Proposed standard weight (W_s) equations for *Telestes muticellus* (Bonaparte, 1837) in the Tiber River basin

by

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ABSTRACT. - Relative weight (W_r) is a body condition index that enables the evaluation of the wellbeing of fish by comparing the actual weight of a specimen with the ideal weight of a specimen of the same species and same length, in good physiological condition, i.e., standard weight (W_s). Two methods of calculating standard weight are proposed in the literature: the RLP and the EmP method. The main aim of this study was to work out standard weight equations for Italian riffle dace, *Telestes muticellus*, in the Tiber River basin. To this end, length and weight measurements of 9,186 specimens from 57 watercourses in the Tiber River basin were analysed. The resulting standard weight equations were: $\log_{10}(W_s) = -5.085 + 3.081 \log_{10}(TL)$ for RLP method and $\log_{10}(W_s) = -3.706 + 1.685 \log_{10}(TL) + 0.349 [\log_{10}(TL)]^2$ for EmP method. A further aim of this research was to compare the performance of the two proposed methods (RLP and EmP). The use of the EmP W_s equation to compute W_r of Italian riffle dace in the Tiber River basin is suggested, as it proved not to be influenced by length-related bias.

RÉSUMÉ. - Proposition de l'équation pour le poids standard (W_s) pour *Telestes muticellus* (Bonaparte, 1837) dans le bassin du Tibre.

Le poids relatif (W_r) est un indice de l'état corporel qui permet l'évaluation du bien-être des poissons en comparant le poids réel d'un spécimen avec le poids idéal d'un spécimen de la même espèce et de même longueur en bon état physiologique (poids standard, W_s). Deux méthodes de calcul du poids standard sont proposées dans la littérature : les méthodes RLP et EmP. L'objectif principal de cette étude était de mettre au point des équations pour le calcul du poids standard pour *Telestes muticellus* dans le bassin du Tibre. Dans ce but, la taille et le poids de 9186 spécimens de 57 cours d'eau du bassin du Tibre ont été analysés. Les équations permettant le calcul du poids standard résultant sont : $\log_{10}(W_s) = -5{,}085 + 3{,}081 \log_{10}(TL)$ pour la méthode de RLP et $\log_{10}(W_s) = -3{,}706 + 1{,}685 \log_{10}(TL) + 0{,}349 [\log_{10}(TL)]^2$ pour la méthode EmP. Un autre objectif de cette recherche était de comparer les performances des deux méthodes proposées (RLP et EmP). L'utilisation de l'équation EmP pour calculer le W_r de *T. muticellus* dans le bassin du Tibre est ici conseillée, car elle s'est avérée ne pas être influencée par des biais liés à la taille.

Key words. - Cyprinidae - *Telestes muticellus* - Italy - Tiber River basin - Relative weight - Standard weight - Body condition indexes - EmP method - RLP method.

Body condition indexes provide a measure of the health of a fish population, and are based on the assumption that, on considering specimens of the same length, fish of higher weight are in better condition than those of lower weight. Condition indexes have become important tools for fisheries management (Anderson and Neumann, 1996; Blackwell *et al*., 2000); they are widely used because they are not invasive (being based only on length and weight measurements) and allow large numbers of fish to be sampled with minimal mortality (Fechhelm *et al*., 1995). Relative weight (W_r) is one such condition index. Because it is not influenced by changes in body shape, it enables the condition of fish of different lengths and from different populations to be compared. Variations in W_r values may be primarily due to ecological factors (Blackwell *et al*., 2000). Relative weight is based on the comparison between the actual weight of a specimen and the standard weight (W_s) that is the weight of an ideal fish of the same species and of the same length in good physiological condition (Murphy *et al*., 1990). W_s is predicted by a standard weight equation, that is a length-weight regression typical of the species (Wege and Anderson, 1978).

Relative weight has been developed primarily to assess the status of sport fishes (Blackwell *et al*., 2000). However, non-game fish are often affected by the same environmental conditions that limit sport fish and because non-game species are usually not as closely managed measure of their condition could better reflect the status of fish community or aquatic habitat quality (Richter, 2007). According to this, further authors encourage the use of this index in assessment of populations of native, nongame fishes (Bister *et al.,* 2000; Blackwell *et al*., 2000; Didenko *et al.,* 2004; Richter, 2007).

