

Divergent foraging behaviour and
shape in lateral plate morphs of
threespined stickleback
(*Gasterosteus aculeatus*).

Oda Bjærke

Master of Science thesis

2008



Department of Biology
University of Oslo

CEES

Centre for Ecological and
Evolutionary Synthesis

FORORD/PREFACE

Denne oppgaven ble utført ved Universitet i Oslo, i perioden 2007- 2008. Mitt arbeid er en del av et større prosjekt kalt «Adaptive trait transitions and speciation in sticklebacks (*Gasterosteus aculeatus* L.)» ved Senter for Økologisk og Evolusjonær Syntese (CEES).

Gjennomføring av arbeidet har vært med god hjelp fra flere hold. Først og fremst skal Helene M. Lampe og Kjartan Østbye ha en stor takk for ypperlig veiledning gjennom hele prosessen. Konstruktive tilbakemeldinger, gode råd, hjelp og oppmuntring fra dere har vært viktig for oppgaven, og gjort dette til en inspirerende og lærerik periode. Foruten disse er det på sin plass å takke Per-Johan Færøvig for å ha tatt seg bryet med å lære bort hvordan man dyrker alger og dafniekulturer, og dermed faktisk spilt en nøkkelrolle for eksperimentet. Jo S. Hermansen, Ingrid Solberg og Andrea H. Bjærke har lest kritisk gjennom utkast, noe som utvilsomt har forbedret kvaliteten. Tusen takk!

Ellers vil jeg takke alle biologistudentene som har holdt meg med selskap de siste årene, både i og utenfor biologibygningen. Ikke minst har alle de to- og fribente på ”Næsje et al.” på ulikt vis bidratt til høy trivselsfaktor. Min familie skal også ha takk for motivasjon og oppmuntring hele veien.

Oda,

Blindern, Juni 2008.

CONTENTS

FORORD/PREFACE	2
CONTENTS	3
ABSTRACT	4
INTRODUCTION	5
MATERIALS AND METHODS	9
STUDY SITE	9
SAMPLING AND MAINTENANCE OF STICKLEBACKS	11
FORAGING EXPERIMENT	12
<i>Experimental setup</i>	12
<i>Prey</i>	13
<i>Habituation and trial procedure</i>	13
<i>Data acquisition</i>	14
MORPHOLOGY AND GEOMETRIC MORPHOMETRICS	15
STATISTICS	16
RESULTS	18
CONSUMPTION OF PREY	18
FORAGING EFFICIENCY	19
SPATIAL DISTRIBUTION	21
SWIMMING DISTANCE AND VELOCITY	23
SHAPE: GEOMETRIC MORPHOMETRICS	24
EFFECT OF SHAPE ON BEHAVIOUR	27
DISCUSSION	30
PREY SELECTION AND EFFICIENCY	30
SPATIAL SEGREGATION	31
SHAPE	32
EVOLUTIONARY IMPLICATIONS	34
THE PLATE MORPHS	35
LIMITATIONS	36
<i>Experimental setup</i>	36
<i>Effects of parasites</i>	36
CONCLUSION	37
LITERATURE CITED	38
APPENDICES	44
APPENDIX 1 DESCRIPTION OF LANDMARK POSITIONS	44

ABSTRACT

Adaptation to different foraging resources is believed to be an important driving force of divergence between populations. Following colonization of freshwater, threespined sticklebacks (*Gasterosteus aculeatus*) have occupied and adapted to different types of aquatic niches. Adaptation to freshwater often involves, among other things, loss of lateral plates, shift to benthic resource exploitation, and change in body shape. Here, divergence in foraging behaviour and body shape is investigated among different lateral plate morphs coexisting in brackish water, and in a monomorphic population from a river habitat. Foraging behaviour was tested experimentally in the lab by observing fish that were offered benthic and pelagic prey simultaneously, while tracking the movements of the fish automatically. Shape differences were characterized using geometric morphometric Thin plate spline analysis. Significant and corresponding differences in foraging behaviour and shape were found between the plate morphs, and between sticklebacks from river and the lake. The results indicate ecological divergence towards benthic and pelagic habitat use between the plate morphs, resembling that of coexisting benthic-pelagic species pairs. This further suggests a possibility for evolution of ecologically based reproductive barriers between the coexisting morphs, although this remains to be investigated in more detail.

INTRODUCTION

Ecological divergence arises when populations adapt to different biotic environments. Selective forces will vary in a spatially heterogeneous environment, promoting evolution of traits that are advantageous in a population's local habitat (Schluter 2000, Futuyma 2005). Such traits are often related to optimal exploitation of local resources, hence different phenotypes might be seen in populations that exploit different niches (Day et al. 1994, Robinson et al. 1996). Resource polymorphism can evolve in any geographical context; in allopatry, parapatry or sympatry (Schluter 2001). However, sufficient restriction of gene flow is a prerequisite for local adaptation, as gene flow may counteract the effect of divergent selection (Lenormand 2002).

Resource competition is believed to increase divergence by favoring exploitation of alternative resources (Schluter 1994, Boughman 2007). This may also lead to exclusion of individuals with intermediate phenotypes that fail to compete with any of the more specialized individuals (e.g. Futuyma & Moreno 1988). When there is resource competition within a population, opportunity to occupy new underutilized niches is suggested to stimulate such differentiation, favoring individuals that shift to these resources (Simpson 1953, Nosil & Reimchen 2005). Colonization events might provide ecological opportunity, resulting in divergence from the ancestral population due to occupation of empty niches and exploitation of new food sources (Grant & Grant 1995, Schluter 2000)

The threespined stickleback (*Gasterosteus aculeatus*) offers a good example of ecological divergence following colonizations (Schluter 2000), and is therefore well suited for studying these processes (McKinnon & Rundle 2002). Threespined stickleback (hereafter stickleback) is a small euryhaline fish, common and widely distributed in temperate and boreal regions on the northern hemisphere (Bell & Foster 1994). Skeletal armor structures are characteristic for the species, consisting of three dorsal spines, pelvic spines, and lateral bony plates along the sides of the body. These structures function as defense against vertebrate predators by increasing the diameter of the fish, protecting tissue and enhance post-attack escape (Reimchen 1983, 1992, 2000). Marine sticklebacks are phenotypically uniform, and are most likely the ancestral morph, from which the more

variable freshwater forms have derived (Bell & Foster 1994). The marine ancestor is believed to have colonized freshwater habitats repeatedly after the retreat of the land ice approximately 10,000 – 15,000 years ago (Bell & Foster 1994, McPhail 1994). Alternative phenotypes are favored in marine and freshwater habitats as these environments differ profoundly in physical and chemical conditions, as well as predation pressure and foraging opportunities. Accordingly, a suite of phenotypic traits have evolved independently in freshwater populations (McKinnon & Rundle 2002). Such parallel and convergent evolution strongly indicates that the traits are adaptive, evolving as a consequence of natural selection (Schluter 1996, Schluter & Nagel 1995, McKinnon & Rundle 2002).

The most striking morphological transition following freshwater invasion is the parallel rapid reduction of lateral plates (Bell et al 2004, Bell 2001, Marchinko & Schluter 2007, Colosimo et al. 2005, Kristjánsson 2005). Generally, freshwater populations have only few or no lateral plates, whereas marine populations usually are completely plated (Östlund-Nilsson et al. 2007). Bell and colleagues (2004) studied plate morphology in a freshwater population for 12 years after colonization, and found that 75 % had lost most of their plates, mainly during the first 3 years. Several theories attempt to explain the massive loss of plates. The most obvious is a shift in predation regime, and reduced predation by toothed vertebrates in freshwater (Reimchen 1992, 1994, 2000). Being pelagic, the marine sticklebacks are also more vulnerable than the benthic freshwater form, by not having vegetation or other structural cover in immediate proximity (Walker 1997, Bell 2001). Costs involved in producing bony tissue are thought to be particularly high in freshwater due to mineral deficiency (Bourgeois et al. 1994), which might select directly against lateral plates. In sum, it is well established that loss of lateral plates is an adaptive response to freshwater environments (Bell and Foster 1994, Östlund-Nilsson et al. 2007). Three distinct lateral plate morphs are commonly recognized; complete (*Trachurus*), partial (*Semiarmatus*) and low plate (*Leiurus*) (e.g. Wootton 1976). The completes have a continuous row of lateral plates along both sides of the body, forming a pronounced keel on the caudal peduncle, partials have reduced number of plates and a keel which is often weakly expressed. Low plates have a few anterior plates, and the keel is absent (Hagen and Gilbertson 1972, Wootton 1976).

Marine and freshwater forms of sticklebacks are believed to differ in feeding ecology, accompanied by different morphology related to feeding (Wootton 1976). This is attributed to the high relative availability of benthic prey in freshwater habitats and pelagic prey in the sea. (e.g. Delbeek and Williams 1988, Hart and Gill 1994, Walker 1997). The encounter rate with a prey species is decisive for whether or not a stickleback feeds on it (Hart and Ison 1991), as predicted by optimal foraging theory (Stephens and Krebs 1986). The benthic diet consists predominantly of non-evasive macroinvertebrates, the pelagic diet consists of highly evasive zooplankton (Wootton 1994). Consequently, feeding on these prey types requires profoundly different techniques and is enhanced by specific morphological traits. Foraging performance is also largely determined by experience and learning from previous encounters with prey types (Warburton 2003, Ibrahim and Huntingford 1992). Being a visual feeder, sticklebacks select prey using cues in the visual appearance of the prey, i.e. colour, movement and shape (Hart and Gill 1994). Proximate rules used by the sticklebacks to select prey are shown in the field to result in optimal foraging (Ibrahim and Huntingford 1989).

In fish, habitat use and feeding behaviour are closely related to body shape, due to different modes of swimming and movements involved in foraging (Winemiller 1992, Webb 1984). Pelagic feeding entails sustained swimming over long distances in open water, favoring streamlined body shape that reduces pressure drag. In contrast, benthic feeding requires accurate movements and precise turning and tilting in a structurally complex environment, and a deep body profile is assumed to improve swimming performance in this habitat (Walker 1997, Webb 1984, Hart 2003). Thus, the marine sticklebacks are generally streamlined, and the freshwater form more compact and laterally deep (Mattern 2007). However, the latter form is variable according to size of the lake, and predation pressure (Walker and Bell 2000).

Divergent adaptation to different habitats is thought to be fundamental for development of reproductive barriers in sticklebacks (Boughman 2007). Sympatric pairs of benthic and pelagic ecomorphs have diverged by adaptation to different niches, and hybrids are selected against, due to ecological inferiority of phenotypic intermediates (Schluter & Nagel 1995). These distinct sympatric ecomorphs have only been found in 5 lakes (Gow et al. 2008), but there are other more common systems of polymorphic

sticklebacks, which might have a similar, although weaker, divergence pattern (Schluter and McPhail 1992, Cresko and Baker 1996). Sticklebacks with different lateral plate morphology in brackish water might represent anadromous-freshwater divergence, hence being differently adapted to benthic and pelagic resources. Lateral plate polymorphic sticklebacks are, to my knowledge, very little studied with respect to foraging and habitat use (but see Hynes 1950 and Hagen 1967), despite their potential to illuminate the role of resource exploitation in early divergence.

