The ecology of sexual dimorphism in size and shape of the freshwater blenny Salaria fluviatilis

Martin Laporte, Patrick Berrebi, Julien Claude, Dolors Vinyoles, Quim Pou-Rovira, Jean-Claude Raymond, Pierre Magnan

▶ **To cite this version:**

Martin Laporte, Patrick Berrebi, Julien Claude, Dolors Vinyoles, Quim Pou-Rovira, et al.. The ecology of sexual dimorphism in size and shape of the freshwater blenny Salaria fluviatilis. Current zoology, 2017, 64 (2), pp.183-191. 10.1093/cz/zox043 . hal-01920135

HAL Id: hal-01920135
https://hal.umontpellier.fr/hal-01920135v1

Submitted on 1 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Current Zoology, 2018, 64(2), 183–191
doi: 10.1093/cz/zox043
Advance Access Publication Date: 30 June 2017
Article

OXFORD

Article

The ecology of sexual dimorphism in size and shape of the freshwater blenny *Salaria fluviatilis*

Martin Laporte[a,b,*], Patrick Berrebi[a], Julien Claude[a], Dolors Vinyoles[c], Quim Pou-Rovira[d], Jean-Claude Raymond[e], and Pierre Magnan[f]

[a]Institut des Sciences de l'Évolution de Montpellier (ISEM), UMR 5554 UM2-CNRS-IRD, Université de Montpellier II, CC065, Place E. Bataillon, Montpellier, Cedex 5 34095, France, [b]Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Québec City, Canada G1V 0A6, [c]Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Avda. Diagonal 643, Barcelona 08028, Spain, [d]Sorelló, estudis al medi aquàtic, Plaça St. Pere 15, Baixos, 17007 Girona, Spain, [e]Office National de l'Eau et des Milieux Aquatiques (ONEMA), Unité Spécialisée Milieux Lacustres, Pisciculture de Rives, 13, Quai de Rives, Thonon-les-Bains 74200, France, and [f]Centre de recherche sur les interactions bassins versants-écosystèmes aquatiques (RIVE), Université du Québec à Trois-Rivières, C. P. 500, Trois-Rivières, Québec City, Canada G9A 5H7

*Address correspondence to Martin Laporte. E-mail: uni.mlaporte@gmail.com.

Handling editor: Zhi-Yun Jia

Received on 7 February 2017; accepted on 27 June 2017

Abstract

Sexual selection is considered the major cause of sexual dimorphism, but recent observations suggest that natural selection may play a more important role in the evolution of sex differentiation than previously recognized. Therefore, studying the trade-offs between natural selection and sexual selection is crucial to a better understanding of the ecology underlying the evolution of sexual dimorphism. The freshwater blenny *Salaria fluviatilis*, a fish inhabiting lakes and rivers around the Mediterranean Sea, displays strong sexual dimorphism in size, shape, and behavior (i.e., larger body and head size for males and higher swimming requirements for females during the reproductive period). We tested for differences in sexual dimorphism in size and shape between the populations from lake and river habitats with the goal of identifying the trade-offs between natural and sexual selection that underlie variations in sexual dimorphism in this species. Our results show i) differences in sexual size dimorphism (SSizeD) in accordance to Rensch's rule (i.e., larger individuals in rivers associated with higher SSizeD), and ii) a decrease in shape differentiation between males and females in lake populations. Together, this suggests that the different environmental conditions between lake and river habitats (e.g., resource limitations, predation pressure, water velocity) affect the relative importance of sexual selection in the display of sexual dimorphism within the species. This study highlights the importance of considering the environmental conditions to which populations are exposed to better understand the ecology underlying the evolution of sexual dimorphism.

Key words: evolutionary ecology, geometric morphometrics, phenotype–environment interaction, Rensch's rule, sexual dimorphism.

Sexual dimorphism is a common feature found in most animal phyla (Shine 1989). It is thought to have evolved through 2 evolutionary mechanisms: i) sexual selection: competition among members of a same sex that results in variable reproductive success among individuals and ii) natural selection: variations in reproductive and survival success associated with a set of environmental conditions

© The Author (2017). Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

(Slatkin 1984; Shine 1989; Berns 2013). Sexual selection is considered to be the major cause of sexual dimorphism, but recent observations suggest that natural selection may play a more important role in the evolution of sex differentiation than previously recognized (Punzalan and Hosken 2010). For instance, the presence of a trade-off between natural selection and sexual selection could explain sexual dimorphism in the Hawaiian damselfly (Cooper 2010), where sexual dimorphism in body color is strongly correlated with solar radiation levels. At higher elevations, the species shows sexual monomorphism (i.e., red color) that is explained by the superior antioxidant ability of the red pigmentation, which is selected because it can protect from UV damage; at lower elevations, the environmental selective pressure decreases with the decrease of UV radiation and green females are observed. However, males remain red at lower elevations, and a sexual dimorphism is observed and considered to be produced by female preference (Cooper 2010). The importance of such interactions between sexual and natural selections is poorly understood in the wild. Studying the environmental factors that could impact sex differentiation is thus crucial to a better understanding of the ecology underlying the evolution of sexual dimorphism.

Rensch's rule is observed in several taxonomic groups, including amphibians, birds, fish, insects, mammals, and reptiles (Fairbairn 1997, 2005; Colwell 2000; Kratochvill and Frynta 2002; Johansson et al. 2005; Blanckenhorn et al. 2006). This rule states that sexual size dimorphism (SSizeD) increases with body size when males are larger than females, but decreases when females are larger than males (Rensch 1950; Figure 1A). This could be explained by a response of females to sexual selection on males (Fairbairn 1997, 2005): when the average size of males increases or decreases, the average size of females will similarly increase or decrease to a lesser degree. Another hypothesis is that a resource-limited environment will favor smaller body sizes in both sexes and will limit male investment in secondary sexual traits, such as body size (Fairbairn 1997, 2005). In any case, it is expected that in a species where males are larger than females, populations with a higher average size will display a stronger SSizeD.