Telestes muticellus (Bonaparte, 1837) is one of the native species characterizing the fish communities of the "barbel zone" which is typical of the intermediary sectors of the river

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and is the most represented in the Tiber River basin (Lorenzoni *et al*., 2006). However, there is no standard weight equation available for this species in literature.

The main aim of this research was to develop W_s equations for calculating standard weight that would be valid for the populations of *Telestes muticellus* in the Tiber River basin. This species is indigenous to the waterways from the Brenta to the Vomano rivers (Italy, Switzerland) and the river basins from French-Italian border to the Volturno rivers in Italy (Kottelat and Freyhof, 2007).

Two methods of calculating standard weight equations have been proposed in the literature: the regression line percentile (RLP) method (Murphy *et al*., 1990) and the empirical percentile (EmP) method (Gerow *et al*., 2005). The EmP method was proposed in order to compensate for the length-related biases found in W_s equations developed by means of the RLP method (Gerow *et al*., 2004). Ogle and Winfield (2009) recommended using the W_s equation developed with the EmP method to derive W_r values for ruffe (*Gymnocephalus cernuus*). Moreover, Angeli *et al*. (2009), with regard to brown trout (*Salmo trutta*) and Tiber barbel (*Barbus tyberinus*), and Giannetto *et al*. (in press), with regard to European perch (*Perca fluviatilis*), found that the choice of the method used to estimate W_r strongly influences the judgment of the condition of a population. By contrast, Ranney *et al*. (2010) claim that there is little difference between the methods in terms of their relevance to management, and suggest that the RLP technique should remain the standard for developing W_s equations pending the development of an approach that clearly eliminates methodological length bias. As the use of these two methods is still open to debate, a further aim of this research was to use the results obtained in order to compare the validity of the two methods.

MATERIAL AND METHODS

Dataset selection

The area investigated was the Tiber River basin in Central Italy (Fig. 1). The Tiber River has the second largest watershed basin (17,375 km^2) and is the third-longest (405 km) river in Italy. More information on the study area and the fish species present in the Tiber River are available in Lorenzoni *et al*. (2006).

Length and weight data on *Telestes muticellus*, collected from 57 watercourses and 106 locations throughout the Tiber River basin (Fig. 1) in the period 1999-2009, were used in the research. Total body length (TL) was measured to the nearest 0.1 cm, individual total weight (W) was recorded to the nearest 0.01 g. In accordance with both the RLP and EmP methods, the following steps were taken to determine the W_s equation (Angeli *et al*., 2010; Giannetto *et al*., 2011): first, the total dataset was cleared by removing all fish that were large outliers in the TL-W regression of the total sample, since these were probably the result of wrong measure-

Figure 1. - Study area and location of the sampling sites in which populations were collected.

ments; the whole dataset was then divided into populations: data collected in multiple years from the same location were considered as separate population with the exception of locations with small numbers of fish from several years; data from large watercourses derived from separate locations were also considered as separate populations; locations with small numbers of fish (n < 20) were eliminated from the dataset (Ogle and Winfield, 2009). Then a TL-W regression was plotted for each population separately, in order to identify individual outliers (Bister *et al*., 2000). All populations with an r^2 value less than 0.90 or a slope (*b*) value less than 2.5 or higher than 3.5 were excluded (Froese, 2006). By plotting the slopes (*b*) of all populations against all intercepts (*a*) (Pope *et al*., 1995), those populations composed of few specimens or of samples with a narrow length-range were identified as outliers and were excluded (Froese, 2006).

Determination of the minimum total length for the W_s equation

The development of a standard weight requires the determination of a minimum total length to be used in the computation. This is because measuring small specimens in the field carries a high potential error (Murphy *et al*., 1990) and because small fish display high variance due to the differences in growth forms that arise in the juvenile stages. In accordance with Willis *et al*. (1991), the minimum TL was determined as the inflection point in the relationship between the variance/mean ratio for $\log_{10}W$ on 10-mm total length intervals; only fish larger than the minimum total length were included in the analysis. In addition, the EmP method also requires a maximum total length to be used for the standard weight equation. According to Gerow *et al*. (2005) this value is identified as the length-class for which at least three fish populations are present, since three is the smallest sample size that allows estimation of quartiles. Conversely, the RLP method does not have this limit and enables a W_s equation to be developed up to the largest size for which at least one fish in one population is present.

