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How does invasion of a new environment drive body shape? A test of predictions based on Canestrini's goby  
*Pomatoschistus canestrinii* (Ninni, 1883)

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## Abstract

Divergent habitats and an associated divergence in ambient conditions, including physical as well as biotic environmental factors, are considered to have a significant impact on the morphology of many different fish species resulting in intraspecific polymorphism. In course of parallel evolution, similar morphotypes can be found in similar habitats. Vice versa, morphotypes can strongly differ under ecologically different environments. Concerning stream and lake populations, a recently published steady-unsteady swimming performance model revealed that variation in flow regime is one of the major driving forces behind varying morphotypes of free swimming fishes across lotic and lentic habitats. In the present study this model were tested for a benthic species, the small gobiid, *Pomatoschistus canestrinii* (Gobiidae) by using a geometric morphometric approach. Hence, possible adaptive effects on body size as well as on lateral and ventral shape were investigated comparing a native stream population from Jadro River, Croatia, and an introduced lake population from Lake Trasimeno, Italy. Furthermore, sex-related differences in the morphology and allometric effects were analysed. According to the results morphological differences between the river and the lake populations were evident for both, males and females. The stream population was distinctly larger and showed a higher variance in size, regardless of sex. Stream gobies developed more streamlined body shapes, deeper and longer caudal peduncles, smaller and slender heads with smaller eyes, longer upper jaws, narrower gapes and shorter bases of the pectoral fin than lake gobies. Analysis on the sexual dimorphism among both populations showed an increase in size, a larger and broader head, smaller eyes, a broader caudal peduncle, a more superior and wider mouth as well as a longer base of the pectoral fin in males from the stream and the lake populations compared to the females of both habitats. Additionally, males also had the typically longer suction disc (pelvic fins). Possibly as the result of phenotypic plasticity, morphological differences are likely linked to different hydrodynamic constraints in the divergent habitats (lake vs. stream). Concerning body shape it could be demonstrated that the steady-unsteady

swimming performance model is also applicable on benthic gobiid fishes. However, some predicted morphological features did not correspond to the present results for *P. canestrinii*.

Key words: Canestrinii's goby, body shape, phenotypic plasticity, flow regime, geometric morphometric methods, sexual dimorphism

## Zusammenfassung

Unterschiedliche Habitate und der damit verbundene Unterschied in den entsprechenden physikalischen und biotischen Umweltfaktoren, haben signifikanten Einfluss auf die Morphologie verschiedenster Fischarten, welcher zu einem intraspezifischen Polymorphismus führt. Im Zuge der parallelen Evolution, können ähnliche Morphotypen in ähnlichen Habitaten gefunden werden, wohingegen Morphotypen, unter ökologisch unterschiedlichen Bedingungen, stark variieren können. Ein erst kürzlich veröffentlicht und etabliertes „steady-unsteady swimming performance“ Modell zeigte, dass Variationen in der Strömungsgeschwindigkeit einer der wichtigsten Triebkräfte bezüglich der Ausprägung unterschiedlicher Morphotypen freischwimmender Fischarten in fließenden und stehenden Gewässern sind. In dieser Studie wurden, mit Hilfe von geometrischer Morphometrie, ökomorphologische Vorhersagen basierend auf dem „steady-unsteady swimming performance“ Modell für eine relativ kleine epibenthische Fischart, *Pomatoschistus canestrinii* (Gobiidae), getestet. Mögliche adaptive Effekte bezüglich der Körpergröße sowie lateraler und ventraler Körperform wurden durch morphologisches Vergleichen einer natürlichen Population aus dem Fluss Jadro, Kroatien und einer eingeschleppten Population aus dem Trasimener See, Italien, untersucht. Außerdem wurden geschlechtsbezogene Unterschiede in der Morphologie sowie allometrische Effekte analysiert. Gemäß den Resultaten, konnte unabhängig vom Geschlecht, eine deutliche Erhöhung der mittleren Standard Länge und eine erhöhte Größenvarianz in den JAD Individuen festgestellt werden. Morphologische Unterschiede zwischen Exemplaren aus JAD und TRA sind, sowohl für Männchen als auch für Weibchen, ersichtlich. JAD Grundeln entwickelten einen stromlinienförmigen (spindelförmigen) Körper, breitere und längere Schwanzstiele, kleinere und schmalere Köpfe mit kleineren Augen, längere Oberkiefer, schmalere Müuler und kürzere Ansätze der Brustflossen. Eine Untersuchung des Sexualdimorphismus, innerhalb beider Populationen, zeigte eine Steigerung der Körpergröße, größere und breitere Köpfe, kleinere Augen, breitere Schwanzstiele, oberständigere und

breitere Mäuler sowie längere Ansätze der Brustflossen in männlichen Exemplaren aus JAD und TRA. Zusätzlich konnten tendenzielle Reduktionen der Distanzen zwischen dem posterior gelegenen Rand der Bauchflosse und der anterioren Basis der Anals beobachtet werden. Charakteristisch wiesen Männchen außerdem längere ventrale Saugscheiben (Bauchflossen) auf. Möglicherweise sind die morphologischen Unterschiede, als Resultat der phänotypischen Plastizität, an verschiedensten hydrodynamischen Bedingungen in divergenten Habitaten geknüpft (See vs. Fluss). Es konnte gezeigt werden, dass das „steady-unsteady swimming performance“ Modell bezüglich der allgemeinen Körperform auch auf benthisch, gobiide Fischarten anwendbar ist. Einige prognostizierte morphologische Merkmale korrespondierten jedoch nicht mit den Resultaten für *P. canestrinii*.

Schlüsselwörter: Schwarzflecken-Sandgrundel, Strömungseigenschaften, Körperform, phänotypische Plastizität, Morphologie, geometrisch morphometrische Methoden, Sexualdimorphismus

# 1. Introduction

## 1.1 Ecomorphology

Investigation on the morphology of various fish species and how it is influenced by heterogeneous environmental conditions, was the subject of several recently conducted ecomorphological studies (Berner et al., 2008; Bower & Piller, 2015; Cureton & Bourghton, 2014; Douglas & Matthews, 1992; Foster et al., 2015, Kocačić, 2005; Langerhans, 2008; Langerhans & Reznick, 2010; Ramler et al., 2014; Ramler et al., 2016; Stelbrink & Freyhof 2006). Following Langerhans (2008), morphological divergence can be explained by both, phenotypic plasticity and genetic differentiation, which is seen as indicator for an evolutionary response to selection. This assumption is also confirmed in Defaveri and Merilä (2013) analysing Baltic Sea sticklebacks. Abiotic and biotic factors, such as temperature, flow regime, salinity, predation as well as competition are known as major driving forces behind intraspecific polymorphism. Divergent selection pressure affects various “labile” morphological traits, having an impact on the populations’ fitness (Bower & Piller, 2015, Langerhans, 2008; Seehausen & Wagner, 2014). However, the strength and direction of selective agents overwhelming the homogenizing effects of other evolutionary forces like genetic flow and migration, can fluctuate annually, whereby phenotypic plasticity very likely supports rapid morphological change (Blanquart et. al., 2013; DeFaveri & Merilä, 2013; Cureton & Bourghton, 2014). Phenotypic changes in fishes can even occur over a very short period of time and small number of generations. According to Cureton and Bourghton (2014), the magnitude of structural change in a widespread North American stream fish, *Pimephales vigilax* (Cyprinidae), was recorded to be highest in the first 15 generations with a more gradual change afterwards. Especially freshwater fishes, where the number of body shape variations under different environmental conditions tends to be increased, represent a suitable model group to



analyse the relationship between morphology and ecology (Bower & Piller, 2015; Foster et al., 2015).

In general, theory predicts that fishes occupying similar ecological niches develop similar morphotypes, including a parallel evolution of adequate key morphological attributes, while divergent morphotypes occur in divergent habitats (Berner et al., 2008; Bower & Piller, 2015; Cureton & Bourghton, 2014; Foster et al., 2015). Comparing populations from lotic and lentic habitats, a steady–unsteady swimming performance model focusing on morphological trade-offs, was published (Langerhans, 2008; Foster et al., 2015). The swimming performance model, which was established by using geometric morphometrics for two common fish species, *Goodea atripinnis* (Goodeidae) and *Chirostoma jordani* (Atherinopsidae), revealed a morphological relevance of divergent flow regimes on these free swimming fishes (Foster et al., 2015). Head size and positions of mouth, size of fins as well as the length of the caudal peduncle are also considered to be important shape variables discriminating between lotic and lentic populations. In lotic habitats, which are characterized by a high-flow regime and an increase of hydrodynamic drag, the body shape of fishes generally tends to be more streamlined (fusiform) than in lentic habitats. Additionally, individuals from lotic habitats tend to develop more inferior mouths, narrower caudal peduncle, have a higher aspect ratio caudal fin and a shortened analis (Berner et al., 2008; Langerhans, 2008). The head of lotic individuals is predominantly elongated. Such morphological features seem to give advantage to steady swimming under high-flow conditions in order to maintain position or constant locomotion while cruising (searching for food, mates) and migrating (Foster et al., 2015).

Body size and shape are defined as most important factors determining the ecological niche of stream fishes (Bower & Piller, 2015; Douglas & Matthews, 1992). In contrast to lotic habitats, the low-flow regime in lentic habitats usually favours the development of a deeper body shape and a longer caudal peduncle, which improves movement skills such as burst

swimming, predator avoidance, chasing prey and navigating through structurally complex environments (Langerhans, 2008). Hence, the efficiency of swimming and the reduction of energy costs during locomotion can be significantly influenced by body shape (Foster et al., 2015). Additionally, Cureton and Broughton (2014) found out that lake-dwellers show a posteriorly deeper body including a wider caudal peduncle. This morphological feature tends to improve maneuverability and to increase swimming burst speed. Even though, a correlation between phenotypic differentiation and flow regime as well as trophic guild could be confirmed, the substratum was not considered relevant explaining differences in the morphology of the fish (Bower & Piller, 2015). Another study including morphological responses to differences in flow velocity, conducted by Berner et al. (2008), additionally revealed more developed gill structures, but, in contrast to previously mentioned results, more streamlined bodies in lake fishes. Analysis on the diet of the fish showed that limnetic vs. benthic foraging modes and differences in prey resources were mainly responsible for the recorded divergence in the morphology. Accordingly, a shift in diet can have a distinct impact on the body shape. Rapid morphological responses and explosive speciation to a changed diet, occurring over the past 30 years, was found in haplochromine cichlids, whereby phenotypic changes were mainly addressed to the oral jaw (Van Rijssel et al., 2014; Stauffer & Van Snik Gray, 2004). Also in cyprinids, the head shape containing trophic structures like mouth, eyes as well as gill-arch structures, is often modified in order to increase the efficiency of foraging under heterogeneous environmental conditions (Ahnelt et al., 2015). Nevertheless, the role of habitat tends to be more important than trophic dimensions in cyprinids (Douglas & Matthews, 1992). Especially, ambient factors such as predation regime, food, light, parasites and pathogens can strongly differ between stream and lake habitats resulting in divergent selection pressure (Seehausen & Wagner, 2014).

According to published literature, consequences on the evolutionary process, including genetic and morphological differentiation, can also be expected in case of anthropogenic modifications of nature resulting in global warming, introduction of new species, eutrophication and a shift in salinity, flow regime and oxygen level (Cureton & Bourghton, 2014; Langerhans, 2008; Ramler et al., 2014). Long-time monitoring projects including the collection of environmental and species data, represent the best option to test for possible impacts of specific parameters on morphological traits so far (Ahnelt et al., 2015).