In addition to SSizeD, sexual shape dimorphism (SShapeD) is also important to consider because it can affect various functions during an individual's lifetime (e.g., feeding, mating, parental care) (Berns 2013). This is particularly true for fish shape, which has a strong influence on locomotion and foraging but may be involved in other functions related to sex and could generate different trade-offs in performance (Webb 1982, 1984; Langerhans and Reznick 2010; Willacker et al. 2010). As an example, a more slender fish shape is typically found in habitats requiring greater swimming activities (e.g., more disperse prey, higher water velocity) (Proulx and Magnan 2004; Bernatchez et al. 2016; Laporte et al. 2016a, 2016b). Therefore, it is likely that SShapeD differentiation among populations is associated with habitat when swimming requirements differ between the sexes between 2 habitats. In such circumstances, SShapeD could be weak or absent in the environment that does not produce differences in swimming needs between sexes and increase in the environment where the difference in swimming needs increases between males and females.

The freshwater blenny *Salaria fluviatilis* is one of the rare freshwater representatives of the Blenniidae (Zander 1972; Briggs 2010). It is a vulnerable benthic fish endemic to tributaries of the Mediterranean and Black seas (Kottelat and Freyhof 2007; Keith et al. 2011; Laporte et al. 2013). The species shows sexual dimorphism characterized by males having a longer body and a larger head (Roché 2001; Kottelat and Freyhof 2007; Keith et al. 2011; Laporte et al. 2016a). Moreover, during the breeding season, males develop a cephalic crest and 2 anal glands covering the first spinous rays of the anal fin (secondary sexual characteristics); they also exhibit parental care that could be affected by environmental factors such as nest availability and social interactions between males (Fabre et al. 2014).

Interestingly, Lengkeek et al. (2008) found evidence at a fine scale for Rensch's rule for 3 marine blenny species inhabiting the Mediterranean Sea. This relationship appears to be explained by an environmental factor since low genetic variation is expected in populations at close proximity. For the freshwater blenny, fish from river populations generally have a larger body size than those from lake populations (Laporte et al. 2013; Neat et al. 2003). According to Rensch's rule, SSizeD should be higher for river than for lake populations. As mentioned above, different behaviors during the reproductive period (April–August, inclusive) are associated with sexual dimorphism in the freshwater blenny (Freeman et al. 1990; Côté et al. 1999; Vinyoles et al. 1999, 2002; Vinyoles and De Sostoa 2007). In this species, males excavate and inhabit nest cavities under stones and provide parental care (nest protection, egg care) while females swim from nest to nest in search of new mating opportunities (Vinyoles et al. 2002; Vinyoles and De Sostoa 2007). Because females need better swimming ability in rivers, SShapeD may therefore be associated with habitat.

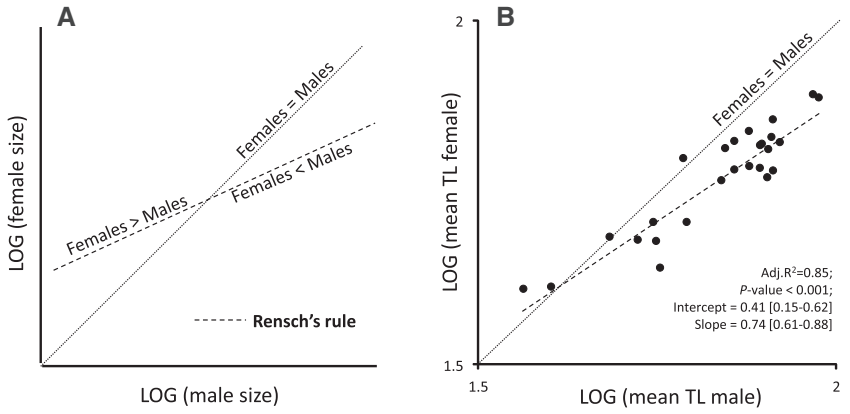

Figure 1. Illustrations of Rensch's rule. (**A**) Theoretical expectation and (**B**) observations of 26 populations of freshwater blenny. The solid lines illustrate unity ($x = y$) and the dashed lines represent the slopes of the relationships between mean male and mean female length (TL).

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

Currently, little is known about the environmental conditions that could affect differences in sexual dimorphism among populations. The goal of this study was to test for the differentiation in size, shape, SSizeD, and SShapeD between river and lake populations of freshwater blenny. The results of this study will ultimately help improve our understanding of how sexual dimorphism could evolved and highlight the importance of ecological factors in the differentiation of sexual dimorphism.

Materials and Methods

Sampling

Between 2002 and 2014, 1,225 freshwater blenny were sampled from 24 sites by electrofishing or snorkeling during the reproductive period (spring and summer months) (Table 1). Five of these sites were lakes (342 fish) and 19 sites were rivers (883 fish; Table 1). Within each site, the same sampling method (electrofishing or snorkeling) and measurement tool (fish measuring board or digital caliper) were used to catch and compare male and female size. Total lengths (TLs, mm) were measured on fish >35 mm and sex was determined. An individual was classified as male when an anal gland and/or cephalic crest were present, and as female otherwise (Figure 2). Any uncertainty during sex determination resulted in the fish being excluded from the analyses.

Size analyses

We first verified that our data conformed to Rensch's rule by testing for homogeneity of the variance between population averages of male and female body size using Levene's test. Under the Rensch's rule, it is expected that the variance in population averages of male body size will show higher variance than that of female (Rensch 1950). In addition, a model II regression (major axis analysis; Legendre and Legendre 1998) was used to test if the slope between the mean female and male sizes was <1, which is also expected under Rensch's rule (Rensch 1950; Figure 1A).

To test the effect of habitat (fixed effect: lake or river), sex (fixed effect: male or female), and their interaction on size, we used a mixed effects linear model using the *nlme* package in R v.3.3.1 with populations nested within habitat as a random effect and individual fish nested within populations [2-way nested ANOVAs; Pinheiro et al. 2015; R Core Team, www.r-project.org (cited at 2010 July 17)]. The interaction between the 2 fixed effects (habitat and sex), if significant, would suggest that size differentiation among sex (SSizeD) would differ among habitat. The occurrence of a "disordinal" interaction was checked by producing interaction plot (not shown), and fixed effects (sex, habitat) were also tested. A significant effect of habitat on size would suggest that habitat is associated with size differentiation, while a significant effect of sex on size would suggest the presence of SSizeD. In addition to this individual level test, we performed a *t*-test to compare SSizeD at population level between lake and river habitat. The SSizeD of a population was calculated by subtracting the logarithm of average male TL by the logarithm of average female TL, following Lengkeek et al. (2008). Two lake populations (Garda and Kournas; Table 1) were included here using previously reported average values of TL for males and females (Neat et al. 2003). Because a small sample size produces a poor estimate (Sokal and Rohlf 1995), we performed the *t*-test on SSizeD for i) all populations and ii) only for populations where at least 15 males and 15 females had been sampled (Table 1 in bold). All computations were done using R v.3.3.1 [R Core Team; www.r-project.org (cited 2010 July 17)].