Here, I study sticklebacks from a young brackish water lake still connected to the sea, consisting of the three plate morphs (*Trachurus*, *Semiarmatus* and *Leiurus*) in sympatry. I also studied a low plate morph from a river upstream of the lake. The aim of this study is to test whether the lateral plate morphs differ in adaptation to freshwater, in terms of foraging behaviour and body shape. Based on the well documented loss of plates in freshwater, I address the hypothesis that low plate morphs are more adapted to benthic feeding than the partial and complete morphs, which are expected to be relatively more pelagic. This leads to four predictions that I test in the present study:

1. Low plate morphs consume more benthic prey and less pelagic prey than the complete morph.
2. The low plate morphs forage more efficiently (rapidly) on benthic prey and less efficiently on pelagic prey than the complete morph.
3. The morphs segregate in the water column according to where they find their preferred prey (the complete morphs in the upper, and low plate morphs in the lower parts of the water column).
4. The morphs differ in shape, reflecting the functional advantages of a streamlined body in pelagic swimming and deep body profile in benthic locomotion. The river morph is expected to deviate from the low plate morph in shape due to water flow in the river.

MATERIALS AND METHODS

Study site

The sticklebacks investigated in this study inhabit River Sandvikselva and Lake Engervann (Figure 1). Lake Engervann is situated near the inner part of the Oslo fjord at 59°53'49"N, 10°32'04"E. It is 0.16 km² large, and the maximum depth is 3m. Lake Engervann is at sea level, and connected to the Oslo fjord by the river Sandvikselva. With high tide, there is influx of seawater to the lake, making the water brackish with variable salinity (conductivity from 60-300 mS/m) according to fluctuations in wind and pressure (Halvorsen et al. 2005). Few organisms are able to adapt to such unstable conditions, hence the biodiversity in Lake Engervann is low, but biomass may be large for those species that succeed. Halvorsen et al. (2005) did a survey of the conditions in the lake: Nine-spined stickleback (*Pungitius pungitius*) is the dominant fish species, followed by threespined sticklebacks. A few species of gobies (Gobiidae spp) are found, and migrating salmon (*Salmo salar*) and marine trout (*Salmo trutta*) might also appear. Lake Engervann is an eutrophic lake with high nutrient content and much vegetation. However, the zooplankton community is extremely poor with low densities and very few species, mainly dominated by copepods. The benthic fauna is more diverse and dense; chironomids, oligochaetes, polychaetes and gastropods being most abundant (see Halvorsen et al. 2005 for details).

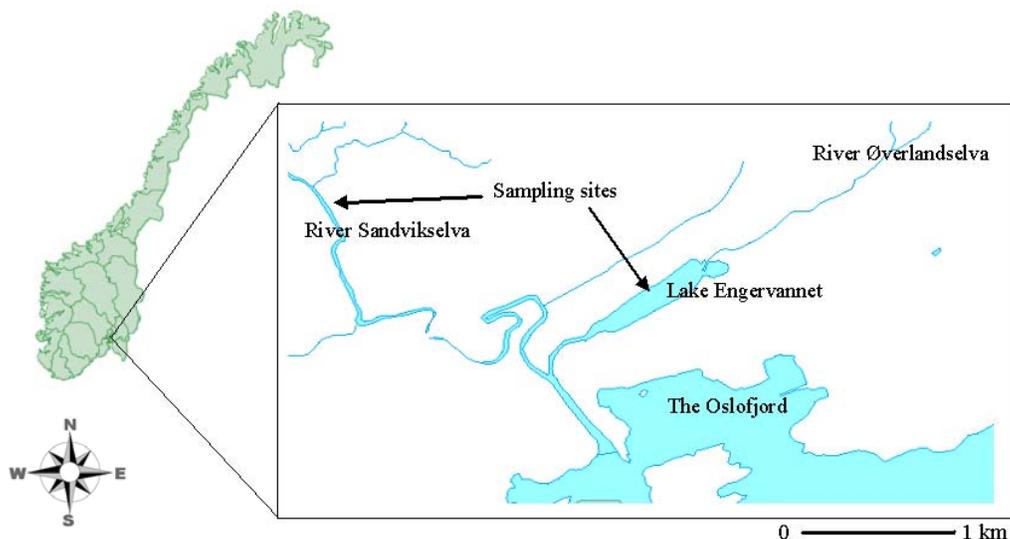


Figure1. Map of Lake Engervann with study sites.

The three-spined sticklebacks in Lake Engervann consist of three sympatric lateral plate morphs; *Trachurus* (complete), *Semiarmatus* (partial) and *Leiurus* (low plate), described in Table 1. The exact frequency of these morphs is not known, but roughly estimated from my samples, there were 5 % low plates, 20 -30 % partial morphs and 60 -70 % complete morphs. Currently, the migration pattern and the extent to which the morphs are stationary in the lake are unclear. That is also the genetic structure of the sticklebacks in Lake Engervann. The other sampling site was in River Sandvikselva, approximately 3 km upstream of the lake. This site is not influenced by sea water. The sticklebacks here are monomorphic for the low plate morph, and assumed not to encounter gene flow with marine sticklebacks. The prey species on this site comprise mainly ephemeropterans and chironomid larvae, accompanied by some gastropods and oligochaetes (Borch et al. 2005). Trout (*Salmo trutta*) and salmon (*Salmo salar*) spawn in the river. The flow of water in this river is on average 3 m³/s, interrupted by seasonal flood with a water flow of 57 m³/s, (Borch et al. 2005).

Table 1. Definitions of the three lateral plate morphs and experimental groups. Sticklebacks on the pictures are stained with alzarin red to highlight the lateral plates and the keel (e.g. Colosimo et al. 2005).

Morph	Definition	Lake Engervann	River Sanvikselva
<i>Trachurus</i>	Complete (continuous row of plates, keel)		
<i>Semiarmatus</i>	Partial (reduction in plates, keel)		
<i>Leiurus</i>	Low (few plates and no keel)		

Sampling and maintenance of sticklebacks

Sticklebacks were collected in March 2007. The main sample was taken in Lake Engervann, from the shore on the north-west side. The river population was sampled ca 3 km upstream of the lake. Plastic fry traps were used to capture the sticklebacks (after Breder 1960), using cheddar cheese to attract the fish. After capture sticklebacks were transported to the laboratory within 30 minutes. They were kept in 500 L fish tanks (EWOS) with through-running water (water temperature 4°C, 12/12h daylight), and fed with red chironomid larvae and pellets (EWOS Modulfôr, Micro 040). The sample from the river was kept in a separate tank, and the fish from Lake Engervann were soon divided into the groups of the three different plate morphs. Inspection of lateral plates was done in the laboratory using a loupe, anaesthetizing with benzocaine (20g/100mL ethanol). Figure 2 illustrates the number of plates in the experimental groups.

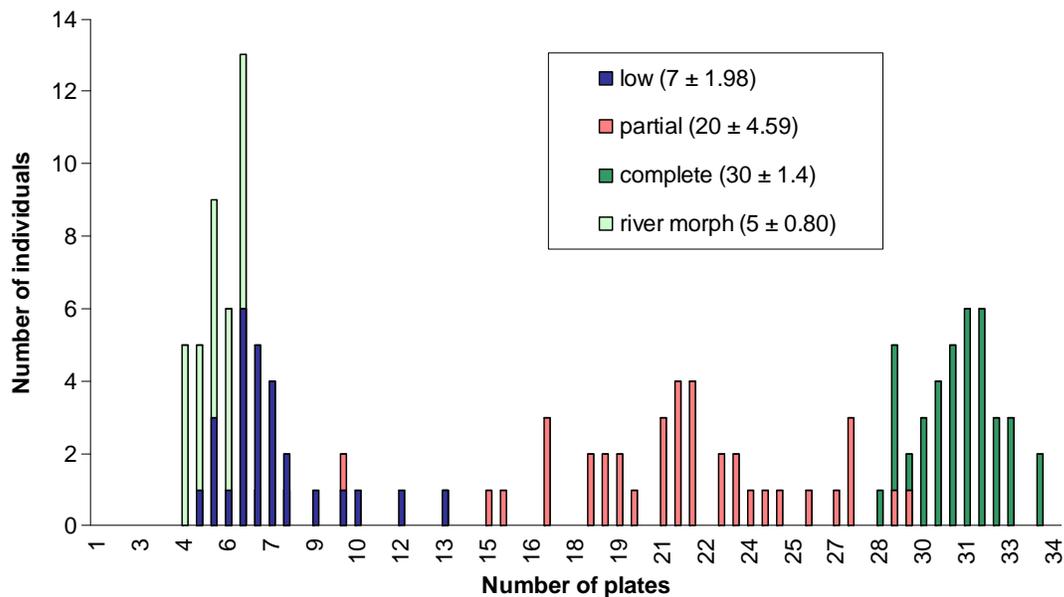


Figure 2. The distribution of lateral plate numbers in the four groups. Numbers in the box are the mean number of plates in each group and standard deviation.

Foraging experiment

The behaviour experiments were conducted in the laboratory at the Department of Biology, University of Oslo, during April and May 2007. The experiments included observation and measurements of foraging and swimming behaviour of individual fish when offered pelagic and benthic prey simultaneously.

Experimental setup

The aquarium used in the experiment was designed for the purpose, with enough space for the fish to choose between open water, sheltered areas and the bottom zone. The aquarium was 122.5 L (70x70x25 cm, height x width x depth). Water temperature was 20°C. The aquarium was illuminated from above (OSRAM 18W/21) and all vertical sides except the front were covered with opaque white plates, facilitating observation and tracking of the fish. Due to automated tracking techniques (described below), the experimental aquarium could not be aerated with a bubbling stone during trials, but was supplied with oxygen before every fifth trial. In the upper left corner there was a small transparent start box, from which the fish were released into the aquarium when a door was pulled up. Coarse gravel covered the bottom, and in the left corner there was an artificial plant area made of bamboos (see Figure 3).

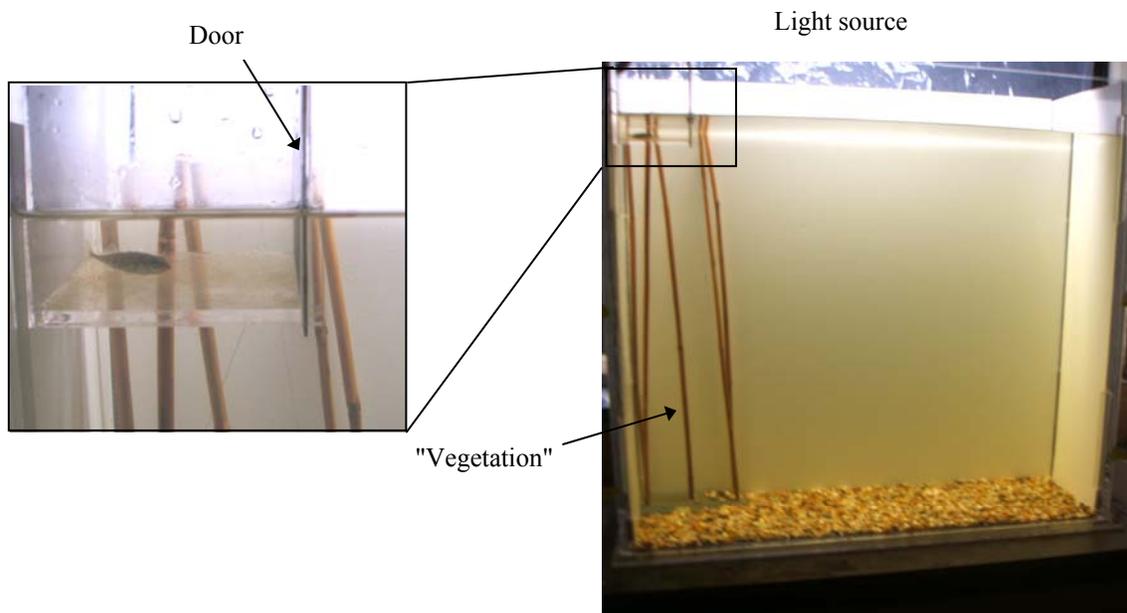


Figure 3. The experimental aquarium and the start box.