Variations in development, life history, locomotion, foraging and reproduction, adapted to the corresponding ecological niche, exist across different fish species. Based on an ecomorphological approach, the morphological trade-offs should not affect all fish species the same way (Langerhans & Reznick, 2010). Comparing high and low flow environments, it is still not clarified, if benthic and pelagic fish show similar morphological adaptations. Due to the fact that gobies (Gobiidae) are the second most species rich family among vertebrates (> 1600 species) and show a strong diversification and a wide geographical distribution, they provide an excellent model group to test for phenotypic divergence (Kocačić, 2005; Tornabene et al., 2013; Vera-Duarte et al., 2017). Nevertheless, regarding to different genera of the taxon Gobiidae, a fundamental lack of ecological, morphological and phylogenetic data still exists. The mechanism in evolutionary process leading to microhabitat association are also not completely understood (Tornabene et al., 2013). Extensive research on the general biology, distribution, population and behavioural ecology of different gobiid fish species, improved crucial scientific understanding of epibenthic and cryptobenthic fishes (Ahnelt & Göschl, 2003; Ahnelt & Scattolin, 2005; Ehrenberg et al. 2005; Kovačić, 2008; Magnhagen & Wiederholm 1982; Vera-Duarte et al., 2017). Nevertheless, only little literature on adaptive morphological and genetic patterns of gobiid fish under local environmental conditions exists.

## 1.2 Canestrini's goby

The small (~55 mm), epibenthic gobiid *Pomatoschistus canestrinii*, is an euryhaline species usually occurring in waters ranging from 5-20 ‰ salinity (Fig.1).



Fig.1. Canestrinii's goby *Pomatoschistus canestrinii*. Photo FishBase

It is endemic to the Adriatic Sea basin and shows a disjunctive distribution along its north and east central region from the Po delta in Italy to the Neretva River in Croatia and in Bosnia and Herzegovina (Miller 1986, Tutman et al. 2016). Especially during the second half of the 20<sup>th</sup> century it has been introduced in different water bodies located in Croatia and Italy, such as Lake Trasimeno in Italy and Ričica River in Croatia (Miller, 2004; Kovačić 2005; Stelbrink & Freyhof, 2006; Tutman et. al., 2013). In its natural distribution area, *P. canestrinii* can be mainly found in estuaries, lagoons and stream habitats, containing smaller and moderate rivers with a constant but low level of flow velocity. This small goby prefers unvegetated sandy and muddy habitats in shallow waters (Miller, 2004) but occurs in Lake Trasimeno also in areas with vegetation and algal cover (Freyhof, 1998, this study). This goby is characterised by a high

phenotypic plasticity in squamation and cephalic lateral-line canal system (Kovačić, 2005; Stelbrink & Freyhof, 2006). Furthermore, *P. canestrinii* is considered to be an opportunistic feeder with a broad diet spectrum (Miller, 2004). During the reproductive period ranging from March to July, female individuals place their adhesive eggs in nests constructed and guarded by males under various available structures like stones plants and shells. Males fertilize the eggs and subsequently defend the clutch in the nest until the hatching of the larvae. A distinct sexual dimorphism in body proportions is evident, whereby males e.g. have deeper caudal peduncles or longer pectoral fins and pelvic discs (Miller, 2004).

### **1.3 Geometric morphometrics**

In ecomorphological studies, geometric morphometric methods, based on Cartesian shape coordinates and a Procrustes superimposition approach, are commonly used to quantify and compare variations of phenotypic traits by setting homologous landmarks (Gunz & Mitteroecker, 2013; Mitteroecker & Gunz, 2009; Ramler et al., 2014; Rohlf & Slice, 1990; Rohlf & Marcus, 1993). Homology criterion is fulfilled by using clearly defined anatomical landmarks, which have to be placed on corresponding locations on each specimen. In order to receive additional information on outlines and surfaces of morphological structures, semi-landmarks without clear anatomical definitions, can be added. Usually, semi-landmarks are positioned between regular landmarks (Ramler et al., 2014). Beside to the Procrustes superimposition algorithm, separating shape from overall size by scaling, from position by translation and from orientation by rotation using centroid, centroid size and the least squared distance method, other techniques like Booksteins' two point baseline registration, sliding baseline registration and resistant fit superimposition can also be applied in order to analyse differences in shape (Mitteroecker & Gunz, 2009). Shape data can either be directly or indirectly acquired resulting in two or three dimensional shape coordinates. Indirect shape data is usually gathered from standardized, digitalized images and model scans of the study object,

whether two or three dimensional, while direct data is deducted from the investigated individuals using technical equipment such as MicroScribe G2X Digitizer. Referring to the landmark coordinates computed by Procrustes superimposition, a reduction in degrees of freedom and a decrease in shape variables is described (Mitteroecker & Gunz, 2009). An iterative statistic procedure, called General Procrustes Analysis (GPA), is basically applied to morphometrically compare shapes and external structures. Moreover, the use of a Principle Component Analysis (PCA) illustrating possible aggregations of data points by linear combination of shape variables, can provide crucial information on the divergence of shape (Mitteroecker & Gunz, 2009). In order to ease the detection of morphological differences between specific groups of interest, extrapolated deformation grids, based on thin-plate spline algorithms (TPS) and computed mean shapes, are strongly preferred (Ramler et al., 2015).

#### **1.4 Study aim**

This study is focusing on the phenotypic responses of *P. canestrinii* to a new environment and an associated divergence in flow velocity. *P. canestrinii*, naturally occurring in running waters (lotic habitat), was unintentionally introduced into a still water (lentic) habitat (Lake Trasimeno) (Freyhof, 1998; Miller, 2004). Investigations are mainly addressed to the question: Are already known systems and models based on comparative morphological data of free swimming fishes suitable for predicting body shape variations across a lotic and lentic population of a benthic fish species? Therefore, a native, flow population from Jadro River (Croatia) and an introduced, still water population from Lake Trasimeno (Italy) was investigated. The steady–unsteady swimming performance model (Langerhans, 2008; Foster et al., 2015) was tested using a geometric morphometric approach. In addition phenotypic variations, which can be explained by sexual dimorphism, were analysed and the relevance of allometric effects influencing shape data was assessed among both populations.

## 2. Material and methods

### 2.1 Study object and sampling

In this study, I investigated two samples of the Canestrini's goby, *Pomatoschistus canestrinii* (Ninni 1883), which is known as endemic to brackish and freshwater inhabiting muddy shallows and sandy substrates along the north and central Adriatic region. One sample of 145 adult specimens was collected at the south shore of Lake Trasimeno (TRA) located in the Tiber River basin, Italy (43°9'11" N, 12°15' E), by Dr. Laura Pompei and Prof. Dr. Massimo Lorenzoni (University of Perugia, Italy) (Fig.2). With a total surface area of 124.3 km<sup>2</sup> and an average depth of 4.72 m, Lake Trasimeno represents the largest lake in the Italian peninsula but the fourth largest lake in Italy. Due to the shallow depth, no thermal stratification exists while water temperature and air temperature are almost congruent. During summer month, the water temperature can reach 30°C and above (Lorenzoni et al., 2010; Pompei et al., 2012). From April to September in 2015, water temperature measured at 15 different sites ranged from 13.60°C to 30°C, showing the highest temperature peak in July. More shallow areas are covered by dense masses of aquatic macrophytes (Pompei et al., 2012). In case of Lake Trasimeno, which contains 19 different fish species dominating by cyprinids, *P. canestrinii* is considered as introduced species (Lorenzoni et al., 2010; Miller, 2004). Nevertheless, it is still not completely clarified, when and how often *P. canestrinii* was introduced in this area. The exact number of the initially introduced individuals is also not clear. All sampling activities occurred during the breeding season in May 2015. In order to avoid any sampling bias, specimens were captured by net fishing at the same depth, using landing nets with a mesh width of approximately 5 mm. They were brought to laboratory, subsequently anesthetized and euthanized by an overdose of MS-222 (tricaine methanesulfonate) and stored in 6% formalin for several weeks. Subsequently the specimens were transferred into 70 % ethanol after passing through an ascending alcohol

series. Shrinkage caused by standard alcohol preserving procedure remains stable after an initial stage of about one month (Moku, 2004). Because digitalization of the individuals from TRA was just conducted 2017, bias by a shrinkage effect can be excluded.

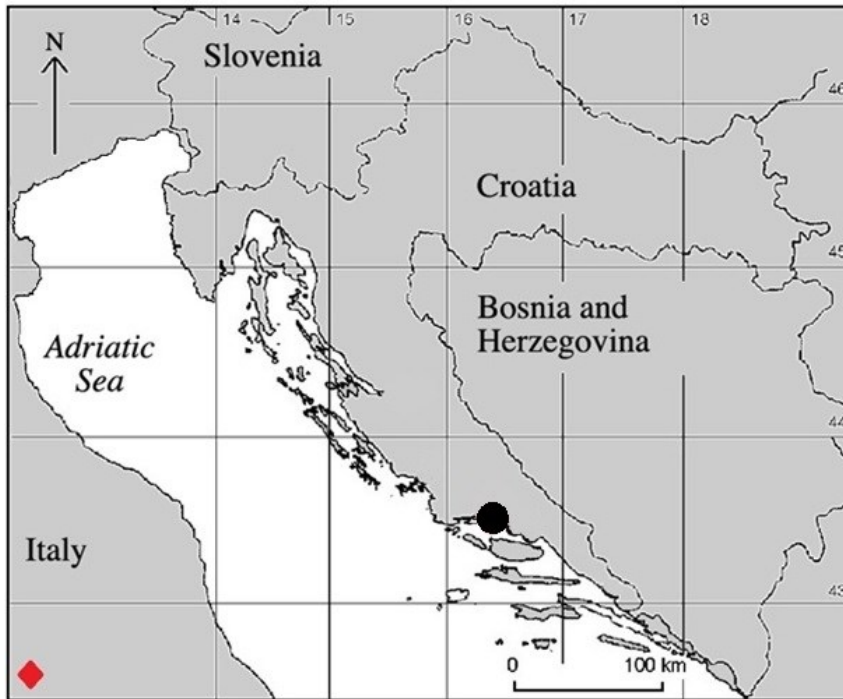


Fig.2. Sampling locations of *Pomatoschistus canestrinii* from the lotic Jadro River population (black dot) and lentic Lake Trasimeno population (red rhomb). Modified from Kovačić (2005).

The second sample consisted of 50 individuals of *P. canestrinii* from Jadro River (JAD), located in the southern part of Croatia, near Split (43°30'N, 16°26'E) (Fig.2). The Jadro River is characterized by an unsteady stratified water flow and represents a native habitat for *P. canestrinii*. With a total length of 4.3 km and an average annual flow rate of  $7.9 \text{ m}^3 \text{ s}^{-1}$ , including a maximum of  $12.8 \text{ m}^3 \text{ s}^{-1}$  and a minimum of  $5.1 \text{ m}^3 \text{ s}^{-1}$ , it is considered as relatively small stream, which discharges in the east coast of the Adriatic Sea through the Bay of Kaštela previously passing the urban region of Solin (Ljubenkov, 2015). Due to the complexity of the ground water system, a continuity of river source can be guaranteed for the entire year. The mean temperature measured in the period 1979/80 and 1984/85 was  $12.55^\circ$  with a minimum of  $10.77^\circ$  and a maximum of  $15.02^\circ$ . The minimum mean profile velocity in the river canyon



reached  $1.5 \text{ ms}^{-1}$  (Bonacci, 2012). Measured at the river mouth, the input of freshwater into the system ranged from  $2.76$  to  $3.24 \text{ m}^3 \text{ s}^{-1}$ . Even  $910 \text{ m}$  upstream, a strong horizontal salinity gradient with a salinity of  $2 \text{ ‰}$  at the surface and  $36 \text{ ‰}$  in the lower layer was observed (Ljubenkovic, 2015).

