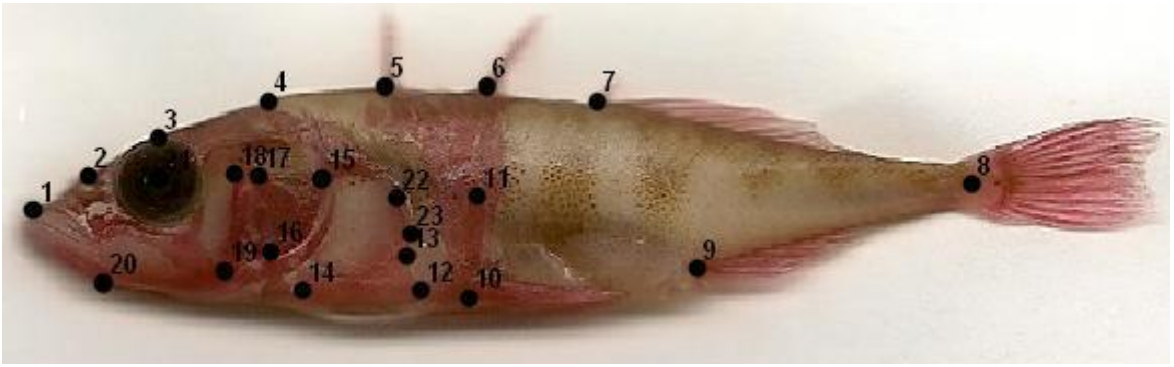

Figure 3. Location of 23 landmarks used for geometric morphometric analysis (Table 1). Landmark number 2 is sliding semi-landmark.

