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PROOF SHORT PAPER

Length-Length, Length-Weight and a Proposed Standard Weight Equation for the Italian Endemic Species *Barbus tyberinus* Bonaparte, 1839

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Abstract

Relative weight (W_r) is an index of condition computed as the comparison between the actual weight of a fish and a standard weight (W_s) that is the weight of an ideal fish at the same length that is in good condition. Data on length and weight of horse barbel *Barbus tyberinus*, an Italian endemic species, were collected throughout the entire distribution range of the species. Data were then used to develop a W_s equation for the species by means of the Empirical Percentile (EmP) method. The EmP W_s equation, calculated on total length (TL), resulted: $\log_{10} W_s = -4.9671+3.0216 \log_{10} TL - 0.0095 (\log_{10} TL)^2$ (TL range 80-480 mm). In addition, length-length and length weight equations were provided. The TL-W equation was calculated as: W = -4.861+2.940 $\log_{10}$ TL. The standard length (SL)-TL equation was TL = 1.149 SL+0.445 while the fork length (FL)-TL was TL= 1.050 FL+0.629. The results provided useful information for the biology of the species and underlined the value of relative weight as a tool to evaluate the condition of fish.

Keywords: Condition index, endemic species, length-weight equation, relative weight, EmP method.

Introduction

Analysis of length-weight relationships provides important insights into the ecology of a species (Anderson and Neumann, 1996). Body condition indexes are useful tools derived from length-weight relationships for a species and provide a measure of the health of a fish population assuming that heavier fish of a given length are in better condition. Body condition indices allow evaluation of the well-being of fish (Blackwell *et al.* 2000) founded on statement that “fatter is fitter” (Glazier, 2000). These indexes have become important tools for fisheries management (Anderson and Neumann, 1996; Blackwell *et al.*, 2000) since they allow to process a large numbers of fish with minimal mortality and they do not require to sacrifice any of the specimens (being based only on measurements of length and weight) (Fechhelm *et al.*, 1995). Relative weight (W_r) (Wege and Anderson 1978) is a condition index computed as the comparison between a measured weight of a fish and a standard weight (W_s) that consists in weight at the same length of an ideal specimen that is in good condition (Murphy *et al.*, 1991). W_s is predicted by a standard weight equation, that is a species-specific length-weight equation (Wege and Anderson, 1978).

Utilization of W_r to perform well-being of fish species has been extensive since its development (Blackwell *et al.*, 2000). However, its applicability is still limited because species-specific W_s equations need to be developed for the application of this index and, for this purpose, a wide sample of specimens collected all over the distribution range of each species is required (Bister *et al.*, 2000).

While this methodology is commonly used in United States, the use of relative weight in other countries is still limited probably because of the lack of specific W_s equations. Standard weight equations were already developed for other Italian endemic fish species such as cavedano chub *Squalius squalus* and brook chub *Squalius lucumonis* (Giannetto *et al.*, 2011; 2012c).

Horse barbel *Barbus tyberinus* Bonaparte, 1839 is a cyprinid species endemic for Italy. Its area of distribution comprises: the Tyrrhenian basin between Magra and Sele drainages; the Adriatic basin from Esino to Ofanto drainages (Lorenzoni *et al.*, 2006a), the Ionian basin in Agri and Basento river (unpublished data)] (Figure 1). In addition to its conservation value, the species is also appreciated for sport fishing. Currently the horse barbel is declining due to the competition and hybridisation with non native species [mainly the congeneric Italian barbel *B. plebejus* Bonaparte, 1839 endemic of Northern Italy

Figure 1. Area of distribution of horse barbel (dark grey area).

and barbel *B. barbus* (Linnaeus, 1758) native of Northern Europe. These species were introduced in the original range of horse barbel by recreational fishermen to improve stock catches (Lorenzoni *et al.*, 2006a). For this reason, *B. tyberinus* was assessed as "Near threatened" in the IUCN Red List of Endangered Species (Freyhof 2011) and "Vulnerable" in the IUCN Red List of Italian Vertebrates (Rondinini *et al.*, 2013).

The main aim of this research was to propose an empirical W_s equation developed for horse barbel. A further aim was to provide the general models of length-length and length-weight for this Italian endemism. The results of the study will represent a useful tool for further studies on this Italian endemic species and will contribute to raise the limited information on ecology and biology of this species.

Materials and Methods

Data Processing and Development of Standard Weight Equation

Data of length [total (TL, mm); standard (SL) and fork (FL) length] and weight (W, g) of horse barbel were collected across its distribution range during different monitoring studies carried on to evaluate the conservation status of the Italian freshwaters fish. For this aim, immediately after measurements were taken all specimens were returned to their environment and no fish was sacrificed.

