Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2011) **78**, 1890–1899
doi:10.1111/j.1095-8649.2011.02952.x, available online at wileyonlinelibrary.com

Proposed standard mass equations for European chub *Leuciscus cephalus* in Italy

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(Received 14 September 2010, Accepted 11 February 2011)

Total length (L_T) and mass measurements of 28 596 specimens of European chub *Leuciscus cephalus*, collected from a variety of waterways across Italy, were used to compute standard mass (W_s) equations by both empirical percentile (EmP) and regression line percentile (RLP) methods. The use of the EmP W_s equation [$\log_{10} W_s = -4{\cdot}79 + 2{\cdot}68 \log_{10} L_T + 0{\cdot}10(\log_{10} L_T)^2$] to compute relative mass (W_r) of *L. cephalus* in Italy is suggested, as it was not influenced by length-related bias (L_T range of application = 70–470 mm).

Key words: EmP and RLP methods; index of condition; relative mass; *Squalius squalus*.

INTRODUCTION

Indices of condition, which enable the well-being of fishes to be evaluated, are based on the assumption that, for a given length, fish of greater mass are in better condition than those of lesser mass. As these indices are based only on length and mass measurements, they are non-invasive. According to Fechhelm *et al*. (1995), the use of indices of condition for monitoring the health of fishes is cost-effective as large numbers of fishes can be sampled with minimal mortality.

The concept of relative mass (W_r) was introduced by Wege & Anderson (1978). Over other condition indices, *i.e.* Fulton's (1911) condition factor and Le Cren's (1951) condition factor, W_r has several advantages as it is easy to calculate and does not change if different units of measurement are used. Its greatest advantage, however, is that it enables the condition of fishes of different lengths and from different populations to be compared, as it is not influenced by changes in body shape. Thus, variations in W_r values will be primarily due to extant ecological factors (Blackwell *et al*., 2000). W_r is calculated as the ratio between the mass of the specimen (W) and the standard mass (W_s): *i.e.* the mass of an ideal fish of the same species and

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of the same length, which is in good condition. Therefore, a W_s equation must be developed for each species.

European chub *Leuciscus cephalus* (L. 1758) is one of the most widespread species in Europe, but despite this the only W_s equation reported for this species is for the River Tiber, Italy (Angeli *et al.*, 2010). The area of distribution of this species includes Europe, Anatolia and the Black and Azov Sea basins (Ladiges & Vogt, 1986), and in Italy it is one of the most common freshwater fishes (Gandolfi *et al.*, 1991). According to Kottelat & Freyhof (2007), however, the Italian populations of *L. cephalus* are genetically distinct from other European populations and should be regarded as a separate species called the cavedano chub *Squalius squalus* (Bonaparte 1837). If this hypothesis is confirmed, Italy would represent the whole area of distribution of the species *S. squalus*.

Two methods of computing W_s equations have been proposed. Murphy *et al.* (1990) introduced the regression line percentile (RLP) method, which uses the 75th percentile of mean masses estimated among populations on the basis of the length and mass regressions of each population. In the past, RLP was the most widely used method of developing W_s equations, and many of the W_s equations present in the literature were developed by means of this procedure (Murphy *et al.*, 1990; Willis *et al.*, 1991; Bister *et al.*, 2000). Gerow *et al.* (2004), however, found length-related biases in W_s equations developed by means of the RLP method. They therefore introduced a new method, the empirical percentile (EmP) method (Gerow *et al.*, 2005), based on the 75th percentile of the observed masses of fishes by 1 cm increments (not masses estimated from regression models, as in RLP). Furthermore, the EmP method uses a curvilinear relationship between length and mass rather than a linear $\log_{10}$ relationship.

The use and validity of the two methods has been discussed for a variety of species and the debate about the choice of the method is still open (Rennie & Verdon, 2008; Angeli *et al.*, 2009; Ogle & Winfield, 2009; Ranney *et al.*, 2010). Consequently, the aim of this study was to develop W_s equations for the Italian area of distribution of *L. cephalus* by comparing the performance of the RLP and EmP methods.

MATERIALS AND METHODS

DATA SET SELECTION

Leuciscus cephalus total length (L_T, mm) and mass (W, g) data were collected from a variety of waterways throughout Italy (https://bio.unipg.it/download/Wr_chub/Appendix1.pdf/).