Development of the W_s equations

The W_s equations were calculated by means of both the RLP (Murphy *et al*., 1990) and the EmP (Gerow *et al*., 2005) methods. With regard to the RLP method, the $\log_{10}W$ at 10-mm length intervals was predicted from the TL-W regression for each population. These values were then transformed to weight and the 75th percentile was calculated. The 75th percentiles were then retransformed to $\log_{10}W$ and regressed on $\log_{10}TL$ by means of a linear model, in order to determine the parameters for the W_s equation (Murphy *et al*., 1990).

Conversely, for the EmP method, the $\log_{10}W$ of the measured (not modelled) sample mean at all 10-mm length intervals from each population was used and the 75th percentiles of weight in each length-interval were regressed against $\log_{10}TL$ by using a weighted quadratic model for the development of the EmP-W_s equation (Gerow *et al*., 2005).

The W_s equations thus obtained were used to calculate the relative weight of each specimen from each population by using the equation provided by Wege and Anderson (1978): $W_r = 100\ (W/W_s)$ where W is the weight of an individual in grams and W_s is the standard weight predicted by the W_s equation.

Comparison between the performance of the RLP and EmP methods

In order to quantify the extent to which the differences between the values of W_r calculated by means of the two methods might influence the judgment of the condition of fish, the performances of the two methods were compared. To this end, in accordance with Giannetto *et al*. (2011), different statistical analyses were used: covariance analysis was applied to the regressions obtained; for both methods, a linear regression between TL-W_r was determined by using the individual W_r values calculated by means of the two methods; the mean relative weight of each population, as calculated by means of the two different methods, was compared; analysis of variance was used to compare the mean values of W_r. Finally, the differences between the values of $W_{r\text{-}EmP}$ and $W_{r\text{-}RLP}$ calculated as the ratio between W_s and the weight obtained from the TL-W regression of the whole sample expressed as a percentage $[(W_{s\text{-}EmP} - W_{s\text{-}RLP}) / W] \times 100$ were analyzed and the trend in these values was plotted as a function of TL (Angeli *et al*., 2010).

Influence of fish length

An important attribute of a good condition index is that, to enable accurate comparison of samples from different fish populations, it should be free from length-related biases (Murphy *et al*., 1990; Anderson and Neumann, 1996; Blackwell *et al*., 2000). Three different methods were used to investigate the potential length bias in the W_s equations derived by means of RLP and EmP methods (Giannetto *et al*., 2011): the Willis method (Willis *et al*., 1991), in which the

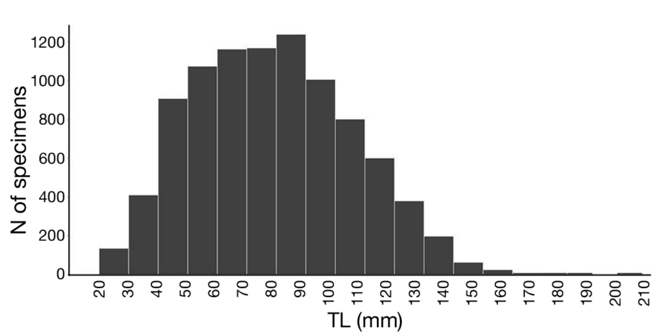

Figure 2. - Distribution of the total sample for 10-mm total length intervals (TL mm) for *Telestes muticellus* in the Tiber River basin.

Table I. - Descriptive statistic of the total sample for *Telestes muticellus* in the Tiber River basin.

	N	Mean	Min	Max	SD
TL (mm)	9186	83	20	210	27.118
W (g)	9186	8.59	0.20	118	8.584

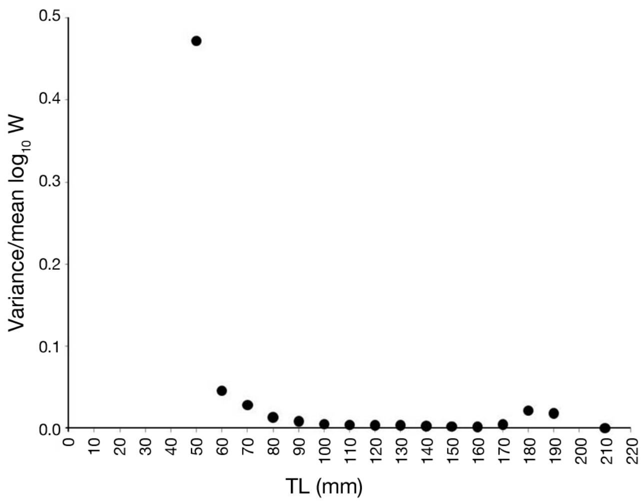

Figure 3. - Relationship between variance/mean ratio for Iog$_{10}$ of weight (W) on 10-mm total length intervals (TL mm) for the determination of the minimum TL for *Telestes muticellus* in the Tiber River basin.