Prey

For each trial, the aquarium contained ten items of benthic and ten items of pelagic prey. Frozen chironomid larvae, approximately 10 mm in size, were used as benthic prey, and live adult daphnia (*Daphnia magna*), approximately 3 mm in size, served as pelagic prey (Fig 4). These prey species were chosen because they are among the most common prey for sticklebacks (Wootton 1994). Daphnia were cultured in the laboratory on green algae and culture medium (50µg P COMBO medium, after Kilhan et al. (1998)).

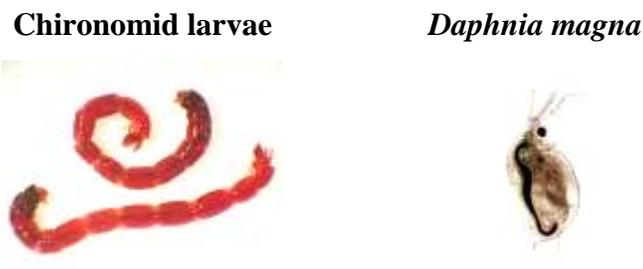


Figure 4. The prey animals used in the foraging experiment: Chironomid larvae and *Daphnia magna*.

Habituation and trial procedure

As the temperature in the experimental room differed from that in the maintenance room, the fish were kept in the experimental room in additional aquaria (2x 430L) for acclimatizing 2-5 days prior to testing. In this period, each fish was introduced to the trial procedure twice in order to familiarize them with the experimental conditions and the available feeding options. I used 147 fish, 40 individuals of each group, except for the low plate morph (n= 27) due to the very low frequency of this group in the lake. The sticklebacks were tested one by one in randomized order. Before each trial the aquarium was supplied with prey so that there were exactly ten daphnia and ten larvae present, and one fish was put into the start box. After 2-3 minutes, when the fish had calmed down and the larvae sunk to the bottom, the door was pulled open. From the moment the fish left the box it was given ten minutes to swim and forage in the aquarium. After each trial, the fish was removed and killed with an overdose of benzocaine (20g/100 mL ethanol), then marked and stored in 70% ethanol.

Data acquisition

All the trials were recorded with a Panasonic B&W CCD camera (3,5-8 mm lens) connected to a computer. EthoVision 3.1 (Noldus Information Technology 2005) was used to obtain all behaviour data. This software produces a track file by continuously recording the precise position of the fish in a two dimensional image, directly as it swims (sample rate: 8,333 samples/sec, capturing 5000 x-y coordinates per trial). Tracking started immediately after the fish left the start box, and stopped automatically after ten minutes. While observing, I manually scored behaviour in EthoVision, in which the exact time of the behaviour event (seconds from start) was recorded. Zones in the aquarium were predefined in EthoVision, for measuring the time each fish spent in benthic and pelagic areas, and in area with structural cover. The benthic and pelagic zones were defined as the lower and upper 25 % of the aquarium respectively, and the cover zone was a rectangular area in the corner where the plant was located, also 25 % of the total area (Figure 5). Table 2 summarizes all behaviours registered in EthoVision.



Figure 5. Definition of zones used to measure differences in spatial distribution associated with morphology.

Table 2. The measurements of behaviour recorded in Ethovision. The number of Daphnia and larvae consumed is daphnia/larvae swallowed- daphnia/larvae spat out.

Manually scored behaviours		Automatically scored
Daphnia swallowed	Larvae swallowed	Swimming velocity (cm/sec)
Daphnia spat out	Larvae spat out	Swimming distance (cm)
Attack failure on daphnia	Attack failure on larvae	Time in zone (sec)

Morphology and geometric morphometrics

Lateral plate morphology formed the basis of the groups that were compared in this study, according to the description in Table 1. Further morphological measures were carried out on dead specimens after finishing behaviour experiments. Body length was measured in cm from tip of the snout to the extent of the caudal fin.

In order to quantify and characterize the variation in body shape among the plate morphs, a geometric morphometric analysis was performed, using thin plate spline (Tps) morphometric software, obtained from Rohlf (2007 a-c). This is a shape analysis based on anatomical landmarks, biologically definable points with correspondence on all specimens. Pictures of right lateral side of individual specimen were taken with a CANON EOS 350D digital camera (90 mm lens, Tamron macro). Individuals were stretched out with needles, minimizing differences in shape due to bending during preservation. A total of nineteen landmarks were digitized using TpsDig2 (Rohlf 2007a), in which x and y-coordinates of the landmarks were captured. Here, the 19 landmarks cover all regions of the body, and were chosen in order to best describe the overall body shape (Figure 6).

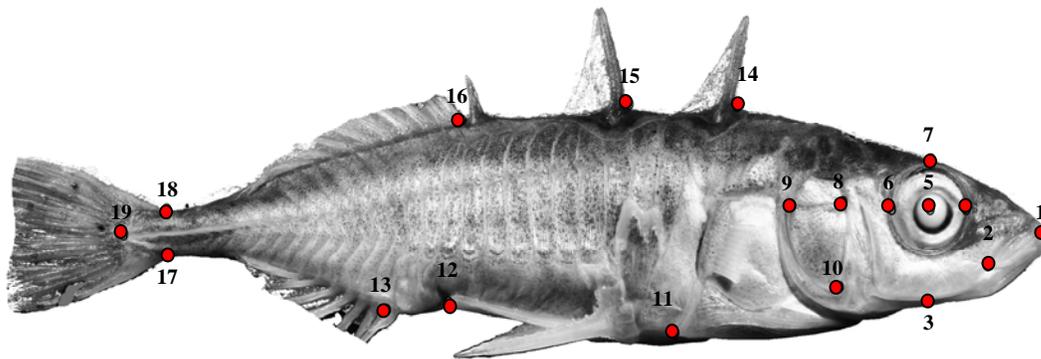


Figure 6. Landmarks used in geometric morphometric shape analysis. See appendix 1 for detailed description of landmark positions.

The pictures with digitized landmarks were first processed in TpsRelw (Rohlf 2007b). Here, information unrelated to shape is mathematically filtered out. This is done with Generalized Procrusters Analysis, which scales and rotates the landmark configurations into a common coordinate system, and a common unit size (Adams et al. 2004). The program then defines the consensus configuration, that is, the "average" shape. From this, TpsRelw computes new individual shape variables: Partial warp scores and uniform scores. The former is deviations in individual landmarks from the consensus configuration (local deformation), the latter is overall deviation from the whole consensus configuration. These variables appear in a matrix which is suitable for multivariate tests (e.g. Adams et al. 2004).

Statistics

All statistics were performed with the statistical software JMP 5 (SAS Institute Inc. 2005). To test for distinguishable variation in shape between the plate morphs, a Discriminant Function Analysis was performed with the matrix of shape variables as predictors and plate morph as grouping. This test attempts to classify individuals into predefined groups (here plate morphs) based on the predictors. The discriminant analysis yields canonical functions, and gives individual scores for each fish in these functions. The canonical functions explain different aspects of shape transformation, and the canonical scores reflect where the fish are positioned along the canonical function axes. Shape transformation figures are produced in TpsRegr (Rohlf 2007c). This program does a multivariate multiple regression of canonical scores against the shape variables obtained in TpsRelw, and then creates transformation grids, visualizing local shape transformations along the canonical axes. One can thus interpret specific shape variations associated with grouping (here plate morphology).

In all behaviour analyses, differences in size were accounted for by including body length as a covariate. When comparing the morphs, Tukey-Kramer *post hoc* analysis was performed to see which morphs differed from each other. ANCOVA was used to analyze swimming behaviour and consumption of daphnia and larvae among the groups. I was also interested in, not only how many prey items that were consumed, but also what kind of prey was eaten most efficiently by the different morphs. Therefore, a survival

regression analysis for larvae and daphnia was carried out. The survival function takes into account at what time during the trial a prey is eaten, and the amount of prey that is not eaten after 10 minutes is censored. A proportional hazard function was chosen, because it is semi-parametric, and the data deviated from normal distribution. Foraging efficiency is often measured as number of strikes per successful prey capture, with the drawback that searching and handling time is not included. By measuring foraging efficiency with a survival analysis, searching- and handling time is implicit.

Time in zones was also analyzed with ANCOVA. Due to skewed data, the times in zone were converted to proportions and arcsine transformed ($\text{Arcsine } \sqrt{(\text{sec in zone} / 600)}$). This transformation stretches out both end of the distribution and compresses the central part. It is a powerful and widely used manner of dealing with skewed proportion data (SAS Institute Inc 2005).

For further investigation of depth distribution, I analyzed the vertical position of individuals over time. While tracking, EthoVision records the exact location of the fish in an x-y coordinate system, where x is the horizontal position and y is the depth in the aquarium. Testing the prediction that low plates are more attracted to the bottom area, I used the y-values to examine the distribution in the water column. For each individual, the mean y-values for 30 second intervals were measured, throughout 10 minute trials. These 20 means were analyzed in a MANCOVA with repeated measures, with body length as covariate. A repeated-measures MANCOVA accounts for lack of independence of the repeated tests. It yields the effect of morph on the depth distribution, and the interaction between time and morph. However, the statistical power for detecting group effect is low (e.g. Gloutney & Clark 1997), and so I also conducted 20 univariate tests with Tukey-Kramer *post hoc* analysis and Bonferroni-adjusted alpha-level ($p < 0.01$). Events of attack failure and spitting out were too few to be analyzed specifically.

RESULTS

Consumption of prey

All fish were observed spending most of the time during the trial searching for food and eating. The total number of prey eaten per individual was 7.51 ± 3.14 (mean \pm SD). Out of 147 individuals, 94 ate both types of prey (63.9%), 51 ate daphnia exclusively (34.7%), and only 2 fish ate larvae exclusively (1.4 %). All morphs ate more daphnia than larvae. The body length was similar for all morphs in the lake, but the river morph was significantly shorter than the low and complete morph from Lake Engervann (ANOVA: $F_{3, 35.8} = 9$, $p = <.0001$). ANCOVAs with body length (cm) as covariate gave no significant differences between the groups in number of daphnia ($F_{3, 2.37} = 2.4$, $p = 0.069$) or larvae ($F_{3, 2.59} = 1.8$, $p = 0.15$) consumed. However, there was a trend that partial and complete morphs consumed more daphnia and fewer larvae than both low plate morphs (Figure 7). The low plate morphs, both from river and lake, were more likely to eat larvae than were the partial and complete morphs. In effect, a weak association between lateral plate morphology and foraging preference was revealed. Body length had a significant effect on prey consumption; larger fish ate more of both prey species (Daphnia: $p = 0.045$, larvae: $p = 0.0027$).

