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### PROPOSED STANDARD WEIGHT EQUATIONS FOR BROWN TROUT (*SALMO TRUTTA* LINNAEUS, 1758) AND *BARBUS TYBERINUS* BONAPARTE, 1839 IN THE RIVER TIBER BASIN (ITALY)

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Abstract: Relative weight is an index of condition that provides a measure of the well-being of a fish population. The index is calculated on the basis of comparison between the actual weight of a specimen and the ideal weight of a specimen of the same species in good physiological condition (standard weight). Two methods forcalculating the standard weight are proposed in the literature: the RLP method and the EmP method. Although the RLP method is widely used, it has some limitations; as it uses the weights derived from the TL/W regressions of different populations to calculate the index, it is influenced by the size distribution of the specimens. The main aim of our research was to work out equations for calculating standard weight that would be valid for two species in the River Tiber basin. To this aim, 91 (N = 18216) different populations of brown trout (Salmo trutta L.) and 64 (N = 12778) different populations of Barbus tyberinus were examined. A further aim was to compare the validity of the two proposed methods (RLP and EmP) of calculating relative weight. For brown trout, the equations calculated with regard to the River Tiber basin are as follows:  $\log_{10}W_s = -5.197 + 3.117 \log_{10}TL$  (RLP method);  $\log_{10}W_s = -5.203 + 3.154 \log_{10}TL$  $-0.015 (\log_{10}TL)^2$  (EmP method), where TL is the total length. The equations calculated by means of the two methods for Barbus tyberinus in the River Tiber basin are as follows:  $\log_{10}W_s = -5.072 + 3.040 \log_{10} TL (\log_{10} TL) (RLP method) and \log_{10}W_s = -4.917 + 2.987$  $\log_{10}TL + 0.003 (\log_{10}TL)^2$  (EmP method).

Key words: Relative weight ( $W_r$ ), index of condition, RLP method, EmP method, length-weight regression.

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### Introduction

A variety of indices of body condition based on length and weight measurements have been developed for fish. Body condition indices are used to describe samples from fish populations and have become important tools for fisheries managers (Anderson and Neumann 1996; Blackwell et al. 2000).

Body condition indices should be free length-related biases from (i.e., any systematic tendency to overor condition underestimate body with increasing length) in order to enable accurate comparisons of samples from different fish populations and assessments of temporal trends in individual fish populations (Murphy et al. 1990; Anderson and Neumann 1996; Blackwell et al. 2000). The relative condition index ( $K_n$ ) (Le Cren 1951) was developed to overcome the length-related biases in Fulton condition factor (Anderson and Neumann 1996). The potential for length-related biases first emerged when the concept of  $K_n$ , as originally proposed by Le Cren (1951), was expanded into the concept of state-wide standards for Alabama fishes (Swingle and Shell 1971). Consequently, relative weight ( $W_r$ ) was developed as a body condition index (Wege and Anderson 1978). Relative weight is calculated by means of the equation:  $W_r = (W/W_s)100$ , (W = weight of the specimen in grams,  $W_s =$ standard weight). Standard weight is determined on the basis of the regression:  $log_{10}W = a' + b log_{10}TL$ .

Wege and Anderson (1978) defined standard weight as the 75th percentile of the weights of a given species within specified length increments. This choice results in "above-average condition" becoming the standard against which to compare fish. A W<sub>r</sub> of 100 for a given fish indicates that the fish is at the 75th percentile of mean weights for the species at that length. Different techniques have been used to model the relationship between Ws and length in order to establish a simple expression of standard weight (Blackwell et al. 2000). Murphy et al. (1990) introduced regression line-percentile the (RLP) method for computing W<sub>s</sub> equations, a technique that has become standard among biologists fisheries (Anderson and Neumann 1996; Blackwell et al. 2000). A W<sub>s</sub> equation should yield approximately the 75th percentile of mean weights among populations of the target species for fish of all lengths within the range of applicable lengths if no length-related biases in W<sub>s</sub> are present (Murphy et al. 1990). If no biases are present, variation in W<sub>r</sub> across length increments in a sample from a given population can be attributed to changes in body condition.

Gerow et al. (2004) found length-related biases in  $W_s$  equations developed by the RLP method for several species. Combining linear regression and extrapolation contributes to biases in  $W_s$  equations developed by the RLP method.

