

COMPARISON OF AGE ESTIMATES FROM SCALES, FIN RAYS, AND OTOLITHS OF THE INTRODUCED PEIPSI WHITEFISH, *COREGONUS MARAENOIDES* (ACTINOPTERYGII: SALMONIFORMES: SALMONIDAE), COLLECTED FROM THE ISKAR RESERVOIR (DANUBE RIVER BASIN)

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Background. Peipsi whitefish, *Coregonus maraenoides* Polyakov, 1874, is an endemic species of Lake Peipsi (Chudskoe) in Estonia and Russia. In the mid-1970s, it was introduced into the biggest Bulgarian artificial body of water—the Iskar Reservoir (Danube River basin). A recent survey confirmed the existence of a self-sustaining population of the Peipsi whitefish in the dam lake. Establishing a suitable method for determining Peipsi whitefish age would allow fishery managers and biologists to extract valuable information on various population parameters. Therefore, the aim of the presently reported study was to test the suitability of scales, pectoral fin rays, and otoliths for the most reliable age determining of Peipsi whitefish.

Material and methods. Age estimates were obtained from transverse sections of sagittal otoliths and pectoral fins, and scales from 54 Peipsi whitefish, collected between October and March 2016–2017. Two readers estimated ages from all three structures independently. The precision and bias of age estimates between readers and among structures were compared using age bias plots, coefficient of variation (CV), percent agreement (PA), and level of readability. Mean consensus ages from two readers for each structure were compared.

Results. Mean consensus age estimates obtained by analysing the scales (2.0 years) were significantly lower than those obtained by analysing the fin rays (2.6) and otoliths (2.7). Between-reader percent agreement was lower and the coefficient of variation was higher for otoliths (PA = 22.2%; CV = 27.6) compared with scales (PA = 46.6%; CV = 10.02) and pectoral fin rays (PA = 67.4%; CV = 10.12). Comparison of age estimates from the different structures revealed the highest PA and the lowest CV values between otoliths and pectoral fin rays (PA = 62.6%; CV = 10.03%), while the lowest PA and highest CV (PA = 35%, CV = 25.03) were observed between age estimates from fin rays and scales (CV, $P > 0.005$). The otolith and scale ages agreed for 46.7% and CV was 21.5. The scales were considered by both readers with the highest level of readability (88.9%) than the pectoral fin rays (70.4%) and otoliths (50%).

Conclusion. The use of fin rays is recommended for the age estimation of *C. maraenoides* from the Iskar Reservoir but further work is needed to validate the accuracy of ageing methodology for small, under one-year old Peipsi whitefish.

Keywords: Peipsi whitefish, Iskar Reservoir, aging structures, precision, bias

INTRODUCTION

Coregonid fish species (fam. Salmonidae) *Coregonus albula* (Linnaeus, 1758), *Coregonus peled* (Gmelin, 1789), and *Coregonus maraenoides* Polyakov, 1874 were introduced into several Bulgarian reservoirs in the mid-1970s (Uzunova and Zlatanova 2007). A recent study found that only the Peipsi whitefish, *Coregonus maraenoides*, has managed to establish a self-sustaining population in the Iskar Reservoir (Uzunova et al. in press**). The Peipsi whitefish is an endemic

species of Lake Peipsi (Peipus, Chudskoe) in Estonia and Russia (Kottelat and Freyhof 2007). The species is of great importance for the local fisheries not only in its native range but also in most places where it has been introduced (Mamcarz 1992, Falkowski and Wołos 1998, Krause and Palm 2000, Pereskokov and Rogozin 2001, Bobyrev et al. 2012, Ševčenko et al. 2014, Mehner et al. 2018).