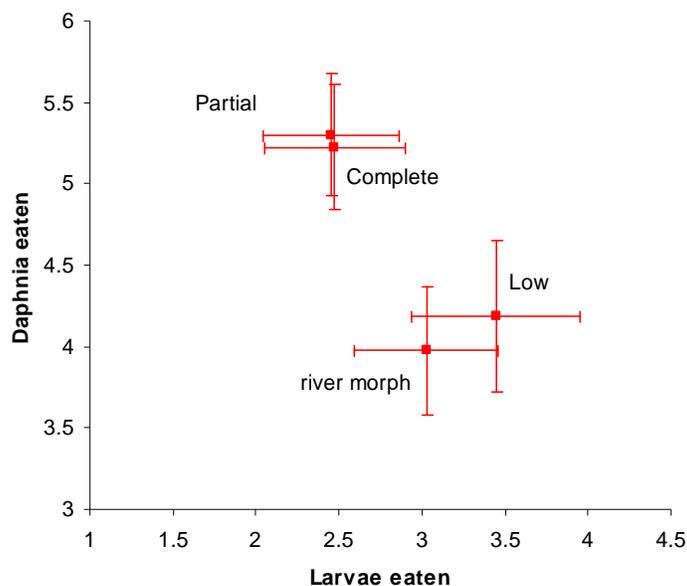


Figure 7. Mean number of daphnia and larvae eaten in the morphs (adjusted for body length), with standard error bars.

Foraging efficiency

Differences in efficiency in eating daphnia and larvae were measured with a survival regression analysis for each of the two prey types. This revealed significant differences between the groups (Table 3). Risk ratios correspond to the risk that the prey will be eaten by a stickleback given its plate morph group. For daphnia, the risk of being eaten is highest when the fish predator is partial or complete morph, and significantly lower when it is exposed to any of the two low morphs. Again, the prediction that a high number of plates correlate with specialization to pelagic foraging is supported. The reverse was the case for larvae, which experienced greatest risk of being eaten when exposed to low plate morphs from Lake Engervann or River Sandvikselva, whereas the complete morphs implied significantly lowest risk on larvae (Figure 8).

Table 3. Results of survival analysis, showing the effect of morph and body length of the fish on the survival of daphnia and larvae.

Daphnia	Source	DF	L-R χ^2	p
	Body length (cm)	1	7.12	0.0076
	Morph	3	17.64	0.0005
Larvae	Source	DF	L-R χ^2	p
	Morph	3	12.94	0.0048
	Body length (cm)	1	34.15	0.0000

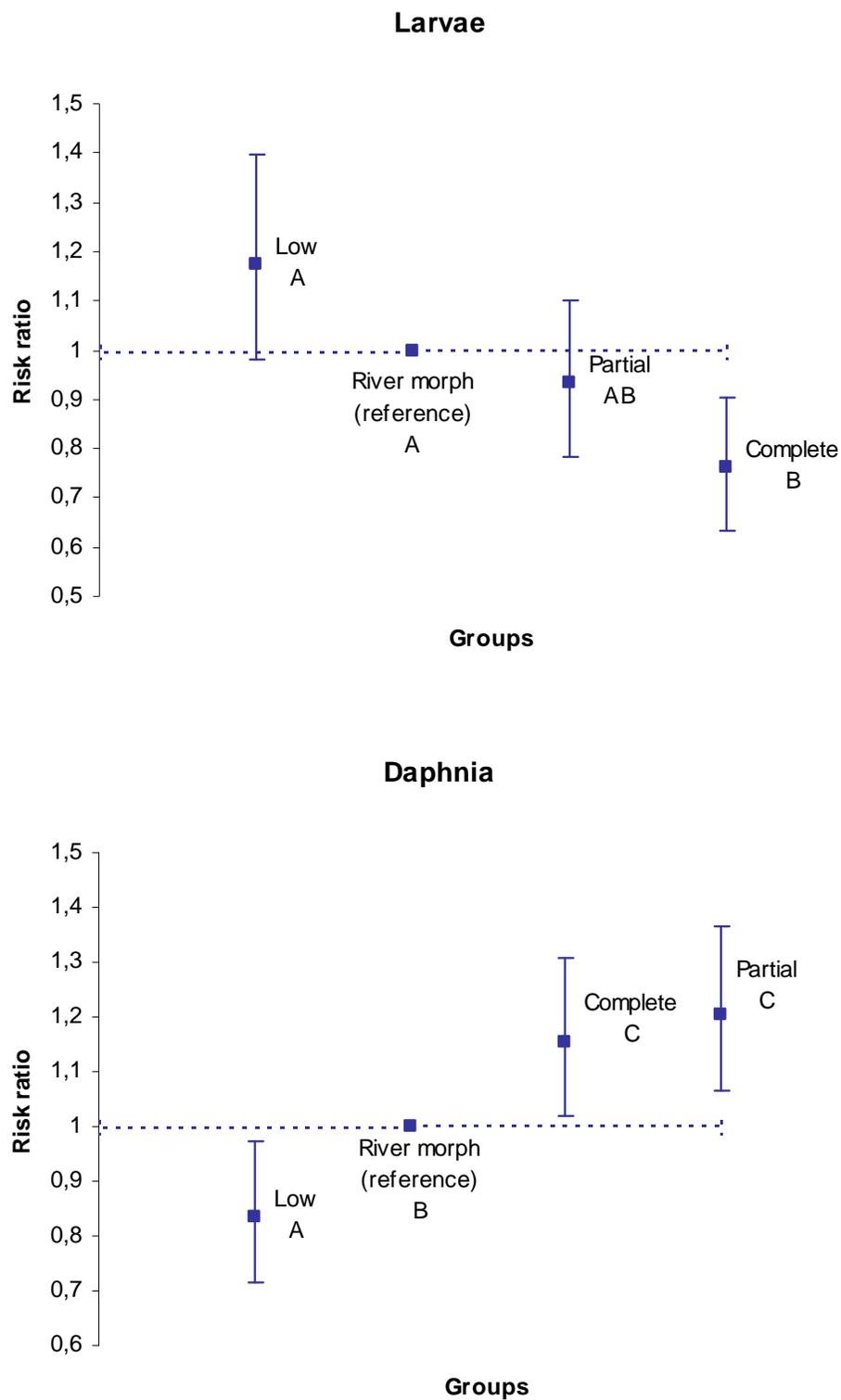


Figure 8. The risk ratios, with confidence intervals, for daphnia and larvae. Values are corrected for body length. Non-overlapping confidence intervals are noted with different letters, which correspond to alpha-level ($p < 0.05$) significant difference between groups.

Spatial distribution

Lateral plate morphology had effect on the time spent in benthic and pelagic zones (Table 4). The low plate morphs from the river spent significantly more time in the benthic zone than partial and complete morphs, shown by Tukey-Kramer HSD analysis. The effect of plate morph was strongest for time spent in the pelagic zone ($p < 0.001$). Here, complete and partial morphs spent more time than the low morphs, but it was only for the river morph that the difference was significant. The emerging pattern indicates that completely and partially plated sticklebacks are attracted to the pelagic area, whereas the low plate morphs seek towards the bottom. The zone with structural cover was equally utilized by all groups.

Table 4. The effect of morph on time spent in a) benthic zone, and b) pelagic zone, using ANCOVA. The zone variables are arcsine transformed proportions of total trial time.

a)				
Source	DF	SS	F	p
Body length (cm)	1	1.71	10.39	0.0016
Morph	3	2.65	5.37	0.0016
Error	141	23.21		
C. Total	145	26.55		
b)				
Source	DF	SS	F	p
Morph	3	3.73	8.20	<0.0001
Body length (cm)	1	1.76	11.63	0.0008
Error	142	21.49		
C. Total	146	25.73		

The MANCOVA, with repeated measures of y-coordinates (depth) over time, revealed a significant effect of morph (Table 5). Univariate ANCOVAs showed that the morph effect was significant the first 6 minutes (Bonferroni-adjusted alpha level). This arises from the river morph being lower in the water column this period (shown by Tukey-Kramer HSD, and also indicated in Figure 9). The interaction between morph and time was marginally significant (Wilk's $\lambda = 0.58$, $p = 0.08$). The river morph tended to seek down towards the bottom after being released much quicker than did the other morphs. However, the morphs from Lake Engervann had a very homogeneous pattern, and towards the end of a trial, all four groups assimilated in depth distribution. In sum, both analyses of spatial distribution show the same; that the river morph is clearly distinguished from the other morphs in being more benthic, and that the low plates from the lake are slightly more benthic than partials and completes, although not significant.

Table 5. Test results of MANCOVA with repeated measures. The significant effect of morph is due to the river morph, the other morphs do not differ from each other (Tukey-Kramer HSD).

f test	Value	DF	F	p
All between	0.15	4	5.23	0.0006
Intercept	0.0008	1	0.12	0.73
Morph	0.11	3	5.12	0.0022
Body length (cm)	0.09	1	12.08	0.0007

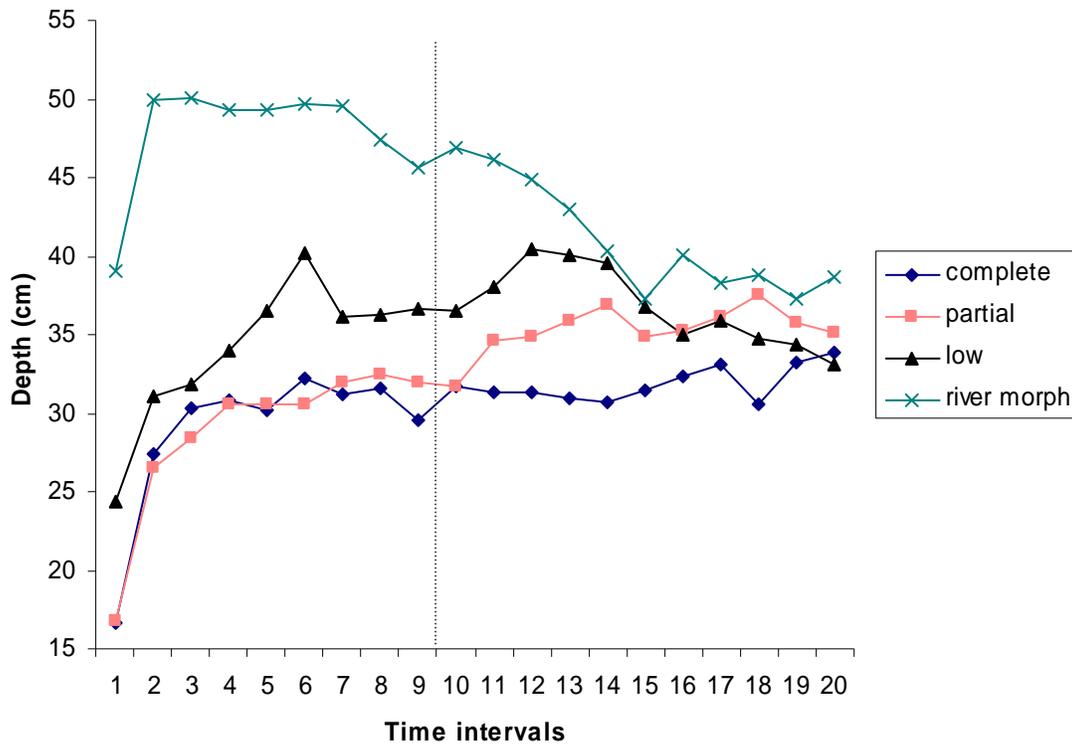


Figure 9. The depth distribution of the groups over time (20 intervals of 30 sec), tested in a MANCOVA with repeated measures. The y-axis refers to the depth at which the fish is positioned, which is increasing with the y value. Points show the mean values within groups for each interval. The dotted line shows the point at which the river morph no longer differs significantly from the others, with Bonferroni corrected alpha level $p = 0.01$.

