

The effect of temperature and diet on stickleback morphology

1. Abstract:

Due to current events throughout the world such as climate change, and increasing anthropogenic influence, organisms across the world are facing fluctuations in their environments. Consequently, ecology, evolution, and development have gained an increased interest over the past decades. In that regard, rapid adaptation is the key for an organism to deal with current global events. Adaptation may include a trait called phenotypic plasticity.

According to literature, three-spined stickleback (*Gasterosteus aculeatus*) are considered to have a high capacity for phenotypic plasticity, which is expressed in response to their environment. Factors such as temperature and diet are known to the sticklebacks phenotype based on its capacity for phenotypic plasticity. The studied stickleback population of Lake Mývatn, Iceland, is known to show a high morphologic divergence despite having no genetic divergence. Lake Mývatn offers a heterogenous environment with spatial variations in temperature, vegetation, and invertebrate communities. This project aimed to study Lake Mývatn's stickleback population's morphology with its capacity for phenotypic plasticity responding to contrasting temperature and diet treatments.

The tested sticklebacks originated from families crossed from a parental generation that has been collected from two areas of Lake Mývatn with contrasting ambient temperatures and invertebrate community compositions. Artificial crosses were created in the lab and each clutch was split in half to be incubated and reared at 13°C or 21°C. Afterwards, the split clutches were split in half again to be fed on cladocerans or bloodworms, resulting in four treatment combinations in total.

The results show that temperature and sex are the main factors for the morphological divergence of the tested sticklebacks in terms of body shape. Despite its highly significant effect size, the diet only contributed little to total variations of the body shape. Whilst the contribution of sex may be explained by the commonly known sexual dimorphism in stickleback, the contribution of the temperature may be linked to the stickleback's ectothermic metabolism. The mismatching with the parental generation's conditions did not affect the morphological variance in the results, which may be explained by the response of phenotypic plasticity to the environment, eventually relieving some possible negative effects from the mismatching conditions.

2. Introduction:

2.1. Plasticity and its response to environments:

Due to current events throughout the world such as climate change, extreme climate phenomena, and increasing anthropogenic influence, fields such as ecology, evolution, and development have gained a lot of interest over the past years. Understanding the biosphere and its ecosystems is of fundamental importance to understand how they may be affected by these current global events. In order to understand, how organisms may deal with those current changes across the globe, it is important to understand the concept of adaptation (Franks and Hoffmann 2012).

Due to the interaction between genes and the environment, different phenotypes may react differently to contrasting or fluctuating environments, respectively, environmental stimuli. Essentially, a phenotype results from the interaction between an individual's genotype with its environment (Plomin, DeFries, and Loehlin 1977; Barr et al. 2003; Williams et al. 2008). The trait phenotypic plasticity particularly describes to which extent an individual may be shaped by its environment (Colicchio 2017; Macagno et al. 2018; Skinner 2016; Álvarez-Quintero et al. 2021; Aiken, Tarry-Adkins, and Ozanne 2016; Duncan, Gluckman, and Dearden 2014). Generally, phenotypic plasticity describes the interaction between the environment and genes. Phenotypic plasticity offers the potential to develop several different phenotypes based on the very same genotype (Matesanz, Gianoli, and Valladares 2010; Price, Qvarnström, and Irwin 2003; Svanbäck, Pineda-Krch, and Doebeli 2009). Phenotypic plasticity becomes particularly meaningful for phenotypic divergence in populations with no genetic divergence, such as the Lake Mývatn stickleback population (Strickland et al. 2022).

2.2. Stickleback as environmental indicators:

The three-spined stickleback is a Holarctic fish species with populations spread over many different latitudes in the northern hemisphere (Fang et al. 2018). Stickleback are known to show sexual dimorphism, *e.g.* in breeding coloration or body shape (Kitano, Mori, and Peichel 2007; Ramler et al. 2014). Male stickleback for example are known to have larger heads in proportion to their body length and show a red breeding coloration during breeding seasons (Kitano, Mori, and Peichel 2007; Ramler et al. 2014; McGee and Wainwright 2013).

Literature provides a lot of examples of stickleback populations that possess a high capacity for phenotypic plasticity (McCAIRNS and Bernatchez 2012; Wund et al. 2008), therefore, stickleback are often studied as indicator for environmental fluctuations due to their high response to their environment (Katsiadaki et al. 2006; Olin et al. 2022). The aim of this project is to study how environmental fluctuations may affect the morphology of the three-spined stickleback (*Gasterosteus aculeatus*). For instance, this project aims to study the response of the stickleback to contrasting temperature and diet treatments.

Many stickleback populations are known to show a high response to their fluctuating environment, resulting in diverging morphologies. For instance, a well-known example would be the divergence of the stickleback into different ecotypes, such as the benthic and limnetic ecotypes (Figure 1). Both ecotypes show diverging morphologies regarding size and body shape (McGee, Schluter, and Wainwright 2013; WILLACKER et al. 2010; Härer, Bolnick, and Rennison 2021). Other studies were able to show how the ambient temperature contributes to diverging stickleback phenotypes (Ramler et al. 2014; Smith et al. 2020). *E.g.*, stickleback are known to be affected in their size by their ambient temperature (Ramler et al. 2014).

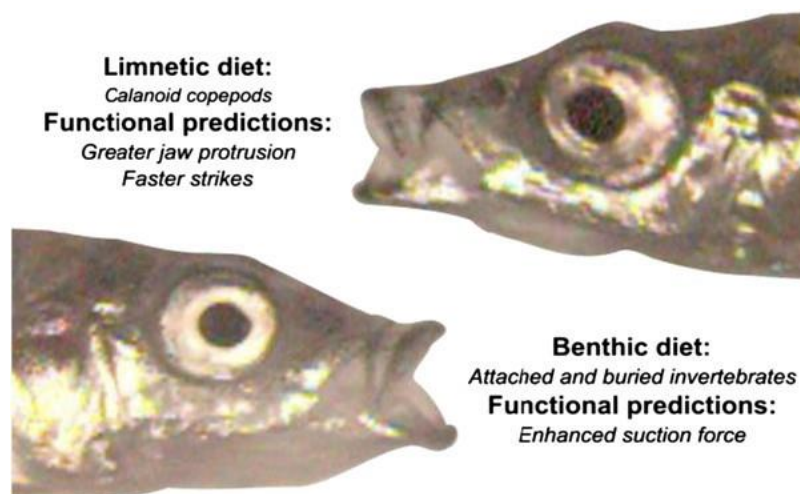


Figure 1: Comparison of limnetic and benthic stickleback ecotype diet and (head) morphology. From (McGee, Schluter, and Wainwright 2013)

2.3. Objectives:

This project aims to study the stickleback morphology of Lake Mývatn in response to contrasting ambient temperatures and diet compositions. Precisely, this project aims to study the relative contributions, respectively effects, of contrasting rearing temperatures and diet compositions to morphological variations between observed sticklebacks. To do so, a

transgenerational experiment with different treatments regarding rearing temperature (13°C *versus* 21°C) and diet (bloodworms *versus* cladocerans) will be conducted. Additionally, this project will also take parental origins into account to study how matching or mismatching rearing conditions in contrast to parental conditions may affect an individual's morphology.

2.3.1. Predictions for contrasting temperature treatments on stickleback:

Both temperature treatments (13°C and 21°C) were not at the optimum range for stickleback according to literature (Ramler et al. 2014; Dittmar et al. 2014; Lefébure, Larsson, and Byström 2011). Generally, the contrasting temperatures should have had an effect on the stickleback size and other morphological traits, since stickleback are ectotherms and rely on the temperature for their metabolic activities (Dittmar et al. 2014). Increased ambient temperatures will certainly increase the metabolic activity of the stickleback, but their nonlinear relationship with temperature (Ramler et al. 2014) makes it difficult to predict, how the contrasting temperature treatments may affect the stickleback size, but we predict that the contrasting temperatures do have an effect on the stickleback morphology.

2.3.2. Predictions for the diet treatments:

The sticklebacks received contrasting diet treatments with the aim to create resemblances to limnetic (cladocerans) and benthic (bloodworms) diets (McGee, Schluter, and Wainwright 2013). Based on the well-known limnetic and benthic ecotypes of the stickleback (McGee, Schluter, and Wainwright 2013), I assume that the diet treatments affected the stickleback morphology, particularly in the head region. I predict that sticklebacks that were fed with bloodworms will show larger body sizes and benthic-like head morphologies, whereas the sticklebacks that were fed with cladocerans will show smaller body sizes and limnetic-like head morphologies (Figure 1). The sticklebacks were fed *ad libitum*, therefore, the hunting/feeding behavior is supposed to be the main contributor to diverging morphologies if the diet treatments have an effect on the stickleback morphologies.

