Morphological variation of pumpkinseed (Lepomis gibbosus) with emphasis on allometry

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Abstract: Pumpkinseed (Lepomis gibbosus) is an exotic fish species widely distributed in Europe and it has expanded its distribution area in Turkey since its first report in 1982. It is well known that invasive species like L. gibbosus adapt to introduced habitats relatively well. In order to evaluate morphological variation within this invasive species, sampling was carried out in June 2012 and May 2013 in the Aegean and Thrace regions, respectively. Body-form variability in sampled populations of L. gibbosus was investigated using landmark-based geometric morphometrics. L. gibbosus showed significant geographic- and habitat-based differences with and without allometric standardization. However, outcomes of these results differed significantly. Differences between locality-based Procrustes distances were found to support habitat-based differentiation. These results suggest that, depending on the hypothesis of the morphometric study, it might be important to implement allometric regression and standardization. Additionally, the range extension of L. gibbosus needs to be monitored carefully. It had previously been reported only from the Thrace and Muğla regions. However, according to our data, it has been sampled from two new basins, Susurluk and Sakarya.

Key words: Invasive species, geometric morphometrics, allometry, new records, Turkey

1. Introduction

Range expansion of invasive fish species is one of the most important problems threatening biodiversity and fisheries in inland waters. According to Gozlan et al. (2010), ‘invasion’ is described as the process whereby an introduced species has established populations and spread rapidly, presenting a risk to native species. Coop et al. (2005) described invasive species as organisms that spread with or without the aid of humans in natural or seminatural habitats, producing a significant change in composition, structure, or ecosystem processes and possibly causing economic losses to human activities.

Pumpkinseed, Lepomis gibbosus (Linnaeus, 1758), is native to parts of North America, but in recent centuries it has been introduced into other parts of the United States, Canada (Scott and Crossman, 1973), and Europe (Holcik, 1991; Garcia-Berthou and Moreno-Amich, 2000). During the 19th century, it was found throughout Europe and South America (Welcomme, 1988), as a sport fish (in France) or as an ornamental fish (in England) (De Groot, 1985; Copp et al., 2004; Povž and Šumer, 2005). In Spain, it was intentionally introduced as prey for native wild stocks that had suffered from heavy predation by previously introduced pike, bass, and catfish (Elvira and Almodovar, 2001). The first record of L. gibbosus in Turkey was given from the Thrace region, in the İpsala Canal (Erk'akan, 1983); soon after, it was also reported from Gala Lake (Baran and Ongan, 1988). Since then, the species’ distribution area is expanding. Recognizing this expansion, researchers have concentrated on the bioecology of L. gibbosus in recent years.

Morphological variation of L. gibbosus has been intensively studied, especially trophic (Wainwright et al., 1991; Robinson and Wilson, 1996; Bhagat et al., 2011a) and sexual (Naspleda et al., 2012) variations. L. gibbosus has been shown to demonstrate inter/intrahabitat-based differences (Bhagat et al., 2006, 2011b), as well as ontogenetic variations (Šumer et al., 2005; Tomeček et al., 2005). In addition to these, morphological variation patterns between lentic and lotic habitats have been studied with hydrodynamic (Webb, 1984) theory-based predictions (Brinsmead and Fox, 2002; Naspleda et al., 2012). These predictions suggest stream forms to be more slender-bodied, with a more robust caudal peduncle and anteriorly placed fins with a large surface area. However, these variation patterns were not consistent.

The aim of this study was to examine lentic/lotic habitat morphological variations of this species and to evaluate the effects of allometric growth patterns on these variations. For this purpose, two regions were selected according
to their invasion background: the Thrace region, from which the first report of pumpkinseed was given, and the southwestern part of Turkey, which was presumably colonized as a result of reintroduction (Barlas et al., 2001). An additional purpose was to report the range extension of the species.

2. Materials and methods
To assess morphological variation of *L. gibbosus*, three populations were selected. In order to identify different growth patterns, body-form variability was demonstrated with landmark-based geometric morphometrics.

