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# MASTERARBEIT

Titel der Masterarbeit

## The effect of temperature on the body shape in threespine stickleback juveniles

Verfasser

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# Abstract

Temperature is a major environmental factor influencing the morphology of fishes and will gain further importance regarding global warming. However, information on thermal effects on shape remains scare. To this end, this study examined the influence of temperature on the body shape in threespine stickleback (*Gasterosteus aculeatus*) juveniles. Laboratory bred sticklebacks, stemming from an anadromous population, have been raised at three different temperatures (13, 17 and 21 °C). Effects on size and shape were assessed and visualized by using modern geometric morphometric methods. In addition, relative linear measurements were inferred from the landmarks. It has also been tested if temperature affects both sexes in the same way. Significant differences were found between the temperatures. Fish reared at 21 °C were significantly smaller than specimens from other groups. Fish reared at 17 °C were on average the largest and also showed the least variance for shape and size, suggesting a canalized development at this temperature. Major differences between the temperature groups were much smaller heads in fish reared at 13 °C and a reduction in size and length of several bony elements in fish reared at 21 °C. General trends across all temperatures were a reduction in length of the median fins, a decrease in size and length of the operculum and the posterior process, an increase in length of the first dorsal fin, and enlargement of the eyes with increasing temperature. Substantial sexual dimorphism was found, however not for size. Deeper bodies, longer median fins, larger heads, and longer posterior processes of the pectoral girdle characterized male sticklebacks. The patterns of shape differences remained the same throughout all temperature groups and did not bias the findings for thermal effects. All findings are put in context with existing literature and implications for the performance of the fish are discussed.

## Keywords

Threespine stickleback – temperature – shape – geometric morphometrics

# Zusammenfassung

Temperatur ist eine der wichtigsten Umweltfaktoren und kann die Morphologie von Fischen beeinflussen. Dieser Umstand wird mit fortschreitender globaler Erwärmung noch zusätzlich an Bedeutung gewinnen. Trotzdem gibt es nur wenig Kenntnis über thermische Effekte auf die Körperform. Zu diesem Zweck behandelt diese Arbeit den Einfluss der Temperatur auf die Körperform von juvenilen Dreistachligen Stichlingen (*Gasterosteus aculeatus*). Im Labor gezüchtete, von einer anadromen Population abstammende Stichlinge wurden bei drei verschiedenen Temperaturen aufgezogen (13, 17 und 21 °C). Effekte auf die Körpergröße und Gestalt wurden ermittelt und visualisiert, durch die Anwendung von Methoden aus der modernen geometrischen Morphometrie. Zusätzlich wurden relative Distanzen zwischen den Landmarken errechnet. Ebenfalls überprüft wurde, ob die Temperatur den gleichen Einfluss auf beide Geschlechter hat. Signifikante Unterschiede konnten zwischen den Temperaturgruppen festgestellt werden. Fische, die bei 21 °C aufgezogen wurden, waren signifikant kleiner als von beiden anderen Gruppen. Fische, die bei 17 °C aufgezogen wurden, waren im Durchschnitt am größten und wiesen auch die geringsten Varianzen in Gestalt und Größe auf, was eine kanalisierte Entwicklung bei dieser Temperatur nahelegen würde. Die auffälligsten Unterschiede zwischen Temperaturgruppen waren deutlich kleinere Köpfe bei Fischen die bei 13 °C aufgezogen wurden, sowie eine Reduktion in Größe und Länge einiger Knochenelemente bei Fischen aus 21 °C. Trends die sich durch alle Temperaturen zeigten waren eine Verkürzung der Dorsal- und Analflosse, eine Verkleinerung des Operculums und des posterioren Fortsatzes des Beckengürtels, eine Verlängerung der ersten Dorsalflosse, sowie eine Vergrößerung der Augen, jeweils bei steigender Temperatur. Ein deutlicher Sexualdimorphismus konnte festgestellt werden, jedoch nicht betreffend der Größe. Charakteristisch für männliche Stichlinge waren hochrückigere Körper, längere Dorsal- und Analflossen, größere Köpfe und längere posteriore Fortsätze. Die Muster der Gestaltsunterschiede waren bei allen Temperaturen die Gleichen und verfälschten daher die Ergebnisse für die Temperatureinflüsse nicht. Alle Ergebnisse werden mit vorhandener Literatur in Kontext gestellt und Implikationen für die Fische werden diskutiert.

## Schlüsselworte

Dreistachliger Stichling – Temperatur – Gestalt – Geometrische Morphometrie

# Introduction

## Temperature

Temperature is known to be one of the most important environmental factors, influencing the development of fishes. It affects overall morphology (MARCIL et al. 2006, GEORGAKOPOULOU et al. 2007, SFAKIANAKIS et al. 2011), meristic counts such as the number of vertebrae or fin rays (HUBBS 1922, LINDSEY 1954, ITAZAWA 1959), growth rate (ALLEN & WOOTTON 1982, HOUDE 1989, RUSSELL et al. 1996, LEFEBURE et al. 2011), mortality, especially at early life stages (HOKANSON et al. 1977, HOUDE 1989, PEPIN 1991), muscle development (VIEIRA & JOHNSTON 1992, JOHNSTON & HALL 2004, KOUMOUNDOUROS et al. 2009), and the occurrence of deformities (BOLLA & HOLMEFJORD 1988, SFAKIANAKIS et al. 2004, ABDEL et al. 2005). The influence of temperature can be direct via altered metabolism (HOUDE 1989, CLARKE & JOHNSTON 1999) or indirect through changes of the physico-chemical properties of water (e.g. viscosity, density, salinity, dissolved oxygen), which may require morphological responses during ontogeny (HAAS et al. 2010, SFAKIANAKIS 2011). The issue of how organisms respond to environmental changes and temperature itself will also gain further importance with regard to global warming (DAUFRESNE et al. 2009, BARRETT et al. 2010, MORAN et al. 2010).

## Threespine stickleback

The threespine stickleback (*Gasterosteus aculeatus* L. 1758) is a small teleost fish, which belongs to the family Gasterosteidae (Fig. 1). It is widely distributed throughout the coastal waters of the Northern hemisphere, with the exception of the Northern coastlines of Russia, Canada and Greenland (WOOTTON 1984, BELL & FOSTER 1994, PAEPKE 2002). The species appears in three ecotypes, marine, anadromous, and resident limnic (WOOTTON 1984, BELL & FOSTER 1994, PAEPKE 2002). The marine and anadromous forms are occasionally grouped as 'oceanic'. Furthermore *G. aculeatus* appears in at least three morphotypes regarding the completeness of the line of lateral bony plates (BAKKER & SEVENSTER 1988, BANBURA & BAKKER 1995), which all members of the stickleback family have instead of scales (WOOTTON 1984, NELSON 2006). When the ice retreated after the last ice age, many freshwater habitats got colonized (reviewed in MCPHAIL 1994, NELSON 2006) and the populations showed rapid radiation in adaptation to distinctive habitats (e.g. stream vs. lake, benthic vs. pelagic; SCHLUTER 1993, 1995, BELL & FOSTER 1994, MCPHAIL 1994). Threespine stickleback thus is commonly referred to as a species

complex (reviewed in BELL & FOSTER 1994). All freshwater populations stem from marine ancestors. While marine sticklebacks are considered rather conserved regarding their genetics and morphology, freshwater forms are very diverse (MCPHAIL 1994, WALKER & BELL 2000, MÄKINEN et al. 2006). Exceptions are North Sea populations, which are known to show higher morphological diversity, e.g. polymorphism in regard to the lateral plate form (MÜNZING 1963, KLEPAKER 1996). Marine and anadromous populations are usually larger than freshwater populations and have adapted to prolonged swimming. Additionally these oceanic forms show large heads, deep bodies, narrow caudal peduncles, anterior, and widely spaced dorsal spines, as well as relatively long median fins (MCPHAIL 1994, SPOLJARIC & REIMCHEN 2007).