The majority of published studies for introduced fishes focused on different population parameters, such as age

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** Uzunova E., Ignatov K., Dashinov D., Tasheva-Terzieva E., Trichkova T. Non-indigenous Peipsi whitefish, *Coregonus maraenoides* (Actinopterygii: Salmoniformes) in the Iskar Reservoir, Danube River basin, Bulgaria. Acta Zoologica Bulgarica (submitted).

structure, growth rate, fish longevity and age at maturity (Carlson et al. 2007, Copp and Fox 2007, Ribeiro et al. 2008). All these parameters depend on correct age estimation, but the accuracy of age estimates obtained from different parts of the fish body is often variable, tend to be species specific and accompanied by different sources of error (Campana 2001). One of the main problems in age and growth studies is the selection of the most suitable structure to age the fish. In general, scales are preferred for age estimation because the method is non-lethal, easy to implement, and require less time and resources than age estimates provided by other structures (Chilton and Beamish 1982, Casselman 1983, 1987, Jerald 1983, Carlander 1987). However, some results suggest that scales commonly yield lower age in comparison to estimates obtained from structures such as fin rays and otoliths, especially in relatively slow-growing and long-lived fishes as coregonids (Mills and Beamish 1980, Barends and Power 1984, Skurdal et al. 1985, Mills and Chalanchuk 2004, Muir et al. 2008a, 2008b, Yule et al. 2008, Herbst and Marsden 2011, Zhu et al. 2015). Obviously, the implementation of validating procedures for the determination of the accuracy of an aging method is needed. However, studies on the validation of age estimations for *Coregonus* spp. are very limited, probably due to the biological features of these species and the difficulty of re-capture (Mills and Beamish 1980, Mills and Chalanchuk 2004, Mills et al. 2004). Therefore, a procedure of selecting an appropriate aging method based on precision and bias age analysis (Campana 1995) has been widely applied (Muir et al. 2008b, Herbst and Marsden 2011, Howland et al. 2004). Assessment of the precision and bias is not equivalent to an accuracy measure of age determination, but provides information about the reproducibility of an individual's age using different structures or comparing the skill level of one reader relative to that of others (Beamish and Fournier 1981, Chang 1982, Campana 1995, Hoenig et al. 1995, DeVries and Frie 1996, Campana 2001). The precision of age estimates is different for various aging structures and is influenced by various biotic and abiotic factors, such as nature of the growth of the particular structure, fish growth rate, food availability, geographic region, level of population exploitation, water pollution, and many others (Hoxmeier et al. 2001, Howland et al. 2004, Quist et al. 2012, Zubova and Kašulin 2014).

Age and growth of Peipsi whitefish, *C. maraenoides*, have commonly been assessed using scales (Krause and Palm 2000, Pereskokov and Rogozin 2001, Ševčenko et al. 2014). However, other calcified structures may be more precise in the age estimation for Peipsi whitefish, but no data is currently available. Therefore, the main goals of our study were to 1) evaluate and compare age estimates of three structures—scales, otoliths, and pectoral fin rays between readers and between pairs of ageing structures and (2) to quantify potential biases of age estimates between readers and between pairs of ageing structures in order to select the most suitable structure for age determinations in Peipsi whitefish from a naturalized population in southern Europe (Iskar Reservoir, Bulgaria).

MATERIAL AND METHODS

Study area and fish sampling. The study was conducted in the biggest Bulgarian artificial body of water—the Iskar Reservoir (42°27'32"N, 023°35'02"E). It is located on the Iskar River, a tributary of the Danube River. The reservoir is located at the altitude of 817.5 m above sea level and its maximum surface area is 3000 ha, the maximum water volume is 0.67 km³, the maximum depth is ≈70 m, and the mean depth is 15 m. The maximum water temperature at the depth of 10 m is 20°C (Kalchev et al. 1993). Commercial use of fish resources is not permitted.

In total, 54 Peipsi whitefish, *Coregonus maraenoides*, ranging from 316 to 519 mm total length (TL) and from 300 to 1755 g total weight (*W*) were collected for the presently reported study. Peipsi whitefish were caught at four sites in the south-eastern part of the reservoir between October and March (2016–2017) using a bottom-set monofilament gillnet, which were 8–10 m deep, 200–400 m long and included panels of 60, 80, 120, and 140 mm stretch mesh sizes. Gillnets with smaller mesh sizes were not used to prevent catching and injuring smaller fishes. Each fishing session lasted for approximately 10 h.

