



Geometric Morphometric Assessment of Shape Sexual Dimorphism in Pikeperch (*Sander lucioperca*)

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Abstract

The present analysis of sexual dimorphism variation is based on 26 specimens (12 females and 14 males) of pikeperch (*Sander lucioperca*) from the same lake. On the left lateral aspect, 19 homologous landmarks were obtained which were studied according to geometric morphometric methods. The utilisation of geometric morphometric techniques in assessing sexual dimorphism in the present study showed statistically significant differences, mainly in length and the dorsal fins of pikeperch, with males having a shorter body and being clearly dorsally-convexed. No differences appeared between sexes for body mass and size.

Key words: body shape; *Percidae*; *Stizostedion lucioperca*; sexual morphology; zander

Introduction

Since Darwin (1871), the study of non-geographic variation in organisms has attracted the interest of biologists. Some authors (Thorpe, 1976; Patton and Rogers, 1983; Mayr and Ashlock, 1991) consider non-geographic variation a function of, among others, differences in sex. Sexual dimorphism and ontogenetic (age-related) variation are some of the sources of phenotypic variation within a species (Weckerly, 1998).

The geometric morphometric (GM) technique is regarded as a powerful means of analysing external morphology and shape differences among organisms (Roth and Mercer, 2000), including fishes (Walker, 1993; Loy et al., 1996), and has also been used to identify fishery stocks (Cadrin, 2000) and to distinguish species of larval fishes (Fulford and Rutherford, 2000), populations and related taxons, as well as to establish phenotypic variation (O'Reilly and Horn, 2004). It also allows the higher resolution of any phenotypic differences.

In the present study, GM analyses were used to assess the nature and extent of sexual dimorphism in pikeperch (*Sander lucioperca*). The pikeperch is a semi-anadromous predatory fish, cool-water percid fish that inhabits both fresh and brackish waters, and is commonly found in estuaries and coastal zones. It is native to Eastern Europe and western Asia. Either by spreading naturally or by introduction, the species has become established in northern to southern European countries, and in Central Asia, Western China and North Africa (M'Hetli et al., 2011).

Pikeperch is a long slender fish that grows to a maximum length of 130 cm, weighs up to 20 kg, and has a lifespan of up to 16 years on average. It has a long slender body with no spines on the gill covers. It has two dorsal fins, one anal fin and a caudal fin. Its coloration is a greyish brown back with light silvery sides marked with between 8 and 12 dark vertical stripes (often broken into spots). Pikeperch reaches a maximum length of 100-130 cm, which corresponds to a weight of about 15-20 kg. The histological gonad development of pikeperch starts at lengths of 5.7 cm TL (Zakes and Demska-Zakes, 1996). Oogenesis starts at lengths of about 7.9 cm in females, while no spermatogenesis has been observed in similarly sized males (Zakes and Demska-Zakes, 1996). To the best of the authors' knowledge, there has to date been no analysis of sexual dimorphism within pikeperch based on GM analysis.

Materials and Methods

Specimens examined

The present analyses of sexual dimorphism and age-related variation were based on 26 specimens (12 females and 14 males) from the permanently flooded Ivars and Vila-sana endorrheic pond (E00°57', N41°40'), which is a no predation environment where this species was introduced some years ago. The basin was drained in 1951, but had recovered by 2009. It is equipped with pumping stations and the water system includes an extensive network of canals. Specimens were randomly collected with nets on the same day of November 2013. Actual body weight was assessed and only individuals with a body weight >100 g were considered. The final specimens selected weighed 125.0–912.9 g (digestive tract included). Sex of specimens was easily determined by macroscopic *post mortem* examination of their gonads. No animal was considered sexually undetermined. As dissections clearly determined sex, it was decided that all specimens had reached full maturity, so it was supposed that the amount of intra-population shape variation based on ontogeny was reduced.