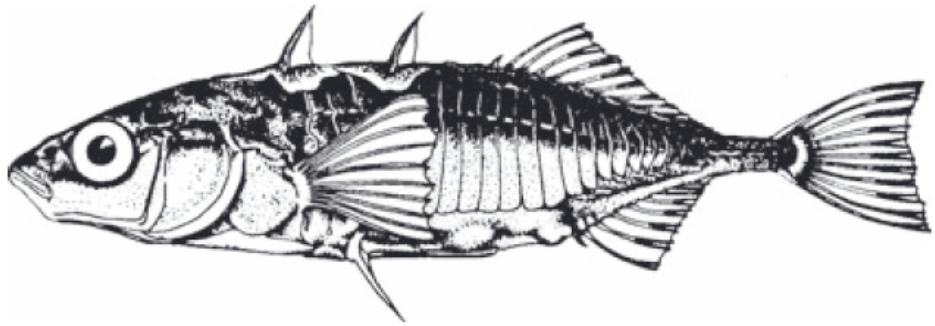


**Figure 1**: Habitus of the threespined stickleback: completely plated oceanic form (WOOTTON 2009).

Similar habitats are often inhabited by sticklebacks with similar morphologies through convergence. Limnetic populations, which primarily feed on zooplankton in the water column, have a slender, small head and mouth, as well as many long gill rakers. Shallower, elongated bodies are characteristic for prolonged swimming (MCPHAIL 1994, WALKER 1997, AGUIRRE 2009). Benthic populations, which feed on invertebrates in the littoral zone, have wider mouths, few short gill rakers, along with deeper bodies suitable for burst swimming as a predator avoidance strategy (SCHLUTER 1993, MCPHAIL 1994, AGUIRRE 2009). Another factor influencing the stickleback body shape is predation. The presence of predators (fishes, birds or also large insect larvae) particularly affects the size of the dorsal and/or pelvic spines and the development of the defensive complex in general (reviewed in REIMCHEN 1994).

All this made *G. aculeatus* a popular model for evolutionary and morphological studies. Numerous papers deal with stickleback morphology, however, most research was made on influences of habitat and predation (reviewed in WOOTTON 2009). Furthermore, the

majority of studies focused on the derived freshwater populations (reviewed in BELL & FOSTER 1994). Likewise, temperature studies on sticklebacks have a long history, but they primarily focused on effects like fecundity or meristics (e.g. CRAIG-BENNETT 1931, HEUTS 1947, LINDSEY 1962). Thermal effects on growth rates were investigated more recently (ALLEN & WOOTTON 1982, LEFEBURE et al. 2011), yet information on shape remains scarce, even with respect to other fish species (BEACHAM 1990, MARCIL et al. 2006, GEORGAKOPOULOU et al. 2007, SFAKIANAKIS et al. 2011).

## Geometric morphometrics

While traditional morphometric methods are restricted to linear measurements and angles (reviewed in ADAMS et al. 2004), modern landmark-based geometric morphometrics uses the coordinates of anatomically definable loci. This loci or landmarks can be two-, or three-dimensional and should represent true homology (BOOKSTEIN 1991, GUNZ et al. 2005), i.e., a given landmark should be placed on the same (corresponding) location on every sample. For information about curves or surfaces with no biologically definable points, semilandmarks can be added, which are allowed to move along the curvature between two regular landmarks. If the structures described by semilandmarks are homologous, they can be treated and analyzed just like classic landmarks (BOOKSTEIN 1997). To get rid of non-shape variation from size, rotation and location, superimposition methods are used, with the Procustes superimposition method being the most commonly applied (Fig. 2). In a first step of this method, the centre of each landmark configuration (i.e. the raw coordinates) is calculated. This centre is the centroid and is the coordinate-wise average of the landmarks of a configuration. All forms are subsequently superimposed on their centroids, which then commonly functions as the origin of the coordinate system. Afterwards the configurations, which are now independent from location, are scaled to a common unit size (centroid size) to get rid of size-depended variation. Independency from rotation is achieved by optimally rotating the forms in a way that minimizes the squared distances of corresponding landmarks. The remaining distances of homologous landmarks account for real shape differences. The resulting coordinates of the landmarks are the Procrustes shape coordinates, which are used for the statistical analysis (summarized in MITTERÖCKER & GUNZ 2009).

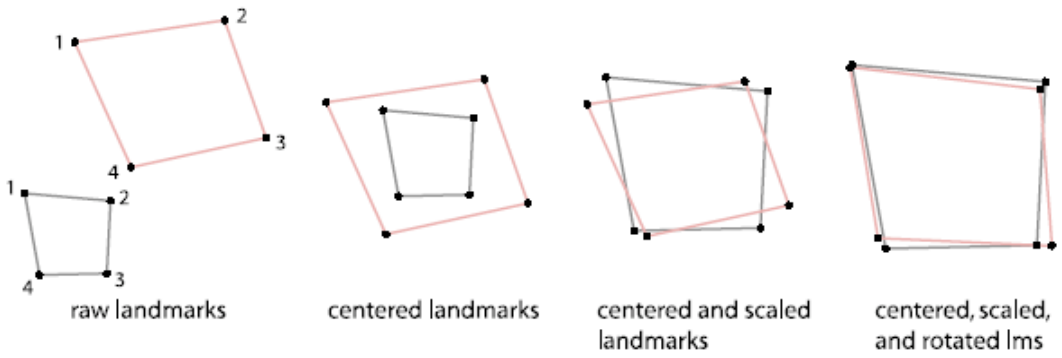



**Figure 2:** Procrustes superimposition method. Landmark configurations are superimposed on their centroids, scaled to a common unit size (centroid size) and optimally rotated to minimize the sum of squared distances between corresponding landmarks. The resulting coordinates from the landmarks are the Procrustes shape coordinates which are used for statistical analyses (MITTERÖCKER & GUNZ 2009).

Graphical visualization of the results is one of the major strengths of geometric morphometrics. Shape differences are usually presented by deformation grids, in which one configuration, most commonly the mean shape, is deformed into another, using thin-plate spline (TPS) algorithms (BOOKSTEIN 1991). The deformation of a regular grid represents the shape differences between two configurations, e.g., the mean shapes of two populations from different environments (MITTERÖCKER & GUNZ 2009).

In the last years, geometric morphometric methods became increasingly popular and are now widely applied to biological questions. One research field, where geometric morphometrics was and is heavily used, is cichlid fish evolution in the Great Eastern African Lakes (summarized in KERSCHBAUMER & STURMBAUER 2011). Geometric morphometrics proved to be a powerful tool for the detection of adaptations (STEWART & ALBERTSON 2010), sexual dimorphism (HERLER et al. 2010), or examples of convergent evolution leading to ecomorphological equivalency (KASSAM et al. 2003). Geometric morphometric methods have high statistical power and can be used to detect small differences between populations of a species complex (KLINGENBERG et al. 2003, POSTL et al. 2008).

## Aim

By using modern Geometric Morphometric methods I want to investigate how temperature affects the shape of juvenile threespine sticklebacks. To reduce other environmental effects than temperature I use laboratory-reared sticklebacks, raised at three different temperatures. I compare overall shape, size and relative distances of traits of all temperature groups to look for trends and patterns of shape changes. The

same is done for the sexes to examine if both males and females are equally affected by temperature. Additionally, I assess the variances of shape and size to check for canalization processes.

# Material & Methods

A total of 466 specimens of threespine stickleback (*Gasterosteus aculeatus*) juveniles were examined. Wild fish have been caught in the Sylt-Rømø Bight (54°52' to 55°10' N, 8°20' to 8°40' E; Fig. 3), which is a part of the Wadden Sea, an intertidal zone in the southeastern part of the North Sea. The bight is a closed basin and is bordered by the coastline of the two islands and the mainland of Denmark and Germany. It is approximately 406 km$^2$ in extent and has a mean depth of 4.2 meters. The tides are semidiurnal and the tidal range is on average 2 meters. A deep runway between the two islands is the only connection to the open sea (ASMUS & ASMUS 2000, KELLNREITNER et al. 2012).