After applying the L_T and W regression to the total sample, all specimens that appeared to be large outliers (value of L_T or W that diverged by more than double from the expected value) were excluded, as they were probably derived from wrong measurements. The total data set was then divided into populations. Data derived from separate locations on large waterways were considered to refer to separate populations and data collected in different years from the same location were also regarded as referring to separate populations, with the exception of locations with small numbers of fish ($n < 20$).

Plotting the $\log_{10} L_T$ and $\log_{10} W$ for each population, all anomalous values of L_T or W (deviating by more than double the expected value by the regression curve) were identified and removed. Then, on the basis of the $\log_{10} L_T$ and $\log_{10} W$ equations thus obtained, all populations with an r^2 value $<0{\cdot}90$ or a slope (b) value outside the range of $2{\cdot}5–3{\cdot}5$

were excluded (Froese, 2006). The next step was to plot b of all populations against all intercepts (a) (Pope *et al*., 1995) to identify outliers represented by populations composed of few fish or of samples with a narrow L_T range (Froese, 2006).

DETERMINATION OF THE APPLICABLE L_T RANGE FOR THE W_S EQUATION

To develop a W_s equation, an applicable L_T range has to be determined (Gerow *et al*., 2005). The minimum L_T is required because small fishes generally display a high variance due to the potential error that may occur in weighing them in the field (Murphy *et al*., 1990). According to Willis *et al*. (1991), the minimum L_T was determined as the inflection point in the relationship between the variance:mean ratio for $\log_{10} W$ by L_T intervals of 10 mm; only fish larger than the minimum L_T were included in the analysis.

The EmP method also requires the determination of a maximum L_T, identified as the L_T class for which at least three fish populations were available (Gerow *et al*., 2005). Herein lies a further difference between the RLP and EmP methods; indeed, unlike EmP, RLP enables the calculation of a W_s equation for fish L_T up to that of the largest individual in the population. To allow comparison between the RLP and EmP methods in this study the same applicable L_T range was used to determine both the RLP and EmP W_s equations.

DEVELOPMENT OF THE W_S EQUATION

The W_s equations for *L. cephalus* were calculated by means of both the RLP method (Murphy *et al.,* 1990), which uses masses derived from the L_T and W equation for each population, and the EmP method (Gerow *et al*., 2005), which, unlike RLP, is based on quartiles of measured mean masses of fishes (not masses estimated from regression models) in a given length class among sampled fish populations.

For each W_s equation (RLP and EmP), the W_r of each specimen from each population was calculated using the equation provided by Wege & Anderson (1978): $W_r = 100\ W\ W_s^{-1}$.

COMPARISON BETWEEN THE PERFORMANCE OF RLP AND EmP METHODS

The RLP and EmP methods were then compared, first using the L_T and W_r linear regression; these regressions were then compared by means of ANCOVA. Second, the mean W_r values were calculated for each method and compared by means of a t-test. Finally, the ratio between the difference of $W_{s\text{-EmP}}$ and $W_{s\text{-RLP}}$ and the mass obtained by the L_T and W regression of the total sample (W) was calculated and it was expressed as percentage difference $[100(W_{s-\text{EmP}} - W_{s-\text{RLP}})W^{-1}]$; then the trend of these values was constructed as a function of L_T (Angeli *et al*., 2010). This procedure clearly shows the differences between the two methods, as using the ratio between W_s and W enables the determination of whether the differences affect larger or smaller fish. All statistical analyses were performed by using R Development Core Team software (version 2.10.1; www.r-project.org) and the results were considered significant at $P < 0{\cdot}05$.

INVESTIGATION OF THE POTENTIAL L_T BIAS IN THE W_S EQUATIONS

To test the potential L_T bias in the W_s equations derived by the two methods, three different methods were used according to Ogle & Winfield (2009): (1) the Willis method (Willis *et al*., 1991), in which a χ^2-test is used to determine if, from the L_T and W_r regressions for each population, the number of populations showing a significant positive slope is equal to that showing a significant negative slope, (2) the Empirical quartiles method (Gerow *et al*., 2004), as modified by Ogle & Winfield (2009) using the FSA package of R Development Core Team software to determine whether the quadratic regression of the third quartile of the mean masses

FIG. 1. Distribution of the total data set per region of Italy for *Leuciscus cephalus*: the number in each circle indicates the number of specimens caught in each region; the size of the circles is proportional to the size of the sample.

standardized by W_s against L_T intervals of 10 mm had a slope of zero and (3) the analysis of distribution of the residuals (Ogle & Winfield, 2009) to see whether the distribution of residuals of the W_s equation exhibited evident patterns.