Length-related biases in  $W_s$  equations developed by means of the RLP method (Gerow et al. 2004) prompted the development of a new method of computing  $W_s$  equations. The new method, which uses only empirical data, is designated the EmP method. The EmP method is based on quartiles of measured mean weights of fish (not weights estimated from regression models) in a given length-class among sampled fish populations. Since quartiles are not estimated from modelled means, there are no modelling artefacts, such as the bow-tie effect, which influence  $W_s$  equations.

However, standard weight-length relationships have been defined for only a few species. With regard to the brown trout (*Salmo trutta* Linnaeus, 1758), the standard weight proposed in the literature (Anderson and Neumann 1996) is calculated on the basis of equations drawn up for non-European populations. *Barbus tyberinus*, a fish species with limited distribution, is endemic to Central and Southern Italy (Bianco 1995); for this species, no equation has been proposed in the literature.

The aim of the present research was to produce an equation that would be valid for the brown trout and barbel populations in the River Tiber basin. Moreover, the data were used to compare the results yielded by the two methods proposed (EmP and RLP).

# Materials and Methods

The River Tiber is the third-longest river in Italy and has the second-largest watershed. Its source is located on Mount Fumaiolo (about 1270 m a.s.l.). It is 405 km long and is the backbone of the hydrological network in the Umbria Region. The total River Tiber watershed  $(17.375 \text{ km}^2)$  also extends into the Italian Regions of Emilia Romagna, Tuscany, Lazio, Marche, Molise and Abruzzo. The study area was located in the Regions of Umbria, Tuscany and Lazio, from the source of the Tiber to its confluence with the River Aniene. During the research, 91 brown trout populations from 33 waterways and 64 populations of Barbus tyberinus from 35 waterways were examined (Table 1): a total of 18.217 specimens of brown trout and 12.778 of barbel. The total length (TL) (±1 mm) and weight (W) ( $\pm 0.1$  g) of each was recorded (Anderson and Neumann 1996).

	Species	Sample size	Mean	Min	Max	Standard deviation
Weight (g)	Salmo trutta	18216	70,49	0,75	2335	84,49
Length (mm)	Salmo trutta	18216	164,57	20	580	59,42
Weight (g)	Barbus tyberinus	12778	63,17	0,43	1316	82,63
Length (mm)	Barbus tyberinus	12778	156,06	20	502	69,05

Table 1. Descriptive statistics of the sample used to calculate relative weight for the two species.

The techniques used for developing  $W_s$  equations required determination of the minimum total length to be used in the computation. The minimum TL was determined from the relationship of the variance/mean ratio for  $log_{10}W$  by 1-cm intervals of TL (Figure 1). The minimum TL corresponds to the inflection point in the plot (Murphy et al. 1990).

The validity of the data was assessed by plotting the TL-W relationship (mm and g, respectively) for each population separately, so that individual outliers could be identified. In no case did the value of r in these regressions prove to be lower than 0.9; therefore, no population was excluded from the subsequent analyses (Bister et al. 2000).

Slopes (b) were then plotted as a function of intercepts (a) (Pope et al. 1995) to identify and remove population outliers caused by insufficient sample size, narrow length-range, or misidentified length measurements other than TL (Froese 2006). In this case, too, the analysis did not lead to the exclusion of any of the populations examined.

With regard to the procedures used to calculate the standard weight equations, reference was made to Murphy et al. (1990), for the RLP method and to Gerow et al. (2005), for the EmP method. The length-range judged to be suitable for the application of  $W_r$  is divided into J 1-cm TL - classes, each with midpoint  $L_j$  (j = 1, ..., J). TL-W measurements on fish from I fish populations comprise the data.  $\hat{W}_{i,j}$  (i = 1, ..., I) is taken to be the log<sub>10</sub>W at  $L_j$  estimated from a log<sub>10</sub>W on log<sub>10</sub>TL simple linear

regression for the data from fish population i.

The RLP method comprises three steps:

(1) computing  $\hat{W}_{i,j}$  for all combinations of i and j;

(2) computing the 75th percentile (third quartile) of  $\hat{W}_{i,j}$  for each 1-cm TL increment, denoted as  $Q_i$  ( $\hat{W}_{i,j}$ ); and

(3) regressing  $Q_j$  ( $\hat{W}_{i,j}$ ) against log  $L_j$  to obtain the  $W_s$  equation for that species. The standard weight at length  $L_j$  is  $W_s$  ( $L_j$ ).

