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# Morphological comparison of ninespined stickleback (*Pungitius pungitius*) populations in different ecological habitats of northern Germany



## BACHELOR THESIS

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September 2013

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# Zusammenfassung / Abstract

Bei den neunstacheligen Stichlingen (*Pungitius pungitius*) handelt es sich um eine Fischart, die in einer Vielzahl verschiedener Habitate anzutreffen ist. Aufgrund ihrer Ausprägung von unterschiedlichen morphologischen Merkmalen und/oder Körperformen zwischen verschiedenen Populationen, sind sie für die Untersuchung der Mechanismen phänotypischer Variation hervorragend geeignet. Zur Morphologie norddeutscher Populationen gibt es allerdings keine aktuellen Studien. Ebenso ist unklar, wie Umweltbedingungen die natürliche Selektion auf phänotypische Variation in diesen Populationen beeinflussen. Diese Studie hat die Unterschiede in der Körperform und in morphologischen Merkmalen zwischen verschiedenen Populationen norddeutscher neunstacheliger Stichlinge untersucht. In dieser Studie wiesen Populationen, die einem niedrigen Fraßdruck durch Raubfische in ihrer Umwelt ausgesetzt waren, im Vergleich zu Fischen, die einem hohen Fraßdruck durch Raubfische in ihrer Umwelt ausgesetzt waren, eine erhöhte Körpergröße und eine verstärkt ausgeprägte Ausstattung an Stacheln auf. Außerdem wiesen Fische, die insgesamt ähnlichen Umweltbedingungen ausgesetzt waren, eine höhere Übereinstimmung betreffend ihrer Morphologischen Merkmale auf als Fische aus Populationen die geographisch näher zueinander lokalisiert waren. Diese Ergebnisse zeigen, dass ein hoher Fraßdruck, wahrscheinlich hervorgerufen durch sympatrische Raubfische, die Variation von Körperform und morphologischen Merkmalen zwischen verschiedenen Populationen stark beeinflusst. Darüber hinaus scheint die Umwelt einen höheren Einfluss auf phänotypische Variationen zu haben, als geographische Entfernungen von Populationen zueinander. Dennoch bedarf es weiterer Forschungen um einen vollständigen Eindruck des Zusammenspiels von Umweltbedingungen zu erhalten, die die phänotypischen Variationen neunstacheliger Stichlinge beeinflussen. Der Vergleich einer erhöhten Anzahl von Populationen, welche den gleichen Umweltbedingungen ausgesetzt sind und ein ausgewogenes Geschlechterverhältnis aufweisen, könnte helfen um sexuelle Unterschiede besser analysieren zu können. Eine präzise Untersuchung des Nahrungsangebotes und der Wasserzusammensetzung einzelner Habitate könnte darüber hinaus Aufschlüsse über den Einfluss dieser beider Faktoren auf phänotypische Variationen bringen.

Nine-spined sticklebacks (*Pungitius pungitius*) are a species of fish which are widely distributed throughout a range of different habitats. Their expression of different morphological traits and/or body shapes among populations makes them an excellent model to understand mechanisms of phenotypic variation. Currently, there are no recent studies about the morphology of northern German populations as well as how environmental conditions drive natural selection on phenotypic variation in these populations. In this study divergences in morphological traits and body shape between populations of northern German nine-spined sticklebacks were investigated. Populations inhabiting an environment with a low predation pressure of piscivorous fish showed a larger body size and a decreased spine-armory in comparison to fish inhabiting an environment with a high predation pressure of piscivorous fish. Moreover, fish from populations exposed to similar environmental conditions showed a higher similarity to each other concerning their morphology than fish, that are located near each other geographically. These results demonstrate that differences in predation pressure, likely caused by sympatric piscivorous fish species, strongly influence morphological and body shape variation between different populations. In addition, the environment seems to have a higher effect on phenotypic variation than geographical distances between populations. Nonetheless, more work is needed to get a better impression about the interaction of environmental conditions affecting phenotypic variation in nine-spined sticklebacks. A comparison of more populations, inhabiting similar environments with a balanced sex ratio of specimens could be helpful to analyze sexual divergence. Additionally, a precise analysis of differences in food availability and water chemistry could help elucidate the influences of these factors on phenotypic variation.

# Introduction

## Investigated species

The nine-spined stickleback (*Pungitius pungitius*, LINNAEUS, 1758) is a common teleost distributed throughout temperate to arctic regions of the northern hemisphere (Wootton 1976; ÖSTLUND-NILSSON et al., 2007). They inhabit a range of different environments like seas, lakes and rivers as well as small creeks and ponds (e.g. BĂNĂRESCU & PAEPKE, 2001; ÖSTLUND-NILSSON et al., 2007). Additionally, they adapted to life in closed, shallow, weedy and eutrophic waters, depleted in oxygen (LEWIS et al., 1972). Thus the nine-spined stickleback is an excellent model to investigate how morphological characters vary between different environmental conditions.

Regarding their morphology, several studies have shown that nine-spined sticklebacks have a great level of variation concerning their body size (HERCZEG et al., 2009, 2010; MOBLEY et al., 2011) similar to morphological variation in three-spined sticklebacks (*Gasterosteus aculeatus*, LINNAEUS, 1758) (REIMCHEN, 1991). Predation by piscivorous fishes appears to influence body size in fishes (e.g. POPOVA 1967; ZARET 1980; REIMCHEN, 1991; HERCZEG et al. 2009, 2010; MOBLEY et al., 2011). In general, fish size increases in the presence of piscivorous predators (POPOVA 1967, ZARET 1980). For nine-spined sticklebacks, the opposite trend has been reported and large body sizes as well as gigantism are found in isolated ponds that lack piscivorous fish-species (HERCZEG et al. 2009, 2010; MOBLEY et al., 2011). This supports the assumption that predation pressure can also be a reason to keep organisms small due to the increased viability costs like reduced agility and increased detectability (BLANCKENHORN, 2000). Another reason for decreased body size in nine-spined sticklebacks is interspecific competition for food resources or nesting sites with the closely related three-spined stickleback (HERCZEG et al, 2009). On the other hand fecundity selection acting on females is also likely to be one of the main factors influencing gigantism in nine-spined sticklebacks (HERCZEG et al., 2010; HERCZEG et al., 2012). Additionally, sexual selection working through male-male competition and female mate choice often favor large body size (WOOTON, 1979; CLUTTON-BROCK et al, 1982; SHINE 1988, 1989; ANDERSSON 1994).

Predation as well as interspecific competition seems to drive not only selection pressure on body size (HERCZEG et al., 2009; MOBLEY et al., 2011), but also selection on defensive spine armory

and the evolution of the pelvic girdle (e.g. GROSS, 1979; REIMCHEN, 1991; BLOUW & BOYD, 1992; BELL et al, 1993; MOBLEY et al, 2011). In three-spined sticklebacks dorsal and pelvic spines, when fully erect, increase the overall body size as a deterrent to gape-limited piscivorous predators (GROSS, 1978; REIMCHEN, 1991). The pelvic girdle and spines of nine-spined sticklebacks are also thought to function as antipredator structures in nine-spined sticklebacks, even though their smaller size and strength lowers the efficiency of these defenses compared to three-spined sticklebacks that have stronger more robust spines and pelvic girdle (Hoogland et al., 1956).

### **History of the sampling area**

The lakes of the county of Schleswig-Holstein were formed by the deglaciation at the end of the last glacial period more than 10,000 years ago (NIXDORF, 2004; MUUB et al, 1973).

Some of the ponds and lakes of eastern Holstein are the remains of a bigger lake named “Großer Schwentine See”, which covered the valley of the Schwentine in a height of 36 to 39 meters above sea level. The decrease of the water level formed several lakes and ponds, which are all connected by the Schwentine river (NIXDORF, 2004). The largest of these freshwater lakes in Schleswig-Holstein is the Großer Plöner See, which arose by the movement of two ice tongues building a typical tongue-shaped basin (MUUB et al, 1973). Today, two thirds of the shore is built up and the lake is used for fishing by two professional fishery establishments (NIXDORF, 2004). The Großer Plöner See is a part of the so called Schwentine system which connects several ponds and lakes to the river Schwentine. The Schwentine runs to the Baltic through the Kieler Förde. The Grosser Plöner See has a maximum depth of 56 meters and contains a complex community of fish, including several species of piscivorous fish (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein, 1998).

The Kührener Teich is a shallow Pond with a maximum depth of two meters, which is directly connected to the Großer Plöner See by the Schwentine river through a small channel. Although it has been under protection since 1994, it was and still is used extensively for fishing, and the local fishing club continuously introduces Common Carp (*Cyprinus carpio*) and Tench (*Tinca tinca*) to the pond (NLU – Projektgesellschaft mbH & Co. KG, 2010).

An even shallower pond is the Lebrader Teich, where the deepest point is just 1.3m deep. It was made by men out of a moor landscape which was drained by the Kossau stream, by building a small dam in 1685. The pond was used for farming common carp and was drained every year to

collect adult fish. It was only in 1960 that the draining in the late summer seasons was stopped. The farming of the common carp was stopped in 1995 and since 1996 the area of the Lebrader Teich has been leased by the Marius-Böger-Stiftung and has been relatively untouched since. Nevertheless, the area is drained stepwise every year between the end of September and the middle of November to get rid of dissolved nutrients for eutrophication prevention. Certainly there is a minimum level of water left during that time, so the area doesn't dry out totally. It is directly connected by a small stream to the Kossau near the Tresdorfer Teich. The system immediately runs to the Rottensee (NLU – Projektgesellschaft mbH & Co. KG, 2010b).

The Neustädter Binnengewässer was also formed in the end of the last glacial period, when a huge amount of ice sheets in the Norwegian Sea melted, the seawater level rose and landscape subsidence was taking place in Schleswig-Holstein. Seawater flooded the area and flushed away the fen, which was there before, except for a small area that remained. In the lake basin of the Neustädter Binnengewässer, several small freshwater streams (i.e. the Lachsau, the Kremper Au, Lübscher Mühlenbach and other freshwater streams) converge before running to the Baltic through a broad channel. The area was used extensively for fishing before parts of it became a protected area of the county of Schleswig-Holstein in 1984. Additionally, it is an official bird-sanctuary designated by the European community. Today it is only used for fishing by a small fishing club and tourists. The inhabiting fish community is likely to be highly complex including both riverine and Baltic Sea piscivorous species (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein, 2006).