2.3.3. Predictions for the effects of the origin on the stickleback families:

The temperature and diet treatments were either matching or mismatching with the parental conditions from their origin, since the parental generation of the bred and tested F1 sticklebacks was collected from contrasting areas of Lake Mývatn. E.g., the rearing temperature of 13° C was mismatching for those sticklebacks, descending from parents that were collected from warmer areas of Lake Mývatn. The mismatch between these conditions may have been maladaptive due to local adaptations to spatial varying conditions.

However, a strong response by phenotypic plasticity may have relieved some possible negative effects from these mismatching conditions. Stickleback are known to have a high capacity for phenotypic plasticity (McCAIRNS and Bernatchez 2012; Wund et al. 2008), consequently, I assume that the origin, or respectively mismatching conditions between the parental and F1 generation will cause no differences in the morphology of the tested sticklebacks.

3. Methods:

3.1. Study system: Lake Mývatn

After the last ice-age, only few species were able to colonize Iceland. For instance, only six freshwater fish species were able to colonize Iceland (Sigursteinsdóttir and Kristjánsson 2005). The low biodiversity combined with the harsh environment in Iceland results in relatively simple habitats and ecosystems (Lörz et al. 2021). This also applies to the Icelandic Lake Mývatn. The lake is a shallow eutrophic lake in north-eastern Iceland (65°36'N, 17°00'W), that formed around 2300 years ago following a volcanic eruption (Figure 2). Its 37km² area is divided into two main basins: The smaller, spatially more heterogeneous, and deeper (1 - 6m depth) north basin (N basin, Ytri flói) and the more homogenous and shallower (2 - 3.2m depth) south basin (S basin, Syðri flói). Furthermore, there is spatial variation in ambient temperature, temperature fluctuations (throughout the seasons), vegetation, and invertebrate communities throughout the lake (A. Einarsson and Björk Örnólfssdóttir 2004).

For instance, the lake's north-eastern parts (Hot Shore – HS, Figure 2) are surrounded by hot springs flow throughout the year which means that this side of the lake has constant ambient temperatures, whereas the north-western parts of the lake (Cold Shore – CS, Figure 2) have

fluctuating conditions throughout the year and seasons (A. Einarsson and Björk Örnólfssdóttir 2004; Á. Einarsson et al. 2004).

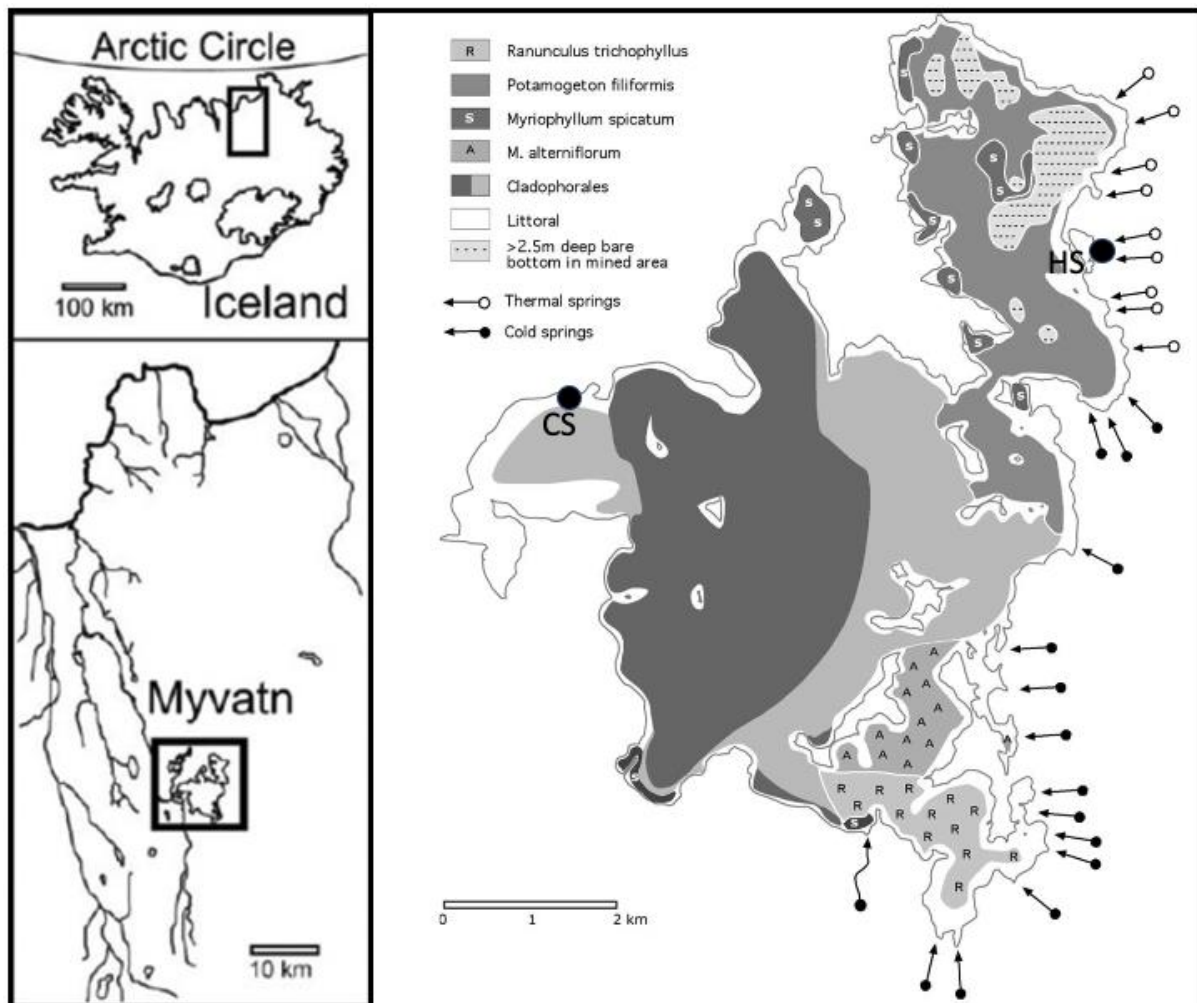


Figure 2: Map of Lake Mývatn, its location within Iceland and the location of the Cold (CS) and Hot Shore (HS) areas of the lake. CS and HS were labelled locations to collect stickleback individuals. From (Á. Einarsson et al. 2004).

3.2. Study system: *G. aculeatus* population in Lake Mývatn

Lake Mývatn is inhabited by three freshwater species: the Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*), and three-spined stickleback (*Gasterosteus aculeatus*), which is the most abundant species within this ecosystem (Á. Einarsson et al. 2004). Due to their high capacity for phenotypic plasticity (McCAIRNS and Bernatchez 2012; Wund et al. 2008), stickleback are known to show strong responses to the environment and environmental fluctuations. Therefore, stickleback are a commonly used species to study the extent of environmental fluctuations.

The local stickleback population of Lake Mývatn shows a high morphologic divergence (Kristjánsson, Skúlason, and Noakes 2002), despite having nearly no genetic divergence within the population (Strickland et al. 2022). Consequently, the diverging morphologies may be a response of the sticklebacks' capacity for phenotypic plasticity (McCAIRNS and Bernatchez 2012; Wund et al. 2008) to the heterogenous ecosystem of Lake Mývatn offers.

3.3. Experimental design:

In June 2020, sexually mature adult sticklebacks were collected from Lake Mývatn to be artificially crossed. The adults were collected from two locations, the Hot Shore (HS) and Cold Shore (CS) of Lake Mývatn (Figure 2). Artificial crosses were created in the lab. Each clutch was split in half and either incubated and reared at 13°C or 21°C. Once the sticklebacks were big enough, each clutch was split in half again and either reared on a benthic diet (bloodworms) or an epibenthic diet (cladocerans). For both diets, the sticklebacks were fed *ad libitum*.