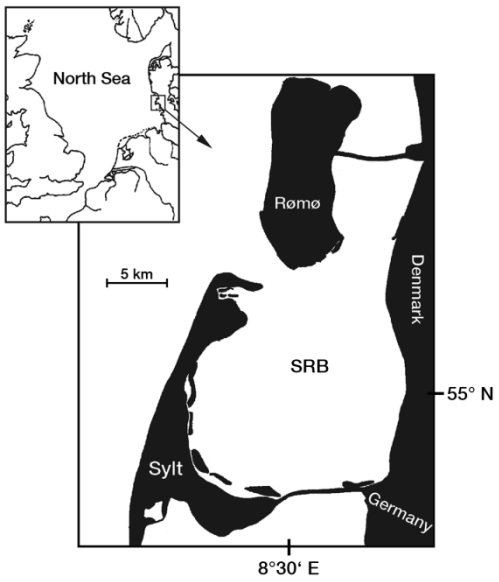



**Figure 3:** Map of the Sylt-Rømø bight (SRB) modified after ASMUS & ASMUS 2000.

Ten families have been formed of the wild caught fish, with 10 dams and 5 sires. Each male fertilized the eggs of two females. Offspring of each family were reared under laboratory conditions at three different temperatures, which were 13, 17 and 21 °C. Fish were 84 to 114 days old (avg. 103 days) when sacrificed and ranged between 21.5 to 35.2 mm standard length (SL, avg. 29.2 mm). Sex was determined genetically. Individuals, for which the sex determination failed, were excluded from the analysis of sexual dimorphism, but included in all other analyses. The numbers of individuals and the ratio of males to females were similar across all temperature groups (Table 1). Rearing, sexing and primary preservation took place at the Wadden Sea Station List/Sylt of the Alfred Wegener Institute for Polar and Marine Research, by the working group of Dr. Mathias Wegner. All further work was carried out by the author at the Department of Theoretical Biology at the University of Vienna.

**Table 1:** Number and sex of specimens, mean standard length (SL), standard deviation ($SL^{sd}$), and range of standard length ($SL^{rg}$) for each temperature group. Ungendered refers to specimens for which sex determination was not possible.

| | All | Males | Females | Ungendered | SL | $SL^{sd}$ | $SL^{rg}$ |
|---|---|---|---|---|---|---|---|
| 13 °C | 159 | 77 | 74 | 8 | 29.62 | 2.87 | 21.5 - 35.1 |
| 17 °C | 154 | 70 | 74 | 10 | 29.85 | 1.97 | 24.3 - 35.2 |
| 21 °C | 153 | 71 | 67 | 15 | 28.06 | 2.28 | 21.5 - 34.2 |
| Sum | 466 | 218 | 215 | 33 | - | - | - |

## Clearing and staining

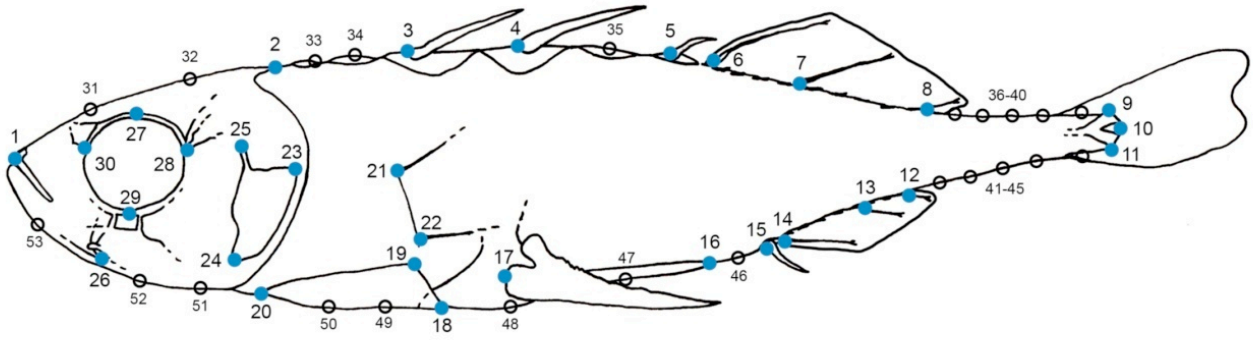
Standard protocols for clearing and staining (POTTHOFF 1984, TAYLOR & VAN DYKE 1985, DARIAS et al. 2010) have been tested and adapted to the specimens used in this study. The specimens were preserved in formalin, which got extracted prior to the chemical treatment by an ascending series of alcohol (dH2O, 20% EtOH, 40% EtOH, 60% EtOH, 2x 70% EtOH). Specimens were rewatered and bleached with hydrogen peroxide to remove pigmentation of the skin. To make the body translucent, a clearing step was included using the digestive enzyme trypsin. In a next step, bones were stained with Alicarin Red S and the specimens got stored in glycerine (see Supplement I for detailed protocol). This method has the benefit that osteological structures, which are stained red, become better observable and distinguishable from other structures.

## Digitalization

All cleared and stained specimens were scanned with a flatbed scanner, following HERLER et al. (2007). For the geometric morphometric analysis, sets of homologous landmarks were set on every image using tpsUtil and tpsDig2 (ROHLF 2010A, ROHLF 2012). Each set consisted of 30 landmarks (1-30) and 23 semilandmarks (31-53; Fig. 4). Besides the traditionally used landmarks (based on WALKER 1997; indicated by bold numbers), additional landmarks and semilandmarks were used for a more comprehensive representation of shape. The landmarks were defined as follows: (**1**) anteriodorsal tip of premaxilla, (**2**) posterior edge of supraoccipital, (**3**) anterior intersection of first dorsal spine (DS) with pterygiophore (PT) III on the dorsal outline, (**4**) anterior intersection of second DS with PT IV on the dorsal outline, (5) anterior intersection of third DS with the PT VI on the dorsal outline, (**6**) anterior base of first dorsal fin ray, (7) base of fifth dorsal fin ray, (**8**) posterior base of last dorsal fin ray, (9) dorsal tip of hypural fan, (**10**) posterior midpoint of hypural fan, (11) ventral tip of

hypural fan, (**12**) posterior base of last anal fin ray, (13) base of fifth anal fin ray, (**14**) anterior base of first anal fin ray, (15) anterior intersection of anal spine with ventral outline, (**16**) posterior tip of ventral process of the pelvic girdle, (17) anterioventral base of left pelvic spine, (**18**) posterior tip of ectocoracoid, (19) dorsal tip of ectocoracoid, (**20**) anterior tip of ectocoracoid, (21) dorsal base of first pectoral fin ray, (22) ventral base of last pectoral fin ray, (23) posteriodorsal edge of operculum, (24) anterioventral edge of operculum, (25) anteriodorsal edge of operculum, (**26**) posterioventral edge of articulare, (27) most dorsal point of the eye outline formed by the frontal, (28) anterioventral tip of sphenotic, (29) most ventral point of the eye outline formed by the suborbitals, (30) posterioventral tip of lateral ethmoid, (31-34) dorsal outline of head, (35) dorsal outline of abdomen, (36-40) dorsal outline of caudal peduncle, (41-45) ventral outline of caudal peduncle, (46-48) ventral outline of abdomen, (49-50) ventral outline of breast, (51, 52) ventral outline of throat, (53) ventral outline of chin.

**Figure 4:** The set of 30 landmarks (blue dots) and 23 semilandmarks (black circles) used for geometric




morphometric analysis (see Material & Methods for detailed location).

## Analysis

The digital images were randomized with tpsUtil (ROHLF 2012). Two-dimensional coordinates were obtained for 53 landmarks, which were set on each image using tpsDig2 (ROHLF 2010A). Landmarks were aligned using Generalized Procrustes Analysis implemented in tpsRelw (ROHLF 2010B), standardizing the landmark configurations for size, orientation, and location. Between-group principal component analysis (bgPCA) was used to assess the distribution of the individual and group mean shapes in shape space. The axes maximize the variation between the group mean shapes and allow for a

low-dimensional representation of the multi-dimensional shape space. In contrast to a normal PCA, the bgPCA projects the data onto the principal components (PCs) of the group means, which usually leads to a better separation of the groups (MITTERÖCKER & BOOKSTEIN 2011). Shape differences were visualized by deformation grids using the thin-plate spline (TPS) algorithm (BOOKSTEIN 1991). Permutation tests were used to test for statistical significance of group mean differences (GOOD 2000). Level of significance was determined at $\alpha$ = 5%. Significances of the relative distance measurements have been Bonferroni-corrected. Mathematica 8 (WOLFRAM RESEARCH INC.) was used for all statistical analyses and the generation of deformation grids.