Shape analyses

Based on the availability of images allowing geometric morphometrics analysis, shape analyses were performed on a subset of samples (i.e., Aiguebelette, Annecy, and Bourget for lakes and Boughzazene, Rizzanese, and Tech for rivers; see column "Morpho" in Table 1). For these populations, fish were anesthetized with clove oil diluted in water and an image of the left side of the fish was captured with a digital camera (Canon, PowerShoot A2000IS; www.canon.com). Fish were placed in the center of the image and on a thin wet sponge to ensure that they remained in a horizontal orientation. Shape analyses were based on geometric landmark coordinates (Claude 2008), and 10 landmarks were digitized for each image (Figure 2) using tpsDig v2.16 (Rohlf 2010). Landmarks were chosen for an optimal coverage of the morphology and to reflect the sexual dimorphism of the species (Roché 2001; Zelditch et al. 2004; Kottelat and Freyhof 2007; Keith et al. 2011; Laporte et al. 2016a). To preserve information on shape differences among fish and to remove information unrelated to shape (i.e., scale, position, and orientation), a partial generalized Procrustes analysis superimposition was first conducted (Rohlf and Slice 1990; Dryden and Mardia 1998). Fish shape was estimated from the superimposed coordinates projected in a principal component analysis using MorphoJ software v1.06 (Klingenberg 2011). The wireframe graph option of MorphoJ was used to display shape changes on informative PC-axes, as indicated by the broken-stick distribution (Frontier 1976; Legendre and Legendre 1998).

To test the effects of habitat (fixed effect, lake or river), sex (fixed effect, male or female), and their interaction on shape, we used a mixed-effects linear model with population nested as a random effect within habitat and individual fish nested within population (2-way nested ANOVAs; Pinheiro et al. 2015). The variable size was added as a covariate to control for the effect of shape allometry. As above, a significant effect of their interaction would suggest that SShapeD differentiation is associated with habitat. The occurrence of a "disordinal" interaction was also checked before testing for fixed effects (sex, habitat). In addition, we examined the average shape differentiation between sexes within a habitat and between habitats within a sex to better visualize the SShapeD between sex and habitat using the R 3.3.1 software (Claude 2008; R Core Team 2013).

Results

Size analyses

Males had a higher variance on their mean size (0.011) than females (0.007), and variances were not homogeneous according to Leven's test ($W = 1.12$; P-value $= 0.295$). Furthermore, the model II regression (major axis) indicated a significant relationship between average male and female sizes (TL) for all populations (P-value < 0.001; adjusted $R^2 = 0.85$; Figure 1B). The slope was less than 1 (0.74; 95% confidence interval: 0.61–0.88) with an intercept greater than 0 (0.41; 95% confidence interval: 0.15–0.62) (Figure 1B).

A significant interaction between habitat and sex was observed ($F_{1,\ 1199} = 21.65$; P-value < 0.001) (Figure 3), suggesting the presence of SSizeD differentiation between habitats. In addition, significant effects of habitat (larger in rivers) and sex (larger in males) on size were observed (respectively, $F_{1,\ 22} = 16.99$; P-value < 0.001 and $F_{1,\ 1199} = 252.26$; P-value < 0.001) (Figure 3), suggesting SSizeD in

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

Table 1. Site, geographic coordinates (latitude N; longitude E), sample year, habitat type, sample size, sex ratio, average size (all individuals, females only, males only), and sexual size dimorphism (SSD)