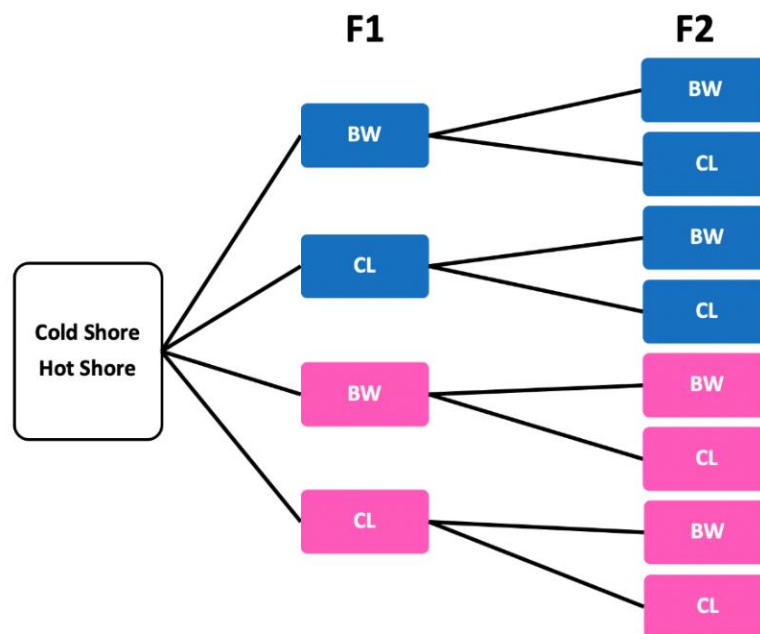


Figure 3: Treatments of the clutches from the F1 and F2 generations. BW = bloodworm diet, CL = cladoceran diet, blue = 13°C rearing temperature, red = 21°C rearing temperature.

The tested sticklebacks were collected after reaching sexual maturity and all adults were terminally sampled. For each stickleback that was sampled, a photo was taken. Afterwards, the fish were fixed in 95% ethanol until further processing. In this project, we included 460 adult fish with approximately 30 fish per treatment x origin x sex combination (Table 1).

Table 1: distribution of the total 460 fish used in this project. This table shows the number of individuals with the same temperature, origin, and diet for each sex.

Cold = 13°C rearing temperature, Warm = 21°C rearing temperature, Bw = bloodworms, Cl = cladocerans, cs = cold shore, hs = hot shore

F1	Male	female
Cold + Cs + Bw	30	34
Cold + Cs + Cl	30	31
Cold + Hs + Bw	24	25
Cold + Hs + Cl	27	27
Warm + Cs + Bw	30	30
Warm + Cs + Cl	30	30
Warm + Hs + Bw	24	29
Warm + Hs + Cl	30	29

3.4. Morphological measurements:

For the morphometrics, the stickleback were compared in their standard length, head proportions, and body shape by using standardized geometric landmarks (Figure 4). The standard length was measured by drawing a line from landmark 1 to landmark 9 (Figure 4) and determining its length based on a reference with known length (e.g. millimeter paper placed under the sticklebacks while taking the photos). For the head proportions, the head length was measured by drawing a line from landmark 1 to landmark 20 (Figure 4) and determining its length similar to the standard length. Afterwards, the head proportion was calculated by dividing the head length by the standard length. The body shape was determined by connecting the landmarks into a shape corresponding to the stickleback shape (Figure 4). The supplementary section (S2) shows, which landmarks were used for the stickleback shapes.

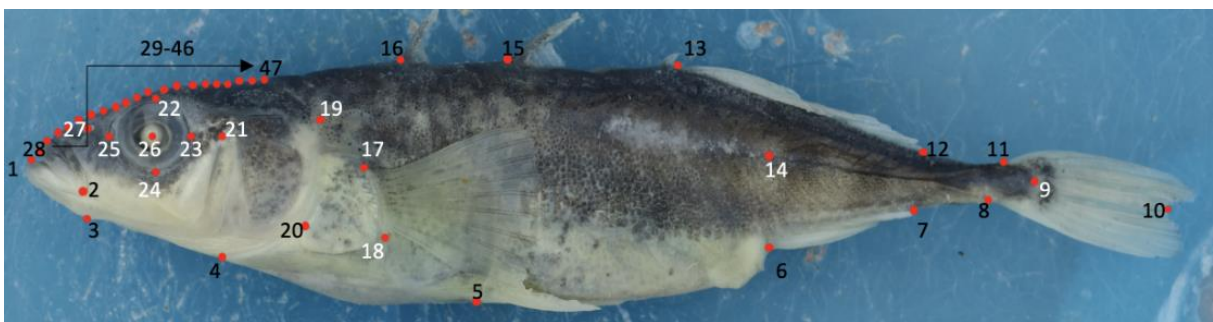


Figure 4: Landmarks used for the morphometrics. The supplementary section (S1) contains a list that explains each of the landmarks by their number.

3.5. Statistics:

After the measurements, the morphologies of the tested sticklebacks were compared by using the *geomorph* package in R. (Baken E, Collyer M, Kaliontzopoulou A, Adams D (2021), Adams D, Collyer M, Kaliontzopoulou A, Baken E (2022)). Generally, the standard length and weight of the sticklebacks were found to be highly correlated (Pearson Correlation: $df = 458$, $p\text{-value} < 2.2e-16$, $cor = 0.95$). In biological terms, it makes more sense for the length to be more relevant to the morphology by determining it based on geometric landmarks. Hence, the standard length will be used for models and visualization of the effects of the treatments on the stickleback morphology.

The following linear regression model was used to study if conditions between the tested sticklebacks and their parental generation from Lake Mývatn had any effects on their standard length (SL): $SL \sim \text{sex} * \text{temperature} + \text{sex} * \text{diet} + \text{origin}$. Similarly, the following model was used to test the same for the head proportions: $\text{head proportions} \sim \text{sex} + \text{temperature} + \text{diet} + \text{origin}$. To be precise, both models were used in an ANOVA. Due to its low and non-significant contribution to the SL and head proportion variances, the origin was excluded from further models to prevent losing power. Afterwards, the following models were used in a linear regression (in R) to analyze the relative contributions of temperature, diet, and sex in the SL and head proportion variations: $SL \sim \text{sex} * \text{temperature} + \text{sex} * \text{diet}$, $\text{head proportions} \sim \text{sex} + \text{temperature} + \text{diet}$.

The body shape was analyzed by running a procrustes analysis (type II) (Demayo, Harun, and Torres 2011) with following model in R: $\text{landmark coordinates (Figure 4)} \sim \text{sex} + \text{temperature} + \text{diet}$. The procrustes analysis served to study the relative contribution of temperature, diet, and sex in the variation of the placed geometric landmarks (Figure 4). Additionally, the R package *geomorph* ((Baken E, Collyer M, Kaliontzopoulou A, Adams D (2021), Adams D, Collyer M, Kaliontzopoulou A, Baken E (2022)) was used to analyze the body shape by performing a principal component analysis, separating the treatment combinations as groups (Abdi and Williams 2010). The shape differences were visualized afterwards by deformation grids (Ramlér et al. 2014).

4. Results:

4.1. Origins of the stickleback families:

The origins of the stickleback families had a low, non-significant effect (*ANOVA*, $df = 453$, $F = 0.5615$, $p = 0.4541$) on the stickleback morphology regarding standard length. Similarly for the head proportions, the origins had a low, non-significant effect (*ANOVA*, $df = 455$, $F = 0.1069$, $p = 0.7438$) on the stickleback morphology as well. The supplementary figures 1-2 (S3, S4) visualize the low effects of the origins on the SL and head proportions. The supplementary tables 1-2 (S5, S6) show the remaining results of the *ANOVAs*.

4.2. Effects of the treatments on standard length:

Table 2 shows the results of the linear regression of the model: Standard length \sim sex * temp + sex * diet. The model's effect size (adjusted R^2) is 0.1891. Consequently, this model may explain up 18.91 % of the total variation in standard length for the tested sticklebacks.

Table 2: Results of linear regression in R by following model: Standard length \sim sex * temperature + sex * diet.

Observations: 460, $df = 454$

R^2/R^2 adjusted: 0.1962/0.1891

Predictors	Estimates	SS	t-value	p-value
Intercept	46.8506	0.3553	131.861	< 0.001 ***
Sex	0.3032	0.4935	0.615	0.5392
Temperature	-0.8047	0.4047	-1.988	0.0474 *
Diet	-2.9748	0.4050	-7.345	< 0.001 ***
Sex * Temperature	-2.1831	0.5662	-3.856	< 0.001 ***
Sex * Diet	1.4365	0.5664	2.536	0.0116 *

The sexes by themselves did not have large effects ($p = 0.539$, Table 2) on standard length (Figure 5). According to the linear regression (Table 2), the diet appeared to have the largest effect on the standard length. For instance, Sticklebacks that received bloodworms generally turned out to be larger, but interestingly, the females that received bloodworms were separated by the temperature treatments (Figure 5), implying the interactions: sex * temperature and sex * diet (Table 2). According to the t-values, the interactions: sex * temperature and sex * diet seem to have a larger effect on the standard length of the tested sticklebacks than the temperature and sex by themselves (Table 2).