# Results

Because the first two PCs only accounted for preservation artefacts (Fig. 5), they were removed from the analysis by projecting the individuals in the subspace perpendicular to the first two PCs. All further analyses were based on these residual data.

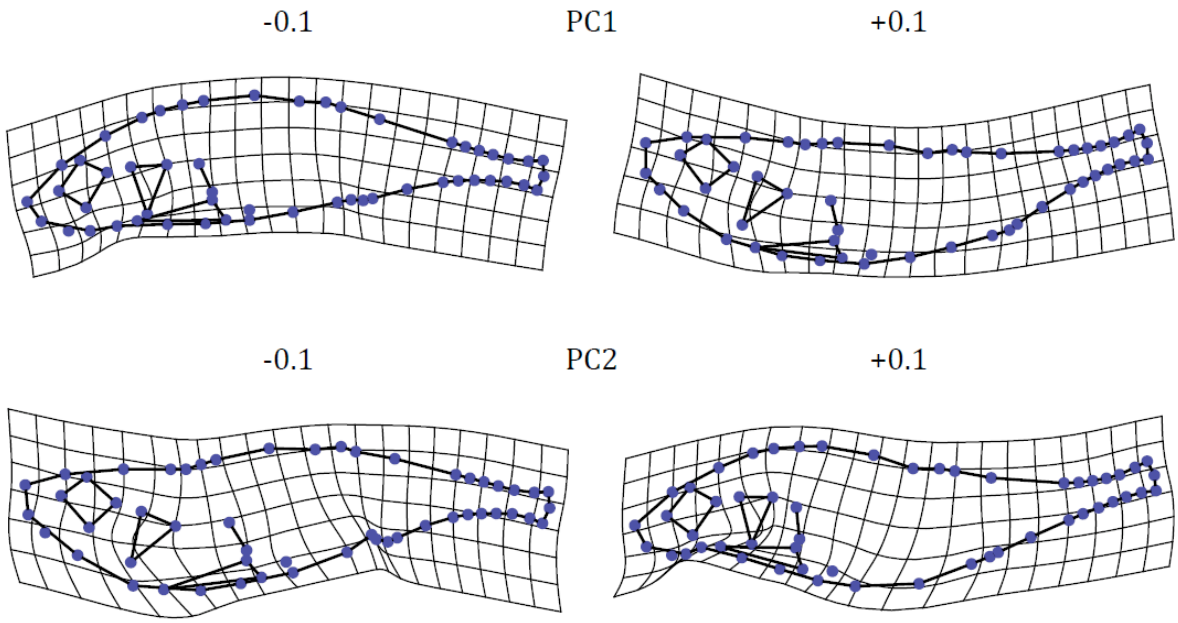



**Figure 5:** Deformation grids of the first (upper row) and second (lower row) principal component, which indicate fixation artefacts.

## Sexual dimorphism

Males and females differ in shape at all temperatures ($p < 0.001$; Fig. 6). In comparison to females, males had deeper bodies, longer median fins, and a shorter free abdominal region. Significant changes were also found in the head region. Males had longer snouts and lower jaws, bigger eyes, as well as generally longer and deeper heads (Fig. 7, Table 2). To ascertain if these substantial differences between the sexes distort the shape changes for the different temperature groups, data was separated by sex and temperature and plotted individually. The patterns of sexual dimorphism remained the same for all temperature groups (Fig. 10). In addition, the ratio of males and females in each temperature group is relatively similar for all temperatures (Table 1). Therefore sexual dimorphism should not blur the findings for the thermal effects on shape. To test if larger sticklebacks differ from smaller ones, the sexual dimorphism in shape was analysed separately for individuals over ($n$ = 192) and under 30 mm SL ($n$ = 274). Both

showed significant differences in shape between the sexes ($p < 0.001$), however the Procrustes Distance, which is a measurement for shape difference, increases with body size (from 0.0111 to 0.0148). No significant differences between the sexes were found regarding size ($p = 0.09$).

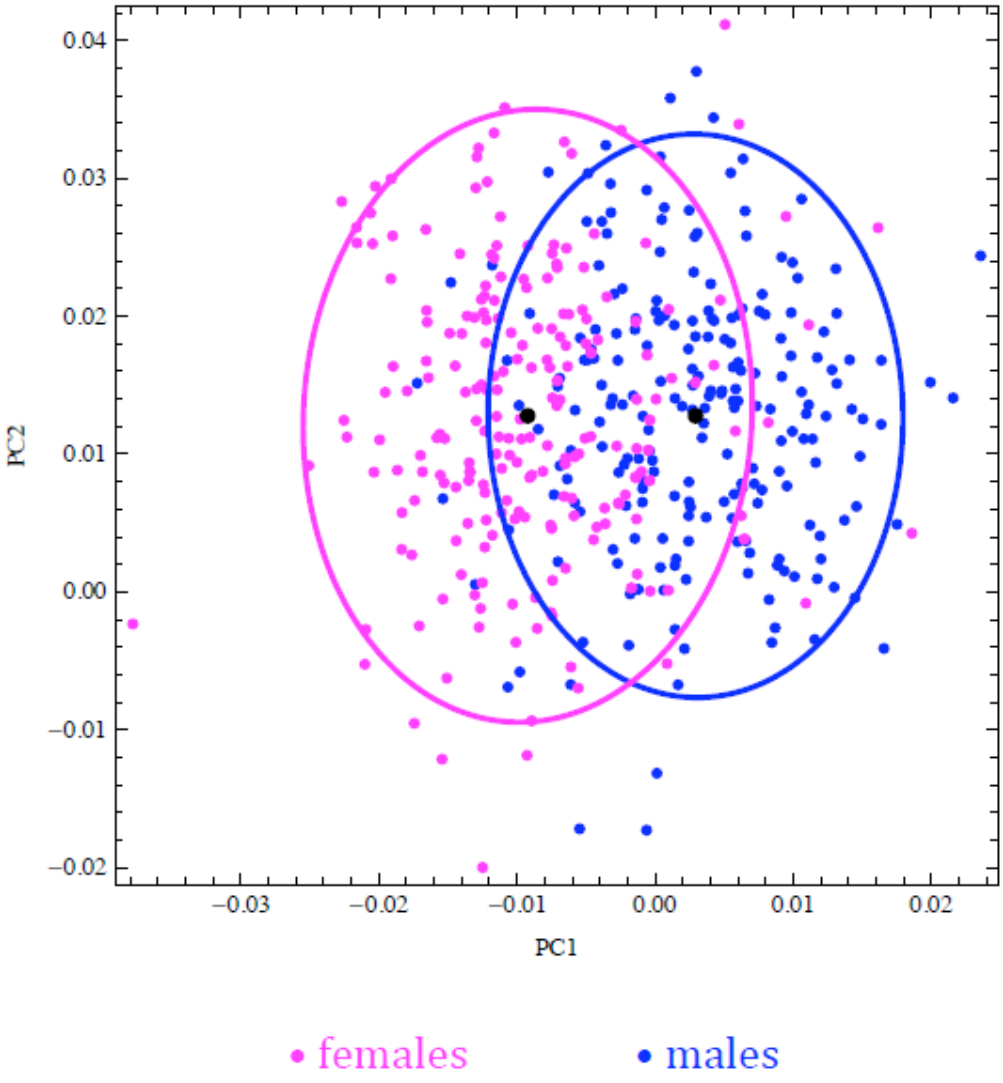



**Figure 6:** Between-group principal component analysis (PCA) of stickleback body shape. The black dots are the mean shapes and the ellipses the 90% equal frequency ellipses for each sex.

