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Body-size Relations and Mouth Dimensions of Fresh and Preserved Freshwater Fish

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Abstract: The relations between fish total (TL) fork (FL) and standard (SL) lengths, between TL and maximum height (H_{max}) and horizontal (HMO) and vertical (VMO) mouth openings and mouth area (MA) of four freshwater fish species (*Alburnoides thessalicus*, *Barbus strumicae*, *B. balcanicus* and *Squalius vardarensis*) are estimated for several rivers of northern Greece. The same relations for *B. strumicae* and *S. vardarensis* are also provided based on preserved samples deposited in the fish collection of the Laboratory of Ichthyology (School of Biology, Aristotle University of Thessaloniki). The relations between different lengths are all linear. The relations between HMO – TL and VMO – TL are either linear or exponential depending on the species and on the water system, while MA is exponentially related to TL for all studied species. Mouth area (MA) increases faster with TL for *S. vardarensis*, which has a higher trophic level than for *B. balcanicus* and *B. strumicae*. Differences are also found for the slope (parameter b) of the above relations between fresh and preserved samples and between the same species from different river systems. Such relations are important for defining the ecological position of each species in the food web and, thus, are used in ecosystem-based models.

Key words: Fish external morphology, mouth area, maximum body height, rivers, collection, Greece

Introduction

The relations among fish body size compartments (i.e. different types of body length) with maximum height and mouth area are widely used to assess fisheries (e.g. gear selectivity, see Reis & Pawson 1999) and ecological (e.g. predator-prey relations, see Karpouzi & Stergiou 2003) issues. Such studies allowing the computation of the above-mentioned proxies to total length are generally lacking for the European (Lammens & Visser 1989) and specifically for Greek (Bobori & Economidis 2006, Bobori et al. 2006, Karachle et al. 2014, Kyritsi & Moutopoulos 2018) freshwater fish.

In the present study, the relations between fish total (TL), fork (FL) and standard (SL) lengths and between TL with maximum body height (H_{max}), horizontal (HMO) and vertical (VMO) mouth openings and mouth area (MA) were estimated for four fish species sampled in several rivers of northern Greece: *Alburnoides thessalicus* Stephanidis, 1950, *Barbus strumicae* Karaman, 1955, *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002 and *Squalius vardarensis* Karaman, 1928. For two of the studied species, body size measurements have been also presented from either other Balkan (i.e. *B. balcanicus*, see Kotlík et al. 2002, Žutinić et al. 2014, Radojković et al. 2019) or Greek freshwater bodies (i.e. *B. strumicae*, see Sapounidis et al. 2015; see also Vasiliou & Economidis 2005 using former names of the same species – *B. peloponnesius* and *B. cyclolepis*). However, no relations between different length types and between TL with H_{max} and MA have been studied so far for the above fish species in Balkan waters.

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The above-mentioned relations, based on preserved museum materials of *B. strumicae* and *S. vardarensis*, were also compared between fresh and preserved samples. Thus, the aim of the study was to examine the body size relationships of four freshwater fish species from Greece and also to test whether the various maintenance techniques affect the length and weight of the fish when different concentrations of formalin solutions were used (see Sagnes 1997, Barros et al. 2018).

Materials and Methods

Fish sampling was conducted in July 2004 in the major rivers of northern Greece: Aliakmonas, Axios, Loudias and Strymonas (Fig. 1) using electrofishing. Preserved, initially in 10% formalin and then in 70% alcohol, samples collected in 1970-2004 and deposided in the fish collection of the Laboratory of Ichthyology (School of Biology, Aristotle University of Thessaloniki) were also studied. For all individuals, TL, FL and SL were measured to the nearest 0.1 cm, H_{max} to mm, VMO and HMO to 0.1 mm and MA to 0.1 cm^2. Relations between TL, FL and SL were linearly expressed with the equation Y=a+bX, where "a" and "b" were the intercept and slope of the regression line, respectively. Considering that the MA has an elliptic form (Erzini et al. 1997), HMO, VMO and MA were estimated as an ellipse (MA=0.25π*VMO*HMO, see Erzini et al. 1997) and were either linearly or log–linearly regressed against TL, based on the R^2 values. MA_{10} and MA_{15}, that corresponded to fixed TL of 10 and 15 cm, respectively, were also estimated from MA-TL relations, depending on the TL range values for each species. The slopes (parameter b) of the above relations were compared between river systems and between fresh and preserved material, using analysis of covariance (ANCOVA, see Zar 1999).