RESULTS

A total of 28 596 specimens were collected during this study to develop W_s equations; the fish ranged in L_T from 20 to 495 mm (mean $\pm$ S.E. $= 154{\cdot}28 \pm 83{\cdot}03$ mm) and in mass from 0·06 to 1855·00 g (mean $\pm$ S.E. $= 76{\cdot}31 \pm 133{\cdot}65$ g).

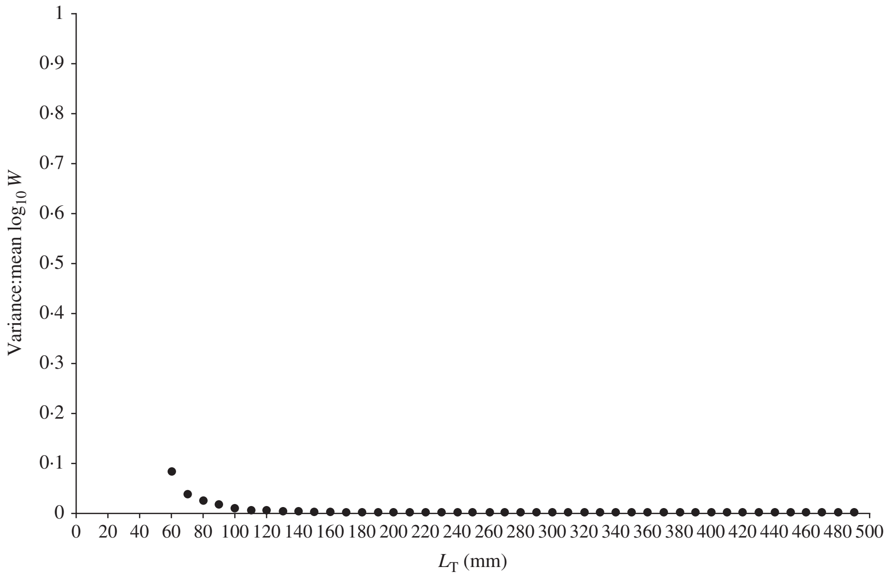

FIG. 2. Relationship between variance:mean for $\log_{10}$ of mass (W) and total length (L_T) at 10 mm intervals for the determination of the minimum L_T of *Leuciscus cephalus* in Italy as indicated by the inflection point in this relationship.

The $\log_{10}$-transformed L_T and W equation calculated on the total sample was: $\log_{10} W = -5{\cdot}26 + 3{\cdot}11 \log_{10} L_T$ ($n = 28\ 596$, $r^2 = 0{\cdot}99$).

The data set comprised 328 populations, distributed throughout the Italian range of *L. cephalus* (Fig. 1).

In accordance with Froese (2006), one population with an r^2 value $<0{\cdot}90$ and a value $b<2{\cdot}5$ was excluded from subsequent calculations, while on the basis of the plot between $\log_{10} a - b$ for all populations, no population was identified as an outlier; the resulting $\log_{10} a - b$ equation was: $b = 0{\cdot}81 - 0{\cdot}44 \log_{10} a$ ($n = 327$ populations, $r^2 = 0{\cdot}98$).

According to Willis *et al*. (1991), 70 mm was assigned as minimum L_T because it was the inflection point in the relationship between the variance:mean ratio for $\log_{10} W$ by L_T intervals of 10 mm (Fig. 2); accordingly, fish <70 mm were removed from the data set. The L_T range of application of the standard equations was 70–470 mm.

The W_s equations calculated by means of the two methods are shown in Fig. 3.

COMPARISON BETWEEN THE PERFORMANCE OF RLP AND EmP METHODS

The L_T and W_r regressions were developed for both methods, the resulting equations being: $W_r = 92{\cdot}54 + 0{\cdot}01\ L_T$ ($n = 24\ 801$; $r^2 < 0{\cdot}01$; $P < 0{\cdot}01$) (EmP method) and $W_r = 87{\cdot}17 + 0{\cdot}03\ L_T$ ($n = 24\ 801$; $r^2 = 0{\cdot}03$; $P < 0{\cdot}01$) (RLP method).