Site	Geographic coordinates	Year	Habitat	N	Sex ratio F:M	Size all (F + M)	Size F	Size M	SSD	Morpho
FR-Aiguebelette	45°33'00" 05°48'13"	2009	Lake	11	1.25	50.16 (1.95)	47.94 (2.99)	52.93 (2.11)	0.043	Yes
FR-Annecy	**45°54'00" 06°07'48"**	**2009**	**Lake**	**54**	**1.29**	**52.95 (1.14)**	**50.86 (1.15)**	**55.65 (2.23)**	**0.039**	**Yes**
FR-Bourget	**45°48'36" 05°49'12"**	**2009**	**Lake**	**30**	**1.00**	**48.39 (0.82)**	**48.41 (1.27)**	**48.36 (1.29)**	**0.000**	**Yes**
GR-Kournas	**35°19'49" 24°16'34"**	**2002**	**Lake**	**395**	**1.37**	**38.98[a]**	**40.68[a]**	**36.66[a]**	**−0.045**	**—**
IT-Garda	**45°33'49" 10°37'44"**	**2002**	**Lake**	**333**	**1.64**	**40.91[a]**	**40.98[a]**	**40.08[a]**	**−0.010**	**—**
SP-Banyoles	**42°07'44" 02°45'35"**	**1999**	**Lake**	**218**	**1.56**	**51.04 (0.62)**	**47.75 (0.66)**	**56.18 (0.98)**	**0.071**	**—**
SW-Léman	46°26'51" 06°29'02"	2014	Lake	35	2.18	62.46 (3.53)	63.00 (4.28)	61.27 (6.55)	−0.012	—
AL-Boughzazene	36°38'24" 05°20'24"	2011	River	32	1.91	70.29 (2.29)	64.92 (1.58)	80.54 (4.68)	0.094	Yes
FR-Abatescu	**41°58'33" 09°24'00"**	**2009**	**River**	**33**	**1.06**	**71.92 (2.66)**	**65.78 (2.44)**	**78.45 (4.35)**	**0.077**	**—**
FR-Bevincu	**42°36'37" 09°26'05"**	**2009**	**River**	**37**	**1.31**	**68.49 (2.49)**	**60.93 (2.13)**	**78.41 (3.88)**	**0.109**	**—**
FR-Bravona	42°13'26" 09°26'49"	2009	River	35	1.50	68.99 (2.70)	60.44 (2.80)	81.80 (2.91)	0.131	—
FR-Fium orbu	42°03'46" 09°22'04"	2009	River	27	4.40	63.01 (2.84)	59.09 (2.28)	80.3 (8.46)	0.133	—
FR-Golu	42°31'10" 09°25'08"	2009	River	31	2.88	61.27 (1.53)	58.48 (1.39)	69.28 (3.05)	0.074	—
FR-Gravona	41°58'48" 08°50'22"	2009	River	14	2.50	66.53 (3.35)	65.09 (4.56)	70.13 (2.86)	0.032	—
FR-Liamone	**42°05'41" 08°45'57"**	**2009**	**River**	**32**	**1.00**	**66.43 (2.70)**	**60.63 (2.48)**	**72.24 (4.42)**	**0.076**	**—**
FR-Rizzanese	**41°38'52" 08°58'08"**	**2009**	**River**	**33**	**0.83**	**75.63 (3.19)**	**66.42 (1.65)**	**83.64 (5.03)**	**0.100**	**Yes**
FR-Taravu	41°35'39" 08°52'08"	2009	River	25	0.56	74.29 (2.49)	66.09 (2.87)	78.91 (3.01)	0.077	—
FR-Taviganu	**42°08'14" 09°27'24"**	**2009**	**River**	**32**	**1.00**	**72.28 (2.26)**	**68.96 (2.95)**	**75.71 (3.32)**	**0.040**	**—**
FR-Tech	42°35'06" 02°58'43"	2010	River	26	0.75	57.21 (2.26)	50.87 (3.05)	61.97 (2.73)	0.086	Yes
SP-A.Balaguer	41°54'37" 00°57'37"	2003	River	55	4.00	64.24 (2.22)	61.34 (2.11)	75.82 (6.34)	0.092	—
SP-Anyà	**41°55'16" 01'06"24"**	**2003**	**River**	**51**	**1.55**	**83.84 (1.91)**	**77.94 (2.03)**	**93.00 (2.67)**	**0.077**	**—**
SP-Fluvià	**42°10'41" 03°04'29"**	**2006**	**River**	**72**	**1.48**	**84.28 (1.77)**	**77.16 (1.54)**	**94.76 (2.77)**	**0.089**	**—**
SP-Matarranya	**40°51'56" 00°04'52**	**2003**	**River**	**48**	**1.18**	**49.73 (1.19)**	**43.65 (0.89)**	**56.91 (1.10)**	**0.115**	**—**
SP-N.Pallaresa	**42°15'58" 01°01'29'**	**2003**	**River**	**50**	**1.78**	**68.68 (1.67)**	**66.69 (2.15)**	**72.22 (2.46)**	**0.035**	**—**
SP-Oliana	42°04'03" 01°18'15"	2003	River	29	1.23	76.21 (2.34)	71.69 (2.39)	81.77 (3.84)	0.057	—
SP-Ter	**42°03'07" 02°57'30"**	**2006**	**River**	**221**	**1.73**	**72.61 (1.10)**	**67.60 (1.14)**	**81.38 (1.94)**	**0.081**	**—**

Notes: "morpho" indicates whether the population was included in the shape analysis. Populations in bold have at least 15 males and 15 females. For all average size measurement, standard error of the mean (SEM) is given in parentheses. AL = algeria; FR = france; GR = greece; IT = italy; SP = spain; SW = switzerland., [a] There were no individual measurements for these populations; average lengths were reported in Neat et al. (2003).

this species in addition to a size differentiation between lake and river populations. Moreover, it is noteworthy that a higher size differentiation was observed between males and females in rivers than in lakes (Figure 3). A significant difference between habitats was observed in SSizeD of populations (all populations: $n = 26$ [7 lakes and 19 rivers], $t = -4.52$, $df = 10.50$, P-value < 0.001; and on populations with at least 15 males and 15 females: $n = 15$ [5 lakes and 10 rivers], $t = -3.17$, $df = 5.41$, P-value $= 0.022$), which shows that

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

Figure 2. Position of the 10 landmarks (top = male; bottom = female). 1: anterior to the snout; 2: above the eyes; 3: top of the head; 4: anterior to the dorsal fin; 5: posterior to the dorsal fin; 6: posterior to the caudal peduncle; 7: posterior to the anal fin; 8: anterior to the pelvic fin; 9: posterior to the lower jaw; 10: anterior to the maxilla.

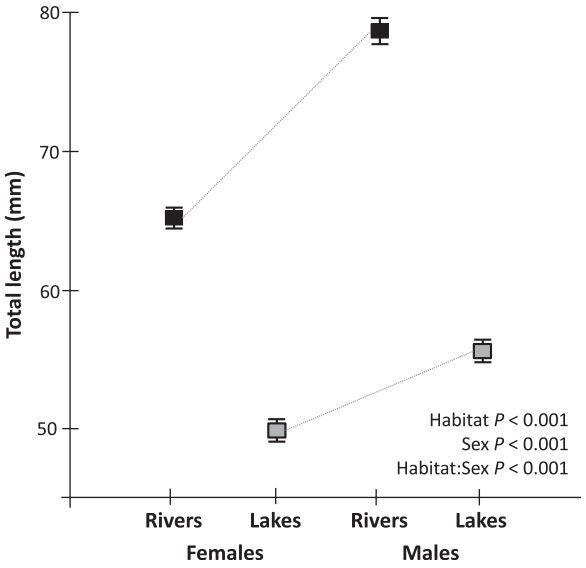

Figure 3. Total length (TL; mm) and 95% confidence interval of female and male freshwater blenny from rivers (black) and lakes (gray). The effects of habitat, sex, and their interaction on TL were all significant.

river populations have a higher SSizeD than lake populations (0.083 for river sites vs. 0.015 for lake sites).

Shape analyses

Based on the broken-stick distribution, only the 2 first shape PCs were considered informative to represent fish shape variation (36.0% and 29.1%, respectively). Because PC2 (but not PC1; see Supplementary Results) showed a clear shape differentiation representing shape sexual dimorphism in this species (Figure 4A), the furthered analyses were performed on only this PC shape axis.