All examined specimens from JAD have been preserved and stored in  $70\%$  ethanol for more than  $100$  years at the Ichthyological collection of the Museum of Natural History in Vienna (NMW). Only fish showing adequate body size ( $\text{SL} > 18\text{mm}$ ) and no deformations or bending effects were selected for further investigation. The valuation of the specimen condition was conducted optically by using a standard stereomicroscope. This historical sample from the Jadro River was selected because *P. canestrinii* was introduced in several water bodies in Croatia during the second half of the  $20^{\text{th}}$  century.

Finally, a total of  $26$  individuals of the native stream population JAD, including  $18$  males and  $8$  females, as well as  $65$  introduced individuals of Lake Trasimeno (TRA), including  $42$  males and  $23$  females, remained for geometric morphometric analysis (Tab.1). Because of an artificial deformation of the right pectoral fin, which did not morphologically affect the left side of the sample, one individual had to be excluded from the ventral morphometric analysis.

Tab.1. Number and, in parentheses, range of SL in mm of investigated individuals included in geometric morphometric analysis. JAD= Jadro River population. TRA= Lake Trasimeno population.

	JAD	TRA
Number of individuals		
<i>n</i> female	8 (18.75 – 44.17)	23 (21.69 – 30.72)
<i>n</i> male	18 (20.66 – 50.54)	42 (21.21 – 37.11)
<i>n</i> total	26 (18.75 – 50.54)	65 (21.21 – 37.11)

Prior to the scanning procedure, sex was determined by a distinct sexual dimorphism of the urogenital papilla, which is longer (overlapping with the anterior base of the anal fin) and slightly narrower in males than in females (Miller, 2004) (Fig.3).

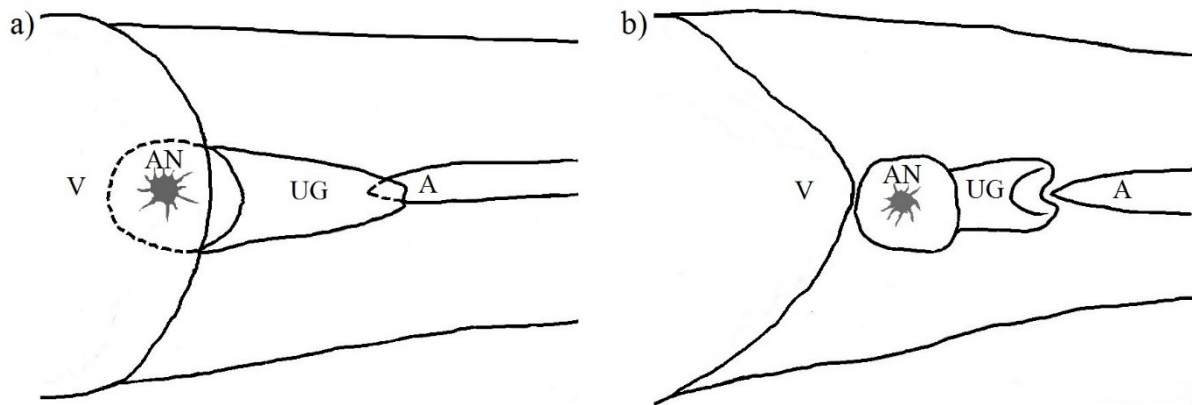


Fig.3. Ventral view. Anal/Urogenital region of *Pomatoschistus canestrinii*. Posterior part of pelvic fins (suction disc) (V), anus (AN), urogenital papilla (UG) and anterior part of anal fin (A) are illustrated. a)= male, b)= female

## 2.2 Digitalization of individuals and landmark setting

The lateral (left side) and ventral side of each individual was digitalized following Herler et al. (2007). Therefore, an Epson Perfection V330 Photo standard flatbed scanner, providing two dimensional data, was used. A testing phase focusing on the improvement of the designed apparatus and applied methods, revealed that a resolution of 4800 dpi and a colour depth of 48 bit were suitable configurations for setting accurate landmarks on digitalized images. Furthermore, brightness and contrast of the images were adjusted using the included standard Epson software tool. Beside to the flatbed scanner, the designed apparatus consisted of a sealed plastic pool, directly placed on the scanner glass and filled with 70% ethanol. The height of the filling (~ 15mm) should be sufficient to ensure total submergence of the specimens in order to avoid any disruptive reflections (Herler et al., 2007). Moreover, needles attached to plasticine (Pelikan 601492) were used to maintain a stable position of the objects. The ventral side of the fish was nearly planar and was adjusted close to the scanner glass. Consequently, also the tail of the body, including the origin of anal fin and the ventral origin of the caudal fin, could be used for morphometric analysis without signal distortion (Fig.4). Additionally, a high quality

of the images was guaranteed by minimizing the disruptive effects of pre-installed light sources using a white background paper cover placed on the upper rim of the plastic pool. A standardized scale beside to the sample was adjusted.

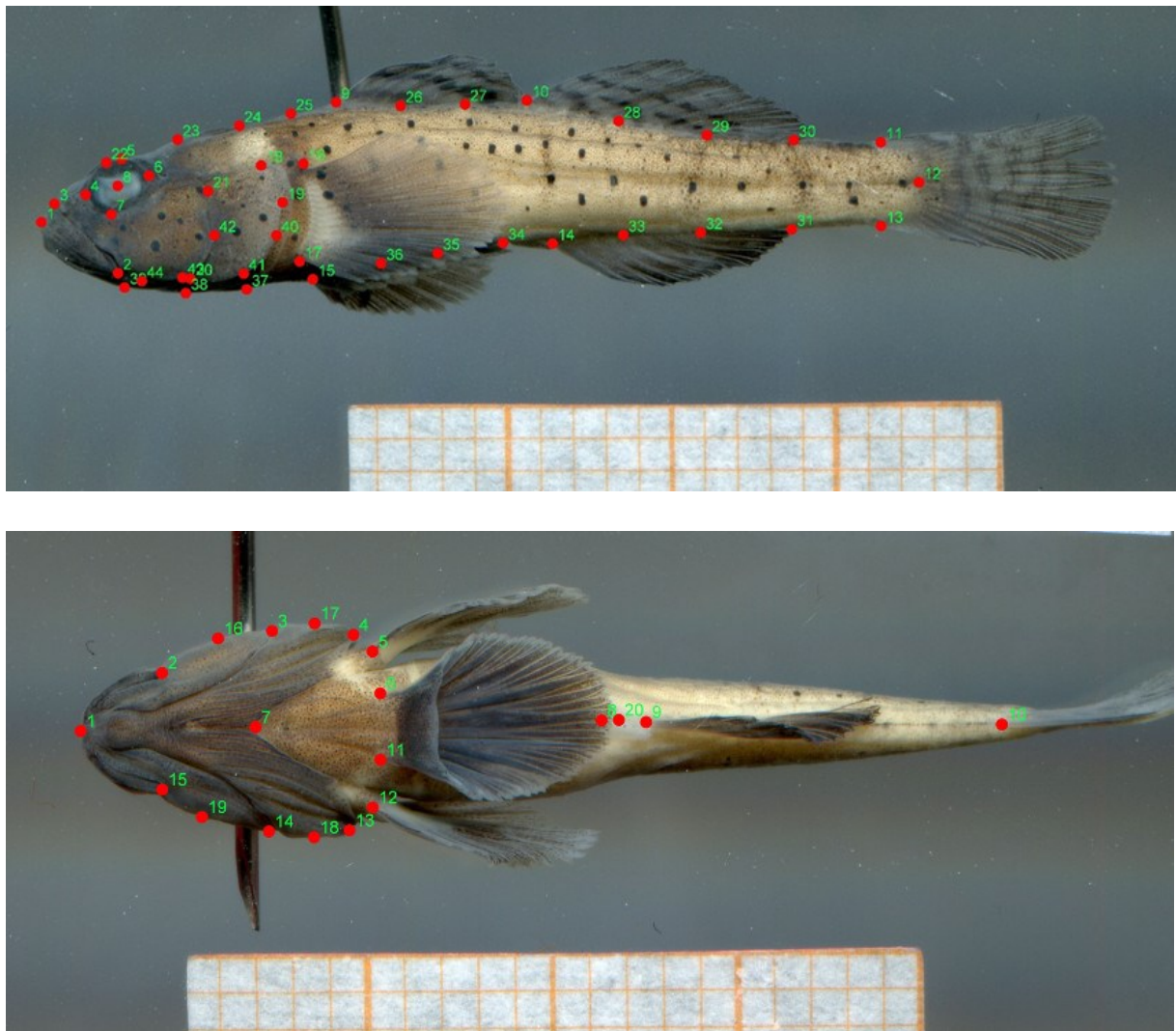


Fig.4. Lateral (above) and ventral (below) view of a digitalized specimen of *Pomatoschistus canestrinii*. Red dots= anatomical landmarks and semi-landmarks.

Prior to the processing of the images by setting landmarks (Cartesian coordinates of measurement points), all scans were randomized in order to exclude an unintended learning effect of the operating person influencing the resulting data. This was achieved by using the

software tool tpsUtil 1.70 (Rohlf, 2016). In a further step, a total array of 21 anatomical landmarks and 23 sliding semi-landmarks was digitalized on each lateral scan. Per specimen, 16 anatomical landmarks as well as 4 semi-landmarks were placed ventrally using tpsDig2 2.26 (Rohlf, 2016) (Tab.2; Fig.5).

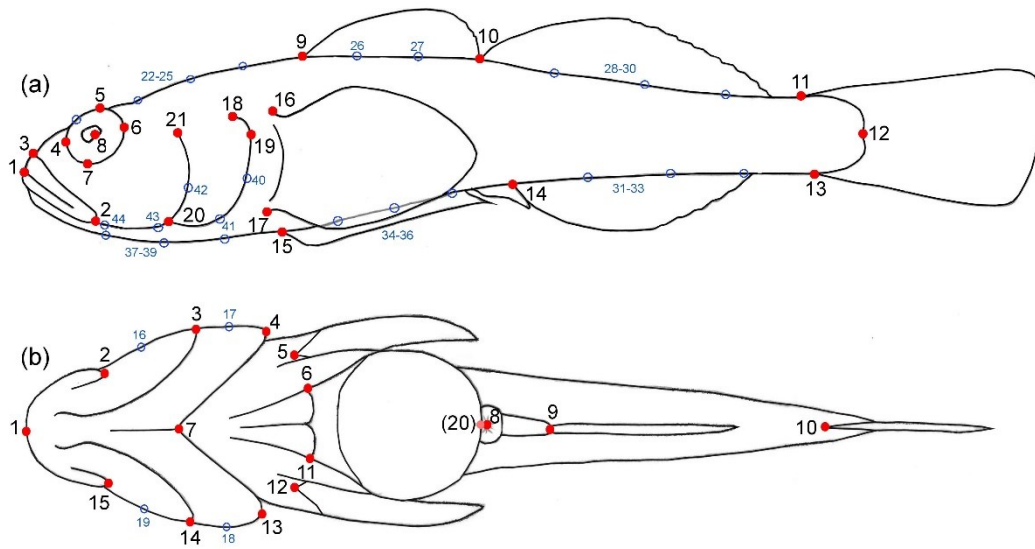


Fig.5. Lateral and dorsal sets of landmarks (red dots) and semilandmarks (blue circles) used for geometric morphometric analysis. (a) Lateral view: 21 landmarks and 23 semilandmarks. (b) Ventral view: 16 (15) landmarks and 4 semilandmarks. Detailed definitions of landmarks are mentioned in table 2.

Because of the highly interfering signals of the ventral landmarks 20 and 8 on the TRA samples, resulting in a disruptive torsion of the deformation grids, only 15 landmarks (landmark 20 excluded) were used for morphometrical analysis of the TRA population. Descriptions of the lateral and ventral landmarks as well as semi-landmarks are given in table 2. Generally, the additional use of non-homologous sliding semi-landmarks should provide information on curves and surfaces of morphological structures, while classic landmarks were homologous and consequently had a higher relevance for morphometric analysis (Gunz & Mitteroecker, 2013; Ramler et al., 2016). The number of semi-landmarks usually depends on the complexity of the

curve (Gunz & Mitteroecker, 2013). Based on the relatively low complexity of the outline of the fish, we assumed a number of 1-4 semi-landmarks between lateral landmarks 3-9, 9-10, 10-11, 13-14, 14-15, 15-1, 19-20, 21-2, and ventral landmarks 2-3, 3-4, 13-14, 14-15 as sufficient to maximize the gain of morphological information (Fig.5).