The EmP method is similar to the RLP method in the use of third quartiles, but computation of the standard weight equation differs.

The EmP method comprises these three steps:

(1) letting  $W_{i,j}$  (measured, not modelled) be the sample mean of  $log_{10}$  weights at length  $L_j$  from fish population i in each of the J 1-cm TL-classes,

(2) computing the third quartile  $Q_j$  ( $\hat{W}_{ij}$ ) in each TL-class, and

(3) regressing  $Q_j$  ( $\hat{W}i,j$ ) against  $log_{10}L_j$  by means of a weighted quadratic model.

The equations thus obtained were used to calculate the relative weight of each specimen from each population. Relative weight ( $W_r$ ) was determined by means of the equation:  $W_r = (W/W_s)100$  (Wege and Anderson 1978), where W is the weight of an individual in grams, and  $W_s$  is the standard weight predicted by the TL - W regressions obtained by means of the RLP and EmP methods.

Subsequent elaborations were aimed at testing the validity of the two methods of calculating standard weight. Specifically, we: 1) calculated the  $TL-W_r$  linear regressions, using the individual  $W_r$  values calculated by means of the two methods; this was done to verify independence from size;

2) used covariance analysis to compare the two regressions obtained;

3) calculated the mean relative weight of each population by means of the two different methods;

4) applied analysis of variance to compare the mean values obtained;

5) compared the responses of the two methods as a function of the size of the specimens examined. This comparison involved analysing the difference between the relative weight yielded by the RLP method (W<sub>s</sub>-RLP) and that yielded by the EmP method (W<sub>s</sub>-EmP), expressing this difference as a percentage of the weight obtained on the basis of the TL – W regression of the total sample 100 (W<sub>s</sub>-RLP - W<sub>s</sub>-EmP)/W and constructing the trend in these values as a function of TL.



Figure 1. Relationship of the variance/mean ratio for log<sub>10</sub>W to log<sub>10</sub>TL, for *Salmo trutta* and *Barbus tyberinus*.

### Results

On the basis of the variance/mean ratio for  $log_{10}W$  (Figure 1), specimens with a total length of less than 8 cm were excluded from the analysis. We defined the minimum specimens size to be the critical point in the variance/mean ratio relationship, where the rate of change began to decrease. Accordingly, 21 specimens of *Barbus*  *tyberinus* and 883 specimens of brown trout were removed from the dataset.

For the *Barbus tyberinus* populations, the TL range used to calculate the relative weight was therefore from 8 to 50 cm for the RLP method and 8 to 42 cm for the EmP method; for *Salmo trutta*, the length-range judged to be suitable for  $W_s$  calculation was from 8 to 58 cm for the RLP method and from 8 to 44 cm for the EmP method.

The TL-W regression of the total sample of brown trout was:

 $\log_{10}W = -1.974 + 3.011 \log_{10}TL (R^2 = 0.98);$ 

for Barbus tyberinus it was:

 $\log_{10}W = -4.965 + 1.297 \log_{10}TL (R^2 = 0.98).$ 

In accordance with the procedures of the two methods, the following standard weight equations were drawn up.

For *Salmo trutta* the equations are (Figure 2):

 $\log_{10}W_s$  = - 5.197 + 3.117  $\log_{10}TL$  (R<sup>2</sup> = 1.00) for the RLP method;

 $log_{10}W_s$  = - 5.203 + 3.154  $log_{10}TL - 0.015$  $(log_{10}TL)^2$  (R<sup>2</sup> = 0.99) for the EmP method.

For *Barbus tyberinus* the corresponding equations are (Figure 3):

 $log_{10}W_s = -5.072 + 3.048 log_{10}TL (R^2 = 1.00) (RLP method);$ 

 $\log_{10}W_s = -4.917 + 2.987 \log_{10}TL + 0.003 (\log_{10}TL)^2 (R^2 = 0.99)$  (EmP method).

The W<sub>r</sub>-TL regressions for *Salmo trutta* were:

 $W_r = 99.067 - 0.021 \text{ TL } (R^2 = 0.008; p = 0.000) \text{ (RLP method)};$ 

 $W_r = 95.779 - 0.005 \text{ TL} (R^2 = 0.0005; p= 0.002)$  (EmP method).