Swimming distance and velocity

In addition to length, the river morph also stands out by shorter swimming distance, and lower velocity than the lake morphs. When correcting for the shorter body length of the river morph, the swimming distance was found to be similar in all groups ($p > 0.05$). For swimming velocity, the difference between the partial morph and the river morph was significant ($p = 0.038$) shown by ANCOVA and correcting for body length. The partial morphs swam fastest (least sq mean = 3.55 cm/sec.) and river morph slowest (least sq mean = 3.00 cm/sec.) of all the morphs.

Shape: Geometric morphometrics

The Discriminant Function Analysis of shape variables was significant (Wilk's $\lambda = 0.046$, $p < 0.001$). Based on the 19 landmarks, it resulted in differences between the morphs, and correctly classified 85.03 % of the sticklebacks into their respective morph group. Almost all the variation was explained by the first and second canonical functions, 88.8% and 7% respectively (Figure 10). On the first axis, the river morph is separated from the three morphs in Lake Engervann ($F_{3,1} = 397.05$, $p = <0.001$). On the second axis, all the sympatric morphs in Lake Engervann differed significantly from each other ($F_{3,1} = 31.52$, $p = < 0.001$). This was confirmed by Tukey HSD analysis in one-way ANOVAs of the canonical scores by the morphs.

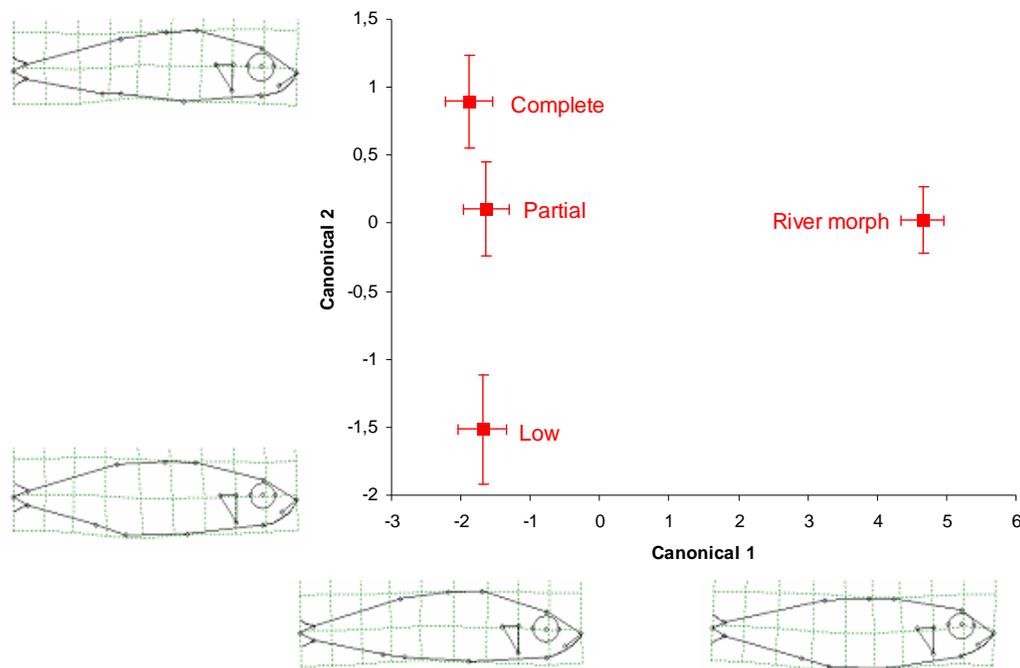
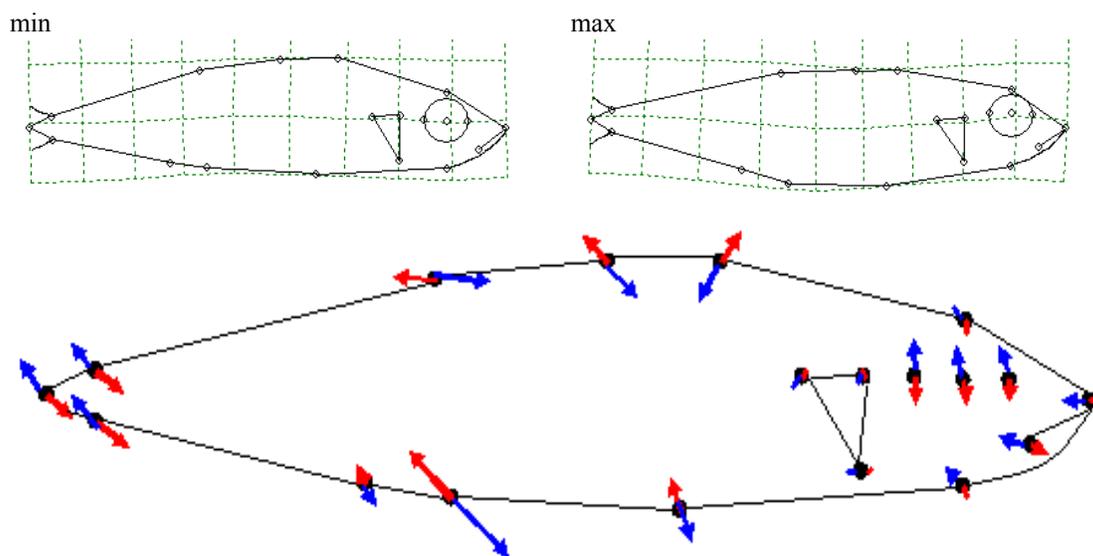


Figure 10. Canonical plot with mean and 2x standard errors of canonical scores in the groups. The first axis explains 88,8 % , and the second axis explains 7 % of the shape variation in the sample. The transformation grids on the figure represent the extreme values on each axis.

The first canonical function (Canonical 1) represents predominantly the difference between sticklebacks in the two sampling sites, Lake Engervann and River Sandvikselva. Specimens from the lake appear on the lower end of the axis and those from the river on the upper end. The main difference seems to be that they are dorsally convex in the lake, and ventrally convex in the river. In addition, the tail region is longer and located higher in the river morph. Also the forehead and the eye is elevated, and the snout is shorter in the river morph compared to specimens from the lake. In the river specimen, the lateral profile has a more equally distributed body depth, whereas in the lake the body depth is tapering backwards from the shoulder region (Figure 11a).

The second canonical function (Canonical 2) characterizes the shape differences among the sympatric plate morphs in Lake Engervann. The low plate morphs and the complete morph are positioned on the lower and upper end of the scale respectively, and the partial morph is located in the upper middle. Here, the major changes occur in the posterior abdominal and dorsal area, which seems distended in the low plate morph. Relative to the complete morph, the low plate shape is overall deeper and more compact. The complete morphs have an elongated ectocoracoid bone, and a slightly longer snout and tail region. In addition, the first dorsal spine is located slightly closer to the head in the complete morph, and that is the point where the shape is deepest. There is also a very small difference in the diameter of the eye, seeming larger in the complete morphs compared to the low plate morph (Figure 11b).

a) First canonical function



b) Second canonical function

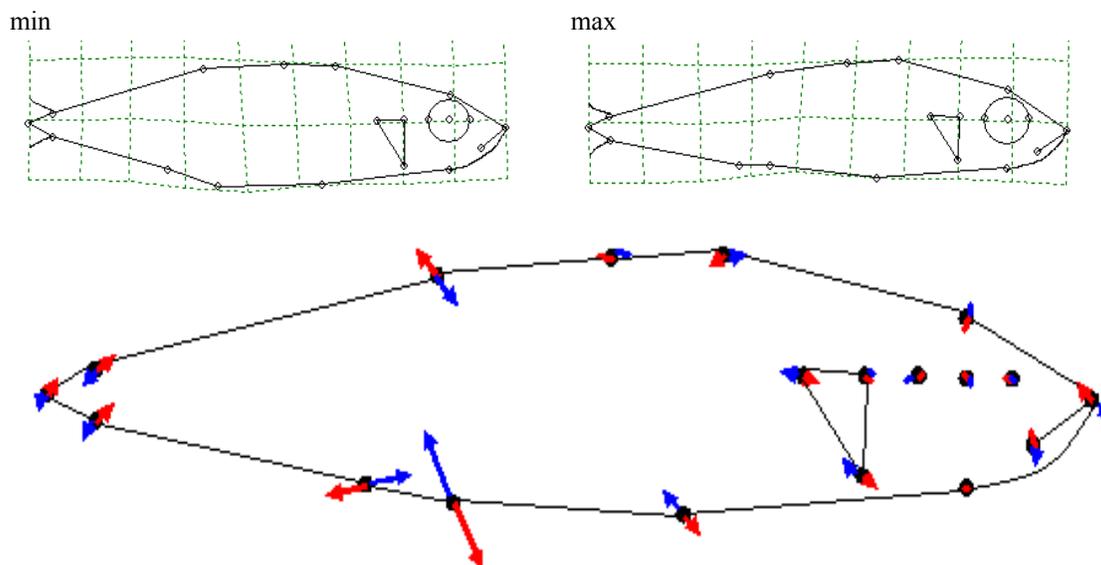


Figure 11. Transformation grid, illustrating variation in shape. The figures are the extreme shape values of the canonical axes. a): the shape variation on the first canonical axis. b): the shape variation on the second canonical axis. Figures with arrows are included to show how each landmark changes position, the base of the arrow is the mean value for the whole sample, and the extent is the extreme low (red) or high (blue) value on the two axes. The deviations shown here, are in the actual scale.

Effect of shape on behaviour

The canonical scores, representing the aspects of shape distinguishing the plate morphs, had significant effect of consumption of larvae (Table 6). The strong effect of Canonical score 1 indicates that the river morph ate more larvae relative to the remaining groups. Correspondingly, the second canonical function was negatively associated with number of larvae eaten, saying that the low plate morphs tended to eat more larvae than the partial and complete morph in Lake Engervann. In sum, this supports the trend of prey selection illustrated in Figure 1. The shape variables did not show any effect on number of daphnia eaten.