Tab.2. Description of 21 anatomical landmarks and 23 sliding semi-landmarks on lateral scans as well as 16 landmarks and 4 semi-landmarks on ventral scans.

<b>Landmarks    Lateral view</b>	
<b>Number</b>	<b>Description</b>
1	anterior tip of premaxilla
2	posterior end of maxilla
3	anterior tip of snout
4	anterior point of orbit
5	most dorsal point of orbit
6	posterior point of orbit
7	most ventral point of orbit
8	center of orbit
9	origin of dorsalis 1
10	origin of dorsalis 2
11	dorsal insertion of caudalis
12	end of hypural plates, base of caudal fin rays
13	ventral insertion of caudalis
14	origin of analis
15	origin of ventralis
16	dorsal origin of pectoralis
17	ventral origin of pectoralis
18	dorsal origin of opercular cleft, most dorsal point of operculum
19	posterior edge of opercle
20	most anteroventral point of opercle
21	conjunction of hyomandibular and operculum
<b>Semilandmarks</b>	
22-25	dorsal outline of the head and trunk
26, 27	dorsal outline between origin of dorsalis 1 and 2
28-30	dorsal outline of caudal peduncle
31-33	ventral outline of caudal peduncle
34-36	ventral outline of abdomen
37-39	ventral outline of head
40, 41	posterior and ventral edge of opercle
42-44	posterior and ventral edge of cheek

Landmarks	Ventral view
Number	Description
1	symphysis of dentaries (lower jaw)
2, 15	posterior end of maxilla, left and right
3, 14	posterior edge of preoperculum, left and right
4, 13	posterior edge of operculum, left and right
5, 12	ventral origin of pectoralis, left and right
6, 11	origin of pelvic fin, left and right
7	confluence of opercular membrane, isthmus
8	centre of anus
9	origin of analis
10	ventral origin of caudalis
20	posterior tip of pelvic disc projected on the horizontal distance between landmark 8 and 10
Semilandmarks	
16-19	lateral outline of head, left and right

### 2.3 Geometric morphometric approach

By means of a Generalized Procrustes Analysis (GPA) and a Procrustes superimposition approach, performed in tpsRelw 1.42 (Rohlf, 2005), the landmark data were aligned. Accordingly, size (by scaling), position (by translation) and orientation (by rotation) of the digitalized objects were excluded from the actual shape (Bower et al., 2015; Mitteroecker & Gunz, 2009; Ramler et al., 2014; Ramler et al., 2016; Rohlf & Slice, 1990; Rohlf & Marcus, 1993). Hence, the generated landmark configurations (Procrustes shape coordinates) were standardized and could be used for morphometrical comparison of the stream and lake populations (JAD, TRA) and statistical analysis of the sexual dimorphism among those populations. Regression analysis of the mean shape differences was accomplished by an implemented software feature in tpsRegr 1.45 (Rohlf, 2016). In order to ease the detection and interpretation of shape variations, differences in body shape were displayed by thin-plate spline deformation grids, based on an image-alignment-interpolation-algorithm, and linearly

extrapolated by a factor 2 (Gunz & Mitteroecker, 2013; Mitteroecker & Gunz, 2009; Ramler et al., 2015; Ramler et al., 2016).

Additionally, a principal component analysis (PCA), performed in PAST 2.17c and PAST 3.14 (Hammer et al., 2001), was applied to the Procrustes shape coordinates to further illustrate the morphological variance and shape differences between the investigated groups in a two dimensional morphospace (Aguirre & Bell, 2012; Bower et al., 2015; Ramler et al., 2014; Untersteggaber, 2013). Based on the within-group-regression of shape on centroid size, allometric effects, which are shape differences caused by variation of size, often closely aligned with the first principal component in shape space and therefore possibly influence the interpretation of morphological divergence between populations and sexes, were tested (Ramler et al., 2016; Mitteroecker et al., 2013). Permutation tests with 1000 permutations each, were used to assess significance for mean shape differences (level of significance  $\alpha = 0.05$ ).

### 3. Results

#### 3.1 Body size

A distinct difference in body size was detected between the stream (JAD) and the lake (TRA) populations, including males and females (Tab.3; Fig.6).

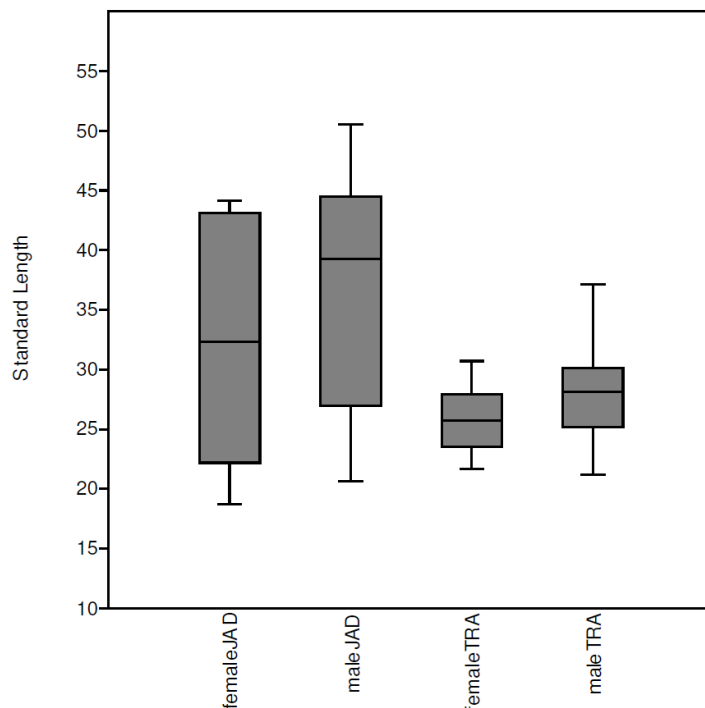


Fig.6. Box plot illustrating standard length variation in male and female individuals of investigated samples. JAD= Jadro River; TRA= Lake Trasimeno

Fish from TRA reached a total mean standard length (SL) of  $27.31 \pm 3.27$  mm, which was 20.73 % smaller than the total mean SL of  $34.45 \pm 9.32$  mm in JAD individuals. JAD females with a total mean SL of  $32.67 \pm 8.88$  mm were 7.29 % smaller than in JAD males ( $35.24 \pm 9.66$  mm). In TRA males, the total mean SL was  $28.08 \pm 3.45$  mm, while it reached



25.9 ± 2.40 mm in TRA females. Consequently, TRA females were 7.76 % smaller than TRA males.

Tab.3. Mean, minimum and maximum standard length (SL) as well as standard deviation (SD) of investigated samples of *Pomatoschistus canestrinii* are listed. JAD= Jadro River population, TRA= Lake Trasimeno population

Standard length SL (mm)	JAD	TRA
total mean SL	34.45	27.31
total SD	9.323	3.274
male mean SL	35.24	28.08
male SD	9.66	3.45
female mean SL	32.67	25.9
female SD	8.876	2.403
male minimum SL	20.66	21.21
male maximum SL	50.54	37.11
female minimum SL	18.75	21.69
female maximum SL	44.17	30.72

### 3.2 Geometric morphometrics and principal component analysis

I found significant shape differences (all  $p < 0.05$ , single  $p$ -values are mentioned below), equally expressed in males and females, between populations from the lotic (JAD) and lentic (TRA) habitat for both body planes (Tab.4; Fig.7; Fig.8). On the lateral side, JAD gobies showed a slender and more streamlined body shape with a clear reduction in body depth. Nevertheless, individuals from JAD had a deeper, slightly longer caudal peduncle resulting in a smoother transition from the abdominal region to the caudal peduncle. Additionally, JAD gobies had smaller heads with shorter head length, smaller eyes, a smaller postorbital region and a slightly longer distance between anterior insertion of the first and the second dorsal fins. In relation to the head size, the length of the upper jaw was increased in both sexes of JAD.

Tab.4. List of major morphological differences between JAD and TRA population.

	<b>JAD</b>	<b>TRA</b>
a) lateral		
body shape	streamlined	increased body depth
caudal peduncle	wide; slightly longer	narrow
head	small; shorter head length	large
eyes	small	large
postorbital region	small	extended
upper jaw	long	short
only males: pectoral fin base	short, more horizontally	long
b) ventral		
gape	narrow, short	wide, long
head	slender	broad
distance between insertion of anal fin and ventral base of caudal fin	long	short
only males: posterior edge of pelvic fin	strongly overlapped with centre of anus	congruent with centre of anus

The morphometrical comparison of JAD males and TRA males, revealed a slightly shorter and more horizontally positioned base of the pectoral fin in JAD individuals (Fig.7). On the ventral side, JAD gobies exhibited a narrower as well as shorter gape and a more slender head. Moreover, only small variation in fin positions arose. The ventral insertion of the pectoral fin of JAD males were placed more anterior, while the base of the fused pelvic fins tended to be more posterior comparing to TRA males. No crucial differences in the length of the base of the pelvic fin as well as the distances between the isthmus and the origins of the pelvic fin, were recorded. Especially in JAD males but also in JAD females, the pelvic disc tend to overlap with the centre of the anus, resulting in a shorter distance between posterior edge of the pelvic fin and anterior insertion of the anal fin, while the posterior edge of the pelvic disc and the centre of the anus were almost congruent in TRA males and did not even reach the anus in TRA females. The distance between origin of anal fin and ventral insertion of the caudal fin was longer in JAD individuals, regardless of sex.

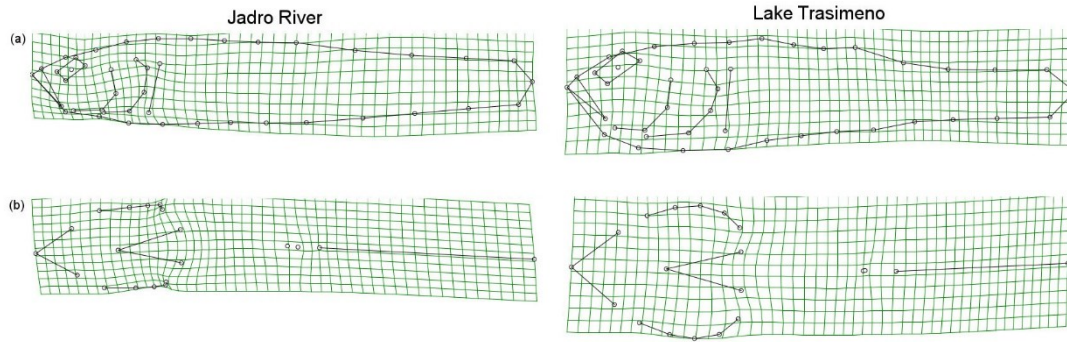


Fig.7. Deformation grids of investigated male individuals of Jadro River and Lake Trasimeno populations. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.001$ ; (b) ventral,  $p= 0.001$

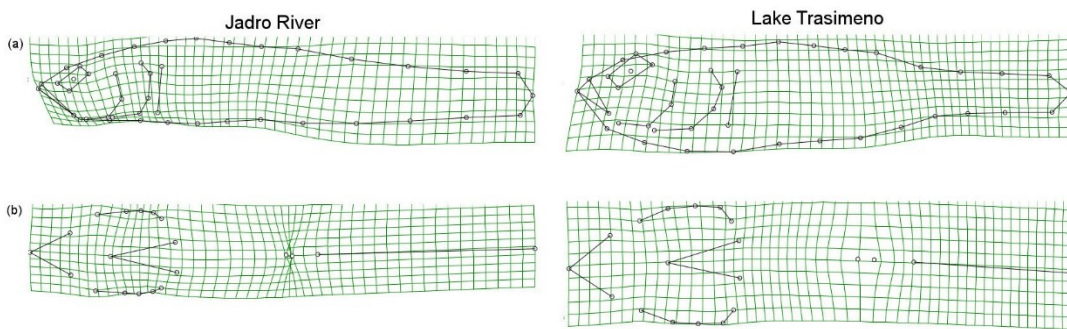


Fig.8. Deformation grids of investigated female individuals of Jadro River and Lake Trasimeno populations. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.001$ ; (b) ventral,  $p= 0.002$

Focusing on the sexual dimorphism, a morphological divergence of body shape was recorded. Basically, shape differences between males and females were equally expressed in both populations (Fig.9; Fig.10). JAD males had larger heads but smaller eyes and thicker caudal peduncles than JAD females. The position of the mouth was more superior and they had longer bases of pectoral fins, which were placed more horizontally. Referring to the ventral plane of the fish, male individuals of JAD had broader heads with wider gapes. A slightly longer distance between the origins of the pelvic fin was detected. The posterior edge of the pelvic fin strongly overlapped with the centre of the anus. This trend was not confirmed in JAD females.