Results

Overall, 518 individuals (241 fresh and 277 preserved) were measured with total length ranging from 6 cm, for *A. thessalicus*, to 24.5 cm, for *S. vardarensis,* both samples from Axios (Table 1). All relations between body lengths (TL, FL, SL) were significantly (p<0.05) linear (Table 1), with the slopes for all studied species being significantly different from null (p< 0.05) and with R^2 values higher than 0.95. H_{max} was significantly (p<0.05) linearly related to TL (Table 2), with R^2 values being higher than 0.73 (for fresh samples of *A. thessalicus* from Axios River; Table 2). HMO and VMO were significantly (p<0.05) linearly related to TL, with the exception of the exponential relations between HMO and VMO with TL for *A. thessalicus* in Loudias (Table 1), whereas for all species MA was significantly (p<0.05) log–linearly related to TL (Table 1).

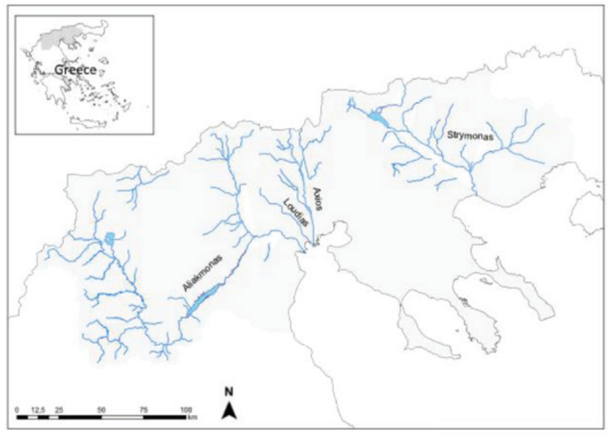

Fig. 1. Map of the sampling area.

Between river system differences (ANCOVA, p<0.05) for the parameter b of the length-length relations were found for 5 out of 15 species-system cases: for *S. vardarensis* (FL–TL, SL–TL and SL–FL relations between Axios and Loudias, Aliakmonas and Loudias and Aliakmonas and Axios, respectively) and for *B. balcanicus* (FL–TL and SL–FL relations between Axios and Loudias). In contrast, few significant differences (ANCOVA, p<0.05) were found for the comparison between fresh and preserved samples: *S. vardarensis* from Aliakmonas (FL–TL, SL–TL, SL–FL), Axios (SL–TL, SL–FL) and Loudias (SL–TL, SL–FL).

The parameter b of the MA–TL equation was spatially differed (ANCOVA, p<0.05) only for *A. thessalicus* between Axios and Loudias and for *S. vardarensis* between Aliakmonas and Loudias. The parameter b of the MA–TL equation was differed between fresh and preserved samples (i.e. *B. strumicae* from Strymonas and *S. vardarensis* from Aliakmonas), whereas the mouth dimensions for species of Group A (e.g. *S. vardarensis*) increased faster with TL than for those of Group B (e.g. *B. balcanicus* and *B. strumicae*; Fig. 2). *Squalius vardarensis* exhibited larger MA (Fig. 3) compared with *A. thessalicus* and *B. strumicae*.