One of the streams of Schleswig-Holstein is the Kossau. This 25 km long stream rises at the Rixdorfer Teich and runs through a small valley before it runs into the Rottensee, where the different streams are uniting into one stream that then runs to the Baltic through the Grosser Binnensee near Hohwacht (NIETZKE, 1937). The Kossau belongs to the carbonate-streams of the northern German lowland. In 1985, its middle portion totaling 15 km was declared a nature reserve (POEPPERL, 1999).

### **Goals of the study**

The aim of this study was to investigate the environmental influences on morphological traits to get an idea about the patterns of natural selection that shape morphological variation. Since the nine-spined stickleback displays phenotypic variation in traits related to predation and habitat, it is an excellent model to investigate patterns of morphological variation. In this study nine-spined sticklebacks of one stream, one lake, one brackish water system and two pond populations were



sampled to investigate if there are morphological divergences between populations inhabiting different types of environments.

Considering the studies mentioned above, it can be assumed that differing levels of competition and predation influence phenotypic divergence. To confirm this, the hypothesis was tested that: (1) fish from sampling areas with a low occurrence of piscivorous predators and a low level of interspecific competition would be phenotypically different compared to fish caught in areas with a high occurrence of piscivorous predators and a high level of interspecific competition. Another interesting part of this study was to see whether there are differences in morphological traits between sexes.

Likewise, there is an interesting fact that all sampled areas of this study are connected through the Baltic. Thus, it cannot be excluded that there is genetic exchange between the sampled areas driven by migration. In this study, it has been suggested that the migration effect is smaller, if the distance between areas is greater. Consequently another hypothesis was tested that: (2) Fish are more morphologically similar in water systems that are directly connected by a relatively short distance, whereas populations that are connected by a long distance are divergent.

# Methods

## Sampling

Nine-spined sticklebacks were sampled in a time period between April 11<sup>th</sup> - May 20<sup>th</sup> 2013 in five locations in the county of Schleswig-Holstein, Germany. To compare different environmental habitats, five sampling areas have been chosen (see table 1 for the main characteristics; see fig. 1 for the geography of the areas):

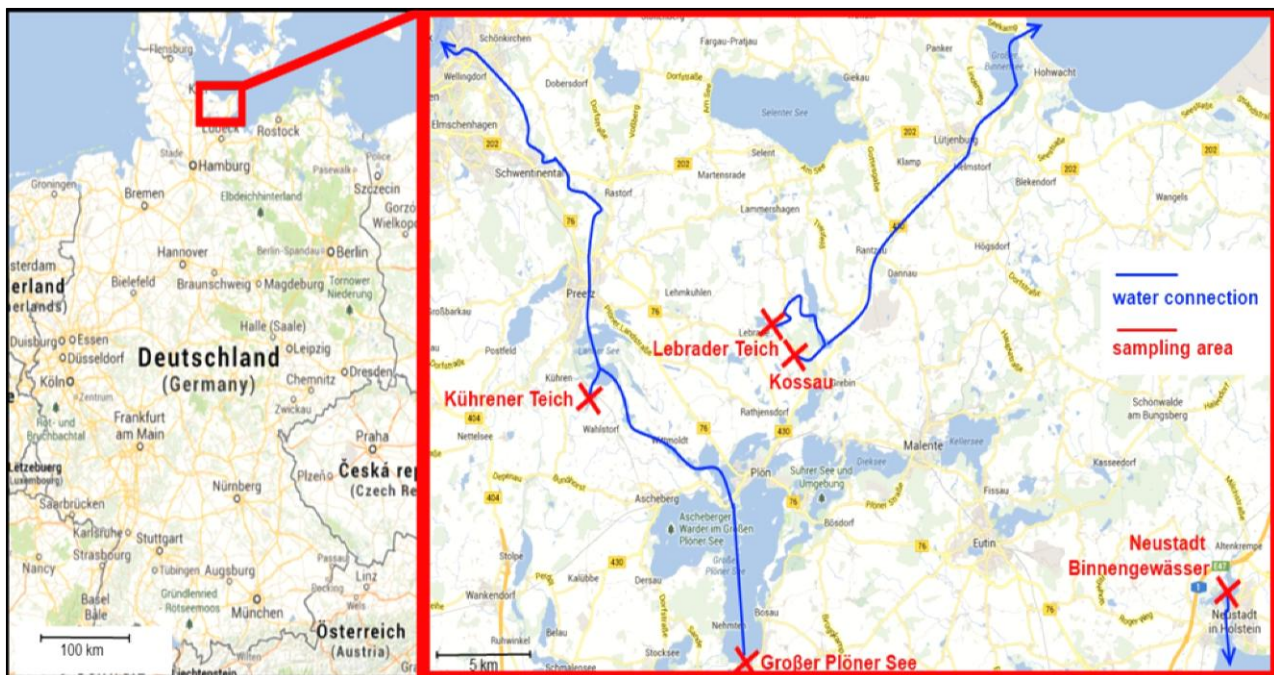


Figure 1: Map of Germany and Schleswig-Holstein, showing sampled areas. Figure created by the aid of google maps.

Großer Plöner See (GPS), Kührener Teich (KT), Lebrader Teich (LT), Neustadt-Binnengewässer (NST), and the Kossau stream (KOS) (see the history of the sampling areas for more information).

## Sampling and preparing of fish:

Collected fish were euthanized with a lethal dose of 1g/L MS222 (tricaine methanesulphonate), dried with a paper towel and weighed using a laboratory balance. To ensure an individual identification, all fish were tagged by a subdermal injection of a unique alphanumeric plastic tag near the caudal peduncle on the right side of the fish (Alpha tag, Northwest Marine Technology). Subsequently, the left side of each fish was photographed with a Canon digital camera (Canon

EOS 650D) under standardized conditions. A piece of scaled graph paper and a small label with the individual given ID of the fish was placed in all photographs.

Sampling site	Date sampled	Number of sticklebacks caught (n)	Number of males	Number of females	Water body surface area [km <sup>2</sup> ]	Maximal depth [m]	Encountered other inhabiting fish species	Coordinates	Salinity [PSU]
GPS	May 2 <sup>nd</sup>	30	15	15	29.1	56.2	BU*, CRO*, CRU*, EP*, NPI*, PCA*, RU*, TSB*, EE*, ZA*, others*	54°04'56.92" N, 10°25'01.14" E	< 0.5 <sup>#</sup>
KT	April 25 <sup>th</sup>	30	11	19	0.313	2.0	EP*, TSB*, CC**, TE**, others	54°11'37.92" N, 10°16'56.80" E	< 0.5 <sup>#</sup>
LT	April 15 <sup>th</sup> - May 9 <sup>th</sup>	30	5	25	0.685	1.3	TE*	54°13'24.10" N, 10°26'31.80" E	< 0.5 <sup>#</sup>
NST	April 11 <sup>th</sup> -12 <sup>th</sup>	30	16	14	1.46	4.3	BG*, EP*, TSB*, EF***, AC***, others	54°12'44.80" N, 10°27'37.40" E	8.8 <sup>*#</sup>
					Length [km]	Mean depth [m]			
KOS	April 15 <sup>th</sup> - May 20 <sup>th</sup>	25	6	19	25	0.55	CRU*, EP*, NPI*, PCA*, others	54°06'41.55" N, 10°48'53.42 E	< 0.5 <sup>#</sup>

Table 1: Main characteristics of sampled areas.

AC = Atlantic Cod (*Gadus morhua*), BG = Black Goby (*Gobius niger*), BU = Burbot (*Lota lota*), CC = Common Carp (*Cyprinus carpio*), CRO = Common Roach (*Rutilus rutilus*), CRU = Common Rudd (*Scardinius erythrophthalmus*), EE = European Eel (*Anguilla anguilla*), EF = European Flounder (*Platichthys flesus*), EP = European Perch (*Perca fluviatilis*), NPI = Northern Pike (*Esox lucius*), PCA = Prussian Carp (*Carassius gibelio*), RU = Ruffe (*Gymnocephalus cernua*), TE = Tench (*Tinca tinca*), TSB = Three-Spined Stickleback (*Gasterosteus aculeatus*), ZA = Zander (*Sander lucioperca*)

other: other fish species assumed

\*caught during the sampling of this project

\*\* NLU Projektgesellschaft mbH & Co. KG, 2010a

\*\*\* present in the western Baltic (H. Möller, 1976);

\* LIMNOPLAN, 2006

\*# LARSON & POHL, 2006

# freshwater salinity level (LEE & BELL, 1999)

After photographing the fish, the caudal fin of each fish was clipped for genetic analyses (not included in this study) and the fish were dissected to identify the sex. To optimize the measurements of bony parts, fish were then stained with Alizarin Red S, which is an anthrachinon dye and accumulates in bony tissues and stains them dark red. This procedure ensures a better viewable contrast of spines and the pelvic girdle. To prepare the staining, all inner organs were dissected out of the abdominal cavity and fish were transferred into 95% Ethanol. All fish of one population were stored together in 250 ml plastic containers and kept in 95% Ethanol for at least 48 hours to ensure their fixation.

To prepare the staining of the pelvic girdle and the spines, fish were rehydrated according to the following scheme: 70% EtOH for 24 hours, 50% EtOH for 24 hours, 20% EtOH for 24 hours and distilled H<sub>2</sub>O for 24 hours. Fish were then transferred into an alkaline Alizarin Red solution (0.1

g/L KOH, 0.425 g/L Alizarin Red in distilled water) for 18 hours and were rinsed in 0.1 g/L aqueous KOH solution for another 24 hours afterwards. Finally, each fish was transferred into a single 15 mL tube with 50 % Isopropanol for long-term storage. After staining, the left side and the ventral side of each individual were photographed again under the same conditions which were used when taking the initial pictures (see above).