The first step was then to validate and screen the total dataset following the procedure recommended by Giannetto *et al.* (2012a).

Then, linear conversion models to convert SL and FL to TL for horse barbel were computed by using the different types of measures collected.

A TL-W equation was developed for the total sample and, to assess the growth pattern of the species (i.e: isometric vs positive or negative allometric growth) (Froese, 2006), the Student's *t*-test was applied to test whether the b value of the TL-W equation was significantly different than 3 (indicating an isometric growth) (Ricker, 1975).

The further preliminary stage for the computation of the W_s equation consisted of the determination of a suitable length range of applicability. A minimum size was determined by plotting the variance/mean of $\log_{10}$W on TL classes of 10 mm as suggested by Willis *et al.* (1991). Then, that length class at which this ratio was less than 0.01 was assessed as minimum TL (Murphy *et al.*, 1990). In accordance with Gerow *et al.* (2005), a maximum TL was identified by checking the length class in the dataset that was present in the dataset with at least three different populations. All specimens smaller or bigger than this size range were excluded from dataset.

The validated dataset was then separated in a larger “development dataset” (used to compute the W_s equation) and a smaller “validation dataset” (utilised to investigate any possible length-related biases in the W_s equation developed) (Ogle and Winfield, 2009; Lorenzoni *et al.*, 2012).

The next step consisted of separating the dataset in different “statistical populations”. To do it, data collected in the same location but in small number (N < 10) were removed from the dataset; data collected in the same location, but in different years, were considered as different statistical samples (Ogle and Winfield, 2009; Giannetto *et al.*, 2012a). A log transformed linear regression between TL and W was accomplished for each population separately, and all the anomalous values (outliers) were removed from the regression since these were probably the result of incorrect measurements (Bister *et al.*, 2000). Then, as suggested by Froese (2006), all the populations owing a R^2 value less that 90% or with a value of *b* fell outer the 2.5-3.5 range were excluded from further analysis because, according to Carlander (1977), anomalous values of *b* or R^2 could derive from statistical populations with narrow length range.

The W_s equation for horse barbel was accomplished through the EmP method. The mean empirical W (for each 10-mm length-group) was calculated by the $\log_{10}$ transformed TL and W of each population of the development dataset; the 3rd quartiles of these mean empirical W were regressed against TL to estimate the EmP W_s equation by using a weighted quadratic model (Gerow *et al.*, 2005).

Validation of W_s Weight Equation

The validation data set was used to validate the proposed W_s equation and to assess the presence of potential bias (Gerow *et al.*, 2005). For this purpose, two different methodologies were used: the analysis of distribution of residuals versus fitted values of the W_s equation (Giannetto *et al.*, 2012a); the empirical quartiles (EmpQ) method proposed by Gerow *et al.* (2004) and calculated through the Fisheries Stock Assessment package (FSA) (version 0.4.1) (Ogle, 2009) by R software (R version 3.0.2 Development Core Team, 2013) to verify if the slope value of the quadratic plot between 3rd quartile of mean W (standardized by W_s) and TL class of 10 mm was 0 (Giannetto *et al.*, 2012a).

Results

A total of 19046 specimens of horse barbel collected throughout Italy were analyzed. The size range was 28-576 mm (mean TL ± SE = 153.22±0.52 mm) whereas the weight range was 0.20-1977 g (mean W±SE = 62.70±0.67 g). The parameters of the log-transformed TL-W equation were reported in Table 1. The value of *b* (2·940) was found different from 3 by *t*-test (t = 1189.097; P<0.01).

The parameters of the linear conversion models computed to convert SL and FL to TL were summarized in Table 1.

The development dataset consisted of 17088 specimens while the smaller validation dataset consisted of 1958 specimens.

The minimum TL was identified to be 80 mm (Figure 2) and the maximum TL was assigned as 480 mm (Table 2). From the 251 statistical populations of the development dataset, 5 populations were removed because the value of *b* was outside the range 2.5 -3.5.

The parameters of the EmP-W_s equation developed for horse barbel were provided in Table 1.

By the plot between residuals and fitted values of the EmP-W_s equation, residuals showed a random distribution indicating no correlation between W_s and TL (Figure 3a). The EmpQ method was applied to the length range 80-340 mm because 340 mm was the length of the longest specimen in the validation dataset. According to this method, the EmP-W_s equation developed for horse barbel did not exhibit apparent patterns (Figure 3b) and the value of slope of both terms resulted not significantly different from zero ($p_{quadratic}$ = 0.063, p_{linear} = 0.537).