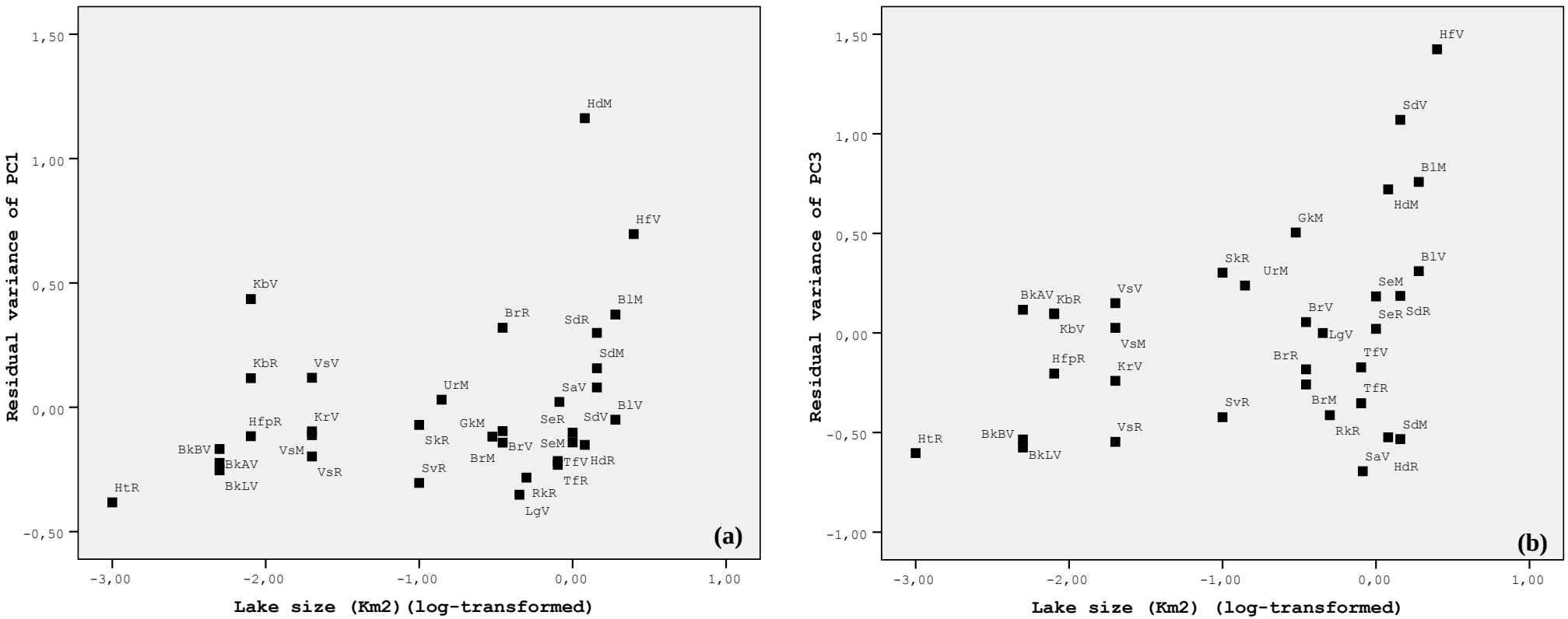

Figure 4. Relationship between residual variance calculated for each PC axis and lake size (using only male stickleback). A positive correlation between residual variance and lake size (km^2) was found for (a) PC1 (b) and PC3 (Spearman's correlation coefficient: PC1: rho = 0.412 and p = 0.017; PC3: rho = 0.405 and p = 0.019, however after Bonferroni corrections the relationship was marginally significant: PC1: p = 0.051 and PC3: p = 0.057). PC1 was mainly associated with body depth and lateral plate length while PC3 with traits that represent the head morphology: snout, jaw and head length. For site abbreviations see Figure 1.

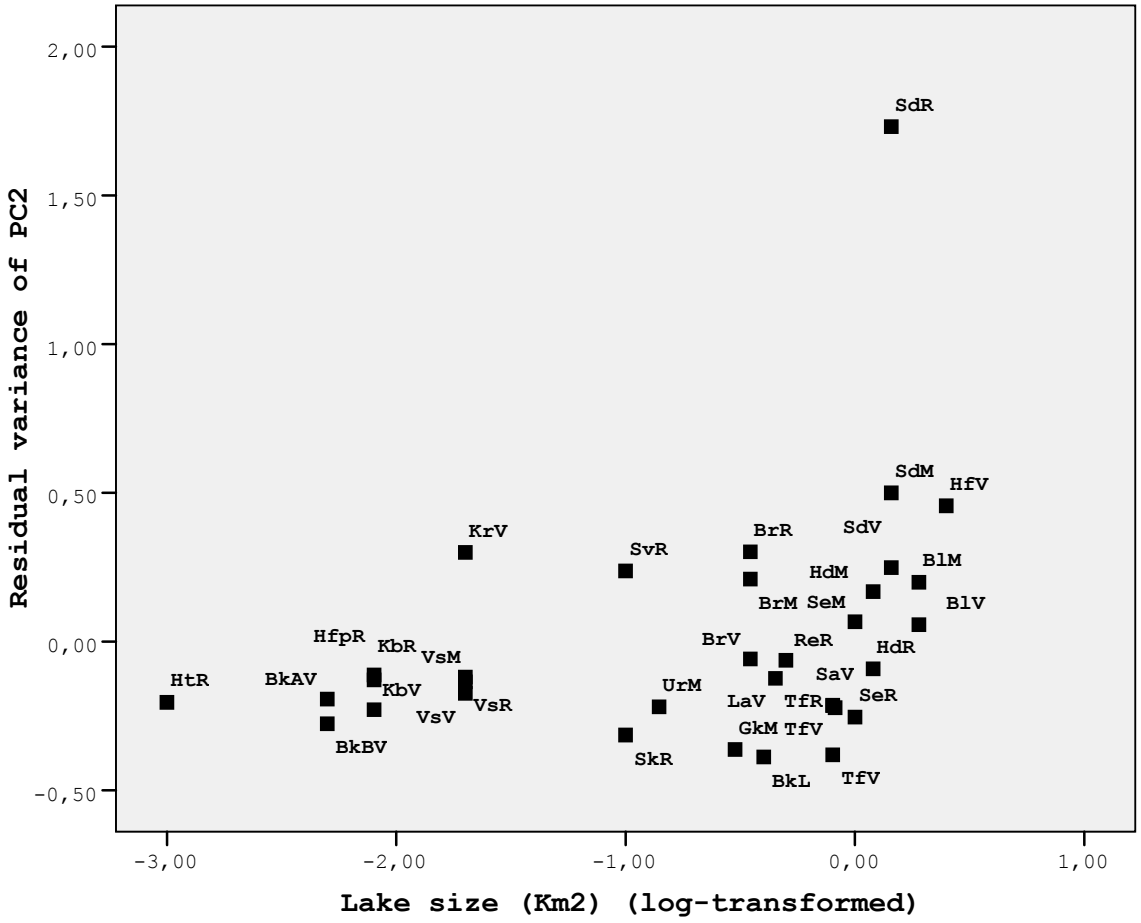

Figure 5. Relationship between residual variance calculated for each PC axis and lake size (using only female stickleback). A positive correlation between residual variance and lake size (km^2) was found for PC2 (Spearman's correlation coefficient: PC2: rho = 0.410 and p = 0.032, Bonferroni corrected). PC2 was mainly associated with pelvic spine, 1st dorsal spine and pelvic girdle length. For site abbreviations see Figure 1.