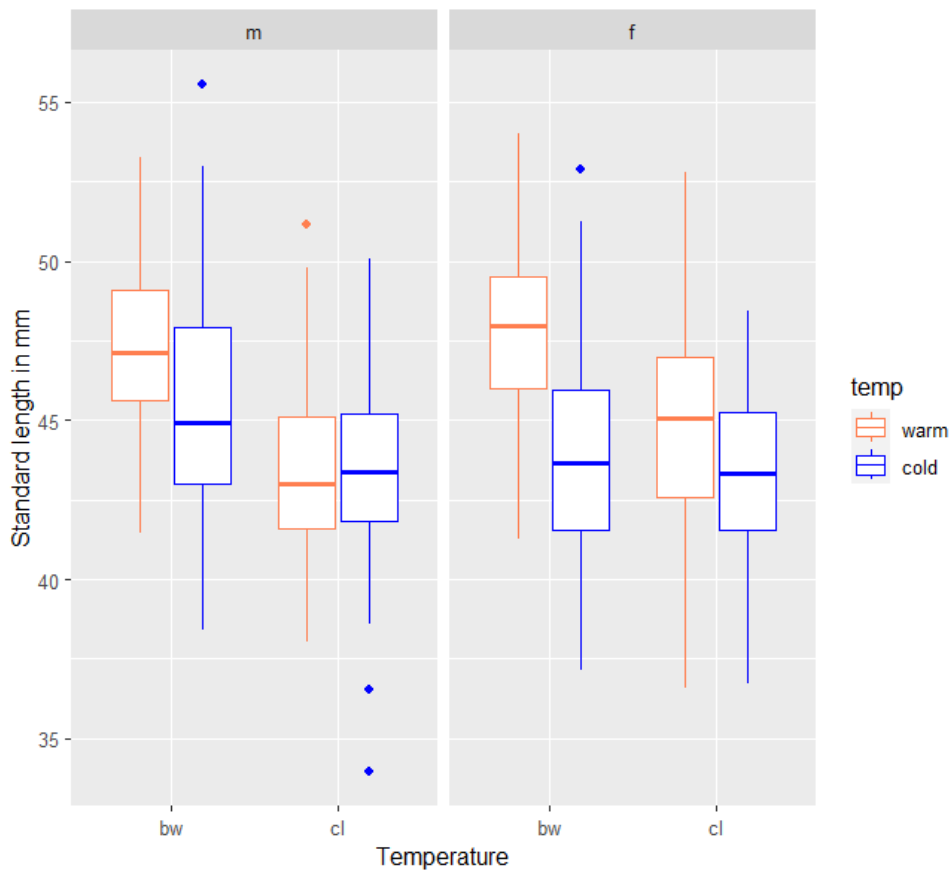


Figure 5: Stickleback standard length (in mm) in response to ambient temperature, diet, and sex. cold = 13°C, warm = 21°C, f = female, m = male, bw = bloodworms, cl = cladocerans

4.3. Effects of the treatments on head proportions:

Table 3 shows the results of the linear regression of the model: Standard length ~ sex + temp + diet. The model's effect size (adjusted R^2) is 0.6733. Consequently, this model may explain up 67.33 % of the total variation in head proportion for the tested sticklebacks.

Table 3: Results of linear regression in R by following model: Head proportion ~ sex + temperature + diet. Observations: 460, df = 456 R^2/R^2 adjusted: 0.6754/0.6733

Predictors	Estimates	SS	t-value	p-value
Intercept	0.0305	< 0.0001	299.555	< 0.001 ***
Sex	-0.0025	< 0.0001	-24.389	< 0.001 ***
Temperature	-0.0016	< 0.0001	-16.299	< 0.001 ***
Diet	0.0009	< 0.0001	8.535	< 0.001 ***

Both, the contrasting temperature and diet treatments, and the sex turned out to have a highly significant effect on the variations in stickleback head proportions (Table 3). Based on the t-values, (Table 3), the sex appeared to have the largest effect on the variations of head proportions, followed by the temperature. The diet has the lowest effect on the variations

based on its t-value (Table 3). Sticklebacks that were reared in the warm (Figure 6) turned out to have larger heads overall independent of the sex. The male sticklebacks generally had larger heads than the female sticklebacks (Figure 6) independently of the treatment combinations (Table 1). Sticklebacks that received bloodworms as diet turned out to have smaller heads in comparison to their standard length than sticklebacks that received cladocerans (Figure 6).

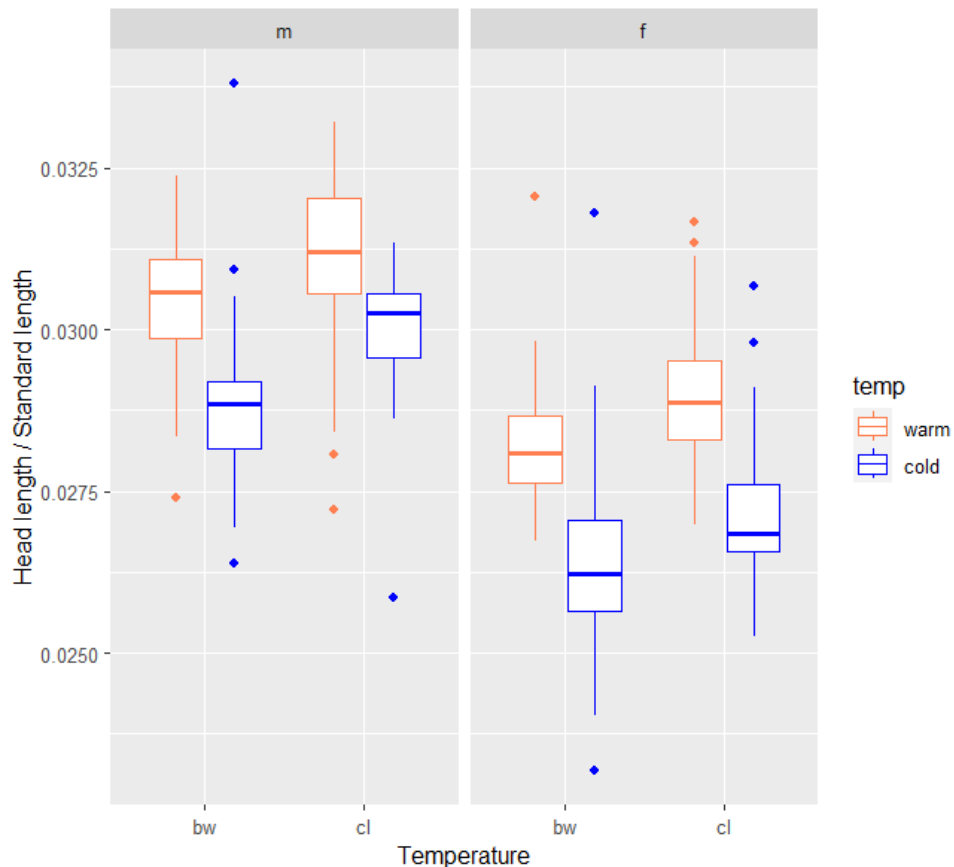


Figure 6: Stickleback head proportion in response to ambient temperature, diet, and sex. cold = 13°C, warm = 21°C, f = female, m = male, bw = bloodworms, cl = cladocerans

4.4. Body shape:

The results of the procrustes analysis show that the sex had the largest effect on the variation of the placed landmarks according to the F-values and corresponding effect sizes (R^2) (Table 4). The sex had an effect size of 15.0 % followed by temperature with an effect size of 14.6% (Table 4). Even though the effects of the diet were highly significant like the effects of sex and temperature, its effect size was comparably low with 3.1% (Table 4).

Table 4: Results of procrustes analysis in R by following model: landmark coordinates \sim sex + temperature + diet.
Observations: 460, df = 456

Predictors	SS	R ²	F-value	p-value
Sex	0.0836	0.1504	102.409	< 0.001 ***
Temperature	0.0812	0.1461	99.485	< 0.001 ***
Diet	0.0170	0.0306	20.865	< 0.001 ***

Figure 7 visualizes how the different treatment combinations (Table 2) were separated by the principal components (PC) 1 and 2. PC 1 explains 33.5 % and PC 2 explains 16.9 % of the variations in body shape. PC 1 particularly separated between the sexes and temperature treatments (Figure 7). For the diet treatments, the effects were more subtle (Figure 7). PC 2 showed the same pattern as in PC 1 but its effects were more subtle overall. For instance, the different treatment combinations were only subtly separated by PC 2 (Figure 7).