First, we found significant effects of the interaction between habitat and sex ($F_{1,\ 171} = 8.58$; P-value = 0.004; Figure 4B), habitat ($F_{1,4}$-value = 22.94; P-value = 0.009), sex ($F_{1,171}$-value = 82.70; P-value < 0.001), and size ($F_{1,\ 171} = 12.70$; P-value < 0.001) on the PC2 shape axis (Figure 4B), suggesting the presence of a difference in SShapeD between habitats, and a difference in fish shape between habitats and sex, as well as an allometry in body shape. According to the shape differentiation on the PC-2 axis (Figure 4A), freshwater blenny from rivers have smaller heads than those from lakes (Figure 4B). The same pattern, but more pronounced, was observed for females in comparison to males (Figure 4B). Shape comparisons between males and females of lake habitat as well as between males from both habitats showed little shape difference (Figure 5A, D). However, in the river habitat, females had smaller heads than males (Figure 5B). Females from river sites also had smaller heads than females from lake sites, but the difference was less marked (Figure 5C).

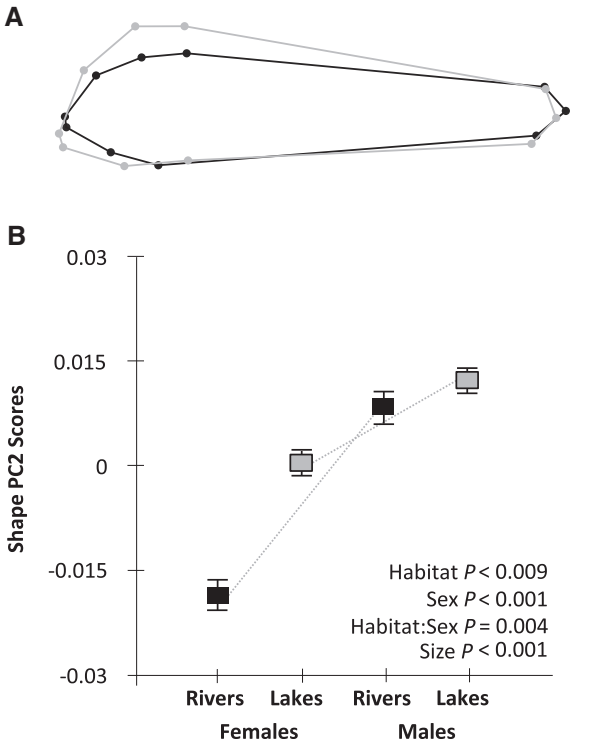

Figure 4. (**A**) Shape differentiation along the second axis of a shape principal component. The black line illustrates the low scores on PC2 and the gray line the high scores. (**B**) Shape PC2 scores and 95% confidence interval of female and male freshwater blenny from rivers (black) and lakes (gray). The effects of habitat, sex, and their interaction on shape PC2 as well as covariate size were all significant.

Discussion

We investigated differentiation in size, shape, and sexual dimorphism among freshwater blenny populations inhabiting lake and river habitats. Together, our observations show a clear pattern in agreement with Rensch's rule: i) males are larger than females, ii) river populations exhibit larger individuals than lake populations, and iii) SSizeD is more pronounced in river than in lake populations. Furthermore, females had smaller heads than males. A more pronounced SShapeD associated with the head was also observed in river compared with lake populations. Considering that the freshwater blenny started its colonization of freshwater habitats in these

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

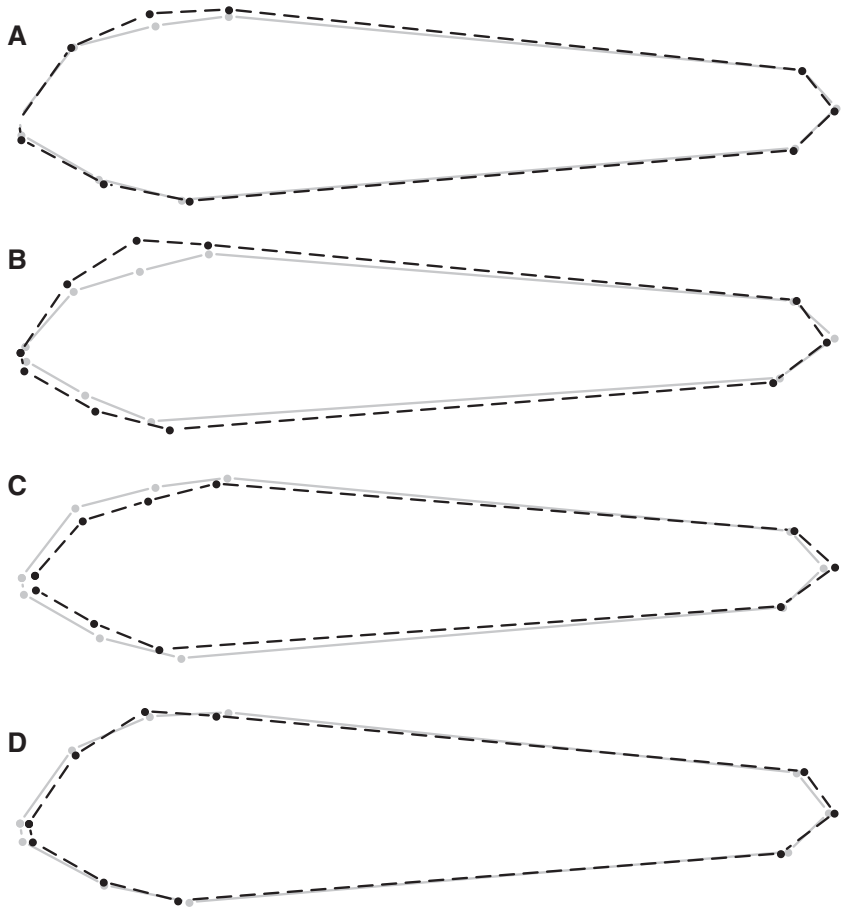

Figure 5. Mean shape comparisons of freshwater blenny and 95% confidence interval: inside a habitat between males (black dashed lines) and females (solid gray lines) in lakes (**A**) and rivers (**B**); inside a sex between rivers (black dashed lines) and lakes (solid gray lines) in females (**C**) and males (**D**).

rivers around 5 million years ago (Almada et al. 2009), than in more inland lakes, these observations suggest a decrease in sexual dimorphism in lake habitats in the species.