Table 6 Summary of ANCOVA canonical scores on number of larvae eaten.

Source	DF	SS	F	p
Body length (cm)	1	80.90	12.70	0.0005
Canonical2	1	41.41	6.14	0.0118
Canonical1	1	39.07	6.50	0.0144

Also for time spent in benthic and pelagic zones were body length, canonical 1 and canonical 2 were significant (Figure 12, Table 7 and 8). The effect of Canonical 1 suggests that the river morph is more benthic and less pelagic than the lake morphs (as shown by the repeated measures MANCOVA and total time in zones). The effect of Canonical 2 confirms that the shape associated with complete and partial plate morphs is more pelagic and less benthic than the shape linked to low plates in Lake Engervann. None of the morphological variables explained variance in time spent in cover zone. In general, the effect of canonical shape variables gives support to the already demonstrated behaviour differences between morphs.

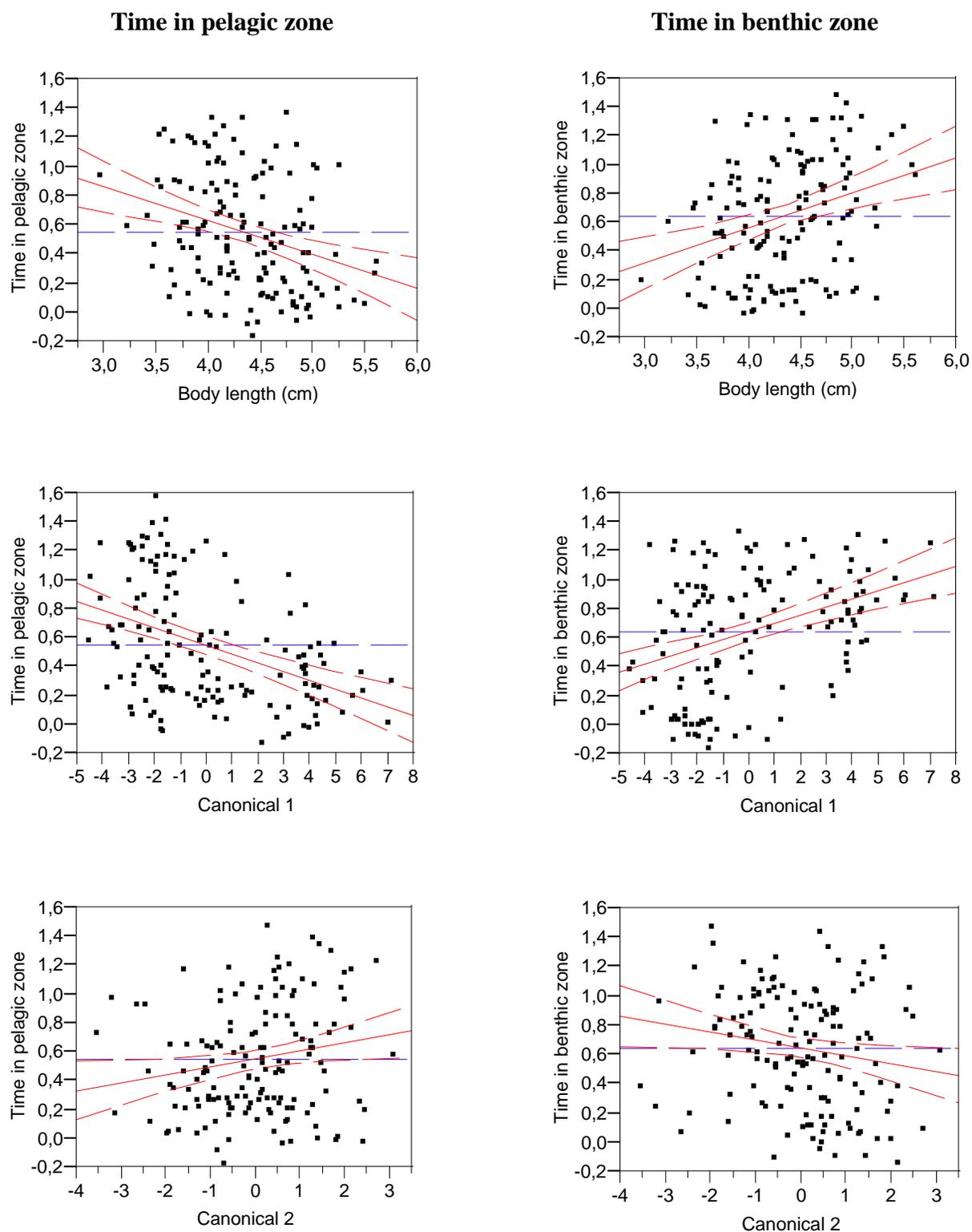


Figure 12. Plots of significant relationships between time in zones and shape variables, when analyzed in an ANCOVA: body length, canonical 1 and canonical 2. The y-values are arcsine transformed proportions of the total trial time.

Table 7. Summary of ANCOVA, showing the effect of canonical scores on time in pelagic zone (arcsine transformed).

Source	DF	SS	F	p
Body length	1	2.05	14.53	0.0002
Canonical 1	1	4.35	30.84	<0.0001
Canonical 2	1	0.71	5.03	0.0265
Error	143	20.21		
C. Total	146	25.73		

Table 8. Summary of ANCOVA, showing the effect of canonical scores on time in benthic zone (arcsine transformed).

Source	DF	SS	F	p
Body length	1	2.21	14.55	0.0002
Canonical 1	1	3.68	24.28	<0.0001
Canonical 2	1	0.68	4.51	0.0354
Error	142	21.54		
Total	145	26.55		

DISCUSSION

My analysis revealed significant behaviour and shape differences between plate morphs. The low plate morphs, both from the river and the lake ate more benthic prey and less pelagic prey than either partial or complete morphs. The low plate morphs also consumed larvae more efficiently and daphnia less efficiently than the partial and complete morphs. The river morph spent the longest time in the benthic zone of the aquarium, whereas the three morphs from the lake did not segregate significantly in the vertical water column. The low plate river morph also differed substantially from all the lake morphs in shape. Smaller, but significant differences in shape were also found between the three coexisting morphs.

Prey selection and efficiency

The differences between plate morphs in number of benthic and pelagic prey eaten are consistent with prediction 1, although the differences were not significant. Earlier studies of diet composition among plate morphs have also demonstrated that complete morphs forage on pelagic prey and low plate morphs feed on prey associated with benthos (Hynes 1950, Hagen 1967). The relative effect of plate morphs on the survival of larvae and daphnia, support prediction 2.

The observed differences may be the outcome of inherited, learned or plastic prey selection according to what are optimal strategies in Lake Engervann and in the sea. The availability and relative abundance of prey species are important for what sticklebacks choose to feed on. Lake Engervann can be considered an extreme benthic habitat. It is shallow with a large littoral area, and it provides almost exclusively benthic organisms as prey for sticklebacks, as the zooplankton community is extremely poor (Halvorsen et al. 2005). Focusing on the most abundant prey is in accordance with optimal foraging (Stephens and Krebs 1986) and might be the reason why the morphs select different amounts of benthic and pelagic prey. Alternatively, the morphs may differ in ability to detect or respond to the very contrasting visual cues of benthic and pelagic prey. Pelagic prey is often small and fast moving, requiring especially sharp vision. In sympatric pairs, the pelagic morph is known to have larger eyes, probably facilitating detection of very

small planktonic prey (Schluter & McPhail 1992, Cresco & Baker 1996). Different abilities to capture and handle benthic and pelagic prey, might also have contributed to the pattern shown here. Performance can be enhanced by morphology and technical skills. In benthic-pelagic pairs, the performance on the two resources is highly related to trophic morphologies, i.e. number and length of gill rakers and mouth width (Schluter 1993). These traits were not measured explicitly here, but might have been a contributing factor. The effect of body shape on foraging performance is discussed later. Proficiency on prey handling is also proved to be influenced by previous experience. In fact, only a few exposures to a certain prey type can drastically improve the foraging performance on this prey (Warburton 2003, Ibrahim & Huntingford 1992). In any case, these results suggest different habitat use of the plate morphs, and might be adaptation to pelagic niche by the partial and complete morphs, and benthic niche by the low plate morphs.

Spatial segregation

The amount of time spent in pelagic and benthic zones in the aquarium partly support prediction 3. The segregation in my study was weaker than expected, since only the river morph was significantly more benthic than the complete morph, and the three morphs in the lake did not differ significantly from each other. The pattern revealed here can be attributed to longer divergence time, hence the stronger adaptation to benthic feeding by the river morph. The river morphs might also prefer the benthic habitat because of reduced currents near the bottom of the river (Alexander 1967), or the benthic zone could be used because this river morph probably experience stronger predation pressure than the other morphs in Lake Engervann. For comparison, sympatric benthic-pelagic ecomorphs segregated considerably into the lower and upper part of the water column when tested in a tank (Larson 1976). This indicates a tight connection between spatial segregation and foraging habitat.

However, the deviating time in benthic zone by the river morphs diminishes towards the end of the trials. One possible explanation is that the river morph realizes the absence in the aquarium of factors that normally can restrict its distribution, i.e. currents and predators. Alternatively, it could be caused by alteration in prey selection as the hunger decreases. In general, feeding rate will decrease as the stomach fills, and a

stickleback with full stomach will select a smaller prey over a larger (Hart & Gill 1992, 1993, Salvanes & Hart 1998). The river morphs might stay close to the bottom until they become satiated, then reject the larvae due to large size, and start to look for the smaller daphnia. If so, it suggests that the river morph seeks down to the bottom in order to search for food, and that consumption of larvae is not an effect of the river morph being in the benthic zone for other reasons (i.e. predator avoiding behaviour, or effect of currents). Also habitat selection can be a learned response in sticklebacks, as they are found to develop preference for habitats in which they previously have encountered profitable prey (Webster & Hart 2006). However, studies on benthic-pelagic species pairs have demonstrated persistence of divergent habitat selection in common garden experiments (see Larson 1967), suggesting that preference for habitat is inherited rather than learned. The latter support the view that the behaviour of the river morphs is the outcome of adaptation. It should be noted that in addition to staying longer at the bottom, the river morph also swam shorter and slower than the other morphs. The swimming behaviour might be explained by the relatively small size of this morph, thus, size differences could also indirectly contribute to the spatial segregation.

Shape

Consistently with prediction 4, there were significant differences in shape between the predefined lateral plate morphs. Two major shape transformations were found; between the river morph and the lake morphs, and between the plate morphs within the lake. Within the lake, the shape changes from deep bodied in the low plate morphs to streamlined and elongated in the partial and complete morphs. These differences are remarkably similar to what have been observed between sympatric benthic-pelagic ecomorphs, and are also consistent with Walker's (1997) prediction that large relative littoral area favour deeper bodied fish.