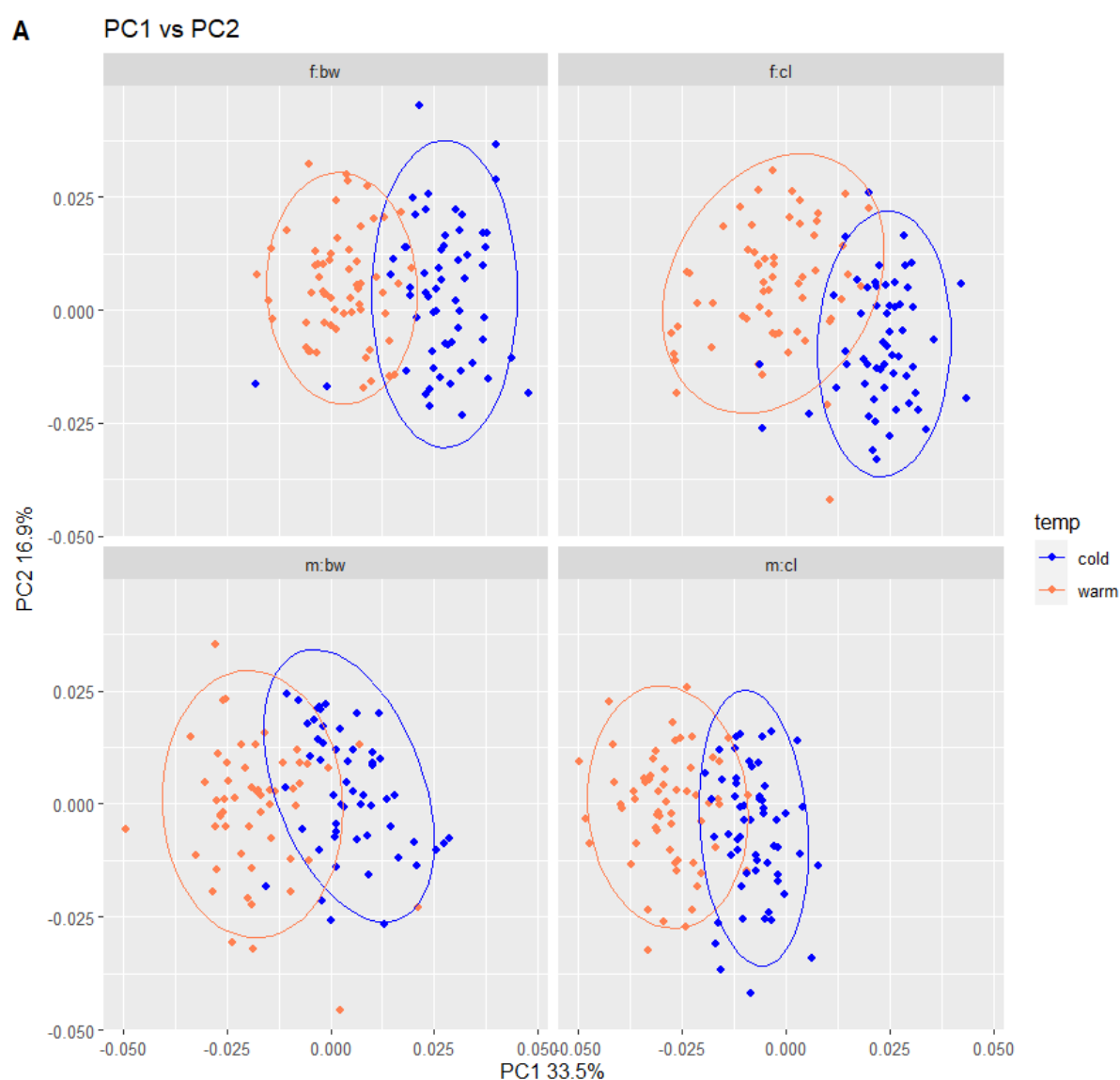


Figure 7: Visualization of principal component analysis and the contribution of the principal components 1-3 to the variance of the coordinates of placed geometric landmarks. The contribution of the principal components was compared by separating between rearing temperature and sex.

The deformation grids (Figure 8) visualize the body shape of the tested sticklebacks based on the highest and lowest score from the principal component analysis (Figure 7). Individuals with lower scores for PC 1 appeared to have longer heads and shorter bodies in proportion to their head length and *vice versa* for individuals with higher scores for PC 1 (Figure 8). Furthermore, individuals with lower scores for PC 1 also turned out to have larger eyes and *vice versa* for individuals with higher scores. Individuals with higher scores for PC 1 also turned out to have to be elongated on their ventral site, particularly around the intestine region (Figure 8). The deformation grids for PC 2 show a similar pattern as the grids for PC 1, but PC 2 effects on the stickleback morphology were more subtle (Figure 8). The head size and elongated ventral site remain the most notable difference between the individual with the lowest and highest score for PC 1 and 2 (Figure 8).

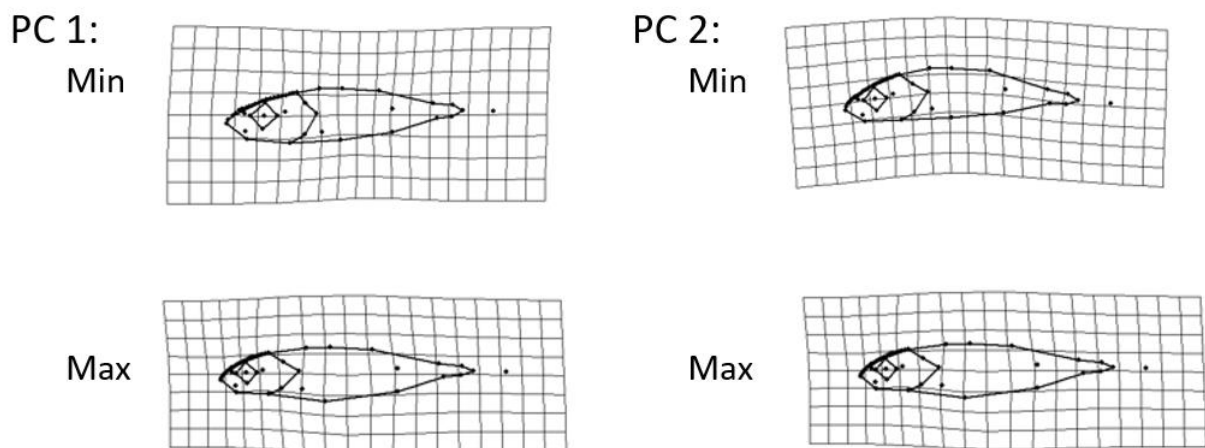


Figure 8: Deformation grids (1.1x magnification) for the visualization of the principal component analysis (Figure 6). “Min” means the observation with the lowest score for the corresponding principal component (PC). Max means the observation with the highest score for the corresponding PC.

5. Discussion:

This project shows an example of how phenotypic plasticity may offer the potential for organisms to alter their phenotype in response to their environment. For instance, this project aimed to study the response of sticklebacks from Lake Mývatn to contrasting factors such as temperature and diet. To do so, we conducted a transgenerational experiment with offspring of Lake Mývatn sticklebacks that were bred in the lab and split into different treatment groups (Figure 3, Table 1). The different treatment combinations were compared in standard length, head proportions, and body shape to study, if contrasting rearing temperatures and diets

affect the stickleback morphologies. The different treatment combinations were also separated by sex (Table 1). The origin was initially taken into account, but due to its low contribution to the standard length and head proportion variations (Supplements S3, S4), it was excluded from further analyses and models. The low, non-significant effect of the origin on the variations emphasizes a high response of phenotypic plasticity, which may have relieved some probable negative effects, selection pressures, etc., from mismatching conditions between the tested F1 generation and their parental generation from Lake Mývatn.