Discussion

In the present study, we report the relations between body size and mouth dimensions for four fish species (*A. thessalicus*, *B. balcanicus, B. strumicae* and *S. vardarensis*), which are considered Balkan endemics. All estimated relations are of high impor-

Table 1. Relations between total (TL, cm), fork (FL, cm) and standard (SL, cm) length and between horizontal (HMO), vertical (VMO) mouth openings and mouth area (MA) and total length (TL) based on fresh and preserved fish material. n – number of individuals, SE_b – standard error of b and R^2 – correlation coefficient. For all length-length relations p <0.001 and R^2>0.95.

Species	River	n	TL range	Length-length	Se_b	Mouth-length*	R^2	Se_b
Fresh samples								
Alburnoides thessalicus	Axios	27	6.0-10.1	FL=0.978TL-0.514	0.025	VMO=1.119 TL- 0.803	0.81	0.110
				SL=0.876TL-0.689	0.029	HMO =1.109 TL – 4.280	0.77	0.124
				SL=0.883FL-0.133	0.035	log(MA) = 0.153 TL + 0.230	0.87	0.012
	Loudias	20	6.2-9.8	FL=0.943TL-0.213	0.027	log(VMO)= 0.554 log(TL) + 0.431#	0.51	0.128
				SL=0.790TL+0.051	0.041	log (HMO) =0.733 log(TL) – 0.117#	0.68	0.118
				SL=0.840FL+0.220	0.034	log(MA) = 0.070 TL + 0.808	0.77	0.009
Barbus strumicae	Strymonas	52	8.9-23.2	FL=0.950TL-3.222	0.016	VMO= 0.807 TL – 1.845	0.94	0.029
				SL=0.845TL-0.479	0.016	HMO = 0.552 TL – 1.997	0.89	0.028
				SL=0.888FL-0.176	0.009	log(MA) = 2.457 log(TL) - 1.200	0.94	0.090
Barbus balcanicus	Axios	39	8.0-17.0	FL=0.942TL-0.229	0.008	VMO = 0.791TL – 1.055	0.89	0.047
				SL=0.828TL-0.301	0.017	HMO = 0.571 TL – 1.559	0.89	0.033
				SL=0.879FL-0.105	0.015	log(MA) =2.449 log(TL) - 1.107	0.93	0.111
	Loudias	28	7.1-15.7	FL=0.943TL-0.150	0.014	VMO = 0.632 TL + 0.741	0.85	0.052
				SL=0.844TL-0.353	0.022	HMO = 0.520 TL – 0.798	0.90	0.034
				SL=0.895FL-0.217	0.019	log(MA) = 0.083 TL+ 0.533	0.91	0.005
Squalius vardarensis	Aliakmo-nas	22	11.5-20.3	FL=0.961TL-0.187	0.018	VMO = 1.017 TL + 0.654	0.88	0.085
				SL=0.851TL-0.385	0.018	HMO =1.058 TL – 6.356	0.86	0.097
				SL=0.882FL-0.159	0.021	log(MA) =2.516 log(TL) - 0.893	0.91	0.178
	Axios	28	9.2-23.5	FL=0.974TL-0.562	0.015	VMO = 1.055 TL + 0.287	0.90	0.069
				SL=0.856TL-0.579	0.014	HMO = 0.923 TL – 5.281	0.89	0.063
				SL=0.875FL-0.047	0.014	log(MA) = 2.527 log(TL) - 0.948	0.94	0.124
	Loudias	25	9.2-15.0	FL=0.913TL+0.174	0.020	VMO = 1.229 TL – 1.727	0.79	0.134
				SL=0.834TL-0.468	0.027	HMO = 0.658 TL – 0.987	0.66	0.098
				SL=0.910FL-0.594	0.027	log(MA) = 2.275 log(TL) - 0.610	0.86	0.191
Preserved samples								
Barbus strumicae	Strymonas	60	6.5-17.5	FL=0.919TL+0.011	0.011	VMO = 0.554TL + 1.004	0.77	0.052
				SL=0.858TL-0.608	0.009	HMO = 0.335 TL +0.177	0.81	0.021
				SL=0.931FL-0.589	0.008	log(MA) = 1.805 log (TL) - 0.549	0.90	0.080
Squalius vardarensis	Aliakmo-nas	46	5.2-21.2	FL=0.947TL-0.098	0.005	VMO = 1.048TL + 0.375	0.97	0.027
				SL=0.830TL-0.455	0.006	HMO = 0.593 TL – 0.253	0.93	0.024
				SL=0.876FL-0.360	0.007	log(MA) = 1.955 log (TL) - 0.253	0.97	0.050
	Axios	23	3.3-24.5	FL=0.941TL-0.130	0.009	VMO = 1.093TL + 0.112	0.98	0.032
				SL=0.813TL-0.486	0.008	HMO = -1.469TL +0.700	0.97	0.029
				SL=0.864FL-0.363	0.008	log(MA) = 2.112 log (TL) - 0.428	0.99	0.053
	Loudias	59	4.2-21.0	FL=0.934TL-0.057	0.003	VMO = 1.093TL + 0.112	0.98	0.019
				SL=0.819TL-0.393	0.004	HMO = 0.584TL -0.253	0.97	0.013
				SL=0.876FL-0.340	0.005	log(MA) = 2.072 log (TL) - 0.372	0.99	0.029