In neither of the two regressions did we observe complete independence from size (p < 0.05); in the case of the EmP method, however, the slope of the line was far less marked. The differences between the two regressions proved highly significant on Ancova (F = 13.92; p = 0.001).

The W<sub>r</sub>-TL regressions for *Barbus tyberinus* were:

 $W_r = 105.163 - 0.034 \text{ TL} (R^2 = 0.022 ; p = 0.000) (RLP method);$ 

 $W_r = 93.714 + 0.003 \text{ TL} (R_2 = 0.001 \text{ ; } p = 0.130) \text{ (EmP method).}$ 

Independence from size was therefore confirmed only for the EmP method; in this case, too, the differences between the two regressions proved highly significant on Ancova (F = 756.18; p = 0.000).



Figure 2. Linear regression between the third quartile of  $log_{10}W$  calculated by means of the RLP method and the  $log_{10}TL$  for *Salmo trutta*.



Figure 3. Linear regression between the third quartile of  $log_{10}W$  calculated by means of the RLP method and the  $log_{10}TL$  for *Barbus tyberinus*.

Figure 4 shows the mean values of  $W_r$  for the single populations for the two species, as calculated by means of the two methods (C.I. 95%); while both trends were very similar for both species, the mean values yielded by the RLP method were, in every case, higher than those yielded by the EmP method. On analysis of variance, the differences between the mean values of  $W_r$ calculated by means of the two methods proved to be highly significant in both species (trout: F =41.298, p = 0.000; barbel: F = 49.813, p = 0.000).

Figure 5 shows the trend in the percentage difference between W<sub>s</sub>-Emp and W<sub>s</sub>-RLP as a function of TL. The trend of the curve is very similar in the two species: the differences between the standard weights calculated by means of the two methods are far more marked for fish of small sizes, when the value of W<sub>s</sub>-Emp exceeds that of  $W_s$ -RLP. The relationships between the  $W_s$ values are inverted for fish of larger sizes  $(W_s-RLP)$  $W_s$ -EmP), while in > the intermediate size-classes the differences cancel each other out. The RLP method therefore underestimates the standard weight of the small specimens; indeed, in the 8 cm length-class, the percentage difference

between the weights calculated by means of the two methods is about 2% for the trout and about 10% for the barbel. At a length of 50 cm,  $W_s$ -RLP exceeds  $W_s$ -EmP by about 4% and 3% for the brown trout and barbel, respectively.

### Discussion

Relative weight is easier to interpret than other condition index, in that it neither increases with increasing length nor varies by species (Ouist et al. 1998). Unlike the condition factor. it Fulton enables comparisons to be made between groups of specimens or populations of different lengths, even in conditions of allometric growth (Blackwell et al. 2000). Indeed, the results obtained seem to show that relative weight is not always independent of the length of the specimens examined, but in part that come from the method (RLP or EmP) that is used in the analysis and maybe from the features of the species examined too. The EmP method nevertheless seems to ensure greater efficacy from this point of view.

The choice of the method used to estimate standard weight strongly influences the results.



Figure 4. Comparison between the RLP and EmP methods: mean values and confidence limits (95%) of relative weight  $(W_r)$  in the 91 populations of trout (a) and in the 64 populations of barbel (b) examined.



Figure 5. Trend in the percentage difference between the standard weights calculated by means of the two methods ( $W_{s-EmP}$  -  $W_{s-RLP}$ ) as a function of size (TL) for the two species examined.

obtained when relative weight is used as an index for condition; indeed, the differences between the two methods of calculating W<sub>r</sub> proved highly significant in both species. For both species, the RLP method tends to attribute markedly higher average W<sub>r</sub> values than those vielded by applying the EmP method to the same populations. This is the result of the underestimation of W<sub>s-RLP</sub> values in smaller specimens; however, it also depends on the age structure of the population, in which younger specimens are generally more abundant. The differences between the W<sub>s</sub> obtained by the two methods, for both species, are evident in specimens with either very low or very high TL values, while among samples of intermediate length, they tend to disappear. This phenomenon could be the consequence of a bow-tie effect (Gerow et al. 2005) caused by using the weights obtained from the TL-W regressions in the W<sub>s</sub> calculation procedure, instead of the values actually measured.

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