### Measurements of morphometric traits

All the pictures were sorted by population and different views. With the help of the software tpsUtil 1.56 (ROHLF, 2013) four TPS files for each population were created. The first TPS file link the photos with view to the unstained left side, the second TPS file link the photos from stained fish with view to the left side, the third TPS file link the photos from stained fish with view to the ventral side and the last TPS file link again the photos from the unstained fish to enable another set of landmarks on these pictures. After the creation of these files, the TPS files were used for the setting of digital landmarks with the aid of tpsDig2 version 2.16 (ROHLF, 2012). Landmarks were set as following:

Set #1 (see fig. 2): 1: midline of the left side of the scale; 2: midline of the right side of the scale; 3: anterior tip of the lower lip; 4: tip of the caudal peduncle; 5: top of the head in the center of the eye; 6: bottom of the head in the center of the eye; 7: very top of the body; 8: lower point of the body perpendicular to point 7; 9: most dorsal point of the eye; 10: most ventral point of the eye.

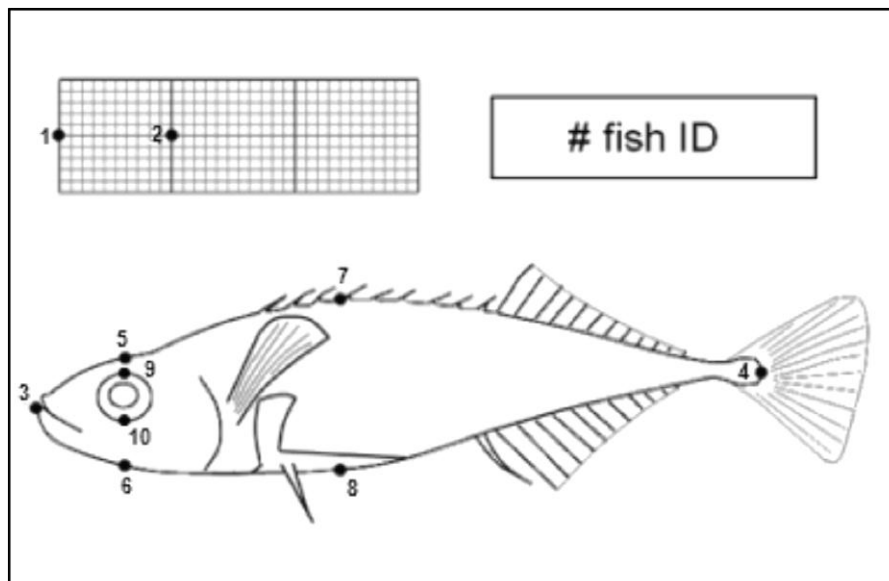


Figure 2: First Landmark Setting. See text for detailed description.

Set #2 (see fig. 3): 1: midline of the left side of the scale; 2: midline of the right side of the scale; 3: dorsal end of the lower jaw; 4: ventral end of the lower jaw; 5: origin of the first dorsal spine; 6: ending of the first dorsal spine; 7: origin of the 4<sup>th</sup> dorsal spine; 8: ending of the 4<sup>th</sup> dorsal spine; 9: origin of the 7<sup>th</sup> dorsal spine; 10: ending of the 7<sup>th</sup> dorsal spine; 11: origin of the anal spine; 12: end of the anal spine; 13: lower posterior origin from the ascending branch of the pelvic girdle; 14 + 15: upper caudal end from the ascending branch of the pelvic girdle; 16: upper cranial end from the ascending branch of the pelvic girdle.

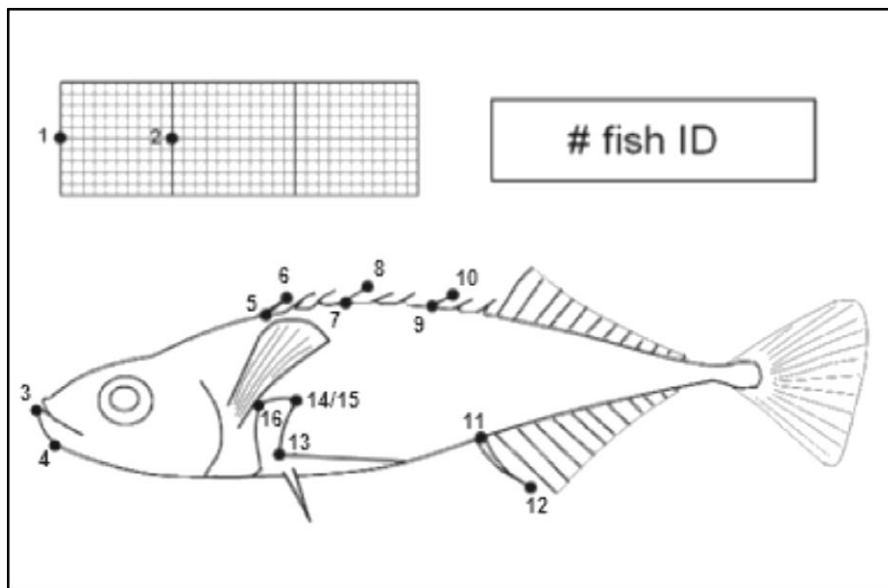


Figure 3: Second Landmark Setting. See text for detailed description.

Set #3 (see fig. 4): 1: midline of the left side of the scale; 2: midline of the right side of the scale; 3: left tip of the anterior process from the pelvic girdle; 4: tip of the posterior process from the pelvic girdle; 5: anterior tip of the left edge of the pelvic girdle; 6: ventral suture of the pelvic girdle; 7: origin of the left pelvic spine; 8: end of the left pelvic spine; 9: origin from the right pelvic spine; 10: end of the right pelvic spine.

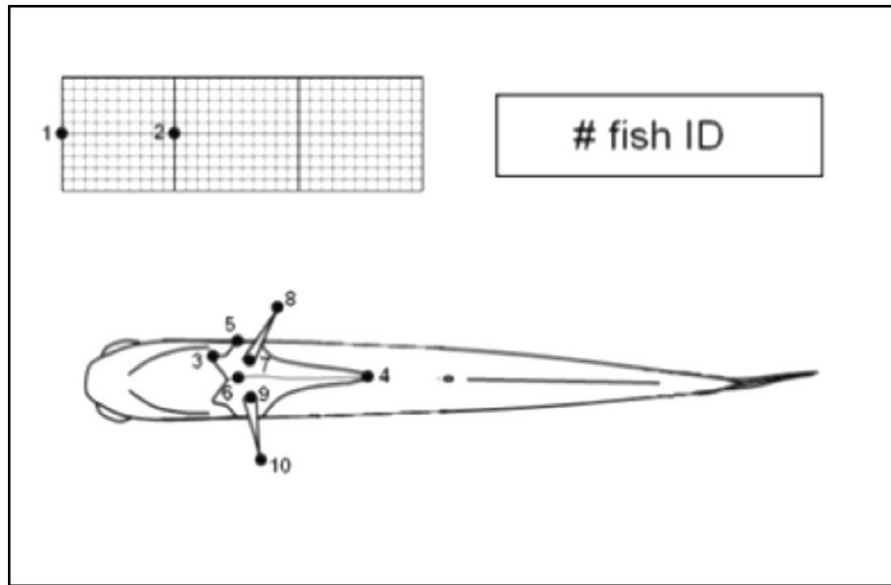


Figure 4: Third Landmark-Setting. See text for detailed description.

The TPS files including the first three settings were then transferred into a self-created program, which uses the ruby-language (Y. Matsumoto, 2002) and measures the distance between all pairs of landmarks. It also scales distances by using the distance between the first two landmarks (which always equals 1cm). Finally, the measuring program produced a comma separated value file for each TPS file, which contained all measured morphological traits of the landmark setting for each individual of the population in question, including their averages as well (see appendix one for the source code of the measuring program).

All measured morphological traits of the first three landmark settings were assembled together into one Excel file. The counted number of dorsal spines was added to the excel file manually after visual inspection of each fish. The sex, which was investigated via inspection of the gonads, was also noted individually. Measured morphological traits were noted as following (see fig. 5): 1: standard length; 2: head depth; 3: body depth; 4: Eye Diameter; 5: Lower Jaw Length; 6: length of the first dorsal spine (= anterior dorsal spine length); 7: length of the fourth dorsal spine (= middle dorsal spine length); 8: length of the seventh dorsal spine (=posterior dorsal spine length); 9: length of the anal spine; 10: length of the left pelvic spine; 11: length of the right pelvic spine ; 12: pelvic girdle length; 13: pelvic girdle width; 14: length of the pelvic ascending branch; 15: width of the pelvic ascending branch; 16: number of dorsal spines.