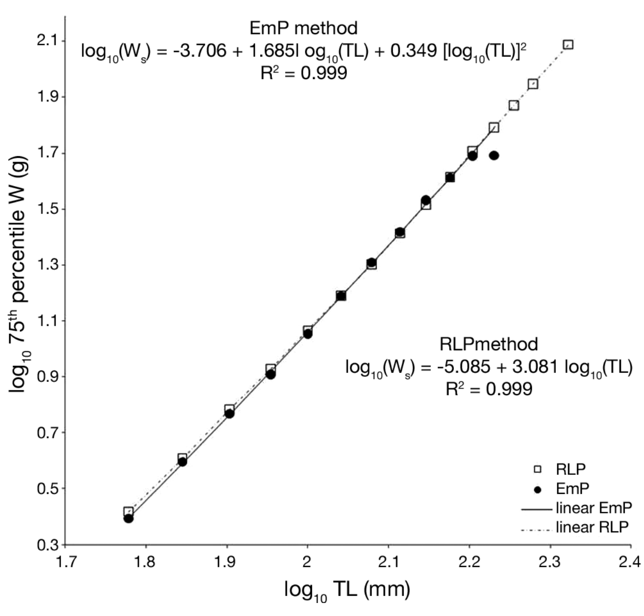

Figure 4. - Plot of the log10 transformed regression of the 75th percentile of the mean weights on 10-mm total length intervals (TL mm). The two equations provided in the figure, represent the Ws equations calculated for both EmP and RLP methods for *Telestes muticellus* in the Tiber River basin. (l = 75th percentile of the mean log10 W at each length class calculated with EmP method for all 82 populations; ● = 75th percentile of the mean log10 W at each length class calculated with RLP method for all 82 populations).

proportions of the significantly positive and negative slopes, from the regression of W_r (calculated with the proposed W_s equation) against TL for each specimen from each population, are compared by means of a chi-square test to determine whether there is a significant deviation from a 50:50 ratio ; the EmpQ method (Gerow *et al*., 2004), as modified by Ogle and Winfield (2009) using the FSA package of R software (R Development Core Team, 2009), in order to establish whether the slope of the quadratic regression of the 3rd quartile of the mean weights standardized by W_s (yielded by the proposed RLP and EmP W_s equations) on the 10-mm TL interval classes has a value of zero; and analysis of the residuals to investigate whether the distribution of residuals of the W_s equation exhibits evident patterns.

RESULTS

The sample examined comprised 9186 individuals with a mean total length of 83 mm (minimum 20 mm; maximum 210 mm) (Fig. 2) and a mean weight of 8.59 g (minimum 0.20 g; maximum 118 g) (Tab. I). The log-transformed TL-W equation calculated on the total sample was:

$\log_{10}$ (W) = -5.1054 + 3.0722 $\log_{10}$ (TL) (r^2 = 0.9409)

The total dataset was divided into 96 populations distributed throughout the Tiber River basin. However, 14 of these populations had an r^2 value less than 0.90 or a *b* value outside the range of 2.5-3.5 and for these reasons were excluded from the subsequent analysis. According to this the dataset was reduced to 82 populations and 7628 specimens. On plotting $\log_{10} (a) – b$ no population was identified as an outlier and the resulting $\log_{10} (a) – b$ equation was:

$b = 0.4954 - 0.5049 \log_{10}(a)$ (r^2 = 0.995)

The minimum total length was determined as 60 mm (Fig. 3), and all fish smaller than this size were removed from the dataset. The length-range judged to be suitable for the W_s equation was 60-210 mm for the RLP method and 60-170 mm for the EmP method. According to this the number of specimens used to develop the W_s equations was 6392 for RLP method and 6387 for EmP method belonging to 82 populations for both methods. The W_s equations thus calculated were (Fig. 4):

$\log_{10} (W_s) = -5.0854 + 3.081 \log_{10}$ (TL) (r^2 = 0.999; p = 0.000) (RLP method);

$\log_{10} (W_s) = -3.706 + 1.685 \log_{10} (TL) + 0.349 [\log_{10} (TL)]^2$ (r^2 =0.999; p = 0.000) (EmP method).

