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Empirical standard mass equation for *Salmo marmoratus*

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Total length (L_T) (range 24–1000 mm; mean \pm s.e. = 170.21 \pm 0.36 mm) and mass (W) (range 0.10–9590 g; mean \pm s.e. = 76.03 \pm 0.87 g) of 36 460 specimens of marble trout *Salmo marmoratus* were used to compute a standard mass (W_s) equation for this species by means of the empirical percentile (EmP) method. The EmP W_s equation calculated was: $\log_{10} W_s = -5.208 + 3.202 \log_{10} L_T - 0.046 (\log_{10} L_T)^2$ (L_T range 90–570 mm) and it is valid throughout the species' area of distribution across Europe.

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Marble trout *Salmo marmoratus* Cuvier 1829 is a salmonid of particular conservation interest, being endemic to a restricted geographical area of the Adriatic Basin, including the Po, Adige, Brenta, Tagliamento and Isonzo River basins in Northern Italy (Turin *et al.*, 2006; Pujolar *et al.*, 2011) and the Adriatic River system of the western Balkans (Povž, 1995; Crivelli *et al.*, 2000). Many authors have reported a progressive restriction of its original distribution area (Sommani, 1961; Tortonese, 1967; Crivelli *et al.*, 2000) and now the species is considered one of the most endangered freshwater fish of the Adriatic Basin (Povž *et al.*, 1996; Crivelli *et al.*, 2000). Specifically, it is listed in Annex II of the European Union Habitats Directive (E.U., 1992) as a species requiring designation of special areas of conservation and considered as endangered according to the Red List of Italian freshwater fishes (Bulgarini *et al.*, 1998; Zerunian, 2002). Hybridization with brown trout *Salmo trutta* L. 1758, displacement by alien rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Vincenzi *et al.*, 2011) and habitat alteration (Crivelli *et al.*, 2000) are the most serious

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causes of concern for the survival of *S. marmoratus*. Accordingly, any management tools that could assist in conserving its populations would be advantageous for the survival of the species. Body condition indices provide a measure of the health of a fish population assuming that heavier fish of a given length are in better condition (Froese, 2006). They have become useful tools for fisheries management because, being based only on measurements of length and mass, they do not require sacrifice of specimens (Anderson & Neumann, 1996; Blackwell *et al.*, 2000). Relative mass (W_r) (Wege & Anderson, 1978) is one of these indices and is based on the comparison between the actual mass of a specimen and the standard mass (W_s), which is the mass of an ideal fish of the same species and same length in good physiological condition predicted by a W_s equation typical of the species. The aim of this research was to develop a W_s equation for *S. marmoratus*. W_r provides a rapid, accessible and non-invasive metric useful to assess the overall health and fitness of this species as well as population-level response to ecosystem disturbance. Until recently, the most widely used method to develop the W_s equation was the regression line percentile (RLP) method (Murphy *et al.*, 1991). Gerow *et al.* (2004), however, found significant length-related biases for W_s equations developed using the RLP method and introduced a new method called the empirical percentile (EmP) method (Gerow *et al.*, 2005), which uses empirical data (instead of modelled data). Currently, the debate on the validity and choice of the method is still open (Gerow, 2010; Ranney *et al.*, 2010), but the results of recent studies suggest that the W_s equation developed by the EmP method is not affected by length-biases and encourage the use of this methodology (Angeli *et al.*, 2009; Ogle & Winfield, 2009; Giannetto *et al.* 2011, 2012).