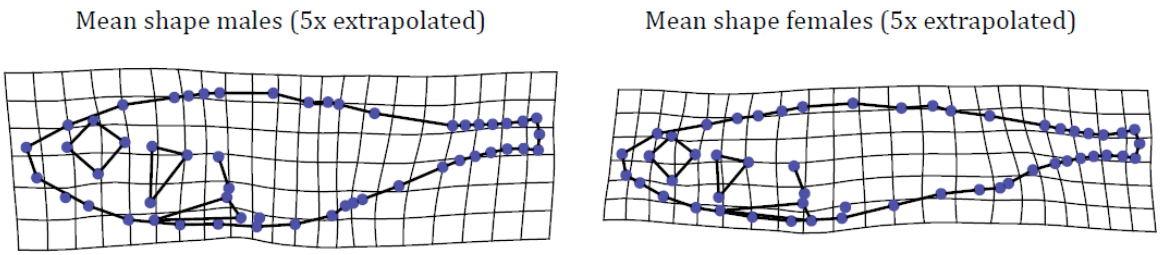



**Figure 7:** Shape differences between males and females, linearly extrapolated by factor of 5.

## Temperature

Fish reared at 21 °C were significantly smaller than both other groups ($p < 0.001$, Table 4). Fish raised at 17 °C did not differ significantly from those of 13 °C regarding size ($p = 0.422$; Table 4). Significant differences between the temperature groups were also found for the variance in size and shape, in which the 17 °C group showed the least values for both (Table 4).

The three temperature groups differed significantly in their shape ($p < 0.001$), however in a non-linear fashion (Fig. 9). Trends across the temperature groups are a reduction in length of both the second dorsal fin as well as the anal fin with increasing temperature. The length of the first dorsal fin increases with temperature, mainly due to a wider spacing of the second and third dorsal spine. The eye size is increasing, while the length of the posterior process of the pelvic girdle and the surface of the ectocoracoid decrease with increasing temperature. In direct comparison, the fish raised at 13 °C have the deepest bodies and caudal peduncles, but show a much smaller head and snout, along with decreased eye size and an anterior shift of the operculum. Fish raised at 21 °C show a reduction in size of bony elements. In addition to the ectocoracoid also the operculum and the ventral process of the pelvic girdle decrease in size of the fish from the highest temperature group (Fig. 8, Table 3).

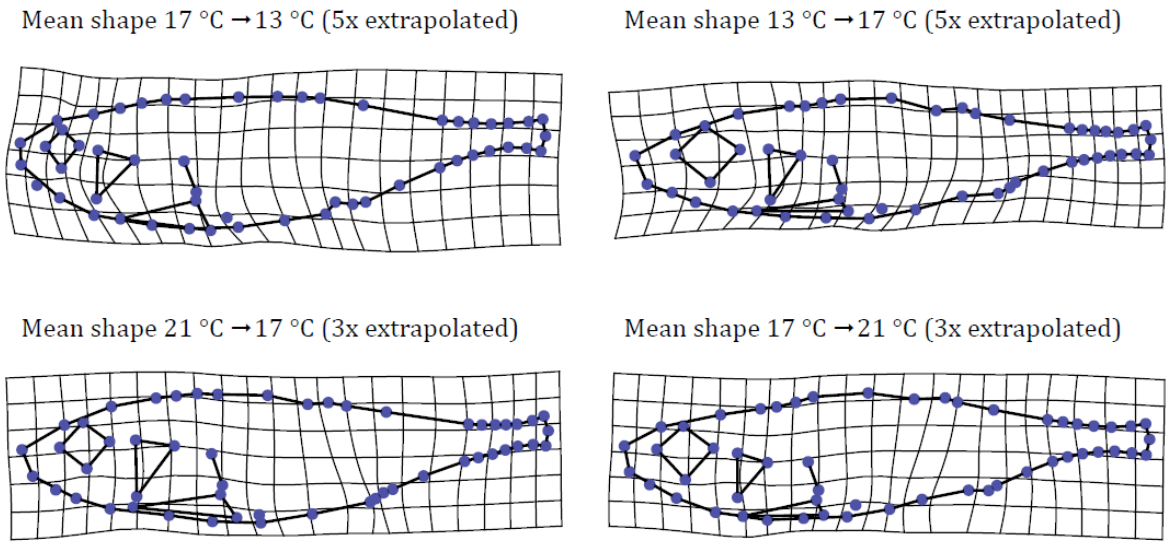



**Figure 8:** Shape differences between 13 °C and 17 °C, as well as between 17 °C and 21 °C, linearly extrapolated by factors of 5 and 3, respectively.

Note that all differences are independent of overall size and therefore are relative and not absolute magnitudes.

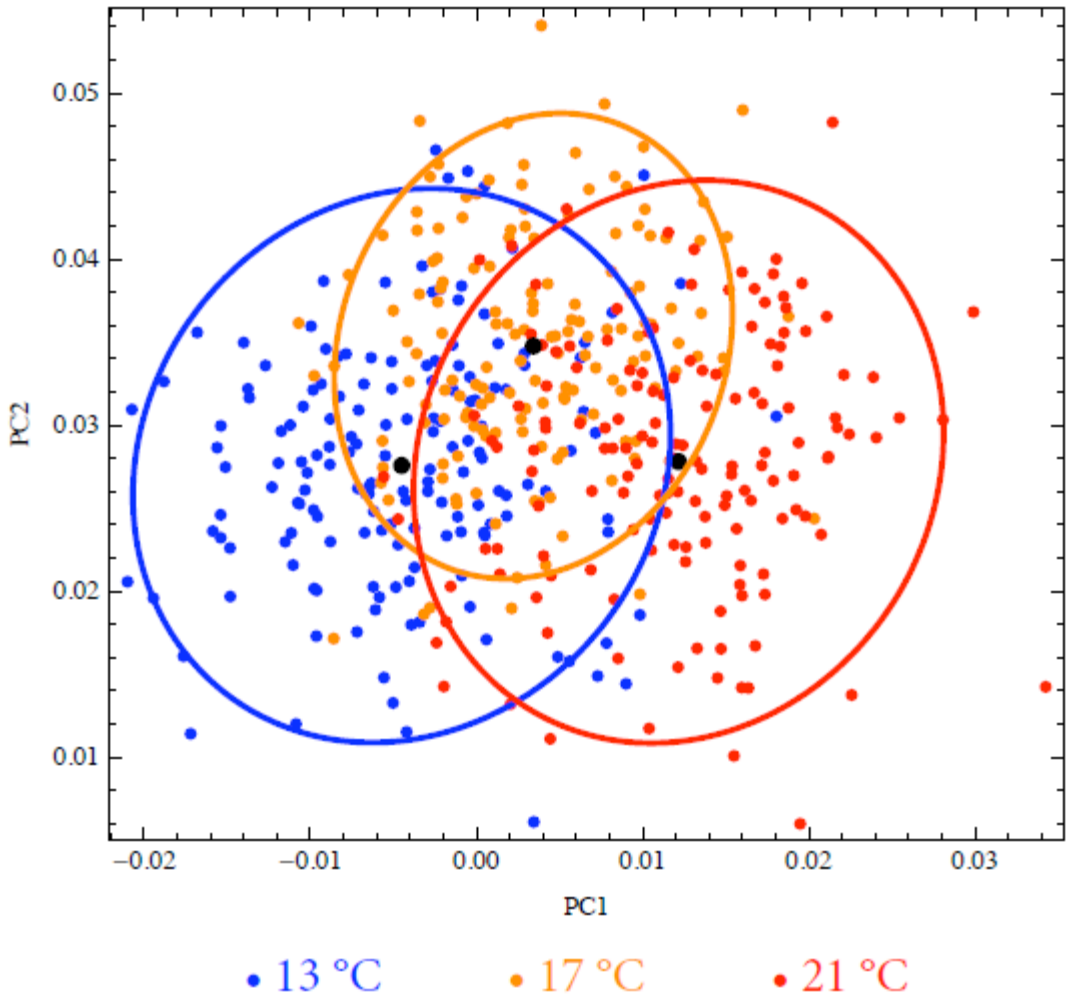



**Figure 9:** Between-group principal component analysis (PCA) of stickleback body shape. The black dots are the mean shapes and the ellipses the 90% equal frequency ellipses for each temperature group.