Comparison between the performances of the RLP and EmP methods

According to covariance analysis (ANCOVA), the mean relative weights calculated by means of the EmP method proved to be greater than those calculated for the same length by means of the RLP method; the differences between

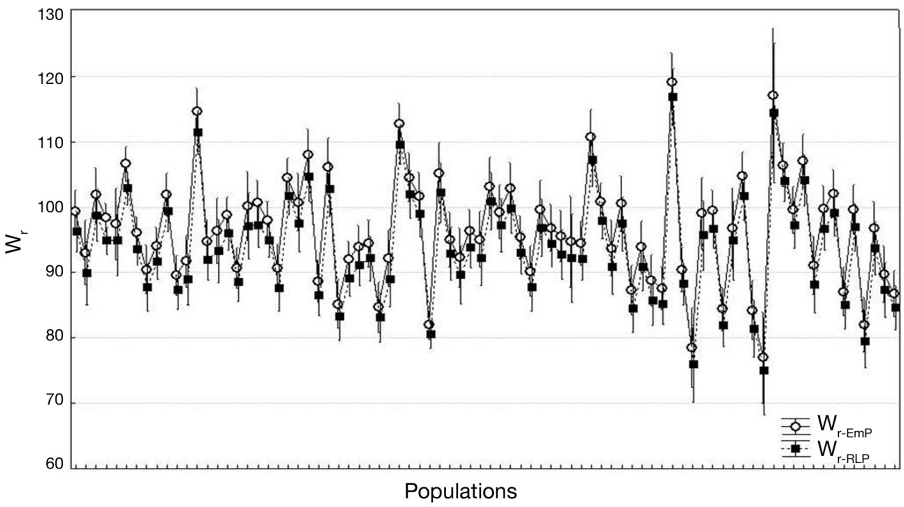

Figure 5. - Comparison of the mean values of relative weight (W_r) (with confidence limits of 95%), calculated by means of both RLP and EmP W_s equations for each of the 82 populations examined for *Telestes muticellus* in the Tiber River basin.

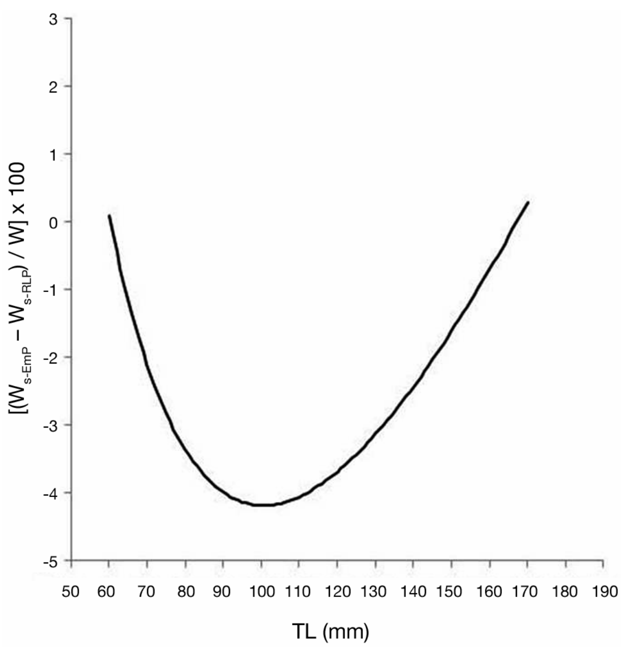

Figure 6. - Percentage difference trend between standard weights calculated with both EmP and RLP methods as a function of total length (TL mm) for *Telestes muticellus* in the Tiber River basin. ($W_{s\text{-EmP}}$ = standard weight obtained by EmP method, $W_{s\text{-RLP}}$ = standard weight calculated by RLP method, W = weight obtained by the length-weight regression of the total sample, 100 = multiplies the value to express it as percentage).

the values were statistically highly significant (mean covariate TL = 115.000 mm, F = 10.099, p = 0.004). The TL – W_r equations calculated for both methods were:

$W_r = 88.463 + 0.088$ TL ($r^2 = 0.012$, $r = 0.111$, $p = 0.000$) (EmP method);

$W_r = 89.512 + 0.048$ TL ($r^2 = 0.004$, $r = 0.062$, $p = 0.001$) (RLP method).