All fish were frozen in individual plastic bags and deposited at the laboratory of the Department of General and Applied Hydrobiology, Faculty of Biology, Sofia University, Bulgaria. Fish were measured for their total and standard length to the nearest 0.1 cm with a digital calliper and body weight was determined to the nearest 0.1 g with an electronic balance.

Collection, preparation, and reading of ageing structures. Scales, sagittal otoliths, and pectoral fin rays were collected from each individual for the age determination.

A minimum of six scales was collected from the left flank of each fish below the middle of the dorsal fin and above the lateral line. The scales were cleaned, dried, and mounted between two glass slides for viewing with a microfiche reader (17.5× magnification using transmitted light). A scale annulus was defined as the complete ridge (circulus) that crosses over a region of incomplete ridges (Fig. 1A). One growth band was defined as an opaque and translucent band pair (Beamish and McFarlane 1983, Casselman 1983, 1987, Muir et al. 2008a).

The entire pectoral left fin from each fish was removed as close as possible to the base and placed in a paper envelope to dry for a month. The first four rays were subsequently placed in a 2 mL tube and embedded in epoxy resin according to the methodology described by Koch and Quist (2007). The rays were then cut into transverse cross sections at an angle of approximately 90° with respect to the longitudinal axis of the rays. Three or four sections (0.7 mm thickness) were cut using a low-speed saw microtome (Leica SP 1600). The sections were fixed to slides in the order they were cut. The age was estimated directly by using a compound microscope at 200× magnification and transmitted light. The annuli appeared as light rings in the fin-ray sections formed during the low growing period (Fig. 1B).

Both sagittal otoliths were extracted from the vestibular apparatus and cleaned with water (Secor et al. 1992). A

preliminary analysis of the whole otoliths showed that they are unreadable. Therefore, one otolith from each pair was preserved in a 50% glycerine solution for one month (Chilton and Beamish 1982) and then was mounted in epoxy using 2 mL microcentrifuge tubes to form a block for sectioning. One or two transverse sections (0.2 mm thickness) through the otolith were cut and mounted on glass slides. The otolith sections were interpreted directly using a compound microscope at 200× magnification with transmitted light. Euparal media was added to improve the clarity. The otolith annuli were defined as the sharp line of transition between the thin, translucent zone and the adjacent broad, opaque zone (DeVries and Frie 1996, Muir 2008a) (Fig. 1C).

Each individual structure was independently examined once by two readers. The Reader 2 (first author) was experienced at estimating the age of fish using all three structures, whereas the Reader 1 (second author) had no previous experience with any of the structures but was trained by Reader 2 and allowed to practice with each structure before estimating the age for the study. The criteria for identifying annuli were adopted jointly by the two readers. Annuli were counted without prior information on length, sex, or date of capture of fish. If the age estimations of a particular structure differed between the two readers, the sample was read for the third time and assigned a final age when a consensus was reached. If consensus was not reached for a particular structure, it was not included in the analysis where the mean consensus age was needed. In the case growth outside the last annulus, the date of capture of fish was checked and specimen was assigned to an age class assuming 1 January as the designated birthday (Jerald 1983).

The readability of each structure (or confidence of the reader) was evaluated in two main categories: the first category covered cases that were classified as having ‘excellent’ or ‘very good’ readability, while the second category included cases that were reported as ‘bad’ or ‘unreadable’.

Data analysis. Age bias graphs were constructed to examine potential biases between readers and between pairs of ageing structures (Campana et al. 1995). To assess bias between readers we plotted the ages estimated by one reader for all fish against the age determined by a second reader. To assess bias between structures we plotted the consensus age for one structure against the consensus age estimated by another structure for all fish. All regression lines were examined for deviations from the 1 ÷ 1 equivalence line. The 95% confidence intervals were calculated and represented by error bars in the resulting figures. For each age bias graph, *t*-tests were used to test the null hypotheses that the slope (β_1) of the regression line and the intercept (β_0) was not significantly different from one and was not significantly different from zero (indicating 1 ÷ 1 agreement in age estimates between reading pairs). A rejection of either hypothesis was interpreted as bias in the age estimates (Long and Fisher 2001).