## Allometry and age

Allometry, as well as age, had significant effects on shape, but explained only 2.9% and 2.3% of the variance, respectively. Smaller individuals had bigger eyes and overall head region along with a deeper body. The size of the operculum, ectocoracoid and posterior process of the pelvic girdle was reduced in smaller fish in relation to bigger ones. The major differences between young and old individuals concerned body depth, median fin length, and length of the caudal peduncle, with young fish having deeper bodies, longer median fins, and shorter caudal peduncles (Fig. 11).

**Table 2:** Sexual dimorphism: Relative distances of linear measurements between landmarks. Asterisks indicate significance (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) after Bonferroni correction ($n=90$). If not otherwise stated the measurements designate the length of a trait. Free abdominal space refers to the gap between the tip of the posterior process of the pelvic girdle and the anal spine. D1 dorsal fin 1; DS dorsal spine.

| | Males | | Females | Landmarks |
|---|---|---|---|---|
| Dorsal fin 2 | 8.67 | ** | 8.45 | 6-8 |
| Anal fin | 5.68 | *** | 5.35 | 12-14 |
| D1 DS1-DS2 | 4.27 | | 4.21 | 3-4 |
| D1 DS2-DS3 | 5.51 | *** | 5.68 | 4-5 |
| D1 DS1-DS3 | 9.75 | | 9.87 | 3-5 |
| Eye horizontal | 4.27 | ** | 4.14 | 28-30 |
| Eye vertical | 4.04 | *** | 3.94 | 27-29 |
| Snout | 2.81 | *** | 2.68 | 1-30 |
| Lower jaw | 4.03 | *** | 3.76 | 1-26 |
| Body depth | 9.89 | ** | 9.73 | 3-18 |
| Base of pectoral fin | 2.56 | * | 2.51 | 21-22 |
| Free abdominal space | 3.34 | *** | 3.61 | 15-16 |
| Caudal peduncle depth | 3.26 | | 3.22 | 8-12 |
| Caudal peduncle length | 7.76 | | 7.59 | 10-12 |
| Head depth | 8.99 | *** | 8.69 | 2-20 |
| Head length | 11.84 | *** | 11.48 | 1-23 |

**Table 3:** Thermal effects: Relative distances of linear measurements between landmarks. Asterisks indicate significance (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) after Bonferroni correction ($n=90$). If not otherwise stated the measurements designate the length of a trait. Free abdominal space refers to the gap between the tip of the posterior process of the pelvic girdle and the anal spine. D1 dorsal fin 1; DS dorsal spine.

| | 13 °C | | 17 °C | | 21 °C | Landmarks |
|---|---|---|---|---|---|---|
| Dorsal fin 2 | 8.81 | *** | 8.63 | | 8.23 | 6-8 |
| Anal fin | 5.67 | | 5.53 | * | 5.32 | 12-14 |
| D1 DS1-DS2 | 4.21 | | 4.22 | | 4.29 | 3-4 |
| D1 DS2-DS3 | 5.45 | | 5.56 | * | 5.78 | 4-5 |
| D1 DS1-DS3 | 9.65 | | 9.75 | *** | 10.05 | 3-5 |
| Eye horizontal | 3.99 | *** | 4.27 | | 4.37 | 28-30 |
| Eye vertical | 3.85 | *** | 4.02 | *** | 4.13 | 27-29 |
| Snout | 2.67 | *** | 2.81 | | 2.75 | 1-30 |
| Lower jaw | 3.87 | | 3.95 | | 3.88 | 1-26 |
| Body depth | 9.90 | | 9.79 | | 9.73 | 3-18 |
| Base of pectoral fin | 2.56 | | 2.55 | | 2.51 | 21-22 |
| Free abdominal space | 3.13 | * | 3.31 | *** | 4.04 | 15-16 |
| Caudal peduncle depth | 3.27 | * | 3.18 | | 3.26 | 8-12 |
| Caudal peduncle length | 7.73 | | 7.55 | | 7.71 | 10-12 |
| Head depth | 8.82 | | 8.87 | | 8.85 | 2-20 |
| Head length | 11.38 | *** | 11.86 | | 11.78 | 1-23 |

# Discussion

## Sexual dimorphism

It has been reported that female sticklebacks are generally larger than males (BAKKER 1994, KITANO et al. 2007, AGUIRRE et al. 2008), however, no differences in size between the sexes could be found in the present study. This may be due to the fact that only premature individuals have been used. Females of a population are often larger than males, which is commonly explained by a larger reproductive success of large females (reviewed in BAKKER 1994). It is also known that courting males show a preference for larger females (ROWLAND 1982, KRAAK & BAKKER 1998). It is therefore possible that size differences would occur at later ontogenetic stages.

Despite no significant differences in size, shape differed significantly between the sexes (Fig. 6), even if split into two size classes (SL larger or less than 30 mm, respectively). This size was chosen, because 30 mm is approximately the minimum size of first maturity in sticklebacks (MCPHAIL 1977). Sexual dimorphism in shape is therefore expressed quite early in the life history of the sticklebacks. The greater Procrustes Distance for the larger size group suggests that sexual dimorphism is being pronounced at later ontogenetic stages. Patterns of shape differences between males and females were the same throughout all temperature groups (Fig. 10) suggesting a genetic basis of sexual dimorphism in shape, without major thermal influence. Larger heads and eyes, along with deeper bodies and larger median fins of male sticklebacks (Fig. 7, Table 2) are in concordance with the existing literature (KITANO et al. 2007, AGUIRRE et al. 2008, AGUIRRE & AKINPELU 2010). These differences are linked to divergent ecological roles of the sexes. In general, males show a more benthic lifestyle, especially during the breeding season, whereas females tend to have a limnetic life in the open water. Benthic prey (invertebrates) is usually larger than limnetic prey (zooplankton), which demands wider gape widths and longer jaws (KITANO et al. 2007, AGUIRRE et al. 2008, AGUIRRE & AKINPELU 2010). Deeper bodies, supported by long median fins, are thought to increase manoeuvrability in the often more complex benthic habitats (SCHLUTER 1993, MCPHAIL 1994, AGUIRRE 2009). Slender bodies are characteristic for prolonged swimming required in open water due to the patchy distribution of the food (MCPHAIL 1994, WALKER 1997, AGUIRRE 2009). Additionally, also divergent reproductive roles affect fish shape. Males need larger mouths for nest building and transportation of the building material, as well as for aggressive behaviour (biting) against competitors during nest

guarding (KITANO et al. 2007, AGUIRRE et al. 2008, AGUIRRE & AKINPELU 2010). Furthermore the pectoral fin has a larger base in males as well as a more caudal insertion than females. Males show brood care behaviour such as nest fanning, for which they use their pectoral fins to bring oxygen to the brood. Larger fins can move a greater volume of water and are therefore generally enlarged in relation to the pectoral fins of female sticklebacks (BRØNSETH & FOLSTAD 1997, BAKKER & MUNDWILER 1999). The longer free abdominal region, or shorter posterior processes of the pectoral girdle, respectively, in females (Fig. 7, Table 2) may be explained by the demand for space for the future eggs, which may be already present in premature females. Previous findings of expanded caudal peduncles in females (AGUIRRE et al. 2008) could not have been confirmed.