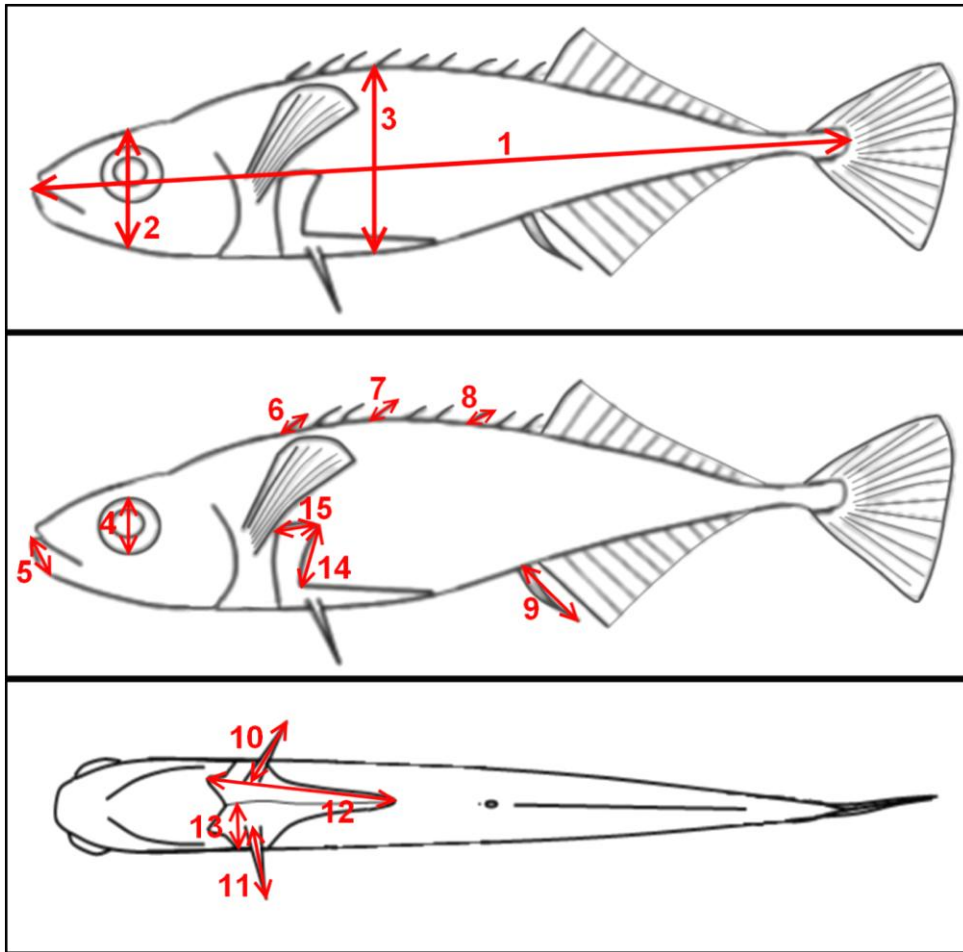


Figure 5: Summary of all measured morphological traits.

To see whether or not the standard length, sex or population (sampling area) affect measured morphological traits, a Multivariate Analysis of Covariance (MANCOVA) was performed on all morphological traits excluding the meristic character (number of dorsal spines) and using standard length as a covariate. Sex and population were used as fixed factors.

To optimize the comparison between populations, all measured values without the standard length and the number of dorsal spines were divided by the body length. So comparisons regarding these traits were made using the related values of the traits in units per standard length ( $SL^{-1}$ ). Furthermore, the data were transferred into JMP 11 (SAS Institute Inc.; Cary, NC) and the significance of the variation between populations of morphological traits was assessed performing an Analysis of Variance (ANOVA) on size corrected measurements. Finally a principal component analysis (PCA) was performed on all measured morphological traits excluding the number of dorsal spines and each fish was labeled by population.

### Shape Measurements

To compare the shape between analyzed populations, a last landmark setting was performed on lateral side photos.

Set #4 (see fig. 6): 1: anterior tip of lower lip; 2: posterior edge of angular; 3: anterior tip of ectocoracoid; 4: posterior tip of ectocoracoid; 5: base of first anal ray on ventral midline; 6: insertion of anal fin membrane on the ventral midline; 7: origin of caudal fin membrane on the ventral midline; 8: tip of the caudal peduncle; 9: origin of caudal fin membrane on the dorsal midline; 10: insertion of dorsal fin membrane on the dorsal midline; 11: base of the first dorsal fin ray on the dorsal midline; 12: anterior junction of the first dorsal spine on the dorsal midline; 13: posterior extent of the supraoccipital head region

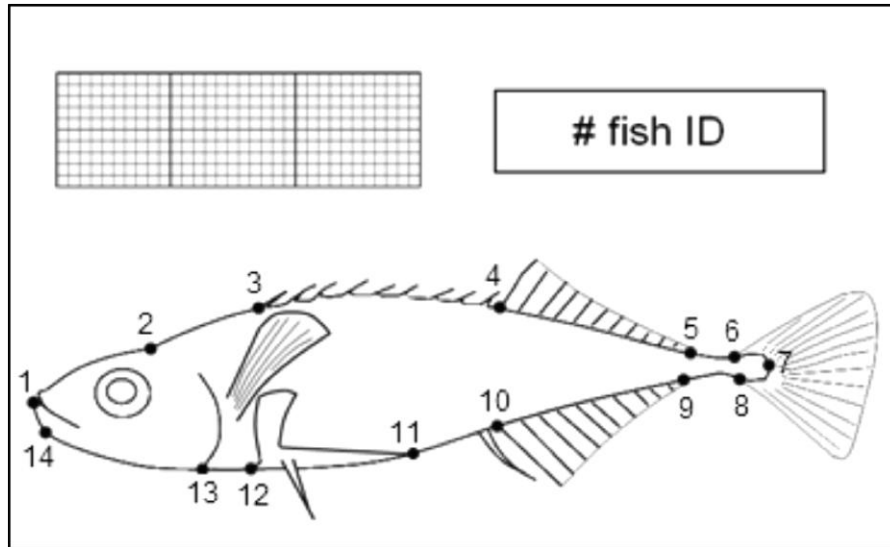


Figure 6: Landmark setting for the 2D shape analysis.

The TPS files of the fourth setting of landmarks, was imported to the program MorphoJ (KLINGENBERG, 2008) building new Datasets. MorphoJ was used to superimpose the digitized landmarks, performing a procrustes fit by aligning landmarks to the general axis (long axis). Then a covariance matrix was created, before performing a canonical variance analysis (CVA). The principal component analysis is one of the widely used methods to analyze shape variation. It decomposes a covariance matrix by orthogonal transformation using linear algebra into a set of so called principal components (PCs). These PCs are a set of linearly uncorrelated variables, whose number reflects more or less the number of original variables. In MorphoJ this number is determined by using a threshold of  $10^{-14}$ , which normally provides the right number of dimensions. The program supplies several outputs. It first shows the eigenvalues of each PC as a percentage of the total variance and as the cumulative percentage of total variance. As a second output it supplies the scores of each PC, computed as the vectors of the deviation from the sample mean multiplied by the eigenvector. Considering the eigenvalues-greater-than-one rule (KAISER, 1960) only eigenvalues greater than one were used for further analyses. Also we used functions of the program to create plots of the computed shape variance.



# Results

## Comparison of morphological traits

With the help of the first three landmark settings, 15 morphological traits were measured and summarized by population in table 2.

	Standard Length	Head Depth	Body Depth	Eye Diameter	Lower Jaw Length	Anterior Dorsal Spine Length	Middle Dorsal Spine Length	Posterior Dorsal Spine Length
	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
<b>GPS</b>	28.3 ± 2.3	3.50 ± 0.22	6.08 ± 0.47	2.22 ± 0.19	2.34 ± 0.21	1.60 ± 0.16	1.51 ± 0.23	1.42 ± 0.19
<b>KOS</b>	50.4 ± 3.6	5.73 ± 0.51	10.43 ± 0.91	3.15 ± 0.23	3.73 ± 0.41	1.92 ± 0.28	1.87 ± 0.26	1.78 ± 0.24
<b>KT</b>	47.9 ± 3.7	5.51 ± 0.50	10.04 ± 0.80	3.18 ± 0.20	3.65 ± 0.48	1.94 ± 0.28	1.96 ± 0.35	1.81 ± 0.22
<b>LT</b>	50.5 ± 4.1	5.60 ± 0.45	11.17 ± 1.32	3.11 ± 0.21	3.50 ± 0.32	2.03 ± 0.26	2.04 ± 0.29	1.88 ± 0.32
<b>NST</b>	33.8 ± 4.0	3.74 ± 0.36	6.18 ± 0.59	2.64 ± 0.23	2.42 ± 0.32	1.68 ± 0.21	1.56 ± 0.21	1.42 ± 0.25

	Anal Spine Length	Left Pelvic Spine Length	Right Pelvic Spine Length	Pelvic Girdle Length	Pelvic Girdle Width	Pelvic Girdle, Ascending Branch Length	Pelvic Girdle, Ascending Branch Width	Number of Dorsal Spines
	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	
<b>GPS</b>	2.11 ± 0.19	3.29 ± 0.37	3.46 ± 0.44	5.50 ± 0.52	1.25 ± 0.17	2.48 ± 0.28	1.44 ± 0.31	9.6 ± 0.61
<b>KOS</b>	2.39 ± 0.39	4.51 ± 0.58	4.61 ± 0.58	8.90 ± 0.80	2.22 ± 0.34	3.30 ± 0.47	1.95 ± 0.51	10.7 ± 0.73
<b>KT</b>	2.11 ± 0.30	4.04 ± 0.49	4.19 ± 0.46	7.79 ± 0.70	1.94 ± 0.28	3.04 ± 0.34	1.63 ± 0.47	9.6 ± 0.57
<b>LT</b>	2.52 ± 0.24	4.63 ± 0.57	4.91 ± 0.62	8.86 ± 0.88	2.20 ± 0.28	3.34 ± 0.48	2.09 ± 0.53	10.1 ± 0.64
<b>NST</b>	2.03 ± 0.29	3.28 ± 0.44	3.46 ± 0.38	6.09 ± 0.66	1.17 ± 0.17	2.31 ± 0.25	1.44 ± 0.42	9.1 ± 0.51

Table 2: Population means for morphological measurements ( $\pm$  one standard deviation) in mm and the mean number of dorsal spines of each population ( $\pm$  one standard deviation).

The results of the MANCOVA showed significant values for all tests, which demonstrated that all three characters (SL, Population and Sex) significantly affect morphological traits (see table 3). Further tests for between subject effects showed P-Values lower than 0.001 for the effect of SL on each investigated morphological trait, showing that SL affects each of the other morphological traits. The P-values for between subject effects of population on the anterior and middle dorsal spine (anterior dorsal spine:  $F_{4,144} = 1.7$ ,  $P = 0.154$ ; middle dorsal spine:  $F_{4,144} = 2.42$ ,  $P = 0.52$ ) indicated that there was no significant effect on these traits. Concerning the effects of sex, between subjects analyses exhibited only 4 significant P-Values lower than 0.05 for the head depth, the eye diameter, the lower jaw length and the pelvic girdle width.

Effect	Wilk's Lambda	F-Value	Among Group DF	Within Group DF	P-Value
<b>Standard Length</b>	0.187	37.67	14	121	P< 0.001
<b>Population</b>	0.111	6.39	56	473	P< 0.001
<b>Sex</b>	0.559	6.82	14	121	P< 0.001
<b>Population * Sex</b>	0.376	2.41	56	473	P< 0.001

Table 3: Results of the MANCOVA.