Image-capturing and landmark digitizing

Image-capturing of geometric morphometric data was performed using a Nikon AF Nikkor® 28-200 mm telephoto lens focused on the left lateral views of body. In setting up the digital camera, care was taken to mount it firmly in place, ensuring that it was perfectly balanced and attached to a tripod stand, and set at maximum zoom. In order to minimise size-related digitising error, the camera was adjusted so that each fish took up approximately the same amount of the frame, regardless of its size. A ruler was used during this process. Fishes were studied fresh and no fish were dissected

prior to being photographed. To assure the reliability of this study, any sample that showed marked fin erosion or developmental deformities was also eliminated. TPS-Dig, v. 2.16 (Rohlf, 2006) was used to digitise and save 19 homologous chosen landmarks (Table I), which provided a comprehensive summary of the general body morphology of the fishes (Figure 1). After digitisation, landmark coordinates were translated to align the centroids of each individual, and were rotated and rescaled to produce Procrustes shape coordinates (using CoordGen6 by H.D. Sheets, available as part of the Integrated Morphometrics Package (IMP) at, <http://www2.canisius.edu/~sheets/morphsoft.html>). Size was scaled as centroid size (CS), which is the square root of the summed square distance from each landmark to the centre of the form.

Shape variation

In order to compare Procrustes to tangent space distances between individuals, a Generalised Procrustes Analysis superimposition (equivalent to a generalised least squares) procedure of Rohlf and Slice (1990) was performed on each data set using TPS-Small 1.20 (Rohlf, 2003). The approximation of shape space by tangent space presented a high correlation (0.999). This high degree of approximation of shapes in the sample (=shape space) by the reference shape (=tangent space) allowed the nature and extent of shape deformations to be accurately captured in subsequent statistical analyses.

Allometry

There has been a great deal of discussion about how to separate size from shape in morphometric studies. Because GM provides a size variable (expressed as CS) that is not correlated with shape variables in the absence of allometry, it is possible to remove variance in shape that is associated with differences in size prior to ordination. As no differences were detected between allometric relationships for males and females (ANCOVA, $F=0.007$, $p=0.931$), specimens were pooled independently of their size (but no sex, evidently) in all subsequent analyses.

Size dimorphism

As said, size was estimated as CS. The two-tailed (Wilcoxon) Mann-Whitney U test was used to test whether the medians of both sexes were different. Total length was obtained from the distance between landmarks 1 and 13 using the scale as the real comparative value.

Shape dimorphism

A one-way non-parametric multivariate analysis of variance (NPMANOVA, also known as PERMANOVA) (Anderson, 2001) was used to assess shape variation between male and female individuals based on Euclidean distances, with Bonferroni corrected p -values. Body-shape variation between males and females was assessed using Principal Component Analysis (PCA) of Procrustes shape coordinates. Attributes of the phenotypic trajectories to determine phenotypic change between sexes were compared when studying partial warps. For plotting partial warps, the amplitude was increased to ten to ease the visualisation of morphological changes between sexes.

These statistical analyses were carried out in PAST- "Paleontological Statistics Software Package for Education and Data Analysis" (Hammer *et al.*, 2001).

Results

Image-capturing device precision and digitizing error

The ANOVA also showed no difference in Procrustes values between the two digitising trials ($p=0.732$).

Sexual dimorphism

Variation of body weights was equal for both sexes ($F=2.310$, $p=0.153$). Based on the results of the NPMANOVA, there was evidence of sexual shape dimorphism ($F=2.613$, $p=0.036$) (Figure 2), although the U -test showed no statistically significant CS differences between the sexes ($U=59$, $p=0.209$). Real body weights were not different between the sexes either ($U=58$, $p=0.190$) (Figure 3). Consequently, only the shape of the lateral view was different between males and females, and was equally sized. Body-shape variation between males and females using PCA of Procrustes shape coordinates is shown in Figure 4. Because of rescaling (i.e. removal of many size effects), the first two components accounted for only 46.1% and 13.6% of the variance, respectively. The respective eigenvalues were 0.00075 and 0.00022. PC I was not correlated with log CS ($r^2=0.035$, $p=0.367$), showing many 'sex overlapped' specimens, most likely due to mere shape variability (not associated with size differences). Landmarks 1 and 13 appeared to be the most discriminative (loadings $>[0.3]$), and both can define body length. If 1-13 distances are compared, statistically significant differences ($p=0.03$) were found between males (282.7 ± 48.6 mm) and females (328.6 ± 44.9 mm). Landmarks 9 to 12 had loadings $>[0.2]$. Correctly sex-classified specimens using landmarks 1 and 9 to 13 (which had loadings $>[0.2]$) were reported at a rate of 92.3%.