A small positive correlation between L_T and W_r was observed for both methods (r: EmP $= 0{\cdot}04$; RLP $= 0{\cdot}17$); in both cases, it was statistically highly significant

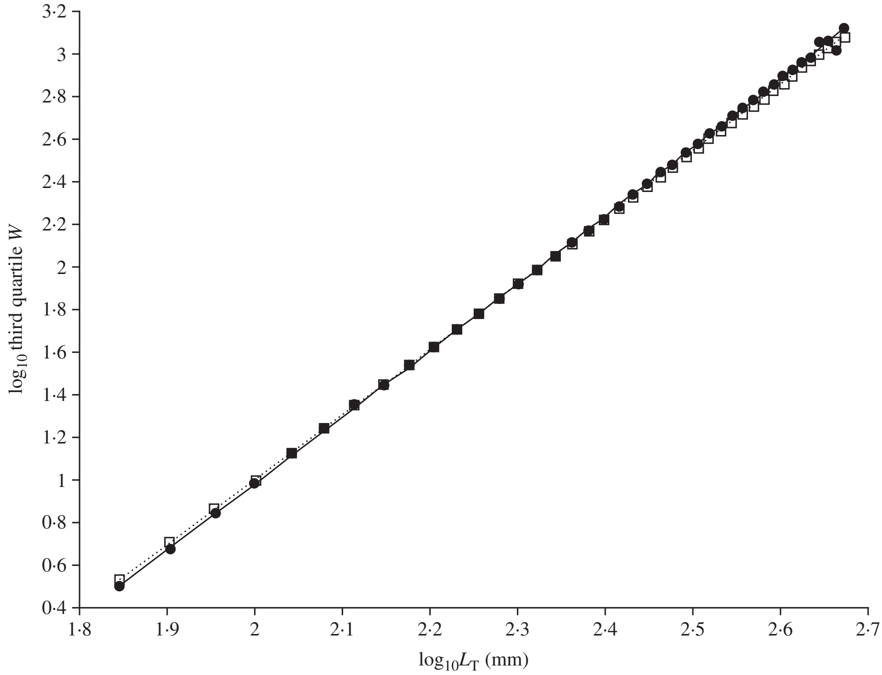

FIG. 3. $\log_{10}$-transformed regression of the third quartile of the mean masses (W) on total length (L_T) at 10 mm intervals. The following equations represent the standard mass (W_s) equations calculated for both empirical percentile [EmP; —●—; $y = -4{\cdot}79 + 2{\cdot}68x + 0{\cdot}10x^2$ ($r^2 = 0{\cdot}99$)] and regression line percentile [RLP; ···□···; $y = -5{\cdot}19 + 3{\cdot}09x$ ($r^2 = 0{\cdot}99$)] methods for *Leuciscus cephalus* in Italy.

($P < 0{\cdot}01$). The values of r and b were smaller for EmP ($b = 0{\cdot}01$) than for RLP ($b = 0{\cdot}03$). The two regressions were analysed and the differences were statistically highly significant (ANCOVA, $F_{1,4948} = 36{\cdot}18$, $P < 0{\cdot}01$; mean covariate: $L_T = 170{\cdot}99$ mm).

The mean W_r value calculated by means of the EmP equation was 93·80, while that calculated by means of RLP was 92·95 (Table I). The differences between these mean values were statistically highly significant (t-test, $t = 5{\cdot}98$, d.f. = 1, $P < 0{\cdot}01$).

The differences between the RLP and EmP methods were more marked for fish <70 mm, which was assigned as the minimum L_T for L. cephalus; for this L_T class, the EmP method yielded higher W_s values than the RLP method (Fig. 4). By contrast,

TABLE I. Comparison between the values of relative mass (W_r) (mean, minimum, maximum and S.D.) estimated by the two standard mass equations calculated by the regression line percentile (RLP; W_{r-RLP}) method and empirical percentile (EmP; W_{r-EmP}) method for *Leuciscus cephalus* in Italy

	n	Mean	Minimum	Maximum	S.D.
W_{r-EmP}	24 742	93·80	41·31	202·85	15·94
W_{r-RLP}	24 742	92·95	40·37	197·24	15·92

n, number of specimens.