The calculated mean body length (Standard Length) was significantly smaller for the GPS population ( $F_{4,144} = 241.42$ ;  $P < 0.0001^*$ ) and the mean body length of the NST population was significantly smaller than the body length of the remaining three populations ( $F_{4,115} = 124.20$ ;  $P < 0.0001^*$ ). The analysis of the quantitative dorsal spine counts showed a clear tendency for the population from NST to have a mean number of nine spines with the lowest amount of variation (mean =  $9.1 \pm 0.51$  SD, Range: 8-10). In contrast, fish from KOS had the highest mean number of dorsal spines with 10-11 spines (Range: 9-12) followed by fish from LT, which had a mean number of ten spines (Range: 9-11). Individuals from KT or GPS showed an intermediate mean number of nine to ten spines (Range: 8-11). All differences in the number of dorsal spines between populations are significant ( $F_{4,144} = 17.8783$ ;  $P < 0.0001^*$ ).

The related head depth as well as the related lower jaw length showed a difference between the GPS population that had higher values than the rest of the populations that seemed to be broadly similar (Related Head depth:  $F = 17.50$ ,  $P < 0.001$ ; related lower jaw length:  $F = 14.85$ ) (see fig. 7 for the comparative graphs). Body depth in relation to body length (SL) showed similar values for populations from GPS, KOS, KT and LT, while fish from NST showed clearly lower values. Comparing the related eye diameter of all populations, GPS and NST fish differed from KT, LT and KOS fish in a higher eye diameter in relation to body size. The values of the related spine lengths (anterior dorsal spine, middle dorsal spine, posterior dorsal spine, anal spine, left & right pelvic spine) demonstrated the significantly highest spine length in relation to body size (standard length) for GPS fish. Also, these measurements consistently showed that the related spine lengths of the brackish water population (NST) are significantly higher than the related spine lengths of the pond populations (KT, LT, KOS). Considering the pelvic girdle, the data analysis demonstrates GPS fish to have longer pelvic girdles and a larger ascending branch (concerning length and width) in contrast to the rest. As opposed to this, brackish water fish from NST showed a higher width of the pelvic girdle.

## Results

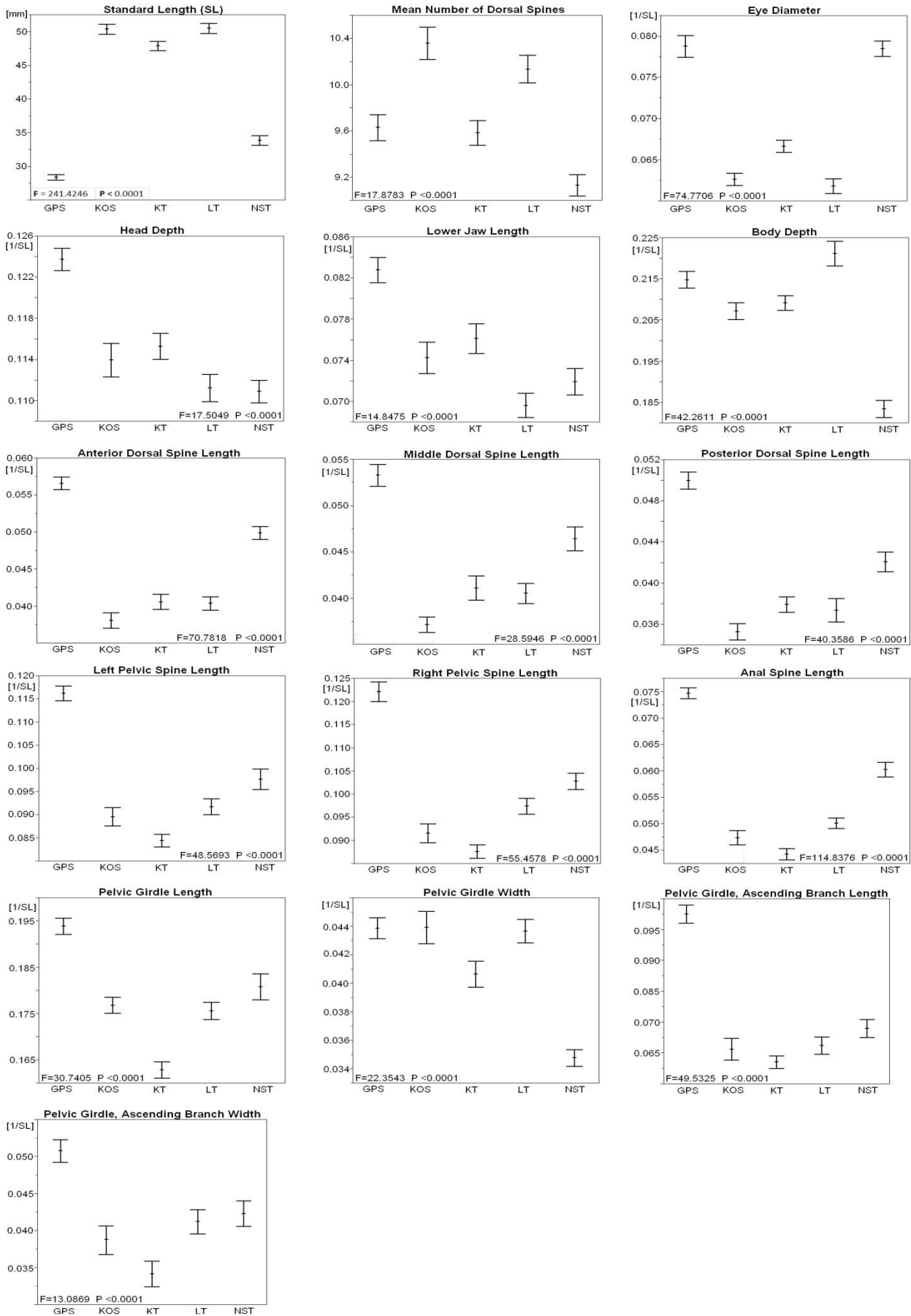


Figure 7: Comparison of the mean values  $\pm$  standard error (SE) between populations for each morphological character corrected for standard length excluding standard length and dorsal spine number, which are not corrected for body size.

### Principal component analysis (PCA)

The PCA for standard length and all other morphological traits showed that there is morphological variance between individuals. A large value, 73.98 cumulative percent of all variation, was explained by the first three principal components axes (see table 4). The 3D scatterplot of the first three PC axes (see fig. 8) indicates that fish from GPS and fish from NST differ significantly from the remaining three populations. This is also shown in the results of the clustering by population (see fig. 11), which indicates that the probability for fish from KOS, LT and KT to be closer related to each other, based on morphological traits, is higher than in comparison to fish from NST or GPS. Fish from KT seem to be more similar to fish from LT and KOS, while fish from GPS and fish from NST differ from the rest concerning their morphological traits.

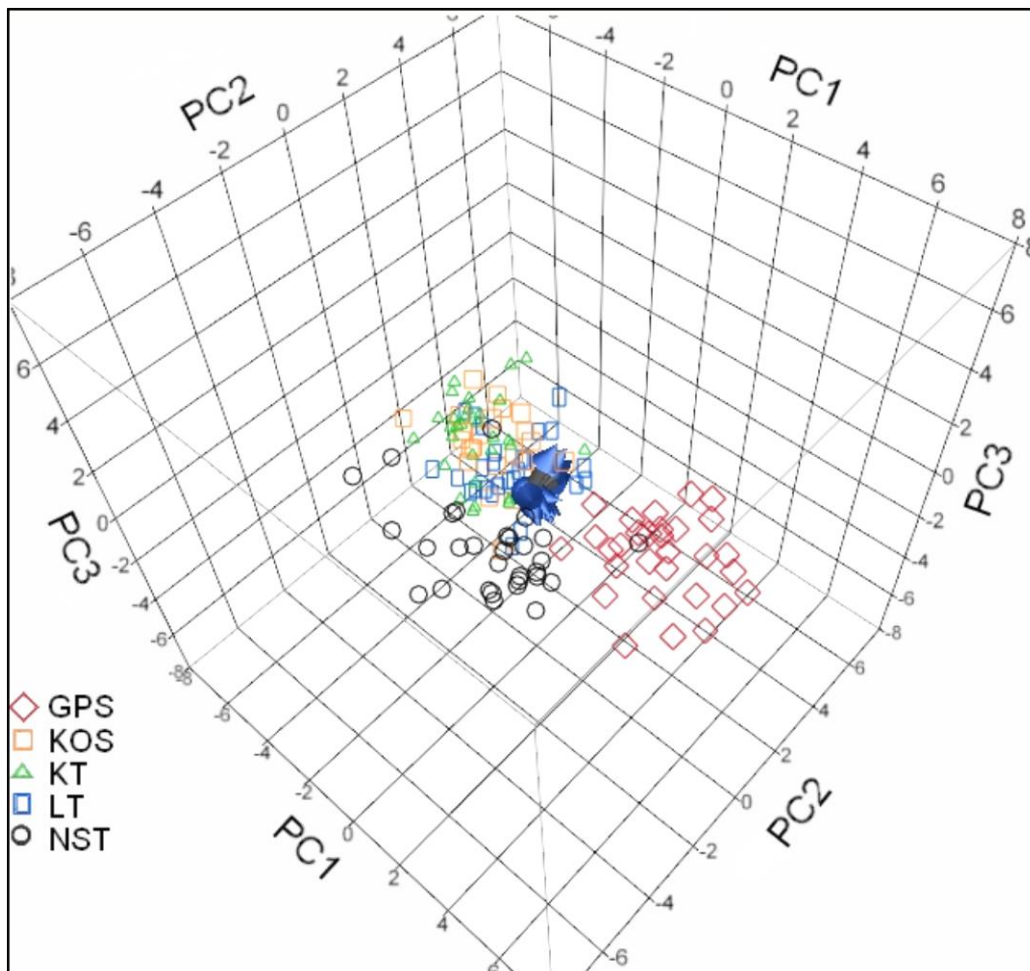


Figure 8: 3D Scatterplot of individual PC scores for the first 3 principal components. Each value is labeled by population (see legend on left side).