Temperature and diet both turned out to have an effect on the morphologies of the tested sticklebacks. For instance, the diet turned out to have the largest effect (based on the t-values) on the standard length (Table 2, Figure 5). This may be related to the prey size, since bloodworms are larger than cladocerans. Hence, more space in the body cavity may be needed to handle larger numbers of bloodworms (McGee, Schluter, and Wainwright 2013). Even though the temperature had a significant effect on the standard length variations (Table 2), its relative contribution was comparably low based on the t-value (Table 2), but we assume that the sex interacted with the temperature and diet, since there is a clear separation between female sticklebacks that received bloodworms as diet (Figure 5). Those females were clearly separated by the temperature treatments, but we do not have an explanation for this observation.

All treatments, respectively temperature and diet, and sex had highly significant effects on the head proportions of the tested sticklebacks (Table 3). Generally, all sticklebacks that were reared in the warm turned out to have larger heads (Figure 6). Male sticklebacks had larger heads in proportion to their standard length overall compared to female sticklebacks (Figure 6). And finally, sticklebacks that received bloodworms, turned out to have smaller heads in proportion to their standard length than those that received cladocerans (Figure 6). Speculatively, the increase in head size in proportion to the standard length may be linked to an increased metabolic rate due to raised temperatures, since sticklebacks are ectotherm (Dittmar et al. 2014). Increased metabolic activity means an increase in the amount of food that is needed for the organism (Killen 2014). Thus, larger heads may help the sticklebacks to catch and process more food. Anyways, the overall larger head sizes for the male sticklebacks may be explained by their higher reliance on head size in general. For instance, male sticklebacks are more reliant on their head size for antagonistic male-male interactions, nest building, etc. (Kitano, Mori, and Peichel 2007).

The results for the head sizes in response to contrasting diets were according to the expectations. Since bloodworms rather resemble a benthic diet, and cladocerans resemble a limnetic diet (McGee, Schluter, and Wainwright 2013), we assumed that sticklebacks that feed on cladocerans will have elongated heads, while sticklebacks that feed on bloodworms will have mouth better optimized for suction (Figure 1). We think that the response to the contrasting diets is based on the contrasting feeding and hunting behavior, similar to the commonly known divergence between the benthic and limnetic stickleback ecotypes (McGee, Schluter, and Wainwright 2013).

According to the procrustes analysis (Table 4), the sex had the largest relative contribution (effect size, respectively R^2) in the variations of the landmark coordinates (Figure 2), respectively body shape. We assume that this may be explained by the sexual dimorphism in stickleback, since male stickleback are known to grow larger heads overall (Kitano, Mori, and Peichel 2007). However, the temperature had a similarly large effect size (Table 4), meaning a strong response of the tested sticklebacks to fluctuating temperatures. The analysis of head proportions showed that the sticklebacks reared at higher temperatures had longer heads in proportion to their standard length (Table 3, Figure 6). The response of the head size to the contrasting temperatures may explain the relatively high effect size, particularly in biological terms, of temperature on the landmark coordinates (Table 4).

The results of the principal component analysis emphasized the results of the procrustes analysis (Table 4). Both principal components (PCs) show the same pattern based on the different separated groups (treatment combinations, Table 1), but the separations by PC 2 are more subtle. Anyways, the male sticklebacks generally had lower scores than the females (Figure 7). Taking the deformation grids into account (Figure 8), individuals with lower PC scores generally have larger heads in proportion to their standard length, which emphasizes the large effect size of the sex (Table 4) on the landmark coordinate variations. Similarly, sticklebacks that were reared in the warm also showed lower PC scores (Figure 7), which resulted in larger head sizes in proportion to their standard length as well (Figure 8).

Anyways, the diet had a larger effect on the standard length (Table 2, Figure 5), but its relative contribution to the landmark coordinate variations was comparably low (Table 4, Figure 7). This observation is emphasized by Figure 7, since the diet treatments were not separated by the principal component analysis. Perhaps, the diet rather affected the jaw morphologies of the studied sticklebacks, hence, conducting a similar experiment dealing with jaw morphology variations in response to the diet may help to study the contribution of diet to the divergence of stickleback morphologies (Nakano et al. 2020).

6. Conclusion:

In conclusion, this project was able to show an example of how contrasting environmental factors such as temperature and diet may affect the phenotype of sticklebacks (in Lake Mývatn). The temperature treatments had a low effect on the standard length variations (Table 2), but large effects on the head proportions and body shapes (Table 3, Table 4, Figure 7). Particularly for the body shapes (Table 4), the effect of the temperature was nearly as large as the sex's, emphasizing how global warming may affect stickleback populations in future by increasing their metabolic rate and therefore their needed amount of food to maintain their metabolism. The increased head size may be linked to a stress response in order to improve the handling of prey. The diet on the other hand had a large effect on the standard lengths (Table 2), but a comparably low effect on the head proportions (Table 3) and body shapes (Table 4, Figure 7), which shows that the varying head proportions contributed more in the body shape differences than standard length. Eventually, the diet could have affected the jaw morphology of the sticklebacks, therefore, another project may be conducted to test, if contrasting diets lead to diverging jaw morphologies of the tested sticklebacks. In that regard, the same sticklebacks that were used for this project can be used for a consecutive study as well, because they are fixed in 95% ethanol.

7. Supplements:

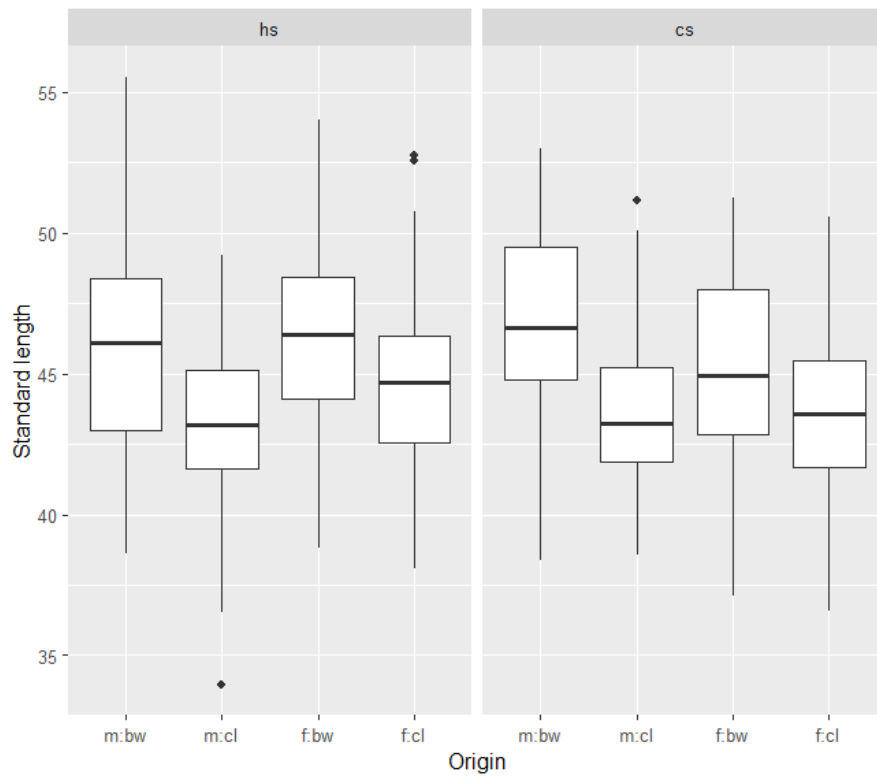
S1:

- Landmarks for the stickleback body shape:
 1. The tip of the lower lip
 2. Posterior end of the mouth
 3. The axis of the jaws
 4. Most ventral point of the operculum
 5. The anterior-most edge of the left pelvic spine
 6. The anterior-most edge of the anal fin
 7. The posterior-most edge of the anal fin
 8. The beginning of the caudal fin, where the membrane contacts the ventral surface
 9. The middle of the hypural fan
 10. Middle of the end of the caudal fin
 11. The beginning of the caudal fin, where the membrane contacts the dorsal surface
 12. The posterior-most edge of the dorsal fin, at the point where it emerges from the dorsal surface
 13. The anterior-most edge of the dorsal fin, at the point where it emerges from the dorsal surface
 14. Placed on the lateral line right above the anterior-most edge of the anal fin
 15. The point where second dorsal spine emerge from the dorsal surface, on the anterior side
 16. The point where first dorsal spine emerges from the dorsal surface, on the anterior side
 17. The uppermost point of the pectoral fin base
 18. The lower point of the pectoral fin base
 19. The uppermost point of the operculum, most dorsal
 20. Where the “turn” of the operculum is at its maximum
 21. The uppermost point of the preoperculum
 22. The uppermost point of the orbital circumference
 23. The posterior-most point of the orbital circumference
 24. The lowermost point of the orbital circumference
 25. The anterior-most point of the orbital circumference