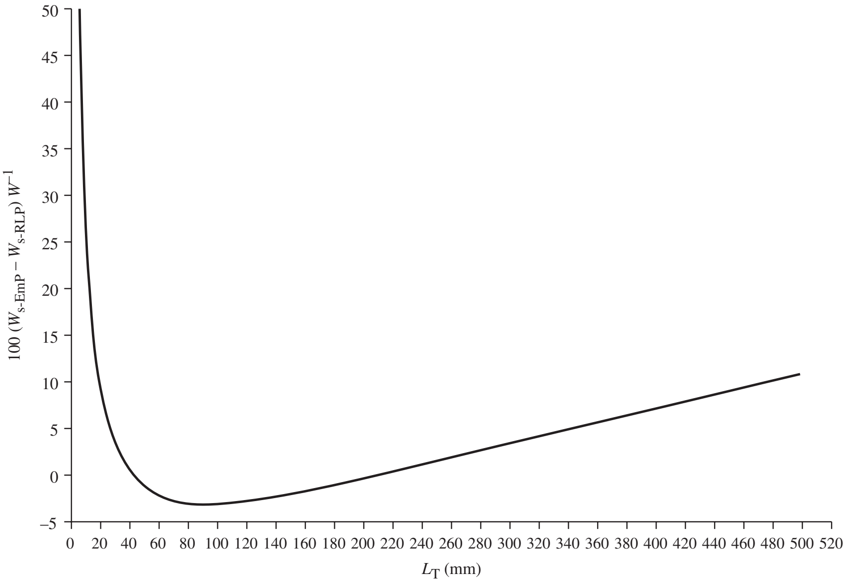

FIG. 4. Trend in the percent difference between standard mass (W_s) calculated with empirical percentile method (EmP; W_{s-EmP}) and regression line percentile (RLP; W_{s-RLP}) method as a function of total length (L_T) for *Leuciscus cephalus* in Italy. W, mass obtained by the L_T and mass regression of the total sample.

for fish of intermediate size, the value of $W_{s\text{-RLP}}$ was higher than that of $W_{s\text{-EmP}}$, the difference between the two methods being c. 3%. For fish >220 mm, the situation changed again ($W_{s\text{-EmP}} > W_{s\text{-RLP}}$) with a difference of c. 10% for the largest fish (Fig. 4).

INVESTIGATION OF THE POTENTIAL LENGTH BIAS IN THE W_S EQUATIONS

On residuals analysis, the EmP W_s equation did not exhibit apparent patterns [Fig. 5(a)], while the RLP W_s equation had residuals showing a clear non-linear trend [Fig. 5(b)]. The EmP W_s equation did not appear to be influenced by L_T according to the empirical quartiles method [Table II and Fig. 6(a)]. According to the Willis method (Willis *et al.,* 1991), 124 of the L_T and W_r relationships had b significantly different from zero ($P < 0{\cdot}05$); of these, 54 populations had positive b and 70 had negative b. The number of relationships with positive b was not significantly different from that of those with negative slopes ($\chi^2 = 2{\cdot}06$; d.f. = 1, $P = 0{\cdot}15$) (Table II).

On applying the empirical quartiles method the RLP W_s equation was influenced by fish length ($P < 0{\cdot}01$ for both linear and quadratic terms of the equation) [Table II and Fig. 6(b)]; according to the Willis method, 132 of the L_T–W_r relationships had slopes significantly different from zero ($P < 0{\cdot}05$); of these, 28 had positive slopes and 104 negative. The number of relationships with positive slopes was highly significantly different from that of those with negative slopes (χ^2 analysis; $\chi^2 = 43{\cdot}76$; d.f. = 1, $P < 0{\cdot}01$) (Table II).

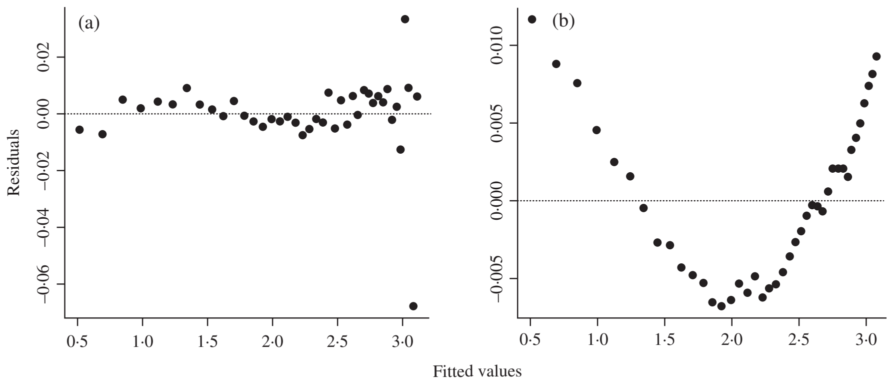

FIG. 5. The distribution of standardized residuals of the regressions used to investigate potential total length (L_T) bias in the standard mass (W_s) equations calculated by the (a) empirical percentile (EmP) method and (b) regression line percentile (RLP) method for *Leuciscus cephalus* in Italy. The horizontal line at 0·0 is shown for reference.