A highly significant ($p < 0.001$) positive correlation between TL and W_r was observed for both EmP and RLP (r = 0.111 for EmP and r = 0.062 for RLP). However, the value of the slope in both equations was very small (b = 0.088 for EmP and b = 0.048 for RLP). The mean value of $W_{r\text{-RLP}}$ (93.803 ± 17.534) was lower than that of $W_{r\text{-EmP}}$ (96.328 ± 17.961). The differences between them resulted be highly significant on *t*-test, (*t*-value = 8.043, p = 0.001). The trends in the mean values of relative weight calculated by means of the two methods in the different populations proved very similar (Fig. 5); however, the values calculated by means of the EmP method were higher than those yielded by the RLP method in every case. At ANOVA the differences between the mean W_r values calculated for each population by means of the two methods were highly significant (F = 85592.754, p = 0.001). Analysing the trend in the percentage differences between $W_{s\text{-EmP}}$ and $W_{s\text{-RLP}}$ as a function of TL (Fig. 6), it emerged that $W_{s\text{-RLP}}$ was higher than $W_{s\text{-EmP}}$ for fish between 60 and 170 mm with a percentage difference between the two methods of about 4% for the length-class of 90 mm (Fig. 6).

Table II. - Results of both Willis and EmpQ methods applied to the W_s equations, calculated by means of EmP and RLP methods for *Telestes muticellus* in the Tiber River basin. (Negative = number of populations showing a significantly negative slope in the TL-W_r regression; positive = number of populations showing a significantly positive slope in the TL-W_r regression; P = P values of significance on chi-square test for the Willis method; P_{linear} and $P_{quadratic}$ = p value of the linear and quadratic terms in the EmpQ method).

	Willis method			EmPQ method	
	Negative	Positive	P	P_{linear}	$P_{quadratic}$
EmP	14	26	0.058	0.676	0.643
RLP	20	16	0.351	< 0.001	< 0.001

Influence of fish length

Applying the Willis method (Willis *et al.*, 1991) to the EmP method, only 40 of the 82 populations showed slopes significantly different from zero ($p < 0.05$) in the TL-W_r rela-