Discussion

Horse barbel is a rheophilic cyprinid that populates the intermediary traits (“barbel zone”) in the secondary water courses in Central Italy (Lorenzoni *et al.*, 2006b). The viability of horse barbel is currently threatened by non native species

Table 1: Parameters of total length- weight (TL-W), total-standard length (SL-TL), total-fork length (FL-TL) and TL-standard weight (W_s) equations developed for *Barbus tyberinus*

Equation	n	R^2	a	Range a (95% CI)	b	Range b (95% CI)	c	Range c (95% CI)
TL (mm)-W	19046	0.993	$0.138\ 10^{-4}$	$0.135\ 10^{-4}$-$0.141\ 10^{-4}$	2.940	2.935 2.945	-	-
SL-TL	187	0.998	0.445	0.356-0.532	1.149	1.139 1.158	-	-
FL-TL	229	0.997	0.629	0.403-0.857	1.050	1.038 1.061	-	-
TL (mm)-W_s	17088	0.999	$0.108\ 10^{-4}$	$0.099\ 10^{-4}$-$0.118\ 10^{-4}$	3.022	2.987 3.056	- 0.009	-0.017-0.002

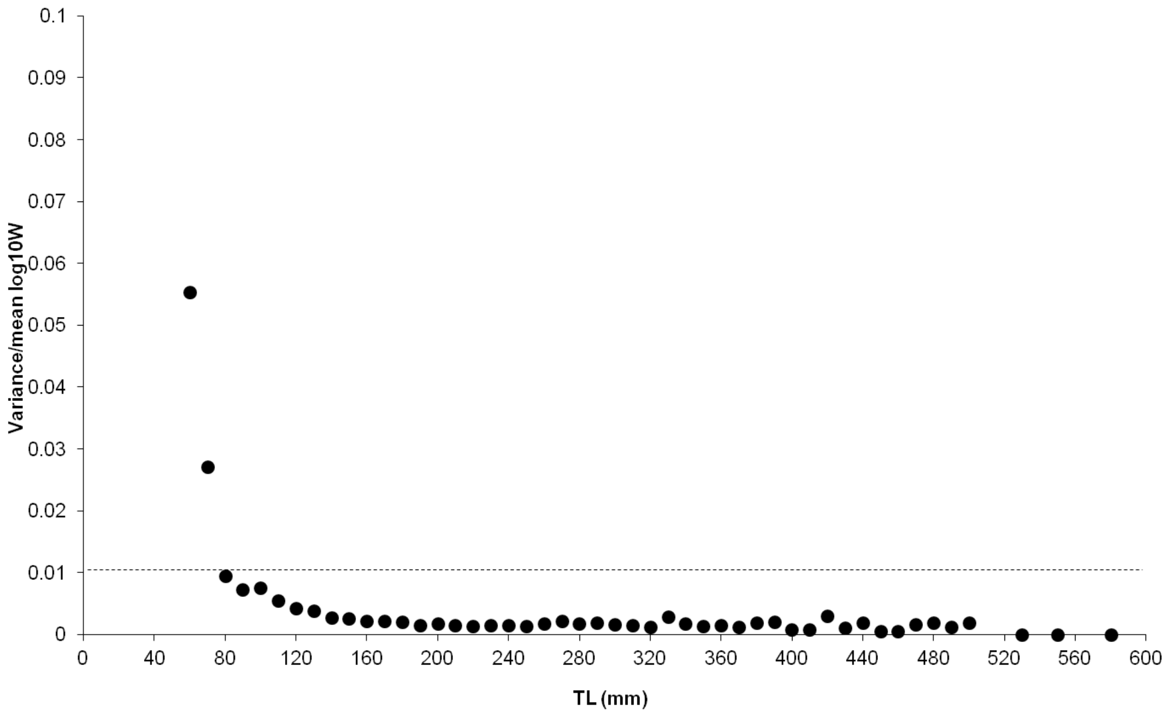

Figure 2. Relationship between variance/mean for $\log_{10}$ of weight (W) and total length at 10-mm intervals for the determination of the minimum total length of horse barbel. The dotted line indicates the value of 0.01.