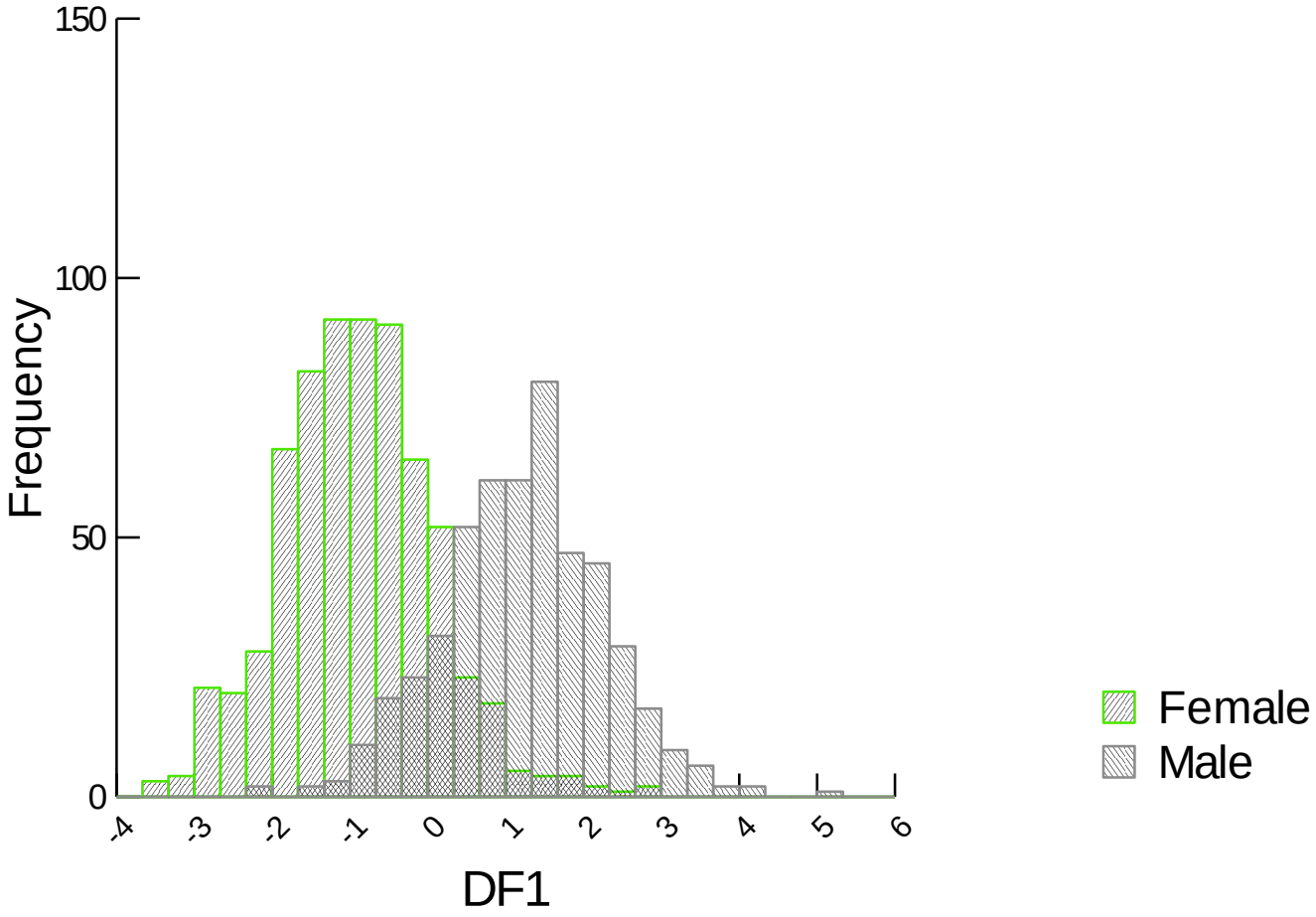

Figure 6. Scores from Discriminant analysis performed on the weight matrix of partial warp scores obtained from sexually mature stickleback. The first axis explained 100% of the variation observed between sexes. Male stickleback exhibited a bias towards positive scores while the opposite trend was observed in females.