2.1. Sampling
A total of 75 specimens were collected from three localities in the Aegean and Thrace regions by electrofishing and line fishing in June 2012 and May 2013. The sampling localities shown in Figure 1 are the Akgedik Reservoir (4800), Sarıçay Stream (4801), and Hayrabolu Pond (5901). The Akgedik Reservoir, a small reservoir with a surface area of 1.5 km², is located in the southwestern part of Turkey. The reservoir was built on Sarıçay Stream and water retention was started in 2009. Hayrabolu Pond is a small pond (<1 km²) located in the northwestern part of Turkey, in the Thrace region. Specimens were fixed in 4% formalin; after a week, the formalin was rinsed off with tap water and specimens were transferred to 70% alcohol solution. Sex was determined visually by examining the gonads.

2.2. Geometric morphometrics
Specimens were positioned on cardboard and photographed from their left side with a millimetric scale placed in the frame. Image acquisition was conducted with a Canon EOS 450D camera equipped with a 55-mm lens in manual focus mode. In order to emphasize the landmarks and for accurate positioning, thin insect fixation needles (No. 3) were used. Thin plate spline (tps) file creation and landmark digitization was conducted with tps series software programs tpsUtil and tpsDig2 (Rohlf, 2015). Landmarks used in this study are summarized in Figure 2. Procrustes superimposition was conducted with the geomorph package (http://cran.r-project.org/web/packages/geomorph/index.html) implemented in R 3.4.0 (http://www.R-project.org); outliers were checked with principal component analysis (PCA). Landmark
contributions to variance were calculated with PCAGen8 (IMP8; http://www3.canisius.edu/~sheets/morphsoft.html). In order to remove shape differences due to varying sizes of the fish, shape coordinates were regressed with centroid size (logarithm), and residuals from this regression were extracted to be used in further analysis. Immature specimens (n = 15) were not represented equally in samples from different populations. Therefore, in order to prevent possible bias, they were removed from the dataset prior to geometric morphometric analysis.

According to preliminary analysis, differences between sexes were not significant; therefore, the sexes were pooled by locality. This analysis was followed by another ANOVA to determine the effects of size on shape. Differences in shape were then examined with multiple-factor ANOVA in the geomorph package in terms of habitat (lentic, lotic) and the three sampling localities. Pairwise comparisons for the three localities were conducted in IMP8 (http://www3.canisius.edu/~sheets/morphsoft.html) as a post hoc test to Procrustes ANOVA. Partial Procrustes distance between means of these localities are reported with their significance according to 1000 bootstrap resamplings.

Five times exaggerated deformation grids from reference specimen to hypothetical mean specimen of the so-called groups were plotted.

3. Results
Following removal of immature specimens, the female to male ratio was 0.62/1.00. Following this removal, sample size (n) and total length (LT) range of the specimens were as follows: for Akgedik Reservoir, n: 17, LT: 6.7–11.1; Sarıçay Stream, n: 10, LT: 5.7–9.3; and Hayrabolu Pond, n: 33, LT: 8.2–14.4.

3.1. Allometry
Regression was conducted in order to quantify the effect of growth on shape differences. According to regression of shape coordinates with centroid size (logarithm), the percentage of variation explained by size was 15.49%. With the increase of centroid size logarithm from 2.0 to 2.8, shape changes were especially observed on landmarks, which are highlighted in black (Figure 3). Analysis showed that the growth rate of the head region and total height is greater according to the growth rate of total length of the specimen.

3.2. Principal component analysis
Morphospace defined by PC1, PC2, and PC3 accounted for 61% of the total shape variance and is discussed in this section. In order to achieve 95% coverage of the variance, 14 principal components must be taken into account. In order to demonstrate the reliability of the landmark digitization, variances when a single landmark is omitted are given in Table 1.

According to the observed decrease in the sum of square loadings when landmarks were taken out, landmarks located in the anterior base of the anal fin (13), first pelvic fin ray (14), anterior base of dorsal fin ray (7), and dorsal base of pectoral fin (16) are responsible for most of the variance. PCA plots and five times exaggerated PCA deformations along the first three principal components are given in Figure 4.