Data on *S. marmoratus* [total length (L_T , mm) and mass (W , g)] were collected across the entire range of the species during different programmes of rehabilitation (Crivelli *et al.*, 2000; Specchi *et al.*, 2004) and monitoring (Turin *et al.*, 2006) carried out to assess the conservation status of the species. Accordingly, all fish were returned to their rivers immediately after measurements. Only specimens recognized as phenotypic *S. marmoratus* were used in calculations. Those showing characteristics of hybrid forms were removed. Body lengths measured only in terms of fork length (L_F) were converted to L_T by applying a general linear conversion model calculated by using all fish from the datasets in which at least two types of length measurement were recorded. The general conversion model used was: $L_T = 1.008 L_F + 4.721$ ($r^2 = 0.999$, $P < 0.01$, $n = 99$). Then the total dataset was cleaned and screened by using the procedure suggested by Giannetto *et al.* (2011). After that, a suitable length range for the application of the W_s equation was calculated. According to Willis *et al.* (1991) by plotting the variance:mean ratio for $\log_{10} W$ on 10-mm L_T intervals, the minimum sample size was determined as the value at which this ratio sharply decreased and was $<1\%$ (Murphy *et al.*, 1991); a maximum L_T was assigned as the length class for which at least three fish populations were present in the dataset (Gerow *et al.*, 2005). All fish outside the suitable length range were not utilized for the next analysis. After this, the dataset was divided into statistical populations: data derived from separate locations on large waterways were considered to refer to separate populations; 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 ($n < 20$) (Ogle & Winfield, 2009). Subsequently, the dataset was divided into two datasets: a large development dataset to

calculate the W_s equation and a validation dataset to assess potential length-bias in the W_s equation (Rypel & Richter, 2008; Ogle & Winfield, 2009). The EmP method proposed by Gerow *et al.* (2005) was applied to the development dataset to estimate the W_s equation for *S. marmoratus*. In order to validate the EmP W_s equation thus obtained and detect potential length-related biases, two different techniques were applied: the empirical quartile (EmpQ) method (Gerow *et al.*, 2004) as modified by Ogle & Winfield (2009) by the FSA package (Ogle, 2009) to determine whether the quadratic regression of the third quartile of the mean W standardized by W_s against L_T had a slope of zero; and the Willis method (Willis *et al.*, 1991), whereby a χ^2 test was used to determine whether the proportion of populations with a significant positive slope in the L_T and W_r equation was equal to the proportion of those with a significant negative slope.

A total of 36 460 specimens was collected throughout the area of distribution of the species (Fig. 1) and analysed. The total sample ranged in L_T from 24 to 1000 mm (mean \pm s.e. = 170.21 ± 0.36 mm) and in W from 0.10 to 9590 g (mean \pm s.e. = 76.03 ± 0.87 g). The \log_{10} -transformed L_T and W relationship of the total dataset is shown in Fig. 2).

The total sample was divided into 197 populations (177 from Italy and 20 from Slovenia), but according to Froese (2006), four populations showing an r^2 value <0.90 or for which a value of slope b fell outside the range of 2.5–3.5 were eliminated. Then, the total dataset was divided into a large development dataset (34 343 specimens and 153 populations) and a small validation dataset (766 specimens and 40 populations) to use a sample as large as possible to calculate the W_s equation



FIG. 1. Data on *Salmo marmoratus* collected throughout the area of distribution of the species: the number in every circle indicates the number of specimens caught in each zone and the size of the circles is proportional to the size of the sample.