Similar tendencies concerning the morphology of the fish, such as larger heads, smaller eyes and distinctly wider caudal peduncles were seen in case of TRA males. The upper jaw of TRA males tended to be not just more superior but also longer than the upper jaw of TRA females. Again, the base of the pectoral fin was longer in TRA males than in females, even though no evidence for a difference in the orientation was found. Additionally, males of both populations had more detached anterior fin bases of dorsal fin 1 and dorsal fin 2, leading to a dorsally depressed outline-appearance in the deformation grids. In correspondence with the male samples of JAD, TRA males showed broader heads with wider gapes and a slightly shorter distance between centre of anus and origin of the anal fin.

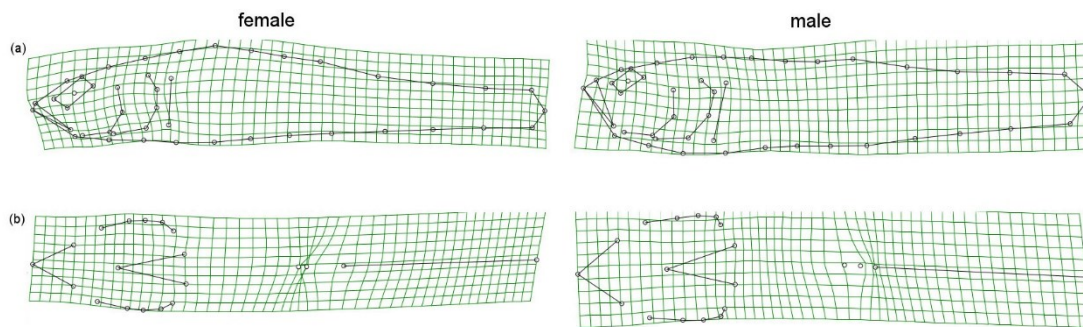


Fig.9. Deformation grids of female and male individuals of Jadro River population. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p=0.142$ ; (b) ventral,  $p=0.347$

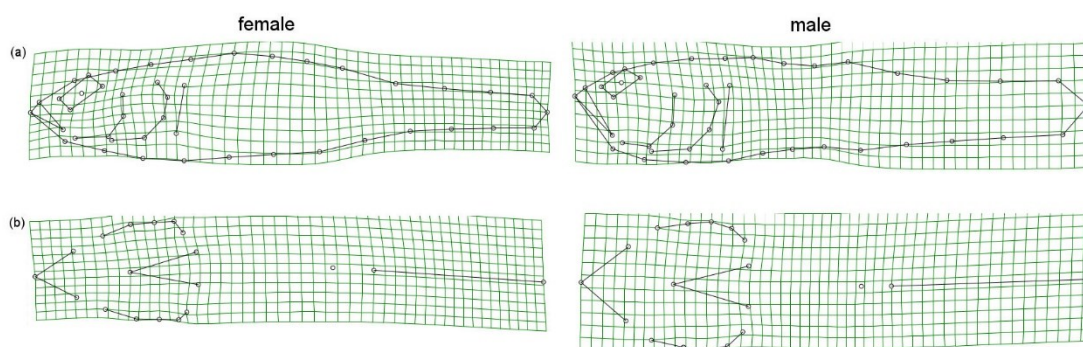


Fig.10. Deformation grids of female and male individuals of Lake Trasimeno population. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p=0.001$ ; (b) ventral,  $p=0.001$

Separately comparing lateral shape data of males (JADm vs. TRAm) and females (JADf vs. TRAf), a  $p$ -value of 0.001, was calculated by performing permutation tests with 1000 random permutations each. Investigating divergence in shape, which can be explained by a sexual dimorphism, a  $p$ -value of 0.001 was recorded in the TRA population, while it reached 0.142 in JAD samples. Consequently, a sexual dimorphism in body shape was considered non-significant in JAD individuals. Permutation tests on the ventral shape data revealed significant shape differences between JAD males and TRA males ( $p= 0.001$ ) as well as between JAD females and TRA females ( $p= 0.002$ ). Again, ventral shape variations were significant between males and females of TRA population ( $p= 0.001$ ), whereas male and female individuals of JAD did not differ significantly ( $p= 0.347$ ).

Allometric effects, were not considered relevant across investigated samples. This assumption was confirmed by the computed deformation grids using the within-group-regression approach treating centroid size (CS) as independent variable (supplementary data, Fig.13 – Fig.16).

The principal component analysis (PCA), illustrating data points (relative warp scores) of male and female individuals of both investigated populations, revealed a high variance in the JAD samples (Fig.11; Fig.12). 95% equal frequency ellipses indicated that female individuals of JAD had the highest variance along most of the principal components, except of PC1 resulting from lateral data (Fig.11). Generally, the highest values of shape variance across all groups included in this study, were observed in the ventral plane, indicating a high phenotypic plasticity of the ventral body shape (Fig.12).

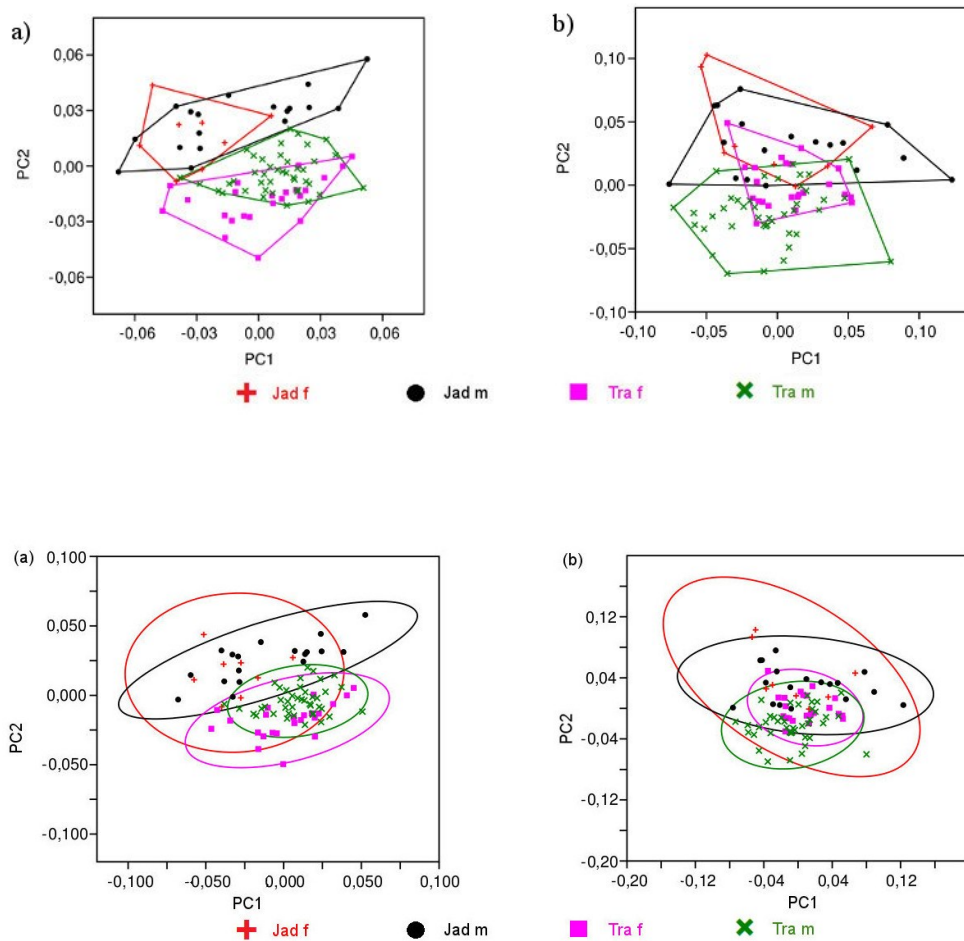


Fig.11. Principal Component Analysis (PCA) of the body shape of *Pomatoschistus canestrinii* comparing female and male individuals of a native and introduced population in Jadro River and Lake Trasimeno. Convex hulls are shown above. Ellipses indicate 95% equal frequency for females and males in both populations (below). PC1 and PC2 are plotted. Females from Jadro River (Jad f) = red plus; males from Jadro River (Jad m) = black dots; females from Lake Trasimeno (Tra f) = pink squares; males from Lake Trasimeno (Tra m) = green crosses; (a) lateral; (b) ventral

Along PC1 resulting from lateral data, variations in body shape, head size, position of mouth and eye as well as length of pectoral fin base was found. Shape differences along PC2 were restricted on body depth, size of the eye, head size, position of anterior dorsal and anal fin bases and depth of caudal peduncle. PC3 included variations in head size, length of the upper jaw, length of pectoral fin base and depth of caudal peduncle as well as differences in the positions of the origin of pelvic fin and the anterior fin base of the anal fin.