3.3. Procrustes ANOVA
Procrustes ANOVA revealed significant effects of habitat and locality (P < 0.01) before and after the standardization procedure (Table 2). Post hoc analysis of the significant locality-based grouping was conducted; the effect of standardization on these is summarized in Table 3.
Partial Procrustes distances between specimens from different localities changed as well, which may seem small but changed the outcome dramatically. Significance of these pairwise distances was high according to 1000 bootstraps. Before standardization, the lentic habitat (Akgedik R.) is positioned closer to its geographically related neighboring lotic habitat (Sarıçay S.), with a partial Procrustes distance of 0.0348. However, following the standardization, it is positioned closer to Hayrabolu Pond (partial Procrustes distance = 0.0218), indicating similar patterns between similar habitats. Effect of standardization is shown in Figures 5 and 6 as five times exaggerated deformation grids, from reference specimen to hypothetical mean specimen of the groups considered in Procrustes ANOVA.

According to these deformation grids, standardization seems to normalize some features such as pectoral fin position, opercular structures, head width, anal fin basal length, (only in Akgedik R.), and caudal peduncle length/width (only in Sarıçay S.). In addition to these normalizations, caudal peduncle features, such as expansion in specimens from Akgedik Reservoir and contraction in Hayrabolu Pond samples, seem to fit the expected pattern after standardization.

As a result, the main differences seem to be related to habitat. In lotic habitats, pectoral and pelvic fins seem to be located anteriorly, whereas they were further back in lentic habitats. In addition to this, the caudal peduncle of lentic fish does not demonstrate a clear difference from the reference specimen; in lotic habitats, it widens posteriorly.

4. Discussion

At least 25 exotic fish have been introduced to the inland waters of Turkey over the last century (Innal and Erk’akan, 2006). The probable pathway of *L. gibbus* to the Thrace
The region is thought to have been via the State Hydraulic Works hatchery located in İpsala, which uses the Meriç River as a water source (Şaslı and Balık, 2003; Balık and Ustaoğlu, 2006). Small pumpkinseeds can be hidden under the opercula of large cyprinids (Villeneuve et al., 2005) and thus become impossible to detect during the introduction of carp.

Variability in body depth, caudal peduncle depth, fin positions, and fin lengths have been addressed and partially determined by some studies (McLaughlin and

| Table 2. Procrustes ANOVA results of shape data. |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                | df       | SS        | MS        | R²        | F         | Z         | p         |
| Centroid size  | 1        | 0.013540  | 0.0135396 | 0.154922  | 13.0718   | 5.4115    | 0.001     |
| Residuals      | 58       | 0.073856  | 0.0012734 |           |           |           |           |
| Sex            | 1        | 0.000823  | 0.0008227 | 0.009414  | 0.5512    | -0.9499   | 0.828     |
| Residuals      | 58       | 0.086573  | 0.0014926 |           |           |           |           |
| Habitat¹       | 1        | 0.009849  | 0.0098493 | 0.11270   | 8.3036    | 4.5069    | 0.001     |
| Locality¹      | 1        | 0.009936  | 0.0099357 | 0.11369   | 8.3764    | 4.8993    | 0.001     |
| Residuals¹     | 57       | 0.067611  | 0.0011862 |           |           |           |           |
| Total¹         | 59       | 0.087396  | 0.0011862 |           |           |           |           |
| Habitat²       | 1        | 0.007730  | 0.0077296 | 0.104657  | 6.9759    | 4.1252    | 0.001     |
| Locality²      | 1        | 0.002969  | 0.0029689 | 0.040199  | 2.6795    | 2.5926    | 0.007     |
| Residuals²     | 57       | 0.063158  | 0.0011080 |           |           |           |           |
| Total²         | 59       | 0.073856  |           |           |           |           |           |


¹ Multiple factor ANOVA results before standardization.