* for all relations p<0.05, # not statistically significant p>0.05

Table 2. Relationship between maximum height (H_{max}, cm) and total length (TL, cm) based on fresh and preserved fish material. a and b = parameters of the length-H_{max} relations, SE – the standard error of the relations, R^2 – coefficient of determination.

Species	River	N	Y = b X + a	R^2	SE_b
Fresh samples					
Alburnoides thessalicus	Axios	27	H_{max} =0.305 TL - 0.756	0.77	0.033
	Loudias	20	H_{max} =0.265 TL - 0.556	0.73	0.038
Barbus strumicae	Strymonas	52	H_{max} =0.148 TL + 0.244	0.91	0.007
Barbus balcanicus	Axios	39	H_{max} =0.185 TL - 0.195	0.89	0.011
	Loudias	28	H_{max} =0.162 TL + 0.082	0.88	0.011
Squalius vardarensis	Aliakmonas	22	H_{max} =0.281 TL - 0.917	0.91	0.020
	Axios	28	H_{max} =0.227 TL - 0.397	0.93	0.012
	Loudias	25	H_{max} =0.250 TL - 0.704	0.85	0.022
Preserved samples					
Alburnoides thessalicus	Aliakmonas	16	H_{max} = 0.272 TL - 0.513	0.94	0.019
Barbus balcanicus	Aliakmonas	28	H_{max} = 0.170 TL + 0.004	0.90	0.011
Barbus strumicae	Strymonas	60	H_{max} = 0.141 TL + 0.145	0.93	0.005
Squalius vardarensis	Loudias	59	H_{max} = 0.231 TL - 0.341	0.99	0.002
	Aliakmonas	46	H_{max} = 0.235 TL - 0.459	0.96	0.007
	Axios	23	H_{max} = 0.209 TL - 0.219	0.99	0.004
	Strymonas	10	H_{max} = 0.232 TL - 0.329	0.99	0.009