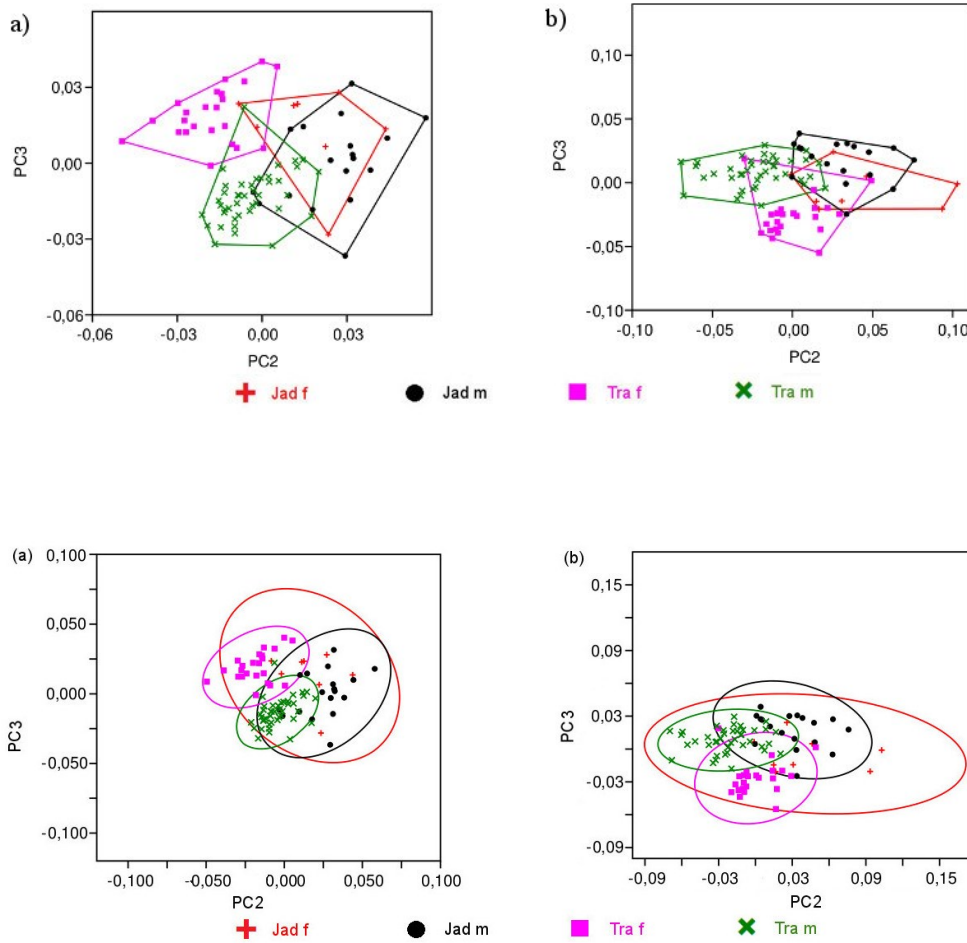


Fig.12. Principal Component Analysis (PCA) of the body shape of *Pomatoschistus canestrinii*. Convex hulls and 95% equal frequency ellipses are illustrated as Fig.11. PC2 and PC3 are plotted. Females from Jadro River (Jad f) = red plus; males from Jadro River (Jad m) = black dots; females from Lake Trasimeno (Tra f) = pink squares; males from Lake Trasimeno (Tra m) = green crosses; (a) lateral; (b) ventral

The plotted values along PC1 ranged from -0.069 to 0.053 in JAD males, from -0.057 to 0.006 in JAD females, from -0.038 to 0.051 in TRA males and from -0.046 to 0.045 in TRA females. According to PC2, the values ranged from -0.003 to 0.060 in JAD males, from -0.008 to 0.044 in JAD females, from -0.022 to 0.020 in TRA males and from -0.050 to 0.005 in TRA females. The corresponding PC3, reached values from -0.040 to 0.032 in JAD males, from -0.028 to 0.027 in JAD females, from -0.033 to 0.032 in TRA males and from -0.001 to 0.040 in TRA females (Fig.11). Shape variations along PC1, resulting from ventral data, included a



strong signal of preservation artefacts, such as lateral bending. Hence, PC1 had limited relevance for describing morphological differences in shape. Therefore, I primarily focused on PC2 and PC3. PC2 revealed differences in head width, gape width, and distance between anterior base of anal fin and ventral insertion of the caudal fin, while PC3 was restricted on differences in the position of the posterior edge of the pelvic fin, successively overlapping the centre of the anus from negative to positive, and differences in the position of the centre of the anus and the anterior insertion of anal fin. In reference to PC1, PC2 and PC3 of the ventral side, JAD males varied between -0.075 and 0.123 (PC1), 0.000 and 0.076 (PC2), -0.024 and 0.040 (PC3). JAD females varied between -0.054 and 0.067 (PC1), -0.001 and 0.103 (PC2), -0.021 and 0.024 (PC3). Focusing on TRA males, the values ranged from -0.073 to 0.080 (PC1), from -0.070 to 0.021 (PC2) and from -0.017 to 0.029 (PC3), while in TRA females values between -0.035 and 0.052 (PC1), -0.020 and 0.049 (PC2), -0.055 and 0.018 (PC3) were recorded (Fig.12). On the lateral side, the percentage of total shape variance explained by PC1 was 33.37% and dropped on 19.12% and 16.52% of total variance in PC2 and PC3. PC1, PC2 and PC3 computed by using ventral shape data, explained 35.00%, 27.25% and 11.46% of total variance in ventral shape data. Consequently, 69.00% and 73.71% of lateral and ventral shape variance could be explained by the first three PCs (Tab.5).

Tab.5. Single values and percent explained for relative warps. Lateral and ventral data is listed.

PC No.	Lateral			Ventral		
	SV	%	Cum %	SV	%	Cum %
1	0.24976	33.37%	33.37%	0.35056	35.00%	35.00%
2	0.18904	19.12%	52.48%	0.30933	27.25%	62.26%
3	0.17573	16.52%	69.00%	0.20054	11.46%	73.71%
4	0.11590	7.19%	76.19%	0.13466	5.16%	78.88%
5	0.08466	3.83%	80.02%	0.12626	4.54%	83.42%
6	0.07702	3.17%	83.20%	0.11604	3.84%	87.26%
7	0.06756	2.44%	85.64%	0.10375	3.07%	90.32%
8	0.06650	2.37%	88.01%	0.07852	1.76%	92.08%
9	0.06094	1.99%	89.99%	0.07302	1.52%	93.60%
10	0.05063	1.37%	91.36%	0.06091	1.06%	94.65%



## 4. Discussion

### 4.1 Morphological divergence and test of *a priori* predictions of swimming performance model

In the present study I analysed differences in body shape of two populations of *P. canestrinii* from habitats with divergent hydrodynamic conditions including running and still water. Distinct patterns of morphological differentiation in size and shape of *P. canestrinii*, could be demonstrated across divergent habitats. Additionally phenotypic differences were highly consistent for both sexes (Fig.7; Fig.8). Comparing the investigated lotic population from Jadro River and the lentic population from Lake Trasimeno, an introduction of *P. canestrinii* in a new environment associated with sharply varying ambient factors such as flow regime, were possibly responsible for recorded phenotypic differences. Phenotypic plasticity is expected to play an important role leading to such rapid morphological changes, which must have occurred over a very short period of time and a small number of generations as has been demonstrated for several fish species (Blanquart et. al., 2013; Cureton & Bourghton, 2014). In Lake Trasimeno, *P. canestrinii* was detected for the first time in 1996 (Freyhof, 1998).

I found a distinct decrease in the body size of the lake gobies, regardless of sex, but a low variance in size. Specimens from the river were larger but variance in size was distinctly higher in males and females (Fig.6). Beside to more streamlined (fusiform) bodies in stream specimens and an increase in body depth of lake specimens, morphological differences were mainly related to the head region, trophic structures including mouth, eye and postorbital structures as well as the base of the pectoral fin and the caudal peduncle (Fig.7; Fig.8). Very likely, the morphological changes were directly related to feeding and swimming. Lake gobies had a less streamlined body, larger and wider heads, larger eyes, shorter upper jaws but wider gapes, larger postorbital regions, longer, slightly more vertically positioned bases of the

pectoral fin and developed narrower and slightly shorter caudal peduncles. Consequently, morphotypes clearly differed between river and lake populations. Morphological traits associated with swimming under divergent water flow conditions appears to be affected by strong selection pressure in *P. canestrinii* as has been shown for species from various fish families (Berner et al., 2008; Bower & Piller, 2015; Cureton & Bourghton, 2014; Foster et al., 2015; Langerhans, 2008).

The present results suggest that the swimming performance model established by Foster et al. (2015) is also applicable to a large extent for the benthic *P. canestrinii*. *A priori* predictions of shape variations derived from the pelagic atherinopsid *C. jordani* (Foster et al., 2015) were consistent concerning a more streamlined body, a slightly longer caudal peduncle and a more inferior mouth position and in stream specimens of *P. canestrinii*. Accordingly, lake individuals developed a deeper body shape, a reduced length of the caudal peduncle and a more superior mouth. Again, these observations were congruent with the swimming performance model of Foster et al. (2015). However, analysis of the goodeid *G. atripinnis*, revealed a shortened head as well as an elongated and deeper caudal peduncle in lentic fishes, which is divergent to the comparison of *C. jordani* populations (Foster et al, 2015). No clear biological signal of more anterior positioned dorsal and anal fins could be detected in the lake population, even though a small variation in the position of pectoral and pelvic fin was existent in males. Nevertheless, it could be predicted that in benthic fish species morphotypes generally fit with the morphological changes caused by habitat associations, even though functional trade off in steady-unsteady swimming in benthic fishes were possibly weak concerning certain morphological attributes like the positions of dorsal, ventral and anal fins (Bower & Piller, 2015; Langerhans, 2008). Those, not directly corresponding, predictions can be explained by species' natural history, which is in this case a benthic mode of life (Langerhans & Reznick, 2010). The impact of divergent flow regimes on the morphology of various fish species inhabiting different microhabitats is still debated.

Generally, adaptation to divergent environmental factors including varying selection pressure forms the base for morphological differentiation. Specific expressions of some phenotypic traits, usually improving swimming performance, can be masked by morphological consequences of other crucial biotic and abiotic ecological factors such as temperature, salinity, light, food consumption, predatory pressure, competition etc. influencing shape (Defaveri & Merilä, 2013; Langerhans & Reznick, 2010; Ramler et al., 2014; Ramler et al., 2016). Observed differences in size and shape of the head as well as associated structures like the eyes, mouth and the opercular region, which were not congruent with the swimming performance model, might reflect the result of divergent foraging strategies and differences in preferred diet, including divergent prey and locomotion activities (Ahnelt et al., 2015; Berner et al., 2008; Ramler et al. 2016; Van Rijssel et al., 2014; Stauffer & Van Snik Gray, 2004). Generally, it has been shown that larger eyes tend to increase visual acuity affecting feeding and predator avoidance (Ramler et al. 2016).

Comparing lotic and lentic population, narrower and slightly shorter caudal peduncles were evident in lake specimens. Usually, the shape of the caudal peduncle is strongly affected by habitat complexity and high predatory pressure favouring improved maneuverability and high acceleration by means of posteriorly deeper bodies with deeper caudal peduncles (Langerhans & Reznick, 2010; Ramler et al. 2016; Seehausen & Wagner, 2014). A potential absence of predators in Lake Trasimeno feeding on *P. canestrinii* can explain a reduced depth of the caudal peduncle in the investigated lake gobies. The variance in body size and shape was increased in the river specimens. This can further indicate predominant habitat heterogeneity in Jadro River resulting in heterogenic local conditions. In this case a high variability and flexibility of essential morphological attributes might enable rapid adaptation to a structurally complex habitats (Langerhans & Reznick, 2010). Nevertheless, Berner et al. (2008) suggested that phenotypic (co)variances are generally lower in stream populations than in lake populations concerning foraging traits.

Beside to phenotypic plasticity and local adaptation to divergent habitats, founder effect and genetic drift seem to induce morphological differentiation across populations as well (Langerhans, 2008). Alternative explanation for a smaller variance in body size and shape in the lake population can be deducted from a loss of genetic variation caused by introduction to a new environment (Ramler et al., 2014). Due to a relatively low level of migration, gene flow as possible contributor to morphological divergence was not considered relevant for the gobiid fish species *P. canestrinii*. In order to test for genetic and morphological divergence, a combination of phylogenetic and morphometric analysis are highly recommended for upcoming projects. Moreover, common garden experiments can be conducted to exclude other driving factors from the variable of interest, which is not the case in investigations of wild specimens being morphologically influenced by a multitude of environmental parameters (Ramler et al., 2014; DeFaveri & Merilä, 2013). Regarding to ecomorphological studies, one of the biggest issues in the past was the lack of standardized framework, which is essential for establishing models providing robust *a priori* predictions by using comparative data (Langerhans, 2008; Langerhans & Reznick, 2010). Hence, further investigations on evolutionary processes promoting divergent morphotypes have to be conducted in order to define solid models predicting body shape variations for a broad spectrum of fish species. This knowledge might help predicting consequences of anthropogenic influenced environments resulting from emissions, eutrophication and building constructions (Ahnelt et al., 2015; Van Rijssel et al., 2016; Cureton & Bourghton, 2014). Additionally, a better understanding of major forces driving body shape and their impact on the locomotory system can facilitate innovations in biomechanics.