Principal component number	Eigenvalue	Percent of explained variation	Cumulative percent of explained variation	Chi Square	Degrees of freedom	P-Value
1	7.0330	50.2	50.236	1561.94	85.838	<0.0001*
2	1.9040	13.6	63.836	699.483	84.920	<0.0001*
3	1.4203	10.1	73.981	474.709	74.267	<0.0001*
4	0.7848	5.6	79.587	272.940	63.953	<0.0001*

Table 4: Eigenvalues of the principal component analysis on SL and other morphological traits related to SL, excluding the number of dorsal spines.

## Shape Analysis

By comparing the graphical 2D visualizations of the wire framed landmark means of each population superimposed on the wire framed means of all specimens, shape differences became obvious (see fig. 9). Brackish water fish from NST showed the greatest differences compared to the mean shape of all specimens (light blue). They seem to have a slim body, while fish from

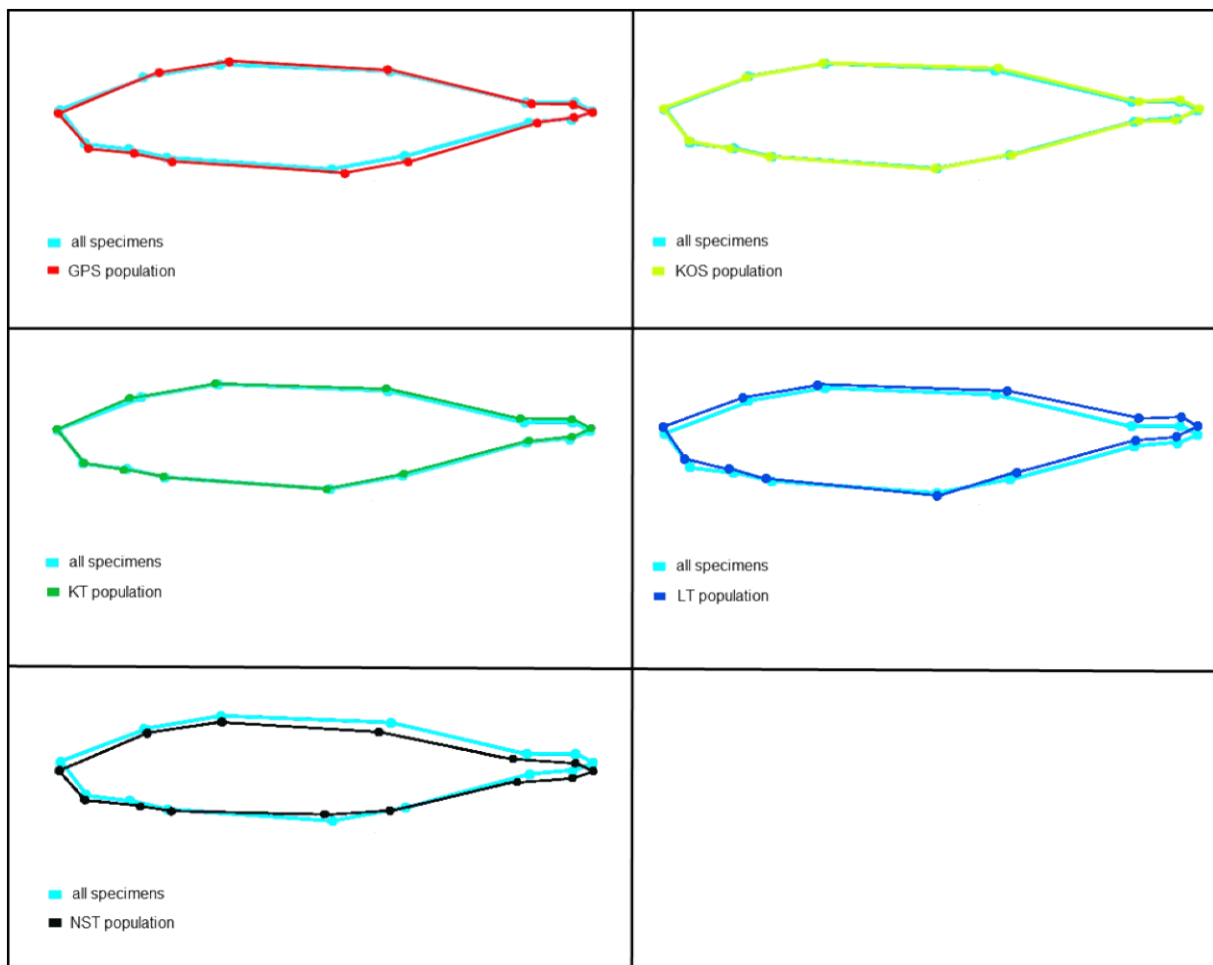


Figure 9: Mean body shape of each population (color varies with population) figured as the wire framed projection of landmark-set no. 4 superimposed on the wire framed landmark means of all specimens (light blue).

freshwater systems (GPS, KOS, KT, LT) are wider and have more voluminous shapes. The Shape of fish from KOS and KT were close to the mean shape of all specimens and they showed the highest similarity to each other. GPS and LT fish differ in their shape from the mean shape of all specimens in some landmarks.

To see whether or not 2D shape data support previous results from the analyzing of measured morphological traits, a canonical variance analysis was performed on the computed procrustes coordinates with population fixed for grouping. As the output shows, 83.61 percent of among group shape variation is explained by the first two canonical variants, which are also the only ones which have Eigenvalues higher than one (see table 5).

Canonical variant number	Eigenvalue	% Variance	Cumulative % of Variance
1	5.03	57.1	57.1
2	2.47	28.1	85.2
3	0.83	9.4	94.7

Table 5: Eigenvalues of the canonical variance analysis on procrustes coordinates of all individuals.

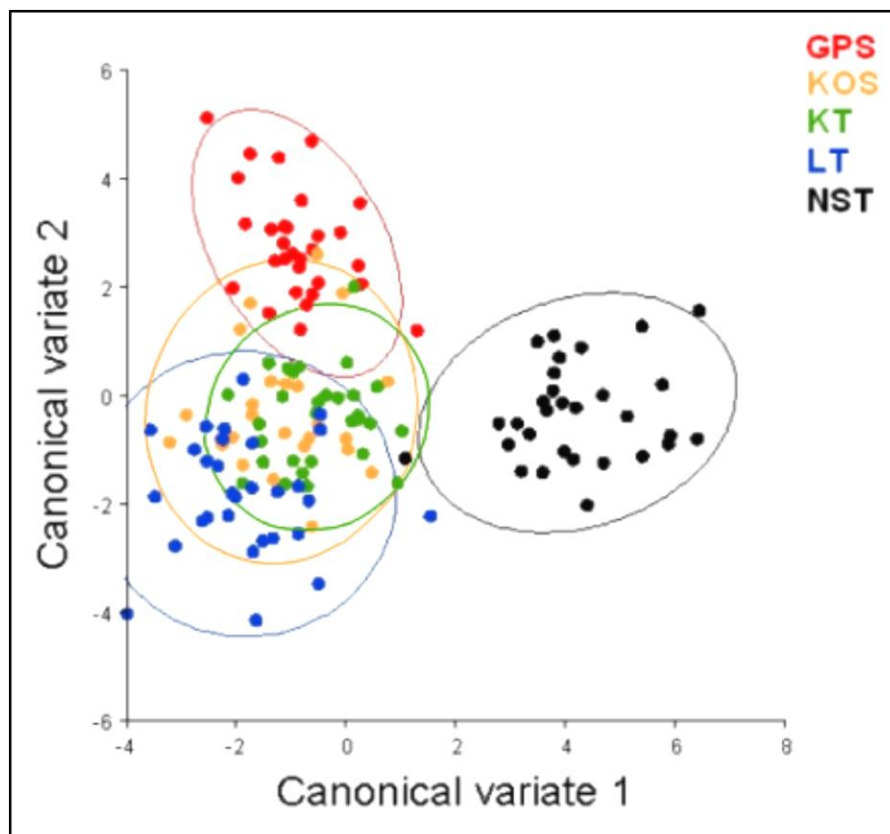


Figure 10: Canonical variant scores of all specimens, labeled by population. The Ellipses are marking the computed area for each population, which includes 95% of all scores for the respective population.

By using these first two canonical variants as axes, the graphical output (fig. 10) shows a significant (see table 6) shape difference for fish from NST and another divergence in shape for GPS fish from the remaining three populations, which seem to have a higher similarity of their body shape. This result agrees with the output of the PC analysis on measured morphological traits.

	GPS	KOS	KT	LT	NST
GPS		0.0246	<0.0001*	<0.0001*	<0.0001*
KOS	0.0246		0.0004	0.0085	<0.0001*
KT	<0.0001*	0.0004		<0.0001*	<0.0001*
LT	<0.0001*	0.0085	<0.0001*		<0.0001*
NST	<0.0001*	<0.0001*	<0.0001*	<0.0001*	

Table 6: Contingency table of the P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups.

## Cluster Analysis

Finally, to analyze the morphological relationships between groups, a clustering analysis was performed on the population values for all morphological traits including the number of dorsal spines. The clustering was performed by using the means, standard deviations and ranges of these traits. Computed distances were visualized in a dendrogram (fig. 11), which shows the most probable similarities between examined populations. As the figure shows, fish from GPS and NST differ from the rest, while KOS and LT seem to be more similar by comparing their morphological characters.