The shape characteristics that I found to be related to plate morph indicate a functional response to feeding in benthic and pelagic habitats. Benthic feeding depends on precise maneuverability, rapid alternation of speed and direction among vegetation and uneven surfaces, and on also the strength to capture relatively large non-evasive larvae (Walker 1997). Hydromechanically, a deep body shape facilitates accurate movements

and locomotion, thereby prey capture success in a benthic environment (Alexander 1967, Walker 1997, Bell & Foster 1994). This shape also improves foraging performance directly; a deeper body facilitate rotation around the vertical axis to snap prey off the substrate (Hart 2003). In contrast, the pelagic environment is usually open, without obstacles, and the pelagic prey is evasive and often patchy distributed (Schluter 1993). Such conditions require steady swimming for longer distances and rapid attack. A streamlined body reduces pressure drag, thereby the costs of sustained pelagic swimming performance (Walker 1997, Taylor & Foote 1991, Webb 1984). In another study, Leinonen et al. (2006) demonstrated deep bodied marine fish and extremely slender fish in freshwater. This is typical when the lake is large and deep, and the sticklebacks utilize the pelagic niche (Walker 1997), which can not be expected in Lake Engervann (Walker & Bell 2000, Walker 1997).

I also found longer snout and larger eyes in the complete morphs than in the low plate morph in the lake. These traits are known to improve feeding performance on pelagic plankton (Larson 1976, Bentzen & McPhail 1984). The longer ectocoracoid bone correspond to the extensive armor in the complete morphs, and is consistent with the theory that bone growth is more costly in freshwater than in the sea (Bourgeois et al. 1994).

High water velocity favours distinct morphologies, due to hydrodynamic implications of locomotion (e.g. Langerhans et al. 2003). The river morph in the present study cope with a different flow regime than the lake morphs, and shape deviations in this morph might therefore stem from the different demands on swimming performance. It is generally predicted that high water velocity favours streamlined shallow body profiles, because this reduces pressure drag, thereby reduce swimming costs and prevent downstream displacement (McGugian et al. 2003, Morinville & Rasmussen 2008, Pakasmaa & Piironen 2001). The flattened dorsal area of the river morph in this study might reduce drag. The river morph also had a relatively elongated caudal region, and compressed central part, which is assumed to improve swimming capacity (Taylor & Foote 1991). The shorter ectocoracoid bone expressed by the river morph is found earlier in fresh water forms, and attributed to calcium deficiency and high costs of bone growth in freshwater (Walker & Bell 2000). Since the river morphs assumingly are isolated from

Lake Engervann and the sea, phenotypic divergence in behaviour and morphology can, if heritable, also have evolved neutrally by genetic drift, but this is not likely due to the support from comparative studies.

The observed body shape might also be subject to environmentally induced plasticity. Several morphological traits related to habitat have been demonstrated to be diet induced plastic responses in sticklebacks (Day et al. 1994, Day & McPhail 1996, Robinson & Parsons 2002). On the other hand, there is evidence for a genetic basis of shape from a recent study, showing that plasticity accounted for less than 10 % of the transformation in shape when populations from extreme benthic and pelagic environments were experimentally transplanted (Spoljaric & Reimchen 2007). Furthermore, a genetic basis for certain aspects of shape, on the head and pelvic region, is recently found to be linked to the major gene locus controlling lateral plate variation (Albert 2008).

The morphometric software used for shape analysis is powerful, and detects extremely subtle shape differences. Thus, when performing a discriminant analysis on the shape variables, there is a risk of accepting a false hypothesis. However, the differences detected here are likely to have considerable functional relevance for the fish. The well demonstrated advantages of the different shapes in benthic and pelagic habitats (Alexander 1967, Webb 1984, Walker 1997), and demonstrated heritability, indicate that shape differences presented here stem from divergent adaptation to different habitats.

Evolutionary implications

Considering behavioural and morphometric traits jointly, the emerging picture is that the complete morph resembles the ancestral form, and partials represent the next step toward freshwater adaptation. Shape variables were significantly associated with number of larvae eaten and time in benthic and pelagic zones. The shape - behaviour link within morphs strengthen the overall hypothesis of ecological divergence. The pattern presented here is similar to that of the distinct benthic and pelagic coexisting ecomorphs, suggesting a potential for development of reproductive barriers in the system.

The ecological theory holds that adaptation to different resources is one of the major driving forces behind speciation (Mayr 1942, Schluter 2000). As foraging is an

important component of fitness, there might be strong disruptive selection, driving phenotypes related to feeding on two distinct resources further apart. This happens because specialized foraging usually entails evolution of heritable traits that improve success on a particular food source, which in turn produce ecologically inferior hybrids. Such ecologically based isolation can potentially generate selection against hybridization, also called reinforcement (Dobzhansky 1951). This is how benthic-pelagic pairs in sympatry are believed to have evolved, with assortative mating for size (Rundle et al. 2000). Sexual selection based on body shape is suggested, but remains to be tested (Boughman 2007). Reproductive isolation can also evolve as a by-product of genetic divergence produced by adaptation to different resources (Dobzhansky 1951). Use of alternative resources allows coexistence of closely related populations, and might thus contribute to divergence in sympatry. The sticklebacks in Lake Engervann will not likely establish as benthic and pelagic ecomorphs in the lake, as the pelagic niche is far too marginal to support a pelagic population. However, divergent foraging ecologies and subsequent evolution of habitat-specific morphology in the morphs might involve a potential for evolution of ecologically based reproductive barriers, preventing the low plate morphs from hybridizing with migrating complete morphs. At the moment this is only speculations with the lack of knowledge about hybridization and gene flow and in the system.

The contribution of plasticity versus adaptation to the differentiation also remains uncertain. The unknown migration history of the morphs makes it difficult to account for the effect of exposure to different resources and the effect of selection. However, phenotypic plasticity may be important during early adaptation. It may facilitate establishment and reproduction in new environments, and thereby allow the time for adaptation (Gahalmbor et al. 2007, Boughman 2007).

The plate morphs

The plate polymorphism in Lake Engervann is large, and encompasses all three described morphs, and high variation within the partial morph. It can be argued that the strict division into three groups used here is artificial and do not represent biologically distinguishable groups. Nevertheless, this division is widely accepted and traditionally

used in previous research, although with some inconsistency and confusion of the terminology (Östlund-Nilsson et al. 2007). The newly discovered genetic basis for plate morphology supports this distinction. Colosimo et al. (2004) discovered that lateral plates are controlled by a single locus gene with incomplete dominance, called Ectodysplasin (Eda). Thus, the partial morph can be produced by heterozygosity.

Limitations

Experimental setup

To make the fish behave as naturally as possible in my experiment, they were acclimatized to the water conditions and habituated to the experimental set-up. However, I had to trade off natural conditions in the experimental aquarium in order to optimize recording and tracking of the fish. Having a more uneven bottom area, i.e. by adding more plants and stones, could possibly have separated differences in swimming performance and foraging success by sticklebacks with different shapes. However, this would have made tracking by EthoVision difficult, as it can only capture the fish in a two dimensional picture. So, to minimize movements not captured by the tracking, I chose to use an aquarium that was relatively compressed. However, this compromise might have undermined spatial segregation between morphs. In order to manually observe foraging events, the daphnia used here had to be larger than many of the zooplankton that sticklebacks probably feed on in the wild. Smaller prey could possibly have resulted in more pronounced differences among the fish in ability to detect and consume this prey. However, even with these limitations I found significant differences between the morphs.

Effects of parasites

Foraging behaviour and morphology is also under the influence of additional ecological factors, as for example parasitism. The stickleback is, due to its trophic ecology, very often heavily parasitized (Barber 2007). Some parasites have considerable impact on feeding behaviour, swimming, habitat use, and morphology, including body shape in sticklebacks (Barber 2007). As the fish used in this experiment were caught in the wild, there is a possibility that some of them were infected by parasites. I can therefore not rule

out that this influenced my results. However, it is unlikely that there are any notable deviations in either behaviour or shape caused by parasites, since the one with known large effects, *Schistocephalus solidus*, is absent in Lake Engervann (K. Østbye, personal communication), and I used only individuals that seemed uninfected.

CONCLUSION

Prey consumption, spatial segregation, and body shape were different among lateral plate morphs, and between the river morph and the morphs in the lake. Together the differences indicated adaptation towards divergent habitat use. I found support for the main hypothesis that low plate morphs are more benthic than partial and complete morphs, thus probably more adapted to the local freshwater conditions. This suggests ecological divergence and a potential for evolution of reproductive barriers. However, the latter requires closer investigations. Further research in this system should therefore be directed towards an assessment of the genetic and plastic components of the divergent phenotypes, revealing whether the differences found here are under selection.

LITERATURE CITED

- Adams D.C., Rohlf F.J., Slice D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*. **71**: 5-16.
- Albert A.Y.K., Sawaya S., Vines T.H., Knecht A.K., Miller C.T., Summers B.R., Balabhadra S., Kingsley D.M., Schluter D. 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution*. **62**: 76-85.
- Alexander R.M. 1967. *Functional Design in Fishes*. Hutchinson Press. London, U.K.
- Barber I. 2007. Host-parasite interactions of the three-spined stickleback. In: *Biology of the three-spined stickleback* (Öslund- Nilsson et al. eds). CRC Press. NW, USA.
- Bell M.A., Foster S.A. 1994. *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford, U.K.
- Bell M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica*. **112-113**: 445-461.
- Bell M.A., Aguirre W.E., Buck N.J. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. *Evolution*. **58**: 814-824.
- Bentzen P., McPahil J.D. 1984. Ecology and the evolution of sympatric sticklebacks (*Gasterosteus*): Specialization for alternative trophic niches in the Enos Lake species pair. *Canadian journal of zoology*. **62**: 2280- 2286.
- Borch H., Haarstad K., Borgstrøm R., Bekken T., Dokk J.G. 2005. Sandvikselva – en forurensingskartlegging av miljøskadelige forbindelser for å avdekke årsaker til redusert reproduksjon av laks og sjøørret. Jordforsk. Rapport nr. 125-04. <http://www.baerum.kommune.no/files/Sandvikselvrapport.pdf>
- Boughman J.W. 2007. Speciation in sticklebacks. In: *Biology of the three-spined stickleback* (Östlund- Nilsson et al. eds). CRC Press. NW, USA.
- Bourgeois J.F., Blouw D.M., Koenings J.P., Bell M.A. 1994. Multivariate-analysis of geographic covariance between phenotypes and environments in the threespine stickleback, *Gasterosteus-aculeatus*. From the cook inlet area, Alaska. *Canadian journal of zoology*. **72**: 1497-1509.
- Breder C.M. 1960. Design for a fry trap. *Zoologica N.Y.* **45**: 55-9
- Colosimo P.F., Hosemann K.E., Balabhadra S., Villarreal G., Dickson M., Grimwood J., Schmutz J., Meyers M., Schluter D., Kingsley D.M. 2004. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*. **307**: 1928-1933.
- Colosimo P.F., Hosemann K.E., Balabhadra S., Villarreal G.Jr., Dickson M., Grimwood