S2:

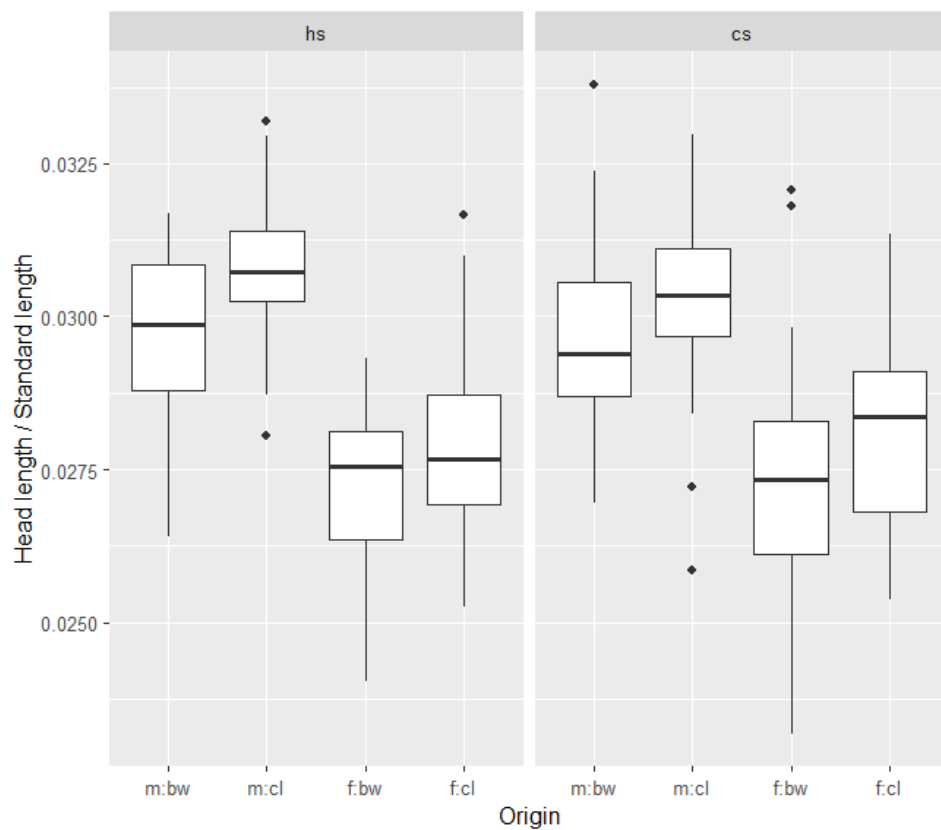
- Body shape landmarks: 1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 19, 21, 23, 24, 25, 28
- Eye shape landmarks: 22, 23, 24, 25
- Head shape landmarks: 1, 3, 4, 20, 17, 19, 47, 37, 29, 28

S3:



S3: Stickleback standard length (in mm) in response to origin, ambient temperature, diet, and sex.
 cs = cold shore, hs = hot shore, cold = 13°C, warm = 21°C, f = female, m = male, bw = bloodworms, cl = cladocerans

S4:



S4: Stickleback head proportions in response to origin, ambient temperature, diet, and sex.
 cs = cold shore, hs = hot shore, cold = 13°C, warm = 21°C, f = female, m = male, bw = bloodworms, cl = cladocerans

S5:

S5: Results of ANOVA of following mode in Rl: *standard length ~ sex * temperature + sex * diet + origin*.
Observations: 460, df = 455

Predictors	SS	F-value	p-value
Sex	0	0.0005	0.9822
Temperature	412	44.9271	< 0.001 ***
Diet	582	62.8023	< 0.001 ***
Sex * Temperature	138	14.9278	< 0.001 ***
Sex * Diet	60	6.4633	0.0114 *
Origin	5	0.5615	0.4541

S6:

S6: Results of ANOVA of following mode in Rl: *head proportions ~ sex + temperature + diet + origin*.
Observations: 460, df = 455

Predictors	SS	F-value	p-value
Sex	< 0.0007	606.8273	< 0.001 ***
Temperature	< 0.0003	267.5216	< 0.001 ***
Diet	< 0.0000	72.6982	< 0.001 ***
Origin	< 0.0000	0.1069	0.679

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9. References:

- Abdi, Hervé, and Lynne J. Williams. 2010. 'Principal Component Analysis'. *WIREs Computational Statistics* 2 (4): 433–59. <https://doi.org/10.1002/wics.101>.
- Aiken, Catherine E., Jane L. Tarry-Adkins, and Susan E. Ozanne. 2016. 'Transgenerational Effects of Maternal Diet on Metabolic and Reproductive Ageing'. *Mammalian Genome* 27 (7): 430–39. <https://doi.org/10.1007/s00335-016-9631-1>.
- Álvarez-Quintero, Náyade, Alberto Velando, Jose C. Noguera, and Sin-Yeon Kim. 2021. 'Environment-Induced Changes in Reproductive Strategies and Their Transgenerational Effects in the Three-Spined Stickleback'. *Ecology and Evolution* 11 (2): 771–83. <https://doi.org/10.1002/ece3.7052>.
- Barr, C. S., T. K. Newman, M. L. Becker, C. C. Parker, M. Champoux, K. P. Lesch, D. Goldman, S. J. Suomi, and J. D. Higley. 2003. 'The Utility of the Non-Human Primate Model for Studying Gene by Environment Interactions in Behavioral Research'. *Genes, Brain and Behavior* 2 (6): 336–40. <https://doi.org/10.1046/j.1601-1848.2003.00051.x>.
- Colicchio, J. 2017. 'Transgenerational Effects Alter Plant Defence and Resistance in Nature'. *Journal of Evolutionary Biology* 30 (4): 664–80. <https://doi.org/10.1111/jeb.13042>.
- Demayo, Cesar G, Stephenson Abadies Harun, and Mark Anthony J Torres. 2011. 'PROCRUSTES ANALYSIS OF WING SHAPE DIVERGENCE AMONG SIBLING SPECIES OF *Neurothemis DRAGONFL*'.