Table 2: Number of populations (N pop) of horse barbel used to develop the standard weight equation per 10-mm length class (TL). (as suggested by Gerow (2005), the TL classes marked with an asterisk were not used in the determination of the W_s equation since 3 is the minimum number that allows the calculation of quartiles)

TL (mm)	N pop	TL (mm)	N pop
80	136	310	71
90	145	320	44
100	151	330	45
110	174	340	30
120	159	350	29
130	158	360	23
140	177	370	18
150	180	380	13
160	188	390	7
170	189	400	11
180	186	410	10
190	187	420	10
200	191	430	3
210	184	440	3
220	175	450	4
230	158	460	5
240	148	470	3
250	135	480	4
260	126	490	1*
270	101	500	1*
280	111	530	1*
290	86	550	1*
300	72	580	1*

that have been widely introduced into its distribution range. The high risk of hybridisation and the negative interaction between horse barbel and the other species of barbel were reported by several authors (Bianco and Ketmayer, 2001; Lorenzoni *et al.*, 2006b; Giannetto *et al.*, 2012b).

In this contest, a chance to evaluate the condition of specimens, and compare the condition of different populations by means of a W_s equation valid for the whole distribution area of the species, could

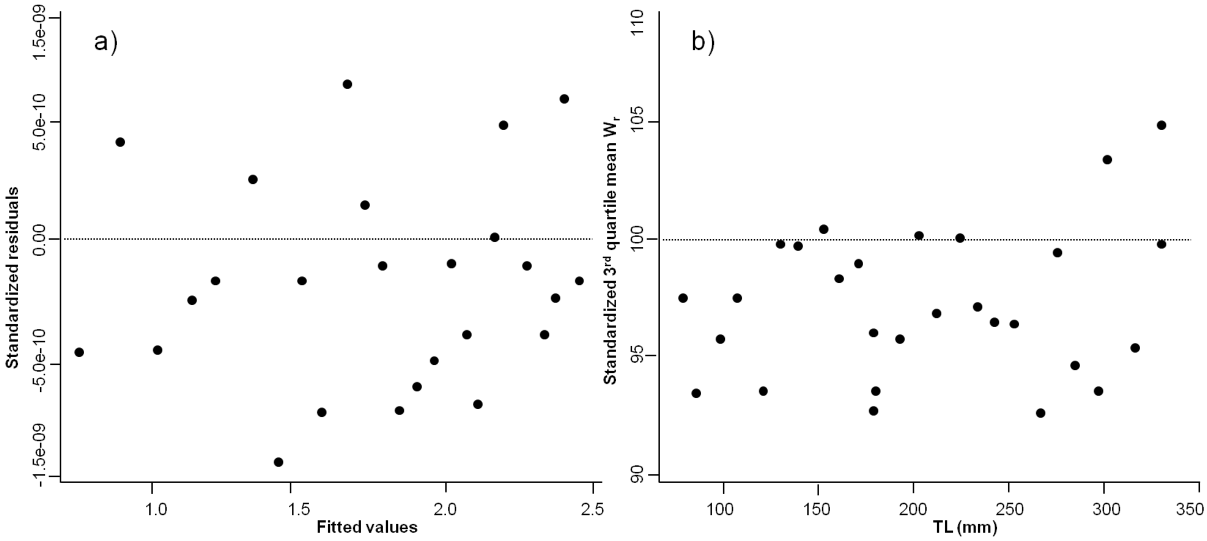

Figure 3. Plots showing the distribution of the residuals (a) and the results of the application of the Empirical Quartiles (EmpQ) method (b) used to investigate potential length-bias in the standard mass (W_s) equation for horse barbel. (Residuals= standardized residuals of the regression; Fitted values= values obtained by the model fit; Standardized 75° percentile mean W = standardized 75° percentile mean weights calculated by W_s equation; TL (mm) = total length in mm).

represent a precious tool for the conservation and management of the species. Comparisons between the data of different periods, and different populations, may allow to detect any decline in the condition and then to identify potential at risk populations of horse barbel.

The EmP W_s equation developed in this study was not affected by length-bias and this suggested that it can be used for evaluation of W_r across the entire distribution area of horse barbel.

Moreover, a general total length-weight equation was also provided for horse barbel. The b value of the TL-W (2.94) was significantly lower than 3 (value indicating an isometric growth) and this supported the assumption of a negative allometric growth for horse barbel.

In conclusion, the results obtained will be useful for further studies on ecology and biology of this Italian endemic species. Moreover, the W_s equation proposed for horse barbel, together with the W_s equations already developed for other Italian species, will represent a precious tool to assist in the evaluation of fish communities' ecological status required to all European union members in accordance with the mandatory of the Water Framework Directive 2000/60/EU (European Parliament and Council, 2000).

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