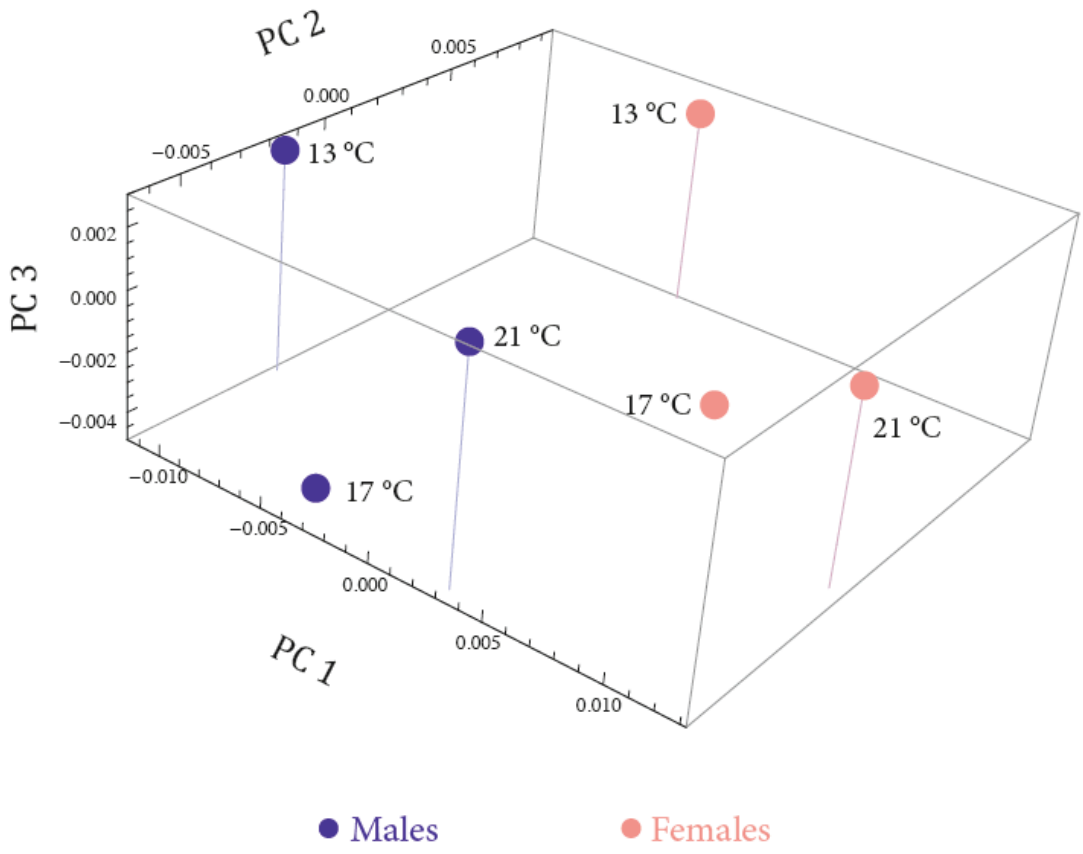



**Figure 10**: PCA of male (blue dots) and female (pink dots) mean shapes for each temperature. Males and females are distant, but the pattern of shape change is very similar.

## Temperature

The lengths of the median fins (second dorsal and anal fin) decrease with increasing temperature. In addition, the lowest temperature group shows the deepest bodies (Fig. 8, Table 3). Total body and caudal depth are therefore reduced at higher temperatures, which imply changes in swimming abilities. Large median fins increase the depth of the

trunk without an increase in drag and are often observed in open water stickleback populations that have to combine prolonged swimming for prey capture and burst swimming for predator escape (HEUTS 1949, WALKER 1997).

Studies on other fish species consistently found deeper bodies at higher temperatures, leading to the suggestion that this may be a general trend in fishes (BEACHAM 1990, MARCIL et al. 2006, GEORGAKOPOULOU et al. 2007, SFAKIANAKIS et al. 2011). However, the present findings show that this is not necessarily the case.

The decreasing size of the ectocoracoid with increasing temperature (Fig. 8) could indicate an effect on locomotory abilities. Sticklebacks have a labriform style of swimming, exclusively using their pectoral fins for low-paced swimming, hovering and also for nest fanning. Axial undulation is only used for quick acceleration for predator escape or prey capture (TAYLOR & MCPHAIL 1986, BELL & FOSTER 1994). One of the main muscles of the pectoral fin used for the swimming stroke arises from the ectocoracoid (WESTNEAT & WALKER 1997, WALKER & BELL 2000). The power generated by a muscle is proportional to its cross-section area. Less area for muscle attachment could lead to reduced power of swimming strokes.

One of the most apparent differences between the temperature groups is the smaller head of the lowest temperature group (Fig. 8, Table 3). Relatively smaller heads, along with smaller eyes and gape width is a typical feature of female sticklebacks (KITANO et al. 2007, AGUIRRE et al. 2008, AGUIRRE & AKINPELU 2010). The patterns of shape changes remain the same though, even if the temperature groups are further separated by gender (Fig. 10). Also the proportion of males and females are similar across ($n$ = 218 and 215, respectively) and within (mean $n$ = 73 and 72, respectively) all groups. Thus, the shape changes observed are unlikely to be biased by sexual dimorphism. The reason for the smaller heads, however, remains unclear.

A distinct feature of the 21 °C temperature class is that all bony elements measured (operculum, ectocoracoid, posterior process of pelvic girdle) are reduced in size or length (Fig. 8, Table 3). This goes along with a significantly smaller mean size of this group, whereas 13 °C and 17 °C did not differ significantly in size (Table 4). This leads to the suggestion that the fish raised at 21 °C already suffer from the elevated temperature. Further research is needed to test if the reduction in size holds true for other bony body parts as well, especially those of the defensive complex like dorsal and pelvic spines, the pectoral girdle as a whole, or lateral plates. It is known that cold tolerance is heritable and is able to evolve very rapidly. In only three generations, cold tolerance can evolve to

a value 2.5 °C lower than in the ancestral population (BARRETT et al. 2010). However, it is not known if these findings can be simply assigned to heat tolerance as well. Further multi-generational experiments would be needed to test if the sticklebacks can adapt to elevated temperatures and compensate the decrease in size. Interestingly the present findings support the hypothesis of DAUFRESNE et al. (2009), who proposed reduced body size as the third universal ecological response to global warming, besides migration to higher latitudes and altitudes. The temperature-size rule (TSR) predicts smaller sizes at maturity, but higher growth rates at higher temperatures for ectotherms (ATKINSON 1994, WALTHER et al. 2002). This could imply a change in phenology of the threespine stickleback under global warming, which in turn may lead to a decoupling of life cycles with interdependent species, e.g. predators and prey (DURANT et al. 2005, 2007, JONZEN et al. 2007). It is known for threespine sticklebacks that growth rate increases with temperature (ALLEN & WOOTTON 1982, LEFEBURE et al. 2011), but additional research is needed to reveal if this is accompanied with smaller sizes at maturity, thus supporting the TSR.

The differences between the temperature groups are non-linear (Fig. 9), which shows, that the effects of temperature on morphology are not trivial. It is therefore not easy to make predictions for other temperatures. This non-linearity was also found by SFAKIANAKIS et al. (2011) in a study on zebrafish (*Danio rerio*). However, what is particularly interesting in the present findings is that the fish reared at 17 °C, which were on average the largest ones, also showed the least variances for shape and size (Table 4). This reduced variances suggest that the development at or close to 17 °C is most canalized. Canalization is defined as a reduction in the variability of a trait, which is commonly assessed by the reduction in observed variance of a trait (GIBSON & WAGNER 2000). A strong directional selection, as may caused by changing environmental factors like temperature, forces a population from “flat” (i.e., canalized), to “steeper” areas on the phenotype landscape, which means that the influence of variation (genetic and developmental) increases. In these “steep” regions hidden genetic variance gets expressed, leading to greater variances of a trait (GIBSON & WAGNER 2000, MITTERÖCKER 2009).

**Table 4:** Averages and variances of standard length (SL) and total shape variance for the three temperature groups. Asterisks indicate significance (* p<0.05. ** p<0.01. *** p<0.001).

| | 13 °C | | 17 °C | | 21 °C |
|---|---|---|---|---|---|
| Average SL[mm] | 29.62 | | 29.85 | *** | 29.06 |
| Variance SL | 8.23 | *** | 3.90 | * | 5.21 |
| Variance shape | 7.85 | * | 7.20 | ** | 8.38 |

Sticklebacks raised at 17 °C were on average the largest (although not significantly different from 13 °C), which, along with the low variances, leads to the conclusion that at or close to 17 °C is also the optimum temperature for development. This temperature is lower than previous estimates of 19 °C (ALLEN & WOOTTON 1982) and 21.7 °C (LEFEBURE et al. 2011), respectively. It should be noted though that both studies primarily focused on growth rate and did not account for total size, shape, and their variances. As mentioned above, if the TSR holds true, it is also possible that fish show higher growth rates at higher temperatures, but still end up with smaller total sizes. It should also be noted that ALLEN & WOOTTON (1982) used limnic sticklebacks from Wales, while LEFEBURE and his colleagues (2011) used fish from brackish waters of the Swedish coast. Thus, it is possible that the discrepancies result from factors like different environmental influences or variation in growth potential among populations (WRIGHT et al. 2004, MCGUIGAN et al. 2011). It is likely that optima for the development of the threespine stickleback have to be estimated separately for each population and that a generalization on a broader scale is not possible.