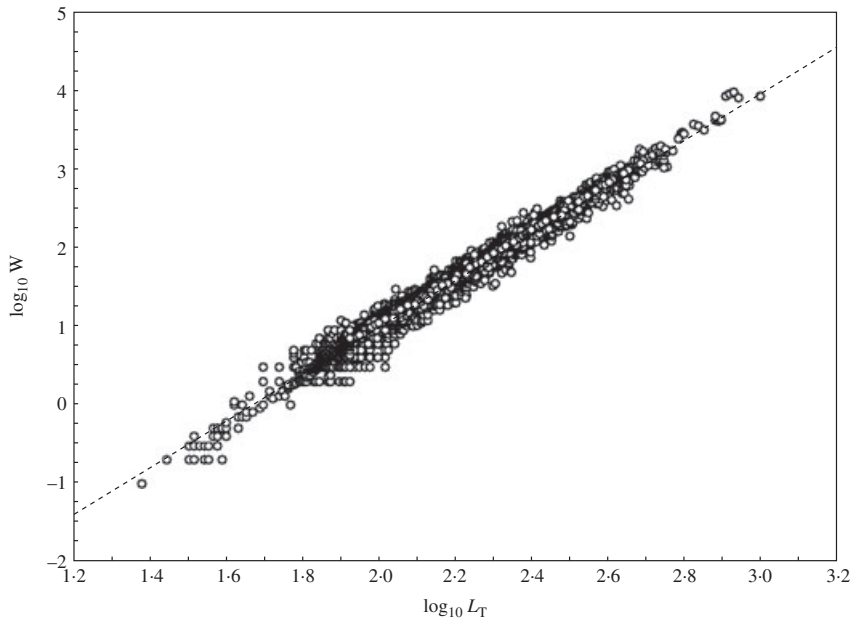


FIG. 2. Log₁₀-transformed regression between total length (L_T) and mass (W) for the total sample of *Salmo marmoratus*. The curve was fitted by: $y = -4.992 + 2.989x$ ($r^2 = 0.988$, $P < 0.001$, $n = 36460$).

(Rypel & Richter, 2008); the data were selected so that both datasets contained populations distributed geographically throughout the range of the species (Ogle & Winfield, 2009). The minimum L_T for the application of the W_s equation was determined as 90 mm, while the maximum L_T was assigned to be 570 mm. The EmP W_s equation calculated for *S. marmoratus* was: $\log_{10} W_s = -5.208 + 3.202 \log_{10} L_T - 0.046 (\log_{10} L_T)^2$ ($r^2 = 0.998$, $P < 0.01$).

Applying the EmPQ method to the validation dataset, even if the plot showed a slight negative slope (Fig. 3) (because of the small number of populations in the higher L_T classes), the value of the slope was not significantly different from zero for both terms of the equation ($p_{\text{quadratic}} = 0.85$, $p_{\text{linear}} = 0.22$) indicating that W_s was not influenced by fish L_T . According to the Willis method using χ^2 analysis, the number of relationships with significant positive slopes (eight) was not significantly different from those with significant negative slopes (six) ($\chi^2 = 0.286$; $P > 0.05$).

Salmo marmoratus is a species of great interest for its conservation value and relevance for sport fishing (Vincenzi *et al.*, 2011). The viability of *S. marmoratus* is currently threatened because of its restricted geographical distribution and the risk of hybridization with the introduced *S. trutta* (Povž *et al.*, 1996). Since the late 1980s, rising awareness about the decline of *S. marmoratus* have led to the development of rehabilitation projects in both Italy (Specchi *et al.*, 2004) and Slovenia (Crivelli *et al.*, 2000). For these reasons, the use of a measure of body condition such as W_r could be very useful to increase the knowledge on the population ecology of the species and, in conjunction with other demographic measures and life-history traits (*e.g.* age and growth), to provide a reference point and an indirect measure of the effects of management conservation actions (Murphy *et al.*, 1991; Blackwell *et al.*,

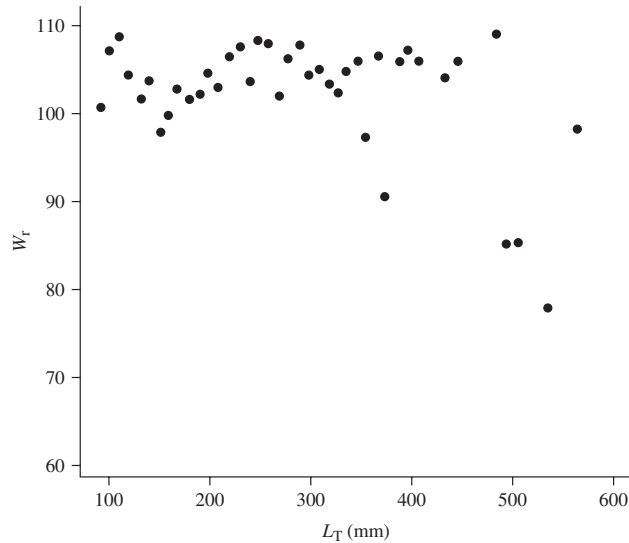


FIG. 3. Plots showing the results of the application of the empirical quartiles (EmpQ) method used to investigate potential length bias in the standard mass (W_s) equation for *Salmo marmoratus*. (W_r , standardized 75th percentile mean masses calculated by W_s equation; L_T , total length).

2000). The EmP W_s equation calculated in this study was free from length-related bias and its use to compute W_r for *S. marmoratus* is suggested.

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