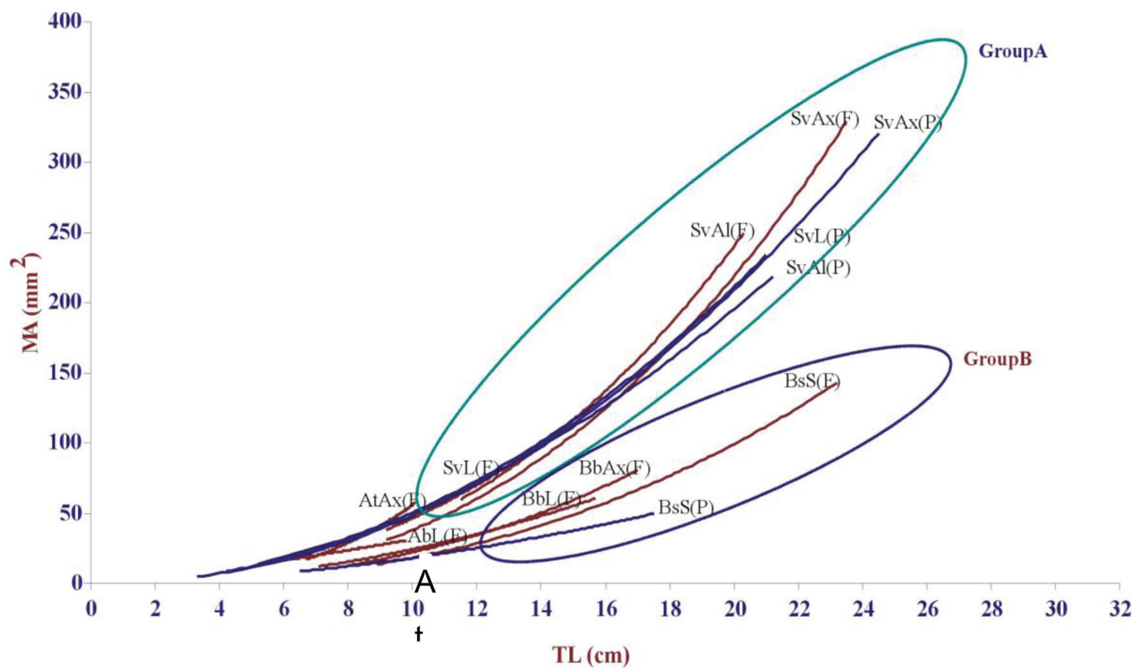

Fig. 2. Mouth area (MA, mm^2) – total length (TL, cm) regressions for fresh (F) and preserved (P) fish samples. AtAx: *Alburnoides thessalicus* from Axios, AtL: *A. thessalicus* from Loudias. Group A: SvAl: *Squalius vardarensis* from Aliakmonas, SvAx: *S. vardarensis* from Axios, SvL: *S. vardarensis* from Loudias. Group B: BsS: *Barbus strumicae* from Strymonas, BbAx: *B. balcanicus* from Axios, BbL: *B. balcanicus* from Loudias.