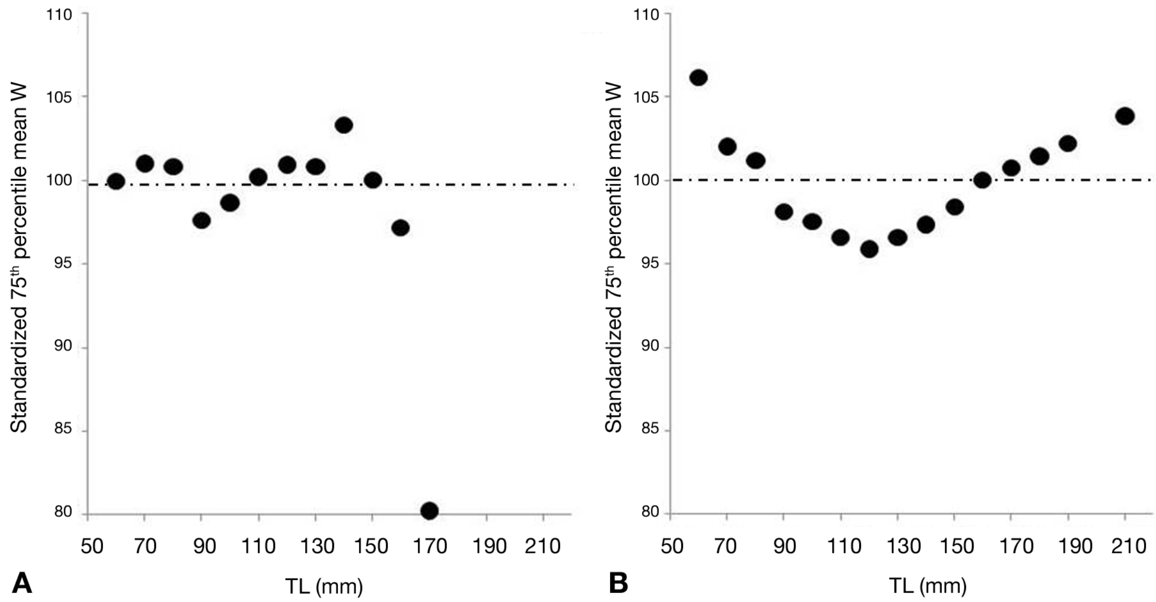

Figure 7. - Residuals plot resulted by applying the EmpQ method to the standard weight (W_s) equations calculated with both EmP method (**A**) and RLP method (**B**) for *Telestes muticellus* in the Tiber River basin. The horizontal line at 100 is shown for reference. (Standardized 75th percentile mean W = 75th percentile of mean weights standardized by W_s equation; TL mm = total length in mm).

tionships. Among those, the number of relationships with a positive slope (26 populations) was not significantly different from those with negative slopes (14 populations) at chi-square analysis ($r^2 = 3.600$, $p = 0.058$) (Tab. II). With regard to the RLP method, the Willis method revealed that 36 of the 82 populations had slope significantly different from zero ($p < 0.05$); among these 16 had a negative slope and 20 a positive slope. These numbers were not significantly different at chi-square analysis ($r^2 = 0.444$, $p < 0.505$) (Tab. II).

According to EmpQ method (Gerow *et al*., 2004), the W_s equation developed by means of the EmP method did not appear to be influenced by fish length (Tab. II; Fig. 7A); by contrast, the RLP W_s equation proved to be influenced by length ($p < 0.001$ for both linear and quadratic terms of the equation) (Tab. II; Fig. 7B).

Analysing the distributions of residuals, the EmP-W_s equation did not exhibit evident patterns, while the RLP-W_s equation had residuals that showed a clear nonlinearity tendency.

DISCUSSION

Comparison of the mean values of W_r calculated for each population by means of both methods revealed similar trends; however, the values of $W_{r\text{-EmP}}$ were higher than those of $W_{r\text{-RLP}}$ for all populations. Moreover, in some cases, for the same population, the W_r value calculated with one of the methods fell outside the target range of 95-105, and according to Anderson (1980), this result indicates fish that are not in good condition. Thus, the choice of the method used to estimate standard weight can significantly influence the judgement of the condition of a population, as observed by Angeli *et al*. (2009) for *Salmo trutta* and *Barbus tyberinus* in the Tiber River and by Giannetto *et al*. (in press) for *Perca fluviatilis*.

On analysing the trend in the percentage differences between $W_{s\text{-EmP}}$ and $W_{s\text{-RLP}}$ as a function of TL, $W_{s\text{-RLP}}$ resulted higher than $W_{s\text{-EmP}}$ for the 60-170 mm length-range, which is the range of application of the $W_{s\text{-EmP}}$ equation. The higher value of W_s calculated by means of the RLP method explains the lower values of W_r yielded by this method. The greatest differences between the two methods were observed for fish of 90 mm, in which a percentage difference of about 4% of the weight of the specimens was detected. These results are in line with what observed Giannetto *et al*. (in press) for *P. fluviatilis*, in which the RLP method yielded W_s values higher than the EmP method.

It is important to underline that the mean values of W_r for each population depends on its age structure and, because the younger specimens are generally more abundant, this could explain because mean $W_{r\text{-EmP}}$ is higher than $W_{r\text{-RLP}}$ for each population.

One of the properties of a good body condition index is that it should be free from length-related biases in order to enable accurate comparison 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). Indeed, our results show that relative weight is not always independent of the length of the specimens examined, and that it depends in part on the method (RLP or EmP) used in the analysis. Specifically, with regard to Italian riffle dace, the equation developed by means of the EmP method proved not to be influenced by fish length according to the Willis method, the EmpQ method or analysis of the residuals of the equation. By contrast, the W_s equation developed by means of the RLP method proved to be influenced by fish length according to both the EmpQ method and analysis of residuals, but not the Willis method.

On the basis of the results obtained, the use of the EmP equation to determine W_r for the Italian riffle dace in the Tiber River basin is suggested.

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