## 4.2 Sexual dimorphism

The observed sexual dimorphism of *P. canestrinii* comprised differences in size and shape (Fig.6; Fig.9; Fig.10). Males of both populations were larger, had larger and wider heads but smaller eyes, more superior mouths with wider gapes, deeper caudal peduncles, longer bases of pectoral fins and longer suction discs than females. In addition to wider gapes, the upper jaw in the lake males was longer than in females. A deeper caudal peduncle and a longer suction disc in males was also described by Miller (2004),

Reproductive behaviour and divergent modes of foraging likely lead to a sex-related variation in pectoral fin base length, head size as well as proportions of trophic structures, including mouth, eyes and opercular region (Berner et al., 2008; Kerschbaum et al., 2014; Ramler et al., 2016). Larger heads, wider gapes and longer bases of pectoral fins of the males seemingly support nest preparation including the cleaning of a sheltered area and parental care including protecting, cleaning and fanning of the egg clutch (Tavolga, 1954). It is tempting to assume that in river habitats fanning is less important. This assumption was supported by a longer pectoral fin base length in lake males. However, the pectoral fin base was also elongated in river males compared to river females.

Feeding behaviour and preferred prey coincide with shape differentiation of body, head and trophic structures (Berner et al., 2008). Interestingly, the size of the eyes was decreased in males, even though they developed larger heads. Ahnelt et al. (2016) suggested that larger eyes in cichlids are often linked to a benthic food preference and might give advantages in predator avoidance. An increase in eye size among female individuals of *P. canestrinii* could indicate a more benthic foraging mode and an increased need of predator avoidance. A possibly different feeding mode in males is indicated by a more superior positioned mouth and a longer upper jaw which potentially enables the access to alternative food sources located in open water. The sex-related difference in the upper jaw length was more distinct in lake specimens, which may

indicate that, in case of Lake Trasimeno, this morphological feature was affected by stronger selection pressure.

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## Supplementary data

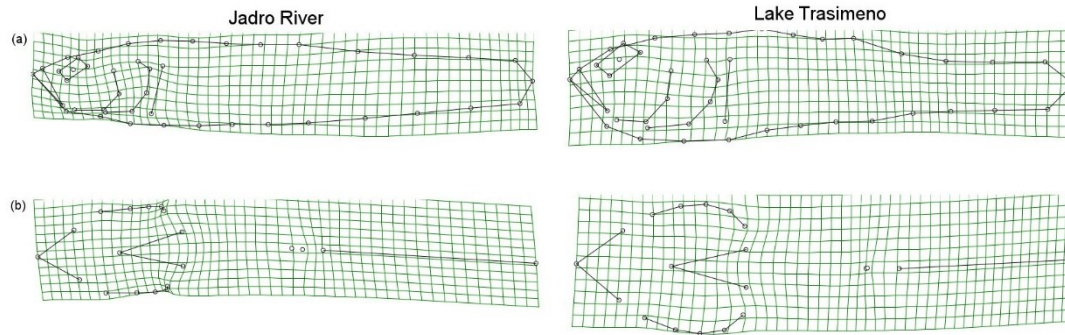


Fig.13. Allometric analysis using within group regression approach. Morphometrical comparison of male individuals of Jadro River and Lake Trasimeno populations. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.001$ ; (b) ventral,  $p= 0.001$

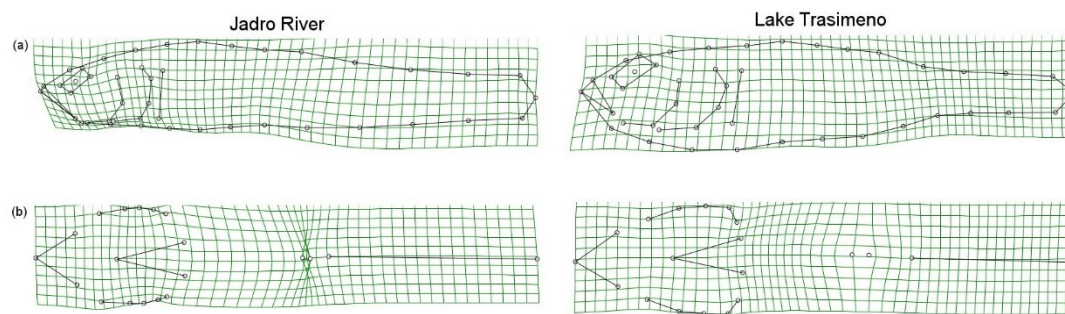


Fig.14. Allometric analysis using within group regression approach. Morphometrical comparison of female individuals of Jadro River and Lake Trasimeno populations. Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.001$ ; (b) ventral,  $p= 0.001$

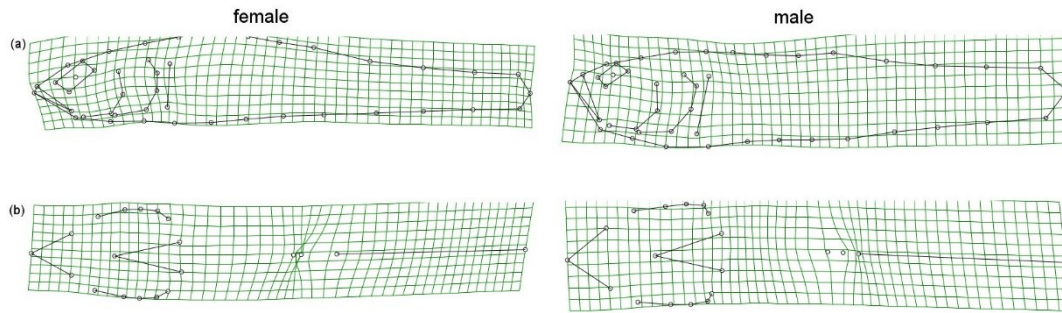


Fig.15. Allometric analysis using within group regression approach. Morphometrical comparison of female and male individuals from Jadro River (JAD). Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.270$ ; (b) ventral,  $p= 0.015$

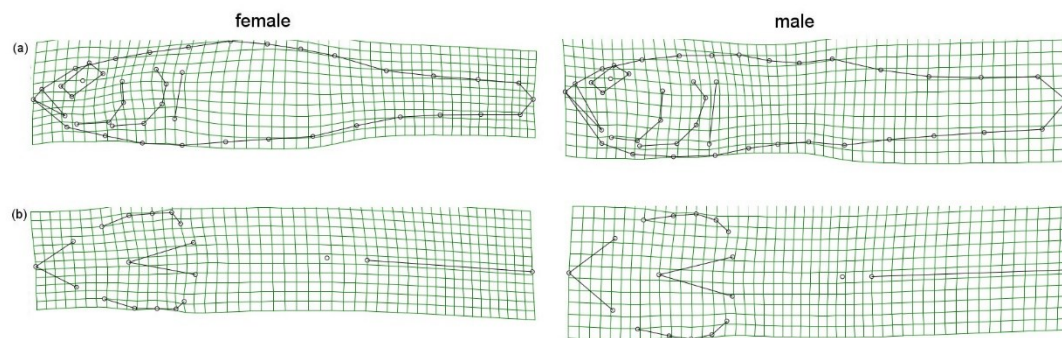


Fig.16. Allometric analysis using within group regression approach. Morphometrical comparison of female and male individuals from Lake Trasimeno (TRA). Shape differences were linearly extrapolated by factor 2. (a) lateral,  $p= 0.001$ ; (b) ventral,  $p= 0.001$

Tab.6. Single values and percent explained for relative warps. Lateral and ventral data is mentioned.

Lateral male			
PC No.	SV	%	Cum%
1	0.19384	36.53	36.53
2	0.15647	23.80	60.33
3	0.08874	7.66	67.99

Lateral female			
PC No.	SV	%	Cum%
1	0.16178	41.27	41.27
2	0.11431	20.61	61.87
3	0.08024	10.15	72.03

Ventral male			
PC No.	SV	%	Cum%
1	0.31193	42.78	42.78
2	0.24518	26.43	69.22
3	0.11895	6.22	75.44

Ventral female			
PC No.	SV	%	Cum%
1	0.20048	43.35	43.35
2	0.13258	18.96	62.31
3	0.10074	10.95	73.26

Lateral JAD			
PC No.	SV	%	Cum%
1	0.17121	46.05	46.05
2	0.09878	15.33	61.38
3	0.08750	12.03	73.41

Lateral TRA			
PC No.	SV	%	Cum%
1	0.17023	30.94	30.94
2	0.15364	25.20	56.14
3	0.09553	9.74	65.89

Ventral JAD			
PC No.	SV	%	Cum%
1	0.26103	54.07	54.07
2	0.14956	17.75	71.82
3	0.09292	6.85	78.67

Ventral TRA			
PC No.	SV	%	Cum%
1	0.56248	68.72	68.72
2	0.23617	12.12	80.84
3	0.16640	6.01	86.85

## Lateral

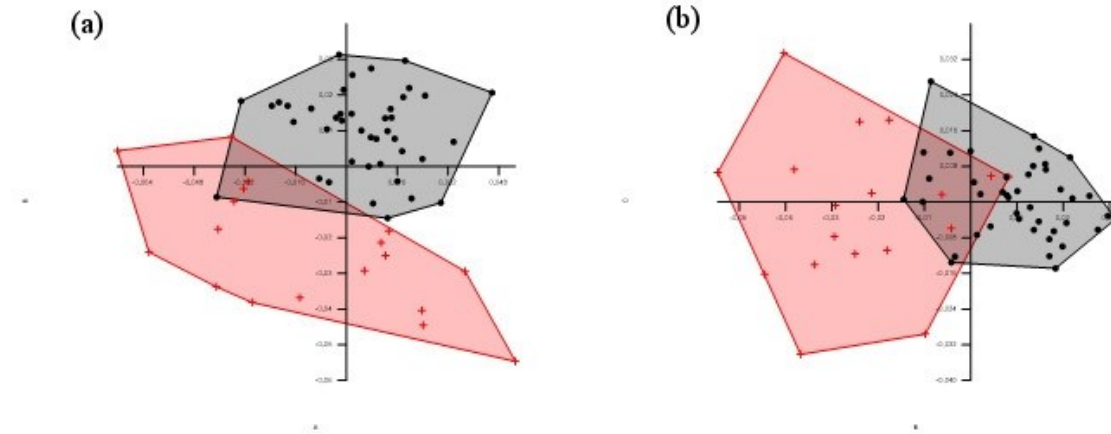


Fig.17. Principal component analysis (PCA) of the lateral body shape of *Pomatoschistus canestrinii* comparing males from Jadro River (red crosses) and Lake Trasimeno (black dots). (a): PC1 and PC2; (b): PC2 and PC3 are plotted.

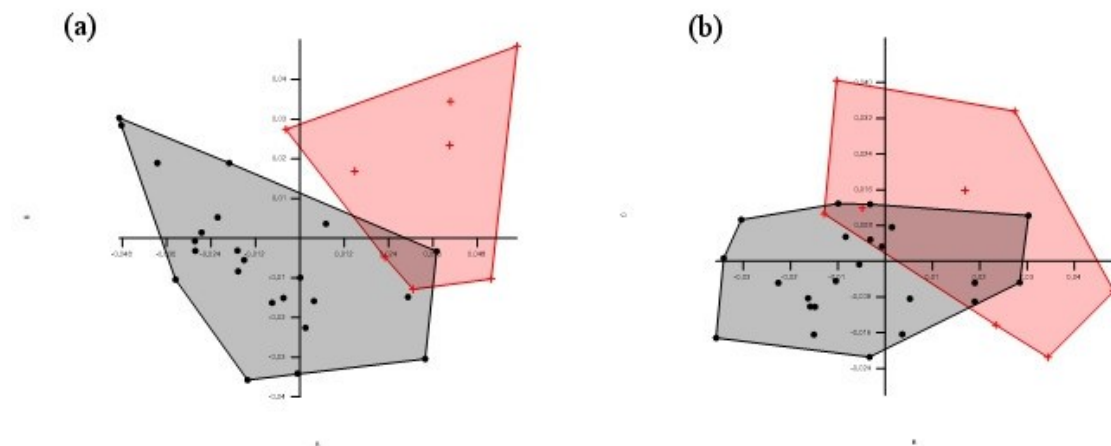


Fig.18. Principal component analysis (PCA) of the lateral body shape of *Pomatoschistus canestrinii* comparing females from Jadro River (red crosses) and Lake Trasimeno (black dots). (a): PC1 and PC2; (b): PC2 and PC3 are plotted.