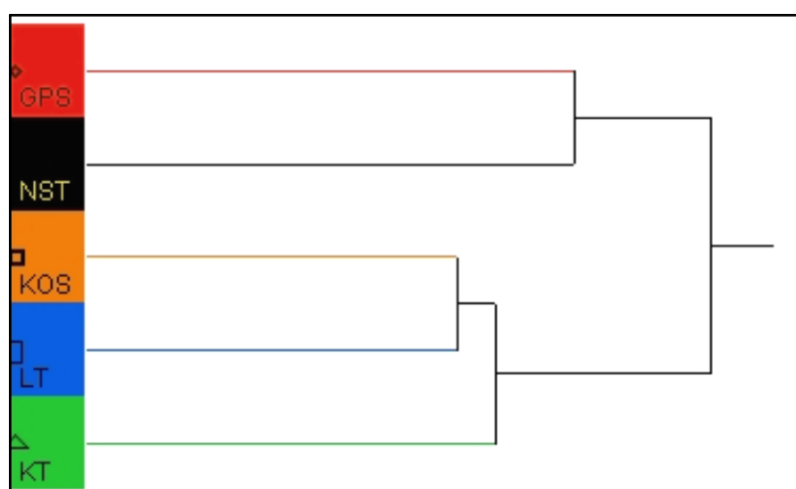


Figure 11: Dendrogram output of the cluster analysis on morphological traits showing relationships between populations.

# Discussion

## Comparison of morphological traits

Before comparing single morphological traits between populations, it is worth asking whether or not fish from different sampled areas really differ considering their morphological traits. This question was answered by the results of the MANCOVA. With a low value of 0.111 for wilk's lambda, population (=sampling area) had the biggest effect on morphological traits compared to the other investigated characteristics.

Significant P-values for the effect of SL on all the other measured morphological traits indicated that the complete morphology is dependent to the body size of the fish. Therefore a small nine-spined stickleback is more likely to have smaller spines than a larger one. Thus these results show that it was necessary for a realistic comparison of morphologic traits between populations to relate the other morphological traits to the standard length.

Comparing the SL itself between fish from different populations, the results showed that the mean SL from GPS as well as the mean SL from NST fish is lower than the SL of the other populations. Body size has been reported before to vary between populations of nine-spined sticklebacks under different environmental conditions (HERCZEG et al. 2009, 2010; MOBLEY et al., 2011). One reason for smaller body sizes might be interspecific competition i.e. with the three-spined stickleback (*Gasterosteus aculeatus*) (HERCZEG et al. 2009). This assumption is supported by the results of this study which shows that populations, where nine-spined sticklebacks are non-sympatric to three-spined sticklebacks (KOS and LT), have the largest body size. Also, nine-spined sticklebacks from GPS and NST, which are sympatric to three-spined sticklebacks, showed the smallest body size (see table 2). An exception was found in fish from KT, which exhibits a tendency for larger body sizes while living sympatric to three-spined sticklebacks. This leads to the hypothesis that other factors influence their body size. Another factor which seems to have a strong effect on body size is predation (e.g. POPOVA 1967; ZARET 1980; REIMCHEN, 1991; HERCZEG et al. 2009, 2010; MOBLEY et al., 2011). Regarding the predation regime of all sampled areas, investigated individuals from populations sympatric to lots of piscivorous fish species (GPS and NST) (see table 1) had the lowest body sizes. In contrast, nine-spined sticklebacks from LT, where no piscivorous fish species could be found, had the largest body size. Fish from KT also show larger body sizes (compared to GPS and NST), although European perch (*Perca fluviatilis*) were caught during sampling. On the other hand only small perches, which showed the same size as the sampled sticklebacks, were caught during



sampling. Additionally, this pond is used extensively for fishing (NLU PROJEKTGESELLSCHAFT MBH & Co. KG, 2010a), which could mean that the larger perch are caught by fishermen. So the predation pressure might be low. Nine-spined sticklebacks from KOS were sympatric to piscivorous fish species like European Perch and Northern Pike (*Esox lucius*). However, only small individuals of these predator species have been caught during sampling time. This leads to the assumption that the Kossau stream might be too shallow for larger individuals of these species and only young perches and pikes are crossing through the Kossau. In this case, larger nine-spined sticklebacks could only be hunted by larger piscivorous fish and hence a larger body size could be an advantage to reduce the total number of possible predators. For three-spined sticklebacks, such a phenomenon is proven, since their size increases when sympatric with piscivorous fish species (POPOVA, 1967; ZARET 1980). This effect might outweigh the viability costs for a large body size. Otherwise it is also possible that a larger body size results from a lower predation pressure like in KT and LT because this study didn't investigate the exact amount of piscivorous fish in the Kossau stream.

Measured body depth showed the lowest mean values for population with a presumably high predation pressure (GPS and NST). When related to standard length, the body depth of GPS, KOS, KT and LT were broadly similar, while fish from NST showed clearly lower values. Thus, this study doesn't indicate a predation pressure escape in nine-spined sticklebacks, as it has been shown for Crucian carp (*Carassius carassius*) (Brönmark & Miner, 1992; Vøllestad et al, 2004). The body depth variation among populations must therefore result from other selective pressures. Since NST is the only brackish water population in this study and body depth is attributed to differences in diet (GROSS, 1979), differences to the freshwater populations may due to differences of available food or other environmental factors.

Regarding the total defensive system of the nine-spined sticklebacks, the results of this study showed variation among populations in the related length of all measured morphological traits. As expected, the largest spines in relation to their body size were measured in populations, inhabiting environments with a high occurrence of piscivorous fish (GPS and NST). Also, populations with a presumably low predation pressure from piscivorous fish had shorter spines in relation to their body size (KT and LT). In fact, nine-spined sticklebacks from KOS had the shortest spines in relation to their body size, which may indicate that the presence of piscivorous fish in the Kossau stream is lower than expected. On the other hand this could also mean that it is a better strategy, in the environment of the KOS stream, to reach a large body size for escaping predation pressure than to increase the spine armory. Likewise, as the community of inhabiting fish is less complex in KOS, KT and LT, it is also imaginable that the density of predatory insects

is higher in these habitats. Consequently, this might lead to smaller spines in these populations as well, since it has been reported that a higher occurrence of predatory insects selects for reduced spine armory (ZIUGANOV & ZOTIN, 1995). On the other hand, the selective advantage of spine loss as a deterrent to dragonfly larvae for example is so small that its effects cannot be distinguished from drift (MOBLEY et al., 2013). Therefore, the effect of predatory insects on the expression of the spine armory is disputable.

The one measured meristic character, the number of dorsal spines, divides the investigated populations in three groups: the group with a population mean number of nine dorsal spines (NST), the group with a population mean number of nine to ten dorsal spines (GPS and KT) and the last group with a population mean of ten dorsal spines (KOS and LT). Since the dorsal spines are a part of the spine armory, they are likely a part of the protection system against predators (HOOGLAND et al, 1956). So it would be conceivable that the efficiency of predation protection increases with the number of dorsal spines. On the other hand, variation in the number of dorsal spines is apparent in each population, which indicates a low selective pressure for the total number of dorsal spines. Furthermore, populations caught in an environment with a high number of piscivorous fish species didn't show the highest number of dorsal spines. Consequently it can be assumed that the exact number of dorsal spines does not affect predation protection as long as there is a minimal number. In conclusion, since the range of the numbers of dorsal spines varies within populations, this trait might be good evidence of genetic drift among populations.

### **Comparison of sexes**

The MANCOVA results of this study showed an effect of sex on morphologic traits like head depth, lower jaw length, eye diameter and pelvic girdle width. However, population was shown to have a bigger effect on measured morphological traits. Hence, individuals from different populations are likely to differ from each other concerning their morphological traits, even if they have the same sex. Thus, to ensure a realistic comparison between sexes, the same number of both sexes must be compared within the same population. Additionally, this comparison should be repeated for a couple of other populations and the results have to be summarized. Furthermore, a minimum amount of 30 individuals per sex should be compared for the study to have sufficient power to show statistical significance. This implies that the results of this study are insufficient to show realistic differences between sexes, since all these conditions are not fulfilled. In conclusion, the results of this study indicate that there could potentially be differences between sexes in regards to the morphological traits mentioned above, but these would need to be considered critically.

## Conclusion

The results from the PCA clearly demonstrated that there are morphological differences between sampled populations. GPS and NST fish differ the most from the other populations (KOS, KT and LT) concerning their morphological traits. These results were confirmed by the canonical variant analysis on landmark procrustes coordinates (shape) showing that GPS and NST nine-spined sticklebacks also differ in shape from the remained populations (KOS, KT and LT), leading to the overall potential for habitat to affect morphology. By comparing the different environmental conditions it is clear that GPS and NST are also the areas that differ the most from the remained areas. Likewise, their environmental conditions differ to each other, since GPS is a deep freshwater lake and NST is a shallower brackish estuary, which is nevertheless deeper than the other areas. Indeed, both have a high occurrence of piscivorous fish species as well as a high level of interspecific competition and both exhibited an increase of their defensive spine armory. They also seem to be reproductively isolated from each other, since their only connection is a relatively long distance through the Baltic and they differ in shape and other morphological traits. Consequently, the results are showing a parallel evolution of similar morphological traits as a response to specific environmental conditions. This strongly implicates natural selection, as genetic drift is unlikely to produce concerted change correlated with the environment (RUNDLE et al., 2000). So if natural selection is driving morphologic variation between sampled populations, it implies that different morphological traits evolve as an adaptation. Further genetic comparisons of the sampled populations using 18 different microsatellites showed a similar result to the morphologic analyses of this study (MOBLEY et al., unpublished data) (see fig. 12), showing that uncovered phenotypic variation is likely not caused by phenotypic plasticity but rather from genetic adaptation. This also leads to the assumption that divergent selection is taking place between the contrasting environments, which might also cause reproductive isolation (SCHLUTER, 2001).

Human disturbances, however, have interrupted the evolutionary processes affecting these populations. Fifty years ago for example, LT was totally drained at the end of each summer. Or the areas were extensively used for fishing, causing changes in the fish community. In addition, the time period between the last glacial period and today is a relatively short time in terms of evolutionary processes and even if there are no large differences detected between sampled populations, constant divergent selection may cause ecological speciation (SCHLUTER, 2001).

Thus uncovered selection pressures may play a role in the big system of natural selection and the process of speciation caused by adaptive radiation.