² Multiple factor ANOVA results after standardization.
According to Naspleda et al. (2012), only greater pelvic and pectoral fin lengths in stream fishes are consistent with hydrodynamic theory, whereas another dataset (Brinsmead and Fox, 2002) shows no significant differences between habitats according to these lengths. On the other hand, slender body (generally), robust caudal peduncle (partly), and anterior positioning of fins (partly) in stream fishes are consistent with hydrodynamic theory (Brinsmead and Fox, 2002). With the aid of geometric

**Table 3.** Pairwise comparisons of partial Procrustes distance between localities with significance values in bold.

<table>
<thead>
<tr>
<th></th>
<th>Akgedik Reservoir</th>
<th>Sarıçay Stream</th>
<th>Hayrabolu Pond</th>
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<tbody>
<tr>
<td>Akgedik Reservoir</td>
<td>-</td>
<td>0.001/0.001**</td>
<td>0.001/0.004**</td>
</tr>
<tr>
<td>Sarıçay Stream</td>
<td>0.0348/0.0353*</td>
<td>-</td>
<td>0.001/0.001**</td>
</tr>
<tr>
<td>Hayrabolu Pond</td>
<td>0.0366/0.0218*</td>
<td>0.0365/0.0274*</td>
<td>-</td>
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*Partial Procrustes distance before standardization/partial Procrustes distance after standardization.
**Significance of distance before standardization/significance of distance after standardization.

**Figure 5.** Five times exaggerated deformation grids from reference specimen to group means (before standardization).
morphometrics, this study showed and visualized different morphometric forms of *L. gibbosus*. In the stream habitat, fins were positioned anteriorly and the posterior part of the caudal peduncle was widened. However, this study failed to determine body depth variability as hypothesized in the previously mentioned studies. *L. gibbosus* is a laterally flattened fish with median and paired fins dependent on swimming; according to Webb (1984), optimal body design for this kind of fish is a deep and laterally flattened body. Therefore, a more slender body in stream habitats might be hard to detect.

Another interesting outcome is response timing to environmental changes. We defined the Akgedik Reservoir (4800) as a lentic habitat, which was formed recently from the neighboring Sarıçay Stream (4801). Water retention in the Akgedik Reservoir only started in 2009, while the Hayrabolu Pond (5901) is an established lentic habitat with insignificant inflows. The Akgedik Reservoir, as a newly established lentic habitat (4800), shows changes between lotic habitat and established lentic habitat. As shown in Figure 6, anterior placement of pelvic and pectoral fins is obvious in Sarıçay Stream specimens, while they are positioned more posteriorly in Hayrabolu Pond (established lentic) specimens. Specimens of the Akgedik Reservoir have anteriorly placed fins (slightly), demonstrating a transition between lentic and lotic habitats. Posterior caudal peduncle depth is smaller in Hayrabolu Pond specimens and slightly larger in Akgedik Reservoir specimens. Both fin placements and caudal peduncle depth gradually change from lotic to lentic habitats, with the newly formed lentic habitat (Akgedik Reservoir) as a transition point. The fish showing fast response

Figure 6. Five times exaggerated deformation grids from reference specimen to group means (after standardization).
as presented in this study suggests that environmental changes seem to be responsible for most of the variation, as suggested by Robinson et al. (2000), Tomček et al. (2005), and Bhagat et al. (2011b). In addition to this, clustering geographically distinct localities (Akgedik Reservoir and Hayrabolu Pond, as shown in Table 3) with different stories of introduction decreases the likelihood of genetic effects on morphometry and favors environmental effects.

The effect of allometry on the results is another subject to discuss. Before standardization, there was no significant clue about the first axis of PCA for length differences, and the proportion of explained variance by size was found to be 15%. Nevertheless, the differences presented here were found to be important, as can be seen in the deformation grids. These results suggest, depending on the hypothesis of the morphometric study, that it might be important to implement allometric regression and standardization.

Showing a potential for developing fast phenotypic changes in different environmental conditions is an important trait for the negative effects of invasive species, which may enhance invasion succession. Therefore, the range extension of L. gibbosus needs to be monitored carefully and removal strategies need to be discussed. Previously, it has been reported only from the Thrace and Muğla regions. However, the species had been sampled by us from two new basins, Susurluk and Sakarya. From the Sakarya Basin, the species had been sampled from Taşkışığı Lake, Sakarya (40°52.256′N, 30°24.124′E), and from the Susurluk Basin, Çınarcık Reservoir, Bursa (40°2.730′N, 28°46.671′E).

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