Differentiation in size and SSizeD

Both female and male freshwater blenny inhabiting rivers were longer than those in lakes. An increase in length could be induced by higher growth rates or longer lifespans (Moss et al. 2016). Food resource availability and predation pressure are 2 important factors affecting growth rate and lifespan (Biro et al. 2004; Dmitriew 2011; Seward et al. 2013; Moss et al. 2016): (i) when food resources are more plentiful, more energy is available for growth and survival rates increase (Dmitriew 2011; Seward et al. 2013); (ii) higher predation pressure could decrease survival rates by increasing mortality in addition to lower growth rates by increasing anti-predator behaviors at the expense of foraging (Biro et al. 2004; Dmitriew 2011). Freshwater blenny generally inhabits the shallow and turbulent environments of small rivers (Freeman et al. 1990; Roché 2001; Laporte et al. 2014). Such environments could harbor lower species diversity compared with lake habitats. In addition, many predators have been introduced to lakes and impoundments throughout the Mediterranean region (Kottelat and Freyhof 2007). For instance, the presence of perch *Perca fluviatilis*, pike *Esox lucius*, eel *Anguilla anguilla*, and grass snake *Natrix natrix* in lake habitats is associated with increased predation on blenny compared with rivers (Neat et al. 2003), although predation of freshwater blenny by the viperine snake *N. maura* has been observed in small rivers (Santos et al. 2006). Estimations of food resources availability for lake and river blenny are presently not available, but the potentially higher species diversity in lakes could lead to higher interspecific competition for food, ultimately reducing the size of freshwater blenny. It should be noted that most Mediterranean rivers are highly productive; freshwater blenny inhabiting these rivers grow fast and reach first sexual maturity earlier than those in lakes (Vinyoles and De Sostoa 2007).

It has been previously reported that males freshwater blenny are larger than females (Roché 2001; Kottelat and Freyhof 2007; Keith et al. 2011). This differentiation is accentuated in river populations, where the largest individuals are males and SSizeD is significantly more pronounced. Interestingly, it was reported that female marine redlip blenny *Ophioblennius atlanticus* move longer distances to find larger males (Côté and Hunte 1989, 1993; Reynolds and Côté 1995). It was also reported that males are larger than females in several other blenny species (Lengkeek et al. 2008; Briggs 2010). Together, this evidence suggests the presence of sexual selection for larger males for reproduction among Blenniidae species. In addition, environmental conditions appear to be the most likely explanation for the observation of Rensch's rule for 3 Mediterranean blenny species (Lengkeek et al. 2008). In agreement with Lengkeek et al. (2008), our results indicate that sexual selection on size could

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

potentially be reduced by environmental factors such as low food availability and high predation pressure. Indeed, a reduction of SSizeD was observed in lake habitats, where predation pressure and interspecific competition may be stronger. Furthermore, a higher mortality of female blenny in fluctuating rivers after reproduction was reported by Vinyoles and De Sostoa (2007), which could also explain differences in the degree of SSizeD between more stable habitats (lakes) versus more fluctuating ones (Mediterranean rivers). Therefore, different age structures in the populations could explain the difference in SSizeD between river and lake habitats due to different survival rates among the 4 groups (habitat × sex). Because fish age was not estimated in this study, we could not test this hypothesis. However, it would be interesting to distinguish the different environmental causes that could explain differences in SSizeD in a further study. Regardless of which environmental factors are the major causes of SSizeD differences between river and lake habitats, overall our results suggest that female mate choice likely drives SSizeD within a population while a response to environmental conditions could produce SSizeD differentiation between populations.

Differentiation in shape and SShapeD

Based on shape differentiation observed along the second PC-axis, river individuals display smaller heads than lakes ones and the difference in SShapeD in rivers is larger than in lakes. These observations could be explained by a difference in swimming requirements between sexes in river habitats and between habitats. Several studies have reported a relationship between swimming requirements and the hydrodynamics of fish, which could result in a smaller head and/or a more slender body (Proulx and Magnan 2004; Langerhans and Reznick 2010; Bernatchez et al. 2016; Laporte et al. 2016b). Thus, the smaller head observed in freshwater blenny of river populations could suggests an adaptation and/or a result of phenotypic plasticity to water velocity, as previously observed for body shape in this species (Laporte et al. 2016a). However, females in the river habitat display a stronger reduction in head size than do males, and this could be related to the fact that females and males have different behaviors during the reproductive period (Vinyoles et al. 2002; Vinyoles and De Sostoa 2007). Indeed, while males are sheltered in their nest and thus experience low water velocity, females, which exhibit a stouter body during their maturing period, must swim nest-to-nest against the current (Vinyoles et al. 2002; Vinyoles and De Sostoa 2007). In highly turbulent water, this behavior could explain—at least in part—why females have a smaller head than males in the river habitat. However, in an environment with lower water velocity and thus lower swimming cost, a less contrasted sexual dimorphism could be expected, as observed in freshwater blenny populations in the lake habitat. Together, these results could suggest that the SShapeD is associated with water velocity and that this association leads to a less striking shape dimorphism in the lake habitat. However, the presence of an ornament, such as the cephalic crest, that is unnecessary for survival is generally a genetically driven feature that is linked to sexual selection. This suggests that female mate choice should promote the presence of head crest and consequently a larger head in males, but that a response to environmental conditions may better explain SShapeD differentiation between river and lake habitats.

Natural selection or adaptive phenotypic plasticity?

Sexual selection for the head crest is not related to environmental conditions, and it is a genetic difference between the sexes. However, the response to environmental conditions that underlies SSizeD and SShapeD differentiation between habitats could be explained by either natural selection (adaptation) or phenotypic plasticity or both. At this point, it is not possible to distinguish between these 2 mechanisms or to estimate their respective contributions. Nevertheless, previous studies have shown that strong genetic differentiation among geographically close populations is present within the freshwater blenny (Laporte et al. 2015a, 2016c). Considering that shape could show strong genetic basis (Laporte et al. 2015b, 2016b), such genetic differentiation could be associated with local adaptation and therefore an adaptation of sexual dimorphism. Alternatively, the species shows high levels of phenotypic plasticity in many traits, such as body shape (Laporte et al. 2016a), reproductive behavior (Fabre et al. 2014; Quirós and Vinyoles 2016), and water salinity tolerance (Plaut 1998, 1999). It is also worth noting that a reduction of water flow in Ebro River that occurred between 2004 and 2011 was associated with an increase in the proportion of small mature males (Quirós and Vinyoles 2016). Considering the generation time of the species (2 years), this suggests that if natural selection has driven the change in average male size in Ebro River, it should happen within a few generations (~3 generations; Kottelat and Freyhof 2007; Vinyoles and De Sostoa 2007; Keith et al. 2011). In any case, this is an intriguing question to be addressed in future studies aimed at better understanding the mechanisms behind both sexual dimorphism differentiation among populations and the ecological basis of sexual dimorphism.