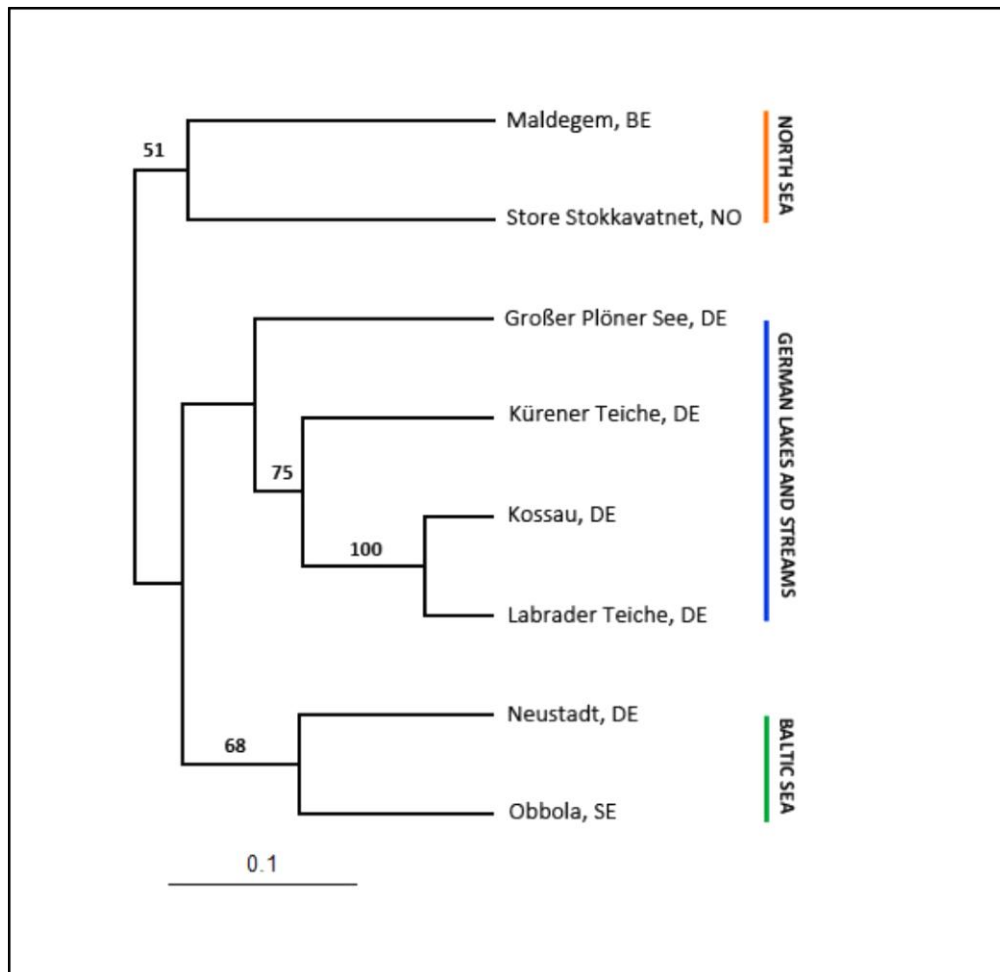


Figure 12: Unweighted pair group method with arithmetic mean (UPGMA) tree of Nei's genetic distance ( $D_A$ ) showing genetic distances based on 18 microsatellite loci (Mobley, unpublished data).

However, the patterns of natural selection are still not clear. The results of this study lead to the assumption that predation is a major factor affecting morphological variation in nine-spined sticklebacks. This confirms the results from a range of other studies both for nine-spined sticklebacks (e.g. GROSS, 1979; HERCZEG et al., 2009, 2010, 2012; MOBLEY et al., 2011; SHIMADA et al., 2011; AIKIO et al., 2013) and in general for phenotypic variation (e.g. REIMCHEN, 1991; VØLLESTAD & VARRENG, 2004; LEINONEN et al., 2006; DINGEMANSE et al., 2009). Interspecific competition to the three-spined stickleback also seems to have an effect on morphological traits and shape, which is in line with the results from other studies (e.g. HERCZEG et al., 2009, 2010; SHIMADA et al., 2011). Consequently, since the populations inhabiting areas with different predation pressure and interspecific competition were not similar, it's difficult to

say which might be more important. Nonetheless, it cannot be excluded that other environmental conditions like different food availability or water chemistry also affect phenotypic variation between populations. In regards to the genetic relationships between populations, KT seems to be closer related to LT and KOS than to GPS. Thus the second hypothesis must be rejected, since it cannot be excluded that the environment has a higher effect on phenotypic variation than geographical proximity, even if only a few populations were analyzed. Furthermore, since the investigated populations differ from another in their morphology and the observed environmental differences are insufficient to explain the entire dimension of variation, this study suggests that in general the habitat (like brackish water estuary, freshwater lake, freshwater stream and freshwater pond) also affect the morphology. Unfortunately this last conclusion need to be considered critically, since the different types of habitats are not replicated in this study. Thus, further investigations analyzing more replications of the same type of habitat are needed to get a better idea about the exact influences of habitat as a whole. Additionally, a higher sampling size of each population including a balanced sex ratio of 30 individuals per sex would help to uncover morphological divergences between sexes. In conclusion, it can be said that more work is needed to be done, since also the exact distribution of the nine-spined sticklebacks in northern German waters isn't fully investigated.

# Acknowledgements

I want to thank Prof. Dr. H. Brendelberger and Prof. Dr. Manfred Milinski for supporting my external bachelor project, which gave me the possibility to work on a scientific research project in the field of evolutionary biology.

Furthermore I want to thank Dr. Kenyon B. Mobley, for supporting me, for his motivation, for his advice, for his patience and because he endured my bad English over the whole time. I really enjoyed being part of his team.

I also want to thank Gerhard Augustin and Daniel Martens for their advice and their help during sampling and Sophie Bodenstein as well as all the other people from the Max-Planck Institute for their advices.

A big thank you goes to my girl-friend Eva Schwanke for her emotional support and her patience.

Finally, I thank my parents, Helmut and Brigitte Buchholtz for believing in me and supporting me in all situations.

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# Appendix

## Linear measurement tool (source code)

```
#head
def splitArray(array,number)
    n=array.size()/number;
    new=Array.new(n);
    insertion=0;
    for i in 0.. new.size()-1
        new[i]=array[insertion,number];
        insertion=insertion+number;
    end;
    return(new);
end;
def scalefactor(array)
    a=Math.sqrt((array[1][0]-array[0][0])**2+(array[1][1]-array[0][1])**2);
    b=1/a;
    return(b);
end;
def measurements(array)
    pos=2;
    new=Array.new((array.size()/2)-1)
    for i in 0.. (array.size()/2)-2
        a=Math.sqrt(((array[pos+1][0]-array[pos][0])**2)+((array[pos+1][1]-array[pos][1])**2))*scalefactor(array);
        new[i]=a;
        pos=pos+2;
    end;
    return(new);
end;
#body
reg=/[1-9]\d+[.]\d+;/
reg2=/LM=\d+;/
reg3=/SCALE.0.\d+;/
puts("Please enter the name of the TPS file:");
name1=gets();
name1=name1.chop()+".tps";
text=File.read(name1);
text=~reg2;
landmarkcount=$&[3,$&.length()-3];
landmarkcount=landmarkcount.to_i();
landmarkcount=landmarkcount*2;
text=~reg;
Array1=Array.new(landmarkcount);
n=0;
while $&!=nil do
    Array1[n]=$&.to_f();
    $'=~reg;
    n=n+1;
end;
Array2=splitArray(Array1,landmarkcount);
for i in 0.. Array2.size()-1
    Array2[i]=splitArray(Array2[i],2);
end;
results=Array.new(Array2.size());
for i in 0.. Array2.size()-1
    results[i]=measurements(Array2[i]);
end;
averages=Array.new(results[0].size());
for means in 0.. results[0].size()-1
    sum=0;
    for samplenum in 0.. results.size()-1
        sum=sum+results[samplenum][means];
    end;
    mean=sum/results.size();
end;
```

```

        averages[means]=mean;
end;
puts("Please enter a name for the textfile, you want to save the measurement-data in:");
name=gets().chop()+".csv";
puts("Please enter the letter in front of the tag-numbers:");
letter=gets().chop().upcase();
puts("Do the letter changes in between the samples? Enter Y (for yes) or N (for no):");
change=gets().chop().downcase();
if change=="y"
    then puts("Please enter the following letter:");
        changeL=gets().chop().upcase();
end;
puts("Please enter the number of the first tag:");
tag=gets().chop().to_i();
puts("Please enter the code of the sample region:");
region=gets().chop().upcase();
measures=Array.new(results[0].size());
puts("Please enter the name of the first measurement:");
measures[0]=gets().chop();
for often in 1.. (results[0].size()-1)
    puts("Please enter the name of the following measurement:");
    measures[often]=gets().chop();
end;
File.new(name,"w+");
f=File.open(name,"w+");
    f.write("#,tag,");
    for measures1 in 0.. measures.size()-1
        f.write(measures[measures1]);
        f.write(",");
    end;
    f.write("\n");
for pos1 in 0.. results.size()-1
    f.write(pos1+1);
    f.write(",");
    f.write(region+"13-");
    if tag>99
        then letter=changeL;
        tag=0;
    end;
    if tag<10
        then f.write(letter+"0"+tag.to_s());
        else f.write(letter+tag.to_s());
    end;
    tag=tag+1;
    f.write(",");
    for pos2 in 0.. results[pos1].size()-1
        if pos2<results[pos1].size()-1
            then f.write(results[pos1][pos2]);
            f.write(",");
            else f.write(results[pos1][pos2]);
            f.write("\n");
        end;
    end;
end;
f.write("\n");
f.write(" ,averages;")
for i in 0.. averages.size()-1
    f.write(averages[i]);
    f.write(",");
end;
f.close();
puts("Measurements of the TPS data file are completed, press ENTER to close");
c=gets();

```

## **Declaration of consent /**

## **Eidesstattliche Erklärung**

I hereby declare that I have written this bachelor thesis on my own and that I have not used any other than the stated sources and aids. All figures or drawings of this thesis were either created by myself or the source is listed in the text. Further I guarantee that the pdf-version of this thesis is identical with the printed version.

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Kiel, 30.9.2013

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