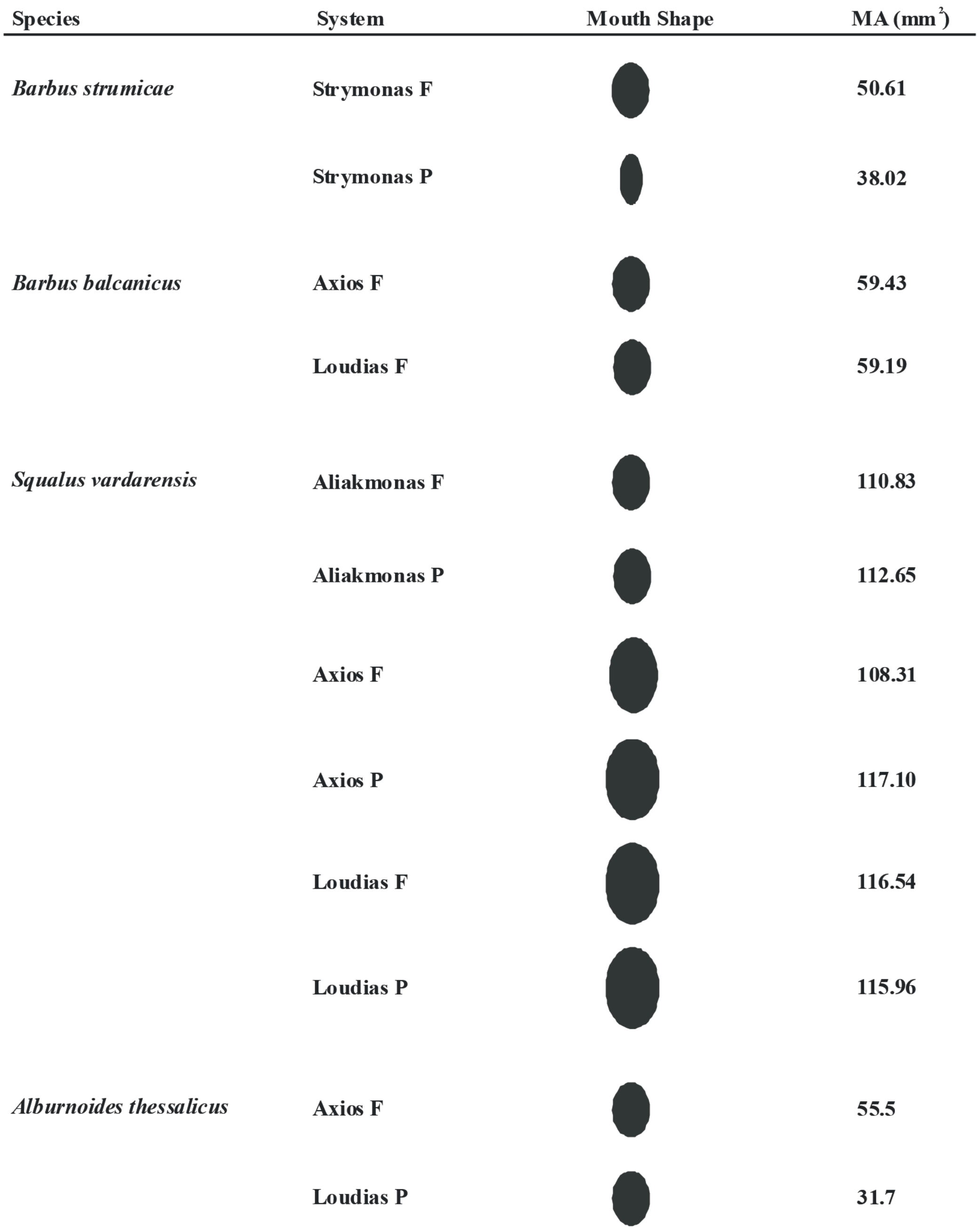

Fig. 3. Mouth shapes (in real dimensions) and mouth area (MA) values corresponding to TL=15 cm based on fresh (F) and preserved (P) fish material. For *Alburnoides thessalicus* (F) MA values correspond to TL=10 cm.

tance as they provide ecological information for the studied species that can be used for between-species comparisons. The faster increase estimated for *S. vardarensis* with TL than for *B. balcanicus* and *B. strumicae* could be related to species trophic habits, because *S. vardarensis*, similar to its sympatric congeneric species *S. cephalus,* has a wide feeding spectrum (i.e. omnivorous) (Ünver & Erk'akan 2011). In contrast, both species of *Barbus* display a quite narrow dietary spectrum and are mostly specialised in larvae of Chironomidae and Ephemeroptera (Sapounidis et al. 2015), a fact that was in agreement with the lowest MA values.

MA-TL relations might be useful as a tool for separating species or genera within families. Moreover, the integration of such relations for a wide range of species with different diet preferences will allow us to estimate safely the species trophic levels. Such estimates from freshwater ecosystems are generally lacking, since diet studies are difficult to be obtained (Bobori et al. 2013). Differences of the estimated parameters from the above-mentioned relations with the corresponding from other systems might be attributed to one or more of the following factors: abiotic (e.g., water temperature) (Wootton 1998), sex (Radojković et al. 2019), observed length ranges (Kyritsi & Moutopoulos 2018), sample size (Cone 1989), trophic status of the water systems (Bobori et al. 2013) and seasonality. These affect gonad activity, which in turn could cause seasonal variations in morphometric relations (Kyritsi et al.2018). Thus, all the estimated relations could be considered as mean annual values and their use should be limited to the observed length ranges. With respect to the nature of the preservative, changes in the fish body size characteristics have been previously described according to the type of preservative used (Sagnes 1997). However, in the present study, most of the morphometric and mouth relations were not significantly biased between preserved and fresh samples.

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