In conclusions, this study shows differences in size, shape, and sexual size and shape dimorphism between lake and river populations of the freshwater blenny. Our results suggest that in addition to sexual selection promoting SSizeD and SShapeD, a response to environmental conditions of lake and river habitats is also associated with both size and shape difference in sexual dimorphism. Further studies will be needed to estimate the relative role of natural selection and phenotypic plasticity behind this response to environmental conditions. Nevertheless, our study highlights the importance of considering the environmental conditions to which populations are exposed when examining causes underlying the evolution of sexual dimorphism.

Authors' Contribution

M.L. designed the study, produced the analyses, and wrote the first draft. All authors participated to the sampling and have revised, improved, and agreed the submitted version of the manuscript.

The authors thanks B. J. G. Sutherland, L. Devine, and A. Kusler for helpful comments on earlier version as well as J. Mattei, C. Albertini, O. Deroche, D. Giorgi, A. Ottavi, L. Torre, S. Lefebvre, and J.-P. Bernier from the Office National de l'Eau et des Milieux Aquatiques (ONEMA); P.-J. D'Agostini and J. Canal from the Fédération de la Corse pour la Pêche et la Protection des Milieux Aquatiques (FCPPMA); M. De-Basquiat; and J. Roussilhes from the Direction Régionale de l'Environnement de l'Aménagement et du Logement de la Corse (DREAL—CORSE) for their invaluable logistical support.

Funding

This work was supported by a post-graduate fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC-471682-2015 to M.L.).

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

References

Almada VC, Robalo JI, Levy A, Freyhof J, Bernardi G et al., 2009. Phylogenetic analysis of peri-Mediterranean blennies of the genus *Salaria*: molecular insights on the colonization of freshwaters. *Mol Phylogenet Evol* **52**:24–431.

Bernatchez S, Laporte M, Perrier C, Sirois P, Bernatchez L, 2016. Investigating genomic and phenotypic parallelism between piscivorous and planktivorous ecotypes of lake trout *Salvelinus namaycush* by means of RADseq and morphometrics analyses. *Mol Ecol* **25**:4773–4792.

Berns CM, 2013. The Evolution of Sexual Dimorphism: Understanding Mechanisms of Sexual Shape Differences, Sexual Dimorphism. In: Moriyama H (Ed.). *Sexual dimorphism*. InTech. [Cited 2010 July 17]. Available from: http://dx.doi.org/10.5772/55154.

Biro PA, Abrahams MV, Post JR, Parkinson EA, 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc R Soc Lond B* **271**:2233–2237.

Blanckenhorn WU, Stillweel RC, Young KA, Fox CW, Ashton KG, 2006. When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution* **60**:2004–2011.

Briggs JC, 2010. A plethora of blennies. *Environ Biol Fishes* **87**:89–92.

Claude J, 2008. *Morphometrics with R*. New York: Springer Science + Business Media, L.L.C.

Colwell RK, 2000. Rensch's rule crosses the line: convergent allometry of sexual size dimorphism in hummingbirds and flower mites. *Am Nat* **156**: 495–510.

Cooper IA, 2010. Ecology of sexual dimorphism and clinal variation of coloration in a damselfy. *Am Nat* **176**:566–572.

Côté IM, Hunte W, 1989. Male and female mate choice in the redlip blenny: why bigger is better. *Anim Behav* **38**:78–88.

Côté IM, Hunte W, 1993. Female blennies prefer older males. *Anim Behav* **46**: 203–205.

Côté IM, Vinyoles D, Reynolds JD, Doadrio I, Percides A, 1999. Potential impacts of gravel extraction on Spanish populations of river blennies *Salaria fluviatilis*. *Biol Conserv* **87**:359–367.

Dmitriew CM, 2011. The evolution of growth rate trajectories: what limits growth rate? *Biol Rev* **86**:97–116.

Dryden IL, Mardia KV, 1998. *Statistical Shape Analysis*. New York: Wiley.

Fabre N, Oliva F, García-Galea E, Vinyoles D, 2014. Plasticity in secondary sexual characteristics in male freshwater blenny *Salaria fluviatilis*. *Can J Zool* **92**:537–543.

Fairbairn DJ, 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu Rev Ecol Syst* **28**:659–687.

Fairbairn DJ, 2005. Allometry for sexual dimorphism: testing two hypothesis for Rensch's rule in water strider *Aquarius remigis*. *Am Nat* **166**:69–84.

Freeman MC, Vinolas D, Grossman GD, De Sostoa A, 1990. Microhabitat use by *Blennius fluviatilis* in the Rio Matarrana, Spain. *Freshw Biol* **24**: 335–345.

Frontier S, 1976. Étude sur la décroissance des valeurs propres dans une analyse en composantes principales: comparaison avec le modèle du bâton brisé. *J Exp Mar Biol Ecol* **25**:67–75.

Johansson F, Crowley PH, Brodin T, 2005. Sexual size dimorphism and sex ratios in dragonflies (Odonata). *Biol J Linn Soc* **86**:507–513.

Keith P, Persat H, Feunteun E, Allardi J, 2011. *Les poissons d'eau douce de France*. Muséum national d'histoire naturelle. Collection inventaire et biodiversité, Paris: Biotope, Mèze.

Klingenberg CP, 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour* **11**:353–357.

Kottelat M, Freyhof J, 2007. *Handbook of European Freshwater Fishes*. Cornol, Switzerland: Publications Kottelat.

Kratochvill L, Frynta D, 2002. Body size, male combat and the evolution of sexual dimorphism in eublepharid geckos (Squamata: Eublepharidea). *Biol J Linn Soc* **76**:303–314.

Langerhans RB, Reznick DN, 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: Domenici P, Kapoor BG eds. *Fish Locomotion: An Etho-Ecological Perspective*. Enfield: Science Publishers, 200–248.