Discussion

Many fish species show sexual dimorphism, a condition where males and females are different in form and/or coloration, meaning that the sexes can be distinguished externally. There are three classes of sexual morphism: a) monomorphic fish shows no differences in form or colour between sexes; b) fish that are temporarily dimorphic or dichromic show colour differences in colour and/or form during the breeding season or differences in colour during courtship and/or spawning; and c) permanently dimorphic or dichromic fish are always different in colour and/or form (Korbuly *et al.*, 2007). For pikeperch, it can be concluded that it presents a clear shape sexual dimorphism, with landmark-based geometric morphometric techniques showing at the same time their usefulness to address questions relating to morphological shape variation. This dimorphism is in accordance with published data, such as Mooj *et al.*

(1999), who described a significant difference in length between adult males and females, and FAO (1984), which stated that females are larger, with the female's belly being full and rounded and the male's belly being flatter. In our research, total length and dorsal conformation accounted for the greatest amount of shape variation between sexes, with females being effectively more rounded and males more slender. Thus, the general pattern of sex shape differences is in contrast to the dorsal shape. No differences in body mass and size appeared. Shape differences do not seem to be related to sensory abilities or feeding, nor the requirements of reproduction and the different roles of males and females in a live bearing reproductive mode.

Sexual dimorphism in shape reflects the effects of sexual selection, ecological differentiation among sexes, or the indirect effects of size dimorphism (Oliveira and Almada, 1995; Fairbairn et al., 2007). As in our study, as ecological differentiation can be discarded (all specimens came from the same pond), and there is no effect of size (demonstrated by the absence of allometry), sexual selection seems the more plausible hypothesis to explain differences between males and females. As small changes in morphology can have profound effects on an animal's functional capabilities (Koehl, 1996), whether there are functional implications of these differences, or if males and females experience different levels of selection pressure from predators could now be investigated.

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Annexure

Table I. Landmarks used.

Number	Anatomical points
1	Anterior tip of upper snout
2	Anterior point of sphenotic at orbit
3	Top of sphenotic at orbit
4	Posterior point of sphenotic at orbit
5	Base of sphenotic at orbit
6	Dorsal point of gill cover
7	Most posterior point on gill cover
8	Base point of gill cover
9	Anterior basal insertion of first dorsal fin
10	Posterior basal point of first dorsal fin
11	Anterior basal insertion of second dorsal fin
12	Posterior basal point of second dorsal fin
13	Midpoint of caudal edge of the hypural plate
14	Posterior insertion of anal fin
15	Anterior insertion of anal fin
16	Posterior insertion of pelvic fin
17	Anterior insertion of pelvic fin
18	Ventral insertion of pectoral fin
19	Dorsal insertion of pectoral fin

Figure 1. Landmarks used in this study. These were recorded on the lateral left surface of *Sander lucioperca*. Landmarks are indicated by circles. The projection of landmark locations for all specimens, after General least square alignment. Nineteen homologous and topologically equivalent landmarks were plotted on the body in order to describe the size and shape.

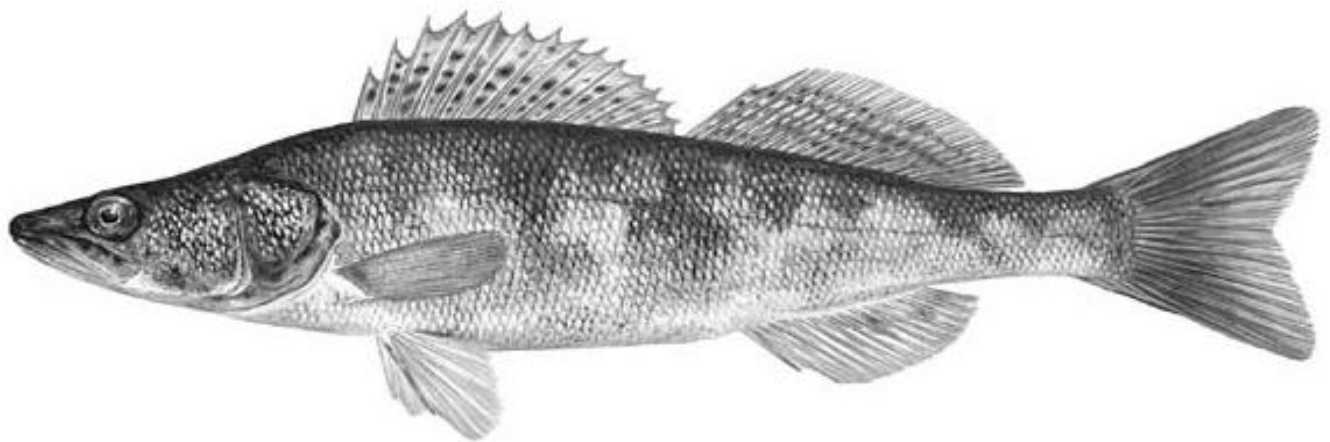
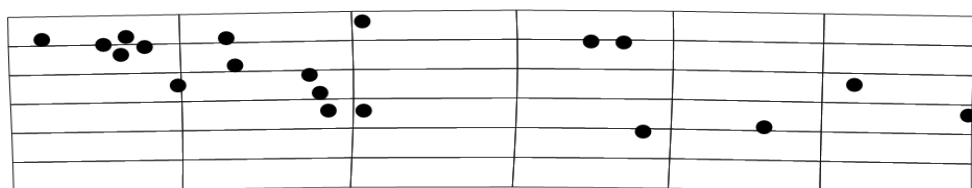