- J., Schmutz J., Myers R.M., Schluter D., Kingsley D.M. 2005. Widespread parallel evolution in stickleback by repeated fixation of Ectodysplasin alleles. *Science*. **307**: 1928-1933.
- Cresko W.A., Baker J.A. 1996. Two morphotypes of lacustrine threespine stickleback, *Gasterosteus aculeatus*, in Benka Lake, Alaska. *Environmental biology of Fishes*. **45**: 343-350.
- Day T., Pritchard J., Schluter D. 1994. A comparison of two sticklebacks. *Evolution*. **48**: 1723-1734.
- Day T., McPhail J.D. 1996. The effect of behavioural and morphological plasticity on foraging efficiency in the threespined stickleback (*Gasterosteus* sp.). *Oecologia*. **108**: 380-388.
- Delbeek J.C., Williams D.D. 1988. Feeding selectivity of 4 species of sympatric stickleback in brackish-water habitats in eastern Canada. *Journal of Fish Biology*. **32**: 41-62.
- Dobzhansky T. 1951. Genetics and the origin of species. 3rd edition. Columbia University Press. New York, USA.
- Futuyma D.J., Moreno G. 1988. The evolution of ecological specialization. *Annual review of ecology and systematics*. **19**: 207-233.
- Futuyma D.J. 2005. Evolution. Sinauer Associates. Sunderland, Mass. USA.
- Gahlambor C.K., McKay J.K., Carroll S.P., Reznick D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*. **21**: 394-407.
- Glutney M.L., Clark R.G. 1997. Nest site selection by mallards and blue-winged teal in relation to microclimate. *The Auk*. **113**: 381-395.
- Gow K.L., Rogers S.M., Jackson M., Schluter D. 2008. Ecological predictions lead to the discovery of a benthic-limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. *Canadian Journal of Zoology*: in press.
- Grant P.R., Grant B.R. 1995. The founding of a new population of darwin's finches. *Evolution*. **49**: 241-251.
- Hagen D.W. 1967. Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). *Journal of the Fisheries Research Board of Canada*. **24**: 1637-1692.
- Hagen D.W., Gilbertson L.G. 1972 Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution*. **26**: 32-51.
- Halvorsen G., Often A., Svalastog D. 2005. Engervannet og Øverlandselva – statusrapport 2005. NINA Minirapport 136.
http://www.jernbaneverket.no/multimedia/archive/01569/16-Status_Vassdrag_1569902a.pdf
- Hart P.J.B., Ison S. 1991. The influence of prey size and abundance, and individual phenotype on

- prey choice by the three-spined stickleback. *Gasterosteus aculeatus* L. *Journal of Fish Biology*. **38**: 359-372.
- Hart P.J.B., Gill A.B. 1992. Constraints on prey size selection by the 3-spined stickleback – energy requirements and the capacity and fullness of the gut. *Journal of Fish Biology*. **48**: 878-890.
- Hart P.J.B., Gill A.B. 1993. Choosing prey size – a comparison of static and dynamic foraging models for predicting prey choice by fish. *Marine behaviour and physiology*. **23**: 91-104.
- Hart P.J.B., Gill A.B. 1994. Evolution of foraging behaviour in the threespine stickleback. In: The evolutionary biology of the threespine stickleback (Bell & Foster eds). Oxford University Press. Oxford, U.K.
- Hart P.J.B. 2003. Habitat use and feeding behaviour in two closely related fish species, the three-spined and nine-spined stickleback: an experimental analysis. *Journal of Animal Ecology*. **72**: 777-783.
- Hynes H.B.N. 1950. The food of fresh-water stickleback (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *Journal of animal ecology*. **19**: 36-58.
- Ibrahim A.A., Huntingford F.A. 1989. Laboratory and field studies of the effect of predation risk on foraging in 3-spined sticeklbacks (*Gasterosteus aculeatus*). *Behaviour*. **109**: 46-57.
- Ibrahim A.A., Huntingford F.A. 1992. Experience of natural prey and feeding efficiency in 3-spined sticklebacks (*Gasterosteus aculeatus* L). *Journal of Fish Biology*. **41**: 619-625.
- Kilhan S.S., Kreeger D.A., Lynn S.G., Goulden C.E., Herrera L. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*. **377**: 147-159.
- Kristjánson B.K. 2005. Rapid morphological changes in threespine stickleback, *Gasterosteus aculeatus*, in freshwater. *Environmental Biology of Fishes*. **74**: 357-363.
- Langerhans R.B., Layman C.A., Langerhans A.K., Dewitt T.J. 2003. Habitat-associated morphological divergence in two meotropical fish species. *Biological Journal of the Linnean Society*. **80**: 689-698.
- Larson G.L. 1976. Social behaviour and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. *Canadian Journal of Zoology*. **54**: 107-121.
- Leinonen T., Cano J.M., Marilä J. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *European Society for Evolutionary Biology*. **19**: 1803-1812.
- Lenormand T. 2002. Gene flow and the imits to natural selection. *Trends in Ecology and Evolution*. **17**: 183-189.
- Marchinko K.B., Schluter D. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution*. **61**: 1084-1090.

- Mattern M.Y. 2007. Phylogeny , systematics, and taxonomy of sticklebacks. In: Biology of the three-spined stickleback (Östlund-Nilsson et al. eds). CRC Press. NW, USA.
- Mayr E. 1942. Systematics and the origin of species: from the viewpoint of a zoologist. Columbia University Press. New York, USA.
- McGuigan K., Franklin C.E., Moritz C., Blows M.W. 2003. Adaptation of rainbow fish to lake and stream habitats. *Evolution*. **57**: 104-118.
- McKinnon J.S., Rundle H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends in Ecology and Evolution*. **17**: 480-488.
- McPhail J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western Columbia. In: The evolutionary biology of the threespine stickleback (Foster and Bell eds.). Oxford University Press. Oxford, U.K.
- Morinville G.R., Rasmussen J.B. 2008. Distinguishing between juvenile and resident brook trout (*Salvelinus fontinalis*) using morphology. *Environmental Biology of Fishes*. **81**: 171-184.
- Noldus Information Technology. 2005. EthoVision version 3.1. Wageningen , The Netherlands.
- Nosil P., Reimchen T.E. 2005. Ecological opportunity and levels of morphological variance within freshwater stickleback populations. *Biological Journal of the Linnean Society*. **86**: 297-308.
- Pakkasmaa S., Piironen J. 2001. Water velocity shapes juvenile salmonids. *Evolutionary Ecology*. **14**: 721-730.
- Reimchen T.E 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution*. **37**: 931-946.
- Reimchen T.E. 1992. Injuries on stickleback from attacks by a toothed predator (oncorhynchus) and implications for the evolution of lateral plates. *Evolution*. **46**: 1224-1230.
- Reimchen T.E. 1994. Predators and morphological evolution in threespine stickleback. In: The evolutionary biology of the threespine stickleback (Bell and Foster eds.). Oxford University Press. Oxford, U.K.
- Reimchen T.E. 2000. Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition. *Behaviour*. **137**: 1081-1096.
- Robinson B.W., Parsons K.J. 2002. Changing times, spaces and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **59**: 1819-1833.
- Rohlf F.J. 2007 a. TpsDig2. version 2.10. Available at <http://life.bio.sunysb.edu/morph/> Ecology & evolution dept., SUNY Stony Brook, NY 11794-5245, USA
- Rohlf F.J. 2007 b. TpsRelw. version 1.45. Available at <http://life.bio.sunysb.edu/morph/> Ecology & evolution dept., SUNY Stony Brook, NY 11794-5245, USA

- Rohlf F.J. 2007 c. TpsRegr. version 1.33. Available at <http://life.bio.sunysb.edu/morph/>
Ecology & evolution dept., SUNY Stony Brook, NY 11794-5245, USA
- Rundle H.D., Nagel L., Boughman J.W. 2000. Natural selection and parallell speciation in sympatric sticklebacks. *Science*. **287**: 306-308.
- SAS institute Inc. 2005. JMP version 5. Cary, NC, USA.
- Schluter D., McPhail J.D. 1992. Ecological character displacement and speciation in sticklebacks. *The American Naturalist*. **140**: 85-108.
- Schluter D. 1993. Adaptive radiation in sticklebacks- size, shape, and habitat use efficiency. *Ecology*. **74**: 699-709.
- Schluter D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science*. **266**: 798-801.
- Schluter D., Nagel L.M. 1995. Parallell speciation by natural selection. *The American Naturalist*. **146**: 292-301.
- Schluter D. 1996. Ecological causes of adaptive radiation. *The American naturalist*. **148**: 40-64.
- Schluter D. 2000. The ecology of adaptive radiation. Oxford University Press. Oxford, UK.
- Schluter D. 2001. Ecology and the origin of species. *Trends in Ecology and Evolution*. **16**: 372-380.
- Simpson G.G. 1953. The major features of evolution. Columbia University Press. NewYork, USA.
- Spoljaric M.A., Reimchen T.E. 2007. 10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. *Journal of Fish Biology*. **70**: 1484-1503.
- Stephens , Krebs 1986. Foraging Theory. Princeton University Press. Princeton, New Jersey.
- Svalanes A.G.V., Hart P.J.B. 1998. Individual variabililty in state-dependent feeding behaviour in three-spined stickleback. *Animal Behaviour*. **55**: 1349-1359.
- Taylor E.B., Foote C.J. 1991. Critical swimming velocities of juvenile sockeye-salmon and kokanee, the anadromous and non-anadromous forms of oncorhynchus-nerka (walbaum). *Journal of fish Biology*. **38**: 407-419.
- Walker J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L, (Gasterosteidae) body shape. *Biological journal of the Linnean Society*. **61**: 3-50.
- Walker J.A., Bell M.A. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation in threespine sticklebacks (*Gasterosteus aculeatus*). *Journal of zoology*. **252**: 293-302.

- Warburton K. 2003. Learning of foraging skills by fish. *Fish and Fisheries*. **4**: 203-215.
- Webb P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist*. **24**: 107-120.
- Webster M.M., Hart P.J.B. 2006. Subhabitat selection by foraging threespine stickleback (*Gasterosteus aculeatus*): previous experience and social conformity. *Behavioral and Ecological Socibiology*. **60**: 77-86.
- Winemiller K.O. 1992. Ecomorphology of freshwater fishes. *National geographic research and exploration*. **8**: 308-327.
- Wooton. R.J. 1994. Energy allocation in the threespine stickleback. In: Evolutionary biology of the threespine stickleback. (Foster and Bell eds.). Oxford University Press, Oxford, UK.
- Wootton R.J. 1976. The biology of the sticklebacks. Academic Press. London, U.K.
- Östlund-Nilsson S., Mayer I., Huntingford F.A. 2007. The biology of the threespine stickleback. CRC Press. NW, USA.

APPENDICES

Appendix 1 Description of landmark positions

Landmark	Landmark position
1	Anterior extent of mouth
2	posterior extent of mouth
3	lowest point under eye
4	anterior extent of orbit
5	center of eye,
6	posterior extent of orbit
7	top of head over eye
8	anteriodorsal extent of operculum
9	posteriodorsal extent of operculum
10	ventral extent of operculum
11	posterior extent of ectocoroid
12	posterior extent of pelvic bone
13	anterior insertion of anal fin
14	anterior insertion of first dorsal spine
15	anterior insertion of second dorsal spine
16	anterior end of dorsal fin
17	ventral insertion of caudal fin
18	dorsal insertion of caudal fin
19	Posterior extent of caudal peduncle.