## Allometry and age

The patterns observed for allometry (Fig. 11) are in concordance with previous findings on sticklebacks and other species. Larger relative head and eye size of smaller individuals are characteristic for vertebrates in general (GOULD 1966) and have also been reported for threespine sticklebacks (MCGUIGAN et al. 2010). The same holds true for increasing size of the operculum (KIMMEL et al. 2008). The operculum is both used for respiratory demands as well as for the jaw opening mechanism. Due to the higher surface-to-volume ratio of larger individuals, they have a higher demand on oxygen, which explains the increase in size of the operculum.

As mentioned above, premature sticklebacks (less than 30 mm SL) already show a sexual dimorphism in shape. The same patterns of shape differences between the sexes

proceed in larger juveniles (greater than 30 mm SL) and eventually in the adults (KITANO et al. 2007, AGUIRRE et al. 2008).

The differences between younger and older sticklebacks (Fig. 11) reflect an ontogenetic niche shift. After hatching, the young sticklebacks initially stay on the benthos of the littoral zone (WOOTTON 1984, BELL & FOSTER 1994, PAEPKE 2002). This zone is usually more complex, offering more places to hide, also from conspecific predators (SILLETT & FOSTER 2000). Deeper bodies, longer median fins, and shorter caudal peduncles account for the higher manoeuvrability needed in complex habitats. Previous studies also reported narrower peduncles, anteriad, widely spaced dorsal spines, and a posteriad pelvis in adult sticklebacks (SPOLJARIC & REIMCHEN 2011). However these shape changes are not confirmed by the present study.

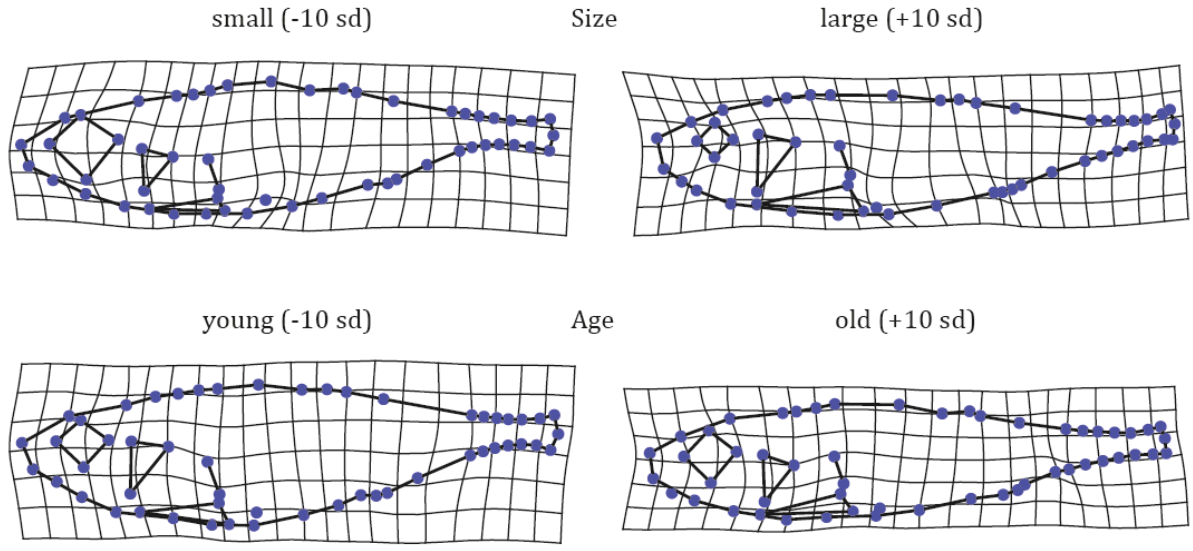



**Figure 11:** Allometry and age effects. Shape differences between small and large (upper row), as well as young and old sticklebacks (lower row). Extrapolated by a factor of ten standard deviations (sd).

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# Supplement I

Protocol for Clearing and Staining of formol-fixated stickleback juveniles

| | |
|---|---|
| <u>Ascending alcohol series</u> | (formol extraction) |
| $dH_2O$ | rinse, 1 h |
| 20% EtOH | 1 h |
| 40% EtOH | 4 h |
| 60% EtOH | overnight |
| 70% EtOH | 2x 24 h and longer |
| <u>Descending alcohol series</u> | (rehydration) |
| 40% EtOH | 1 h |
| 15% EtOH | 1 h |
| $dH_2O$ | rinse, 1 h |
| <u>Bleaching</u> | (removal of pigmentation) |
| 3% $H_2O_2$ + 1% KOH, 1:9 | 60-90 min |
| <u>Clearing</u> | (tissue clearance) |
| $Na_2B_4O_7$*10 $H_2O$ + $dH_2O$, 3:7 | 60 min |
| Add trypsin | 18 h |
| <u>Staining</u> | (staining of bone tissue) |
| $dH_2O$ | rinse, 30 min |
| 0.5% KOH | 30 min |
| Add Alizarin Red S | min. 2 h |
| <u>Ascending glycerol series</u> | (storage) |
| $dH_2O$ | rinse, 30 min |
| 40% glycerol aqu. | min. 3 h |
| 70% glycerol aqu. | overnight |
| 100% glycerol | storage |

Clearing: 0.9 g trypsin per 100 ml water

Staining: 0.15 g Alizarin Red S per 1 l 0.5% KOH solution

Abbreviations: $dH_2O$ distilled water; EtOH ethanol; $H_2O_2$ hydrogen peroxide; KOH potassium hydroxide; $Na_2B_4O_7$*10 $H_2O$ sodium borate decahydrate

# Supplement II

Curriculum vitae

## CONTACT INFORMATION

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## PERSONAL INFORMATION

Date of birth: July 31st, 1987 (St. Pölten)

Nationality: Austrian

## EDUCATION

| | |
|---|---|
| WS 2011 – SS 2013 | Master's study of zoology at the University of Vienna, Austria |
| WS 2010 – ongoing | Master's study of nature conservation and biodiversity management at the University of Vienna, Austria |
| WS 2007 – SS 2010 | Bachelor's study of biology (major: zoology) at the University of Vienna, Austria |
| 2006 – 2007 | compulsory community service |
| 2001 – 2006 | HTBL u. VA St. Pölten (IT department) |
| 1997 – 2001 | Öko-Hauptschule Ober-Grafendorf |
| 1993 – 1997 | Volksschule Weinburg |

## PRACTICAL COURSES (Selection)

| | |
|---|---|
| WS 2012 | Morphometrics: The study of biological forms (Mitteröcker, Mayer; University of Vienna, Austria) |
| July 2011 | Endangered Species Recovery (Durrel Wildlife Conservation Trust; Jersey, UK) |
| SS 2010 | Comparative Anatomy and Morphology of Fishes (Ahnelt; University of Vienna, Austria) |

WS 2009 Project course: Histological methods (Walzl, Handschuh, Rudoll; University of Vienna, Austria)

## CONGRESS CONTRIBUTIONS

**David Ramler, Philipp Mitteroecker, Lisa Shama & Harald Ahnelt**

The effect of temperature on the body shape in threespine stickleback juveniles

Poster presentation at the workshop „Evolutionary potential of marine populations“ at the Wadden Sea Station List/Sylt of the Alfred Wegener Institute for Polar and Marine Research.

## WORK EXPERIENCE

February 2012 intern at the “die umweltberatung” Österreich

September 2008 intern as zookeeper at the Zoo Vienna

## SKILLS AND QUALIFICATIONS

Languages

German (first language)

English (proficient in speech and writing)

Computer literacy

MS Office, statistics (R, Mathematica), GIS (arcGIS10), graphics (Photoshop), basic programming skills (C++, Basic)

Additional qualifications

Exercise instructor for bouldering and sports climbing

Driving license (classes A & B)