Figure 2. Partial warps (relative to a non-deformed grid representing the consensus form of the entire sample for males and females separately) are shown representing females (up) and males (down) individuals, for all landmarks. First warp and amplitude=10.



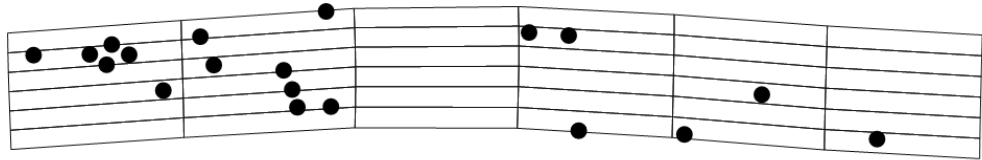


Figure 3. Box plot diagram of body weights for males and females. For each sex, the 25-75 percent quartiles are drawn using the box. The median is shown with a horizontal line inside the box. The minimal and maximal values are shown with short horizontal lines ("whiskers"). No outlier appeared. Body weights for sexes were not different (U=58, p=0.190).

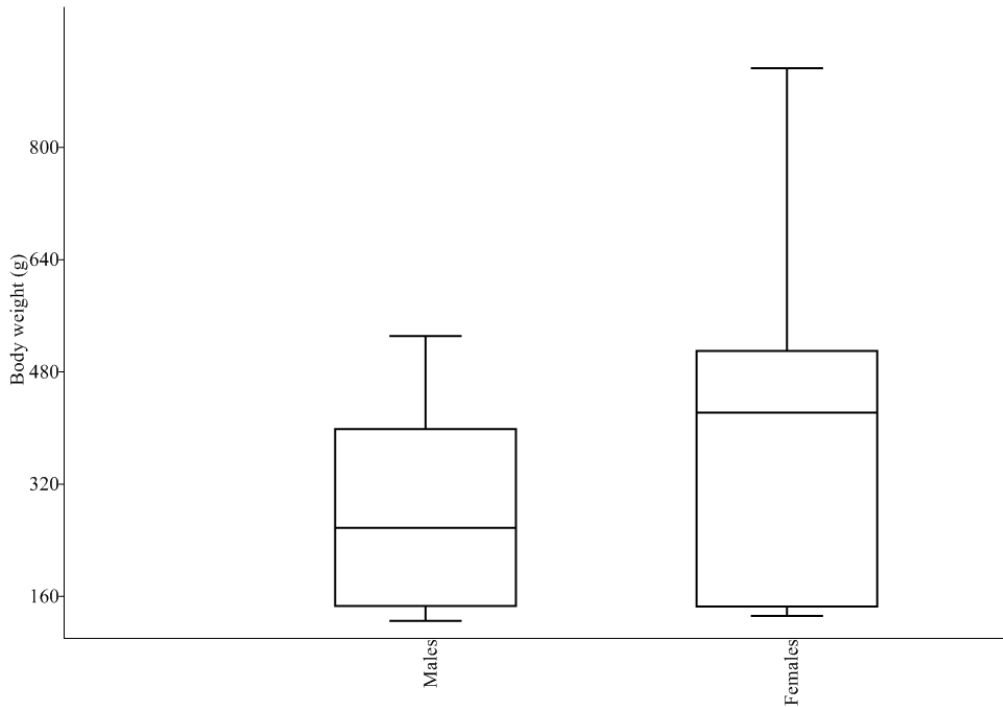


Figure 4. Principal Component Analysis of Procrustes shape coordinates for males (empty squares) and females (filled squares). As a result of rescaling (i.e. removal of many size effects), the first two components account for only 46.1% and 13.6% of the variance, respectively.