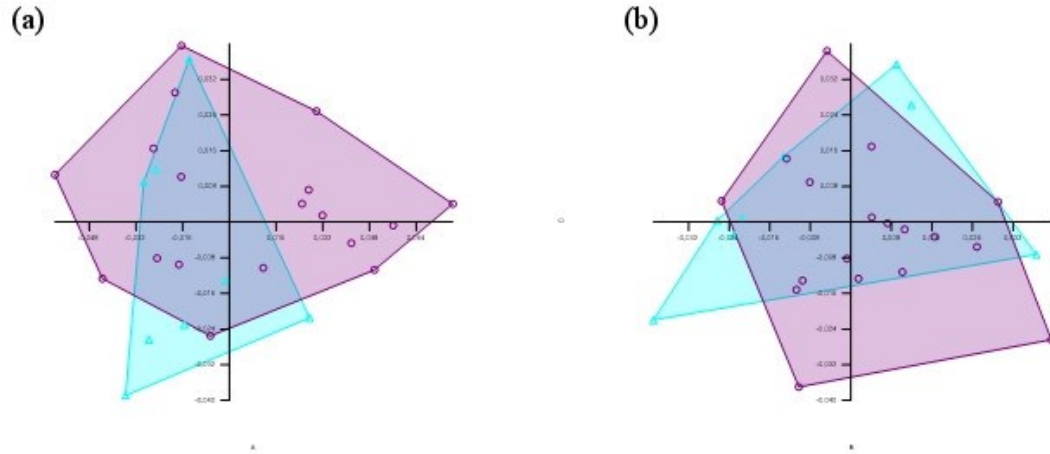


Fig.19. Principal component analysis (PCA) illustrating lateral body shape data of *Pomatoschistus canestrinii* comparing female (cyan triangle) and male (purple circles) individuals from Jadro River. (a): PC1 and PC2; (b): PC2 and PC3 are plotted.

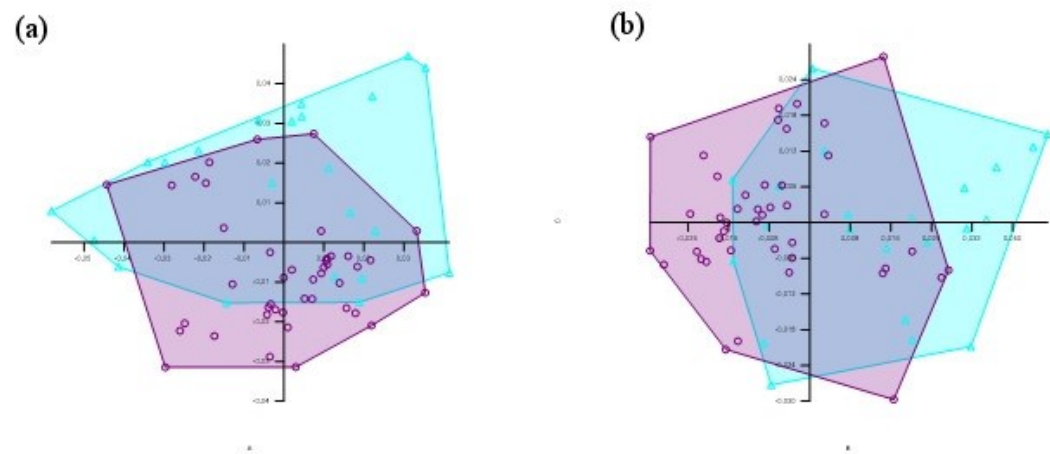


Fig.20. Principal component analysis (PCA) illustrating lateral body shape data of *Pomatoschistus canestrinii* comparing female (cyan triangle) and male (purple circles) individuals from Lake Trasimeno. (a): PC1 and PC2; (b): PC2 and PC3 are plotted.



## Ventral

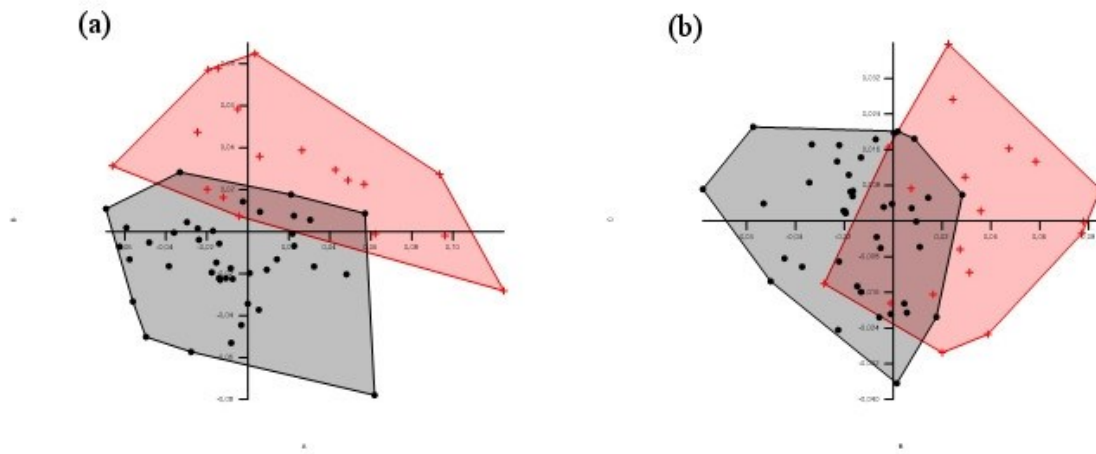


Fig.21. Principal component analysis (PCA) of the ventral body shape of *Pomatoschistus canestrinii* comparing males from Jadro River (red crosses) and Lake Trasimeno (black dots). (a): PC1 and PC2; (b): PC2 and PC3 are plotted.

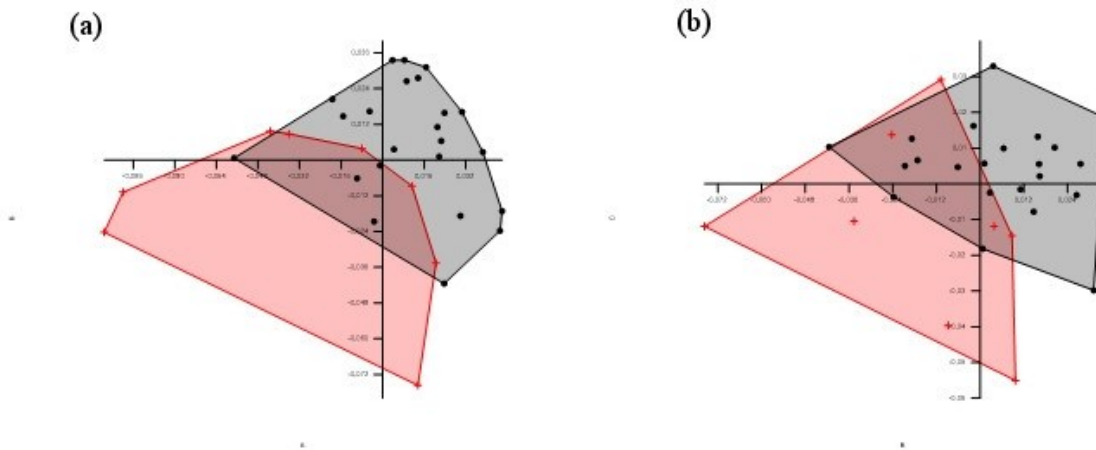


Fig.22. Principal component analysis (PCA) of the ventral body shape of *Pomatoschistus canestrinii* comparing females from Jadro River (red crosses) and Lake Trasimeno (black dots). (a): PC1 and PC2; (b): PC2 and PC3 are plotted.



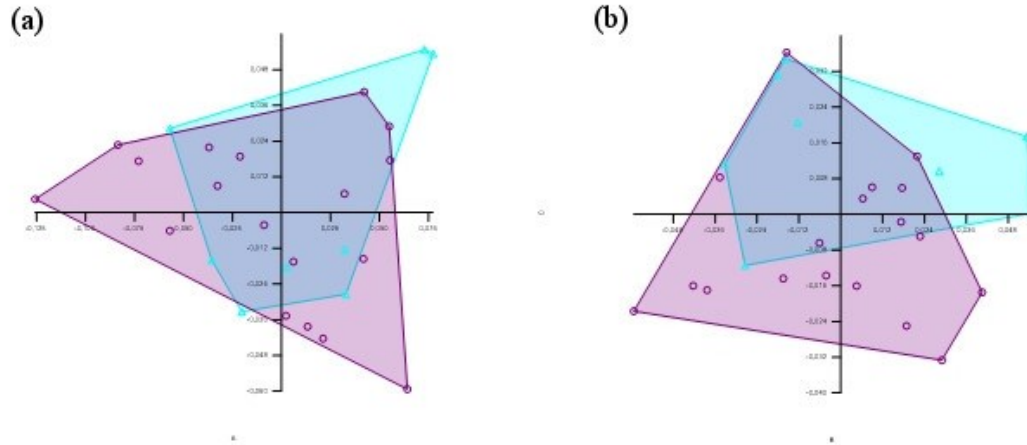


Fig.23. Principal component analysis (PCA) illustrating ventral body shape data of *Pomatoschistus canestrinii* comparing female (cyan triangle) and male (purple circles) individuals from Jadro River. (a): PC1 and PC2; (b): PC2 and PC3 are plotted.

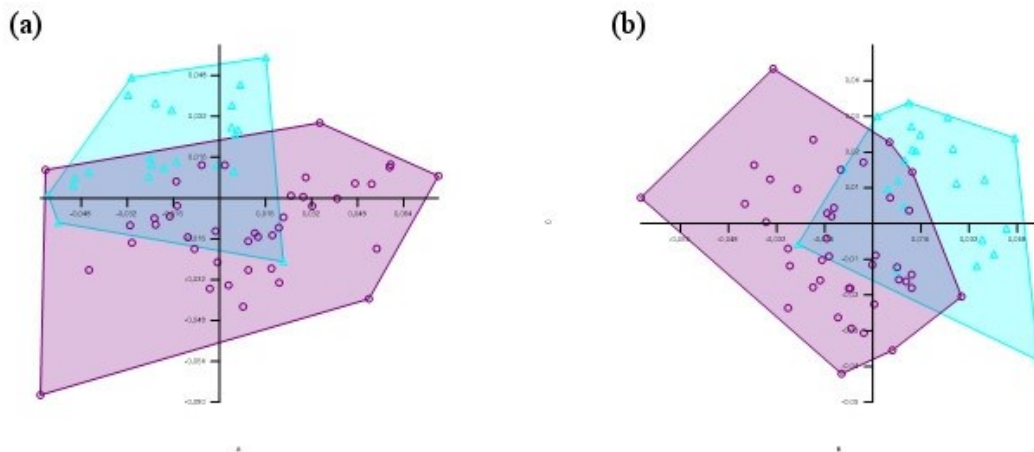


Fig.24. Principal component analysis (PCA) illustrating ventral body shape data of *Pomatoschistus canestrinii* comparing female (cyan triangle) and male (purple circles) individuals from Lake Trasimeno. (a): PC1 and PC2; (b): PC2 and PC3 are plotted.