- Dittmar, Janine, Hannah Janssen, Andra Kuske, Joachim Kurtz, and Jörn P. Scharsack. 2014. 'Heat and Immunity: An Experimental Heat Wave Alters Immune Functions in Three-Spined Sticklebacks (*Gasterosteus Aculeatus*)'. *Journal of Animal Ecology* 83 (4): 744–57. <https://doi.org/10.1111/1365-2656.12175>.
- Duncan, Elizabeth J., Peter D. Gluckman, and Peter K. Dearden. 2014. 'Epigenetics, Plasticity, and Evolution: How Do We Link Epigenetic Change to Phenotype?' *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 322 (4): 208–20. <https://doi.org/10.1002/jez.b.22571>.
- Einarsson, Arni, and Erla Björk Örnólfssdóttir. 2004. 'Long-Term Changes in Benthic Cladocera Populations in Lake Myvatn, Iceland'. *Aquatic Ecology* 38 (2): 253–62. <https://doi.org/10.1023/B:AECO.0000032060.29256.95>.
- Einarsson, Árni, Gerður Stefánsdóttir, Helgi Jóhannesson, Jón S. Ólafsson, Gísli Már Gíslason, Isamu Wakana, Gudni Gudbergsson, and Arnthor Gardarsson. 2004. 'The Ecology of Lake Myvatn and the River Laxá: Variation in Space and Time'. *Aquatic Ecology* 38 (2): 317–48. <https://doi.org/10.1023/B:AECO.0000032090.72702.a9>.
- Fang, Bohao, Juha Merilä, Filipe Ribeiro, Carlos M. Alexandre, and Paolo Momigliano. 2018. 'Worldwide Phylogeny of Three-Spined Sticklebacks'. *Molecular Phylogenetics and Evolution* 127 (October): 613–25. <https://doi.org/10.1016/j.ympev.2018.06.008>.
- Franks, Steven J., and Ary A. Hoffmann. 2012. 'Genetics of Climate Change Adaptation'. *Annual Review of Genetics* 46 (1): 185–208. <https://doi.org/10.1146/annurev-genet-110711-155511>.
- Härer, Andreas, Daniel I. Bolnick, and Diana J. Rennison. 2021. 'The Genomic Signature of Ecological Divergence along the Benthic-Limnetic Axis in Allopatric and Sympatric Threespine Stickleback'. *Molecular Ecology* 30 (2): 451–63. <https://doi.org/10.1111/mec.15746>.
- Katsiadaki, Ioanna, Steven Morris, Christopher Squires, Mark Richard Hurst, Jonathan David James, and Alexander Pickering Scott. 2006. 'Use of the Three-Spined Stickleback (*Gasterosteus Aculeatus*) As a Sensitive in Vivo Test for Detection of Environmental Antiandrogens'. *Environmental Health Perspectives* 114 (Suppl 1): 115–21. <https://doi.org/10.1289/ehp.8063>.
- Killen, Shaun S. 2014. 'Growth Trajectory Influences Temperature Preference in Fish through an Effect on Metabolic Rate'. *Journal of Animal Ecology* 83 (6): 1513–22. <https://doi.org/10.1111/1365-2656.12244>.
- Kitano, Jun, Seiichi Mori, and Catherine L. Peichel. 2007. 'Sexual Dimorphism in the External Morphology of the Threespine Stickleback (*Gasterosteus Aculeatus*)'. *Copeia* 2007 (2): 336–49. [https://doi.org/10.1643/0045-8511\(2007\)7\[336:SDITEM\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2007)7[336:SDITEM]2.0.CO;2).
- Kristjánsson, Bjarni K., Skúli Skúlason, and David L. G. Noakes. 2002. 'Morphological Segregation of Icelandic Threespine Stickleback (*Gasterosteus Aculeatus* L.)'. *Biological Journal of the Linnean Society* 76 (2): 247–57. <https://doi.org/10.1111/j.1095-8312.2002.tb02086.x>.
- Lefébure, R., S. Larsson, and P. Byström. 2011. 'A Temperature-Dependent Growth Model for the Three-Spined Stickleback *Gasterosteus Aculeatus*'. *Journal of Fish Biology* 79 (7): 1815–27. <https://doi.org/10.1111/j.1095-8649.2011.03121.x>.
- Lörz, Anne-Nina, Stefanie Kaiser, Jens Oldeland, Caroline Stolter, Karlotta Kürzel, and Saskia Brix. 2021. 'Biogeography, Diversity and Environmental Relationships of Shelf and Deep-Sea Benthic Amphipoda around Iceland'. *PeerJ* 9 (August): e11898. <https://doi.org/10.7717/peerj.11898>.
- Macagno, Anna L. M., Eduardo E. Zattara, Onye Ezeakudo, Armin P. Moczek, and Cristina C. Ledón-Rettig. 2018. 'Adaptive Maternal Behavioral Plasticity and Developmental Programming Mitigate the Transgenerational Effects of Temperature in Dung Beetles'. *Oikos* 127 (9): 1319–29. <https://doi.org/10.1111/oik.05215>.
- Matesanz, Silvia, Ernesto Gianoli, and Fernando Valladares. 2010. 'Global Change and the Evolution of Phenotypic Plasticity in Plants'. *Annals of the New York Academy of Sciences* 1206 (1): 35–55. <https://doi.org/10.1111/j.1749-6632.2010.05704.x>.
- McCAIRNS, R. J. S., and L. Bernatchez. 2012. 'Plasticity and Heritability of Morphological Variation within and between Parapatric Stickleback Demes'. *Journal of Evolutionary Biology* 25 (6): 1097–1112. <https://doi.org/10.1111/j.1420-9101.2012.02496.x>.

- McGee, Matthew D., Dolph Schluter, and Peter C. Wainwright. 2013. 'Functional Basis of Ecological Divergence in Sympatric Stickleback'. *BMC Evolutionary Biology* 13 (1): 277. <https://doi.org/10.1186/1471-2148-13-277>.
- McGee, Matthew D., and Peter C. Wainwright. 2013. 'Sexual Dimorphism in the Feeding Mechanism of Threespine Stickleback'. *Journal of Experimental Biology* 216 (5): 835–40. <https://doi.org/10.1242/jeb.074948>.
- Nakano, Shigeru, Kurt D Fausch, Itsuro Koizumi, Yoichiro Kanno, Yoshinori Taniguchi, Satoshi Kitano, and Yo Miyake. 2020. 'Evaluating a Pattern of Ecological Character Displacement: Charr Jaw Morphology and Diet Diverge in Sympatry versus Allopatry across Catchments in Hokkaido, Japan'. *Biological Journal of the Linnean Society* 129 (2): 356–78. <https://doi.org/10.1093/biolinnean/blz183>.
- Olin, Agnes B, Jens Olsson, Johan S Eklöf, Britas Klemens Eriksson, Olavi Kaljuste, Laura Briekmane, and Ulf Bergström. 2022. 'Increases of Opportunistic Species in Response to Ecosystem Change: The Case of the Baltic Sea Three-Spined Stickleback'. *ICES Journal of Marine Science* 79 (5): 1419–34. <https://doi.org/10.1093/icesjms/fsac073>.
- Plomin, Robert, J. C. DeFries, and John C. Loehlin. 1977. 'Genotype-Environment Interaction and Correlation in the Analysis of Human Behavior'. *Psychological Bulletin* 84: 309–22. <https://doi.org/10.1037/0033-2909.84.2.309>.
- Price, Trevor D., Anna Qvarnström, and Darren E. Irwin. 2003. 'The Role of Phenotypic Plasticity in Driving Genetic Evolution'. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270 (1523): 1433–40. <https://doi.org/10.1098/rspb.2003.2372>.
- Ramler, D., P. Mitteroecker, L. N. S. Shama, K. M. Wegner, and H. Ahnelt. 2014. 'Nonlinear Effects of Temperature on Body Form and Developmental Canalization in the Threespine Stickleback'. *Journal of Evolutionary Biology* 27 (3): 497–507. <https://doi.org/10.1111/jeb.12311>.
- Sigursteinsdóttir, Rakel Júlía, and Bjarni K. Kristjánsson. 2005. 'Parallel Evolution, Not Always so Parallel: Comparison of Small Benthic Charr, *Salvelinus alpinus*, from Grímsnes and Thingvallavatn, Iceland'. *Environmental Biology of Fishes* 74 (2): 239–44. <https://doi.org/10.1007/s10641-005-0499-2>.
- Skinner, Michael K. 2016. 'Epigenetic Transgenerational Inheritance'. *Nature Reviews Endocrinology* 12 (2): 68–70. <https://doi.org/10.1038/nrendo.2015.206>.
- Smith, C., G. Zięba, R. Spence, T. Klepaker, and M. Przybylski. 2020. 'Three-Spined Stickleback Armour Predicted by Body Size, Minimum Winter Temperature and PH'. *Journal of Zoology* 311 (1): 13–22. <https://doi.org/10.1111/jzo.12766>.
- Strickland, Kasha, Katja Räsänen, Bjarni Kristjánsson, Joseph Phillips, Arni Einarsson, Ragna Snorradóttir, Mireia Bartrons, and Zophonías Oddur Jónsson. 2022. 'Genome-Phenotype-Environment Associations Identify Signatures of Selection in a Panmictic Population of Threespine Stickleback'. Preprint. Preprints. <https://doi.org/10.22541/au.165786488.81262349/v1>.
- Svanbäck, Richard, Mario Pineda-Krch, and Michael Doebeli. 2009. 'Fluctuating Population Dynamics Promotes the Evolution of Phenotypic Plasticity'. *The American Naturalist* 174 (2): 176–89. <https://doi.org/10.1086/600112>.
- WILLACKER, JAMES J., FRANK A. VON HIPPEL, PETER R. WILTON, and KELLY M. WALTON. 2010. 'Classification of Threespine Stickleback along the Benthic–Limnetic Axis'. *Biological Journal of the Linnean Society* 101 (3): 595–608. <https://doi.org/10.1111/j.1095-8312.2010.01531.x>.
- Williams, R. M., L. O'Brien, H. A. Eagles, V. A. Solah, and V. Jayasena. 2008. 'The Influences of Genotype, Environment, and Genotype × Environment Interaction on Wheat Quality'. *Australian Journal of Agricultural Research* 59 (2): 95–111. <https://doi.org/10.1071/AR07185>.
- Wund, Matthew A., John A. Baker, Brendan Clancy, Justin L. Golub, and Susan A. Foster. 2008. 'A Test of the "Flexible Stem" Model of Evolution: Ancestral Plasticity, Genetic Accommodation, and Morphological Divergence in the Threespine Stickleback Radiation'. *The American Naturalist* 172 (4): 449–62. <https://doi.org/10.1086/590966>.