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Article

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

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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

(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.

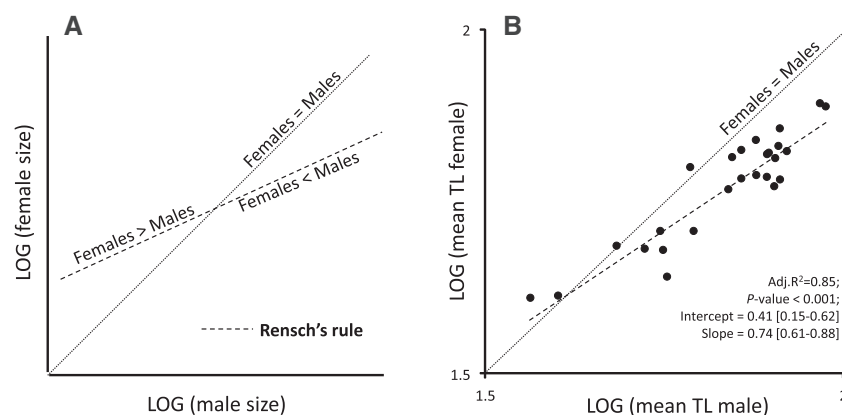


Figure 1. Illustrations of Rensch's rule. (A) Theoretical expectation and (B) observations of 26 populations of freshwater blenny. The solid lines illustrate unity ($x = y$) and the dashed lines represent the slopes of the relationships between mean male and mean female length (TL).

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 Levene'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

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



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.

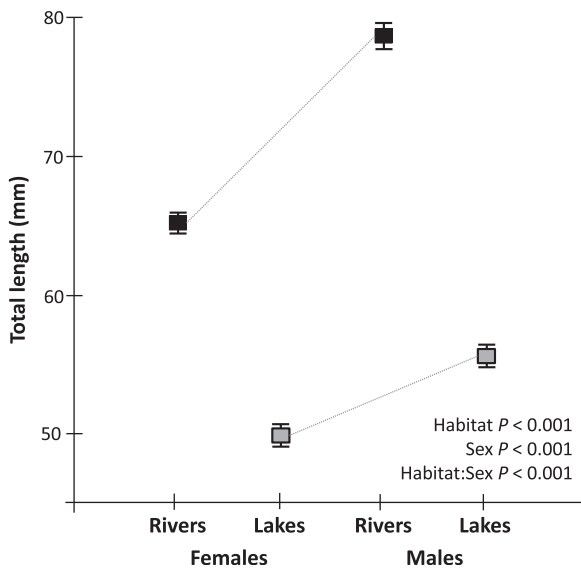


Figure 3. Total length (TL; mm) and 95% confidence interval of female and male freshwater blenny from rivers (black) and lakes (gray). The effects of habitat, sex, and their interaction on TL were all significant.

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 further 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;

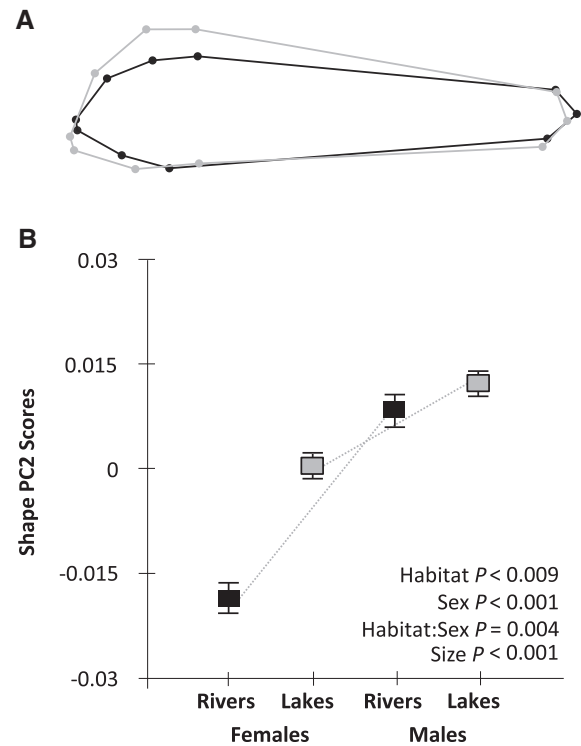


Figure 4. (A) Shape differentiation along the second axis of a shape principal component. The black line illustrates the low scores on PC2 and the gray line the high scores. (B) Shape PC2 scores and 95% confidence interval of female and male freshwater blenny from rivers (black) and lakes (gray). The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

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).

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

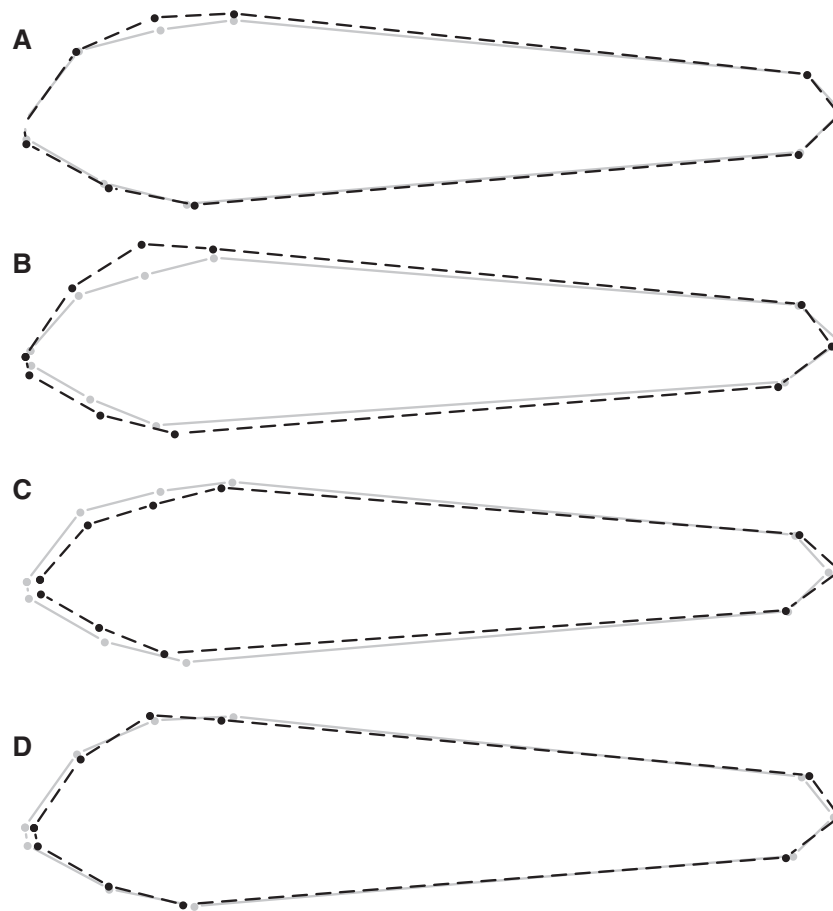


Figure 5. Mean shape comparisons of freshwater blenny and 95% confidence interval: inside a habitat between males (black dashed lines) and females (solid gray lines) in lakes (A) and rivers (B); inside a sex between rivers (black dashed lines) and lakes (solid gray lines) in females (C) and males (D).

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

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 suggest 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.

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Supplementary Material

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

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