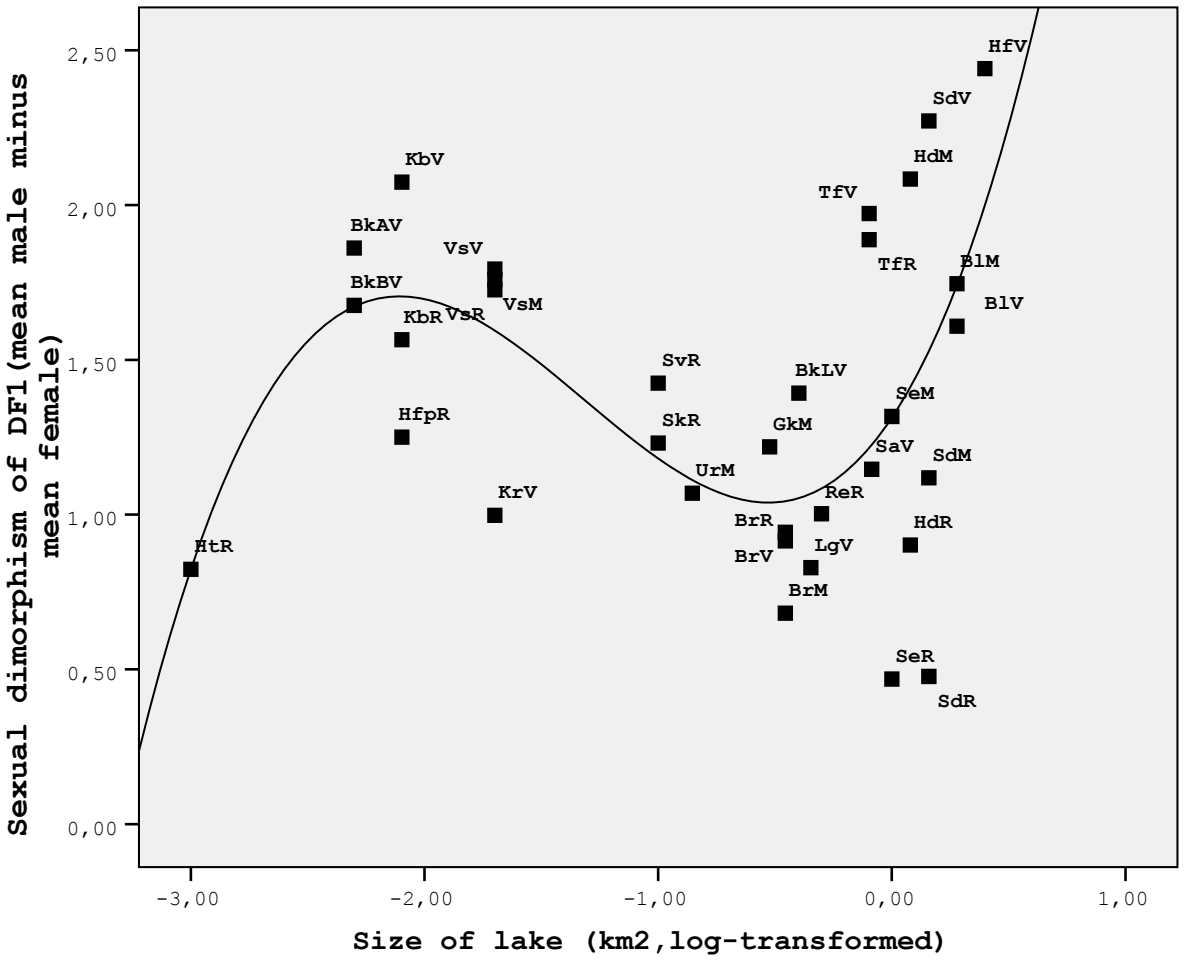

Figure 7. Relationship between lake size (log transformed) and degree of sexual dimorphism of DF1 obtained from discriminant analysis performed on eleven morphometric traits of male and female stickleback. The relationship observed was significantly explained by cubic regression (r^2= 0.317, $F_{(3,29)}$ = 4.490, p = 0.01). For lake abbreviations see Figure 1.