Laporte M, Mattei J, Perret P, Roché B, Vinyoles D et al., 2013. New maximum lengths for freshwater blenny (*Salaria fluviatilis* Asso, 1801) and length comparison between continental and island rivers. *Cybium* **37**:309–313.

Laporte M, Bertolo A, Berrebi P, Magnan P, 2014. Detecting anthropogenic effects on a vulnerable species, the freshwater blenny *Salaria fluviatilis*: the importance of considering key ecological variables. *Ecol Indic* **36**: 386–391.

Laporte M, Leblois R, Coulon A, Bonhomme F, Magnan P et al., 2015a. Genetic structure of a vulnerable species, the freshwater blenny *Salaria fluviatilis*. *Conserv Genet* **16**:571–581.

Laporte M, Rogers SM, Dion-Côté A-M, Normandeau E, Gagnaire P-A et al., 2015b. RAD-QTL mapping reveals different genetic paths and genetic parallelism underlying the parallel evolution of body shape in lake whitefish. *G3* **5**:1481–1491.

Laporte M, Claude J, Berrebi P, Perret P, Magnan P, 2016a. Shape plasticity in response to water velocity in the freshwater blenny *Salaria fluviatilis*. *J Fish Biol* **88**:1191–1203.

Laporte M, Dalziel AC, Martin N, Bernatchez L, 2016b. Adaptation and acclimation of traits associated with swimming activity in lake whitefish ecotypes *Coregonus clupeaformis*. *BMC Evol Biol* **16**:160.

Laporte M, Perrier C, Magnan P, Berrebi P, 2016c. Genetic evidence of recent migration among isolated-by-sea populations of the freshwater blenny *Salaria fluviatilis*. *Conserv Genet* **17**:389–399.

Legendre P, Legendre L, 1998. *Numerical Ecology*. 2nd English edn. Amsterdam: Elsevier Science BV.

Lengkeek W, Didderen K, Côté IM, van Zer Zee EM, Snoek RC et al., 2008. Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Can J Zool* **86**:1173–1178.

Moss DK, Ivany LC, Judd EJ, Cummings PW, Bearden CE et al., 2016. Lifespan, growth rate, and body size across latitude in marine Bivalvia, with implications for Phanerozoic evolution. *Proc R Soc B* **283**:20161364.

Neat FC, Lengkeek EP, Westerbeek EP, Laarhoven B, Videller JJ, 2003. Behavioural and morphological differences between lake and river populations of *Salaria fluviatilis*. *J Fish Biol* **63**:374–387.

Pinheiro J, Bates D, DebRoy S, Sarkar D; R Core Team, 2015. nlme: linear and nonlinear mixed effects models [Internet]. R package version 3.1–128. [cited at 2010 July 17]. Available from: https://cran.r-project.org/web/packages/nlme/nlme.pdf.

Plaut I, 1998. Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiol Biochem* **19**:181–188.

Plaut I, 1999. Effects of salinity acclimation on oxygen consumption in the freshwater blenny *Salaria fluviatilis* and the marine peacock blenny *S. pavo*. *Mar Freshw Res* **50**:655–659.

Proulx R, Magnan P, 2004. Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.). *Evol Ecol Res* **6**:503–522.

Punzalan D, Hosken DJ, 2010. Sexual dimorphism: why the sexes are (and are not) different. *Curr Biol* **20**:R972–R973.

Quirós C, Vinyoles D, 2016. Streamflow reduction induces early parental care in *Salaria fluviatilis* (Asso, 1801) males. *J Appl Ichthyol* **32**:198–203.

R Core Team, 2013. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. [Cited at 2010 July 17]. Available from: http://www.R-project.org/.

Rensch B, 1950. Die abhängigkeit der relativen sexualdifferenz von der körpergrösse. *Bonn Zool Bull* **1**:58–69.

Reynolds JD, Côté IM, 1995. Direct selection on mate choice: female redlip blennies pay more for good mates. *Behav Ecol* **6**:175–181.

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021

Roché B, 2001. *Atlas des poissons d'eau douce de Corse*. Direction régionale de l'environnement Corse. Imprimerie Bastiaise, Bastia, Corse.

Rohlf FJ, 2010. *tpsDig, Digitize Landmarks and Outlines*. Version 2.16. Stony Brook: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ, Slice D, 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst Zool* **39**:40–59.

Santos X, Vilardebó E, Casals F, Llorente GA, Vinyoles D et al., 2006. Wide food availability favours intraspecific trophic segregation in predators: the case of a water snake in a Mediterranean river. *Anim Biol* **56**:299–309.

Seward AM, Beale CM, Gilbert L, Jones TH, Thomas RJ, 2013. The impact of increased food availability on survival of a long-distance migratory bird. *Ecology* **94**:221–230.

Shine R, 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quat Rev Biol* **64**:419–461.

Slatkin M, 1984. Ecological causes of sexual dimorphism. *Evolution* **38**: 622–630.

Sokal RR, Rohlf FJ, 1995. *Biometry*. 3rd edn. New York: Freeman.

Vinyoles D, Côté IM, De Sostoa A, 1999. Egg cannibalism in river blennies: the role of natural prey availability. *J Fish Biol* **55**:1223–1232.

Vinyoles D, Côté IM, De Sostoa A, 2002. Nest orientation patterns in *Salaria fluviatilis*. *J Fish Biol* **61**:405–416.

Vinyoles D, De Sostoa A, 2007. Life-history traits of the endangered river blenny *Salaria fluviatilis* (Asso) and their implications for conservation. *J Fish Biol* **70**:1088–1108.

Webb PW, 1982. Locomotor patterns in the evolution of actinopterygian fishes. *Am Zool* **22**:329–342.

Webb PW, 1984. Form and function in fish swimming. *Sci Am* **251**:58–68.

Willacker JJ, Von Hippel FA, Wilton PR, Walton KM, 2010. Classification of threespine stickleback along the benthic–limnetic axis. *Biol J Linn Soc* **101**: 595–608.

Zander CD, 1972. Evolution of Blennioidei in the Mediterranean Sea. *Rev Trav Inst Sci Tech Pêch Marit* **37**:215–221.

Zelditch ML, Swiderki DL, Sheets HD, Fink WL, 2004. *Geometric Morphometrics for Biologists*. San Diego: Elsevier.

Downloaded from https://academic.oup.com/cz/article/64/2/183/3903110 by BUPHARM user on 01 June 2021