DISCUSSION

Despite the widespread distribution of *L. cephalus*, the only W_s equation reported for this species is for the River Tiber, Italy (Angeli *et al*., 2010). Moreover, if the hypothesis of Kottelat & Freyhof (2007) is confirmed, the W_s equation developed in the present study should assume a greater importance because it would be valid for the whole area of distribution of the species *S. squalus*.

In this study, W_s equations for *L. cephalus* in Italy were developed by means of the RLP method introduced by Murphy *et al*. (1990) and the EmP method developed by Gerow *et al*. (2005). The two W_s equations were then compared to evaluate the differences and the performances of the two methods.

On the basis of the results, the use of the EmP W_s equation is suggested to compute the W_r of *L. cephalus*. This is because, even if the differences between

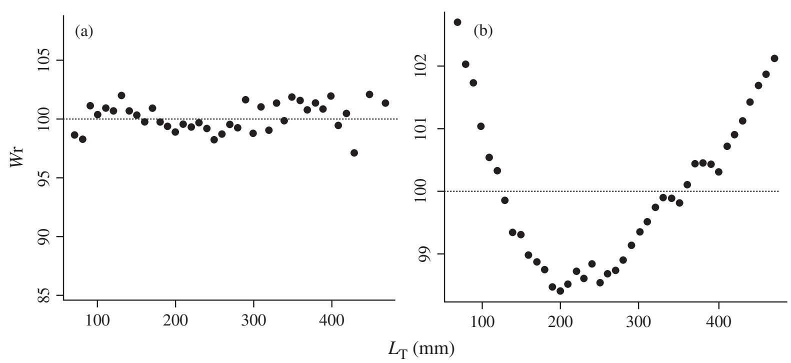

FIG. 6. Residuals plot from applying the empirical quartiles method used to investigate potential total length (L_T) bias in the standard mass (W_s) equations calculated with both (a) the EmP and (b) regression line percentile (RLP) methods for *Leuciscus cephalus* in Italy. The horizontal line at 100 is shown for reference. W_r, standardized 75th percentile mean masses calculated from W_s.

TABLE II. Results of both Willis and Empirical quartiles methods used to investigate potential total length bias in the standard mass (W_s) equations calculated by means of EmP and regression line percentile (RLP) methods for *Leuciscus cephalus* in Italy

	Willis method			Empirical quartiles method	
	Negative	Positive	P	P_{linear}	$P_{quadratic}$
EmP	70	54	0·15	0·88	0·52
RLP	28	104	<0·01	<0·01	<0·01

Negative, number of populations showing significantly negative slopes in the L_T and W_r regression; positive, number of populations showing a significantly positive slopes in the L_T and W_r regression; P, P-values using a χ^2-test according to Willis method; P_{linear}, P-values of the linear term in the empirical quartiles method; $P_{quadratic}$, P-values of the quadratic term in the empirical quartiles method.

the two methods are small and the EmP method requires an additional effort for the sample (because it is based on the observed mass of the specimens), the EmP W_s equation results were not influenced by L_T-related biases. This result has great importance because a good index of condition should be free from length-related biases, to enable accurate comparisons to be made (Murphy *et al.*, 1990; Blackwell *et al.,* 2000). The RLP W_s equation provided in this study was, however, influenced by the size of the specimens (according to the three methods used to investigate the presence of a potential length bias).

As suggested by Murphy *et al.* (1991), the greatest advantage of W_r over other condition indices is the creation of a general standard that allows comparison of the condition of fishes of different lengths and from different populations. For the W_s equation thus developed, further research could evaluate the condition of a population and, combined with other population metrics, *e.g.* age and growth, investigate at local scale the potential causes of low W_r value, *e.g.* poor nutrition, intra- or inter-specific competition and reproductive status, and compare the results with those of other populations.

We are grateful to the following for providing *L. cephalus* length and mass data sets: F. Ciutti (Istituto Agrario di S. Michele all'Adige, S. Michele all'Adige, Trento, Italy); L. Betti (Ichthyologist, Trento, Italy); L. Tancioni (Laboratorio di Ecologia Sperimentale e Acquacoltura, Dipartimento di Biologia, Università degli Studi 'Tor Vergata', Roma, Italy); L. Ciuffardi (Naturalist and Ichthyologist, Genova, Italy); P. Colombari (ARSIAL, Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura del Lazio, Italy). We also are grateful to K. Gerow (U.S.A.) who provided some of his valuable papers and suggestions, and D. H. Ogle (U.S.A.) for the precious FSA package that helped us to elaborate the paper. Thanks also to the two anonymous reviewers and the editorial staff who greatly improved this manuscript with their comments.

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