The precision of age estimates (i.e., among readers and among structures) was evaluated by using the coefficient of variation (CV %) (Chang 1982, Campana et al. 1995)

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where x_{ij} was the *i*th age determination of the *j*th fish, x_j was the mean age estimate of the *j*th fish and *R* was the number of times each fish was aged. The lower values of CV indicated a higher level of precision (Campana et al. 1995). The CV values of the fish studied were processed to produce a mean, standard error, and standard deviation. One-way repeated measures analysis of variance (ANOVA), and pairwise multiple comparisons

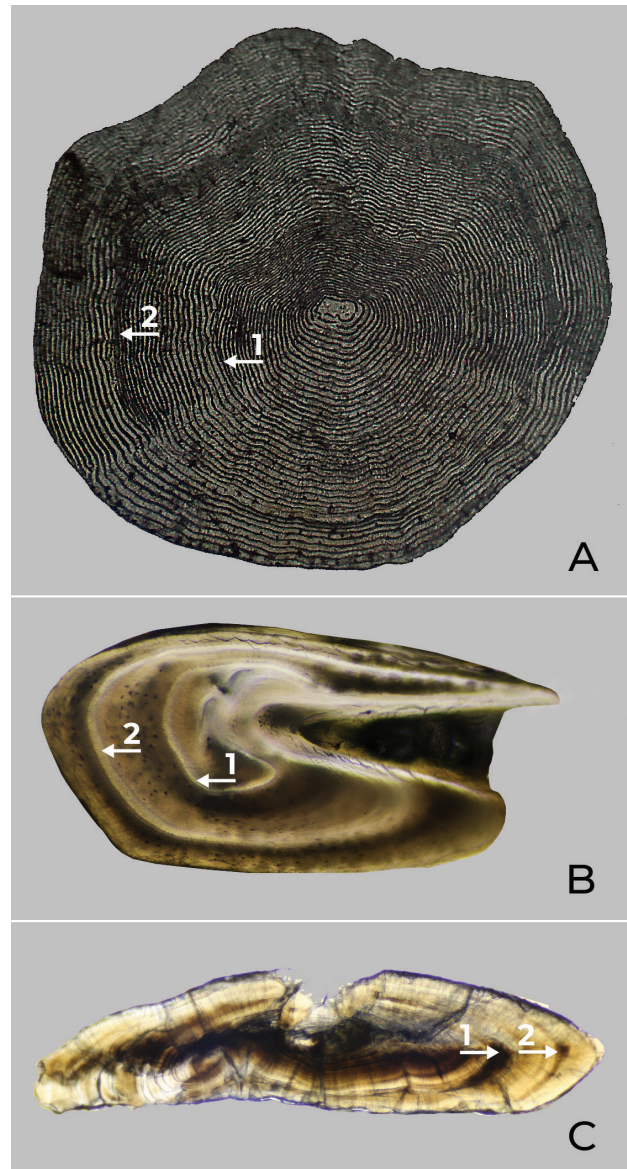


Fig. 1. Scale (A), pectoral fin-ray section (B), and transverse section of a sagittal otolith (C) from a male specimen, 409 g, 350 mm Peipsi whitefish, *Coregonus maraenoides*, captured on 23 December 2016 in the Iskar Reservoir (Bulgaria) that was estimated to be two years old (three summer old) from each structure by both readers; the author's interpretation of annular marks is shown by the arrows on each structure

(Tukey's post hoc test) were used to test whether the CV estimates were significantly different among readers within structures and among structures. Differences were regarded as significant at $\alpha = 0.05$.

Differences between the mean ages (consensus ages) obtained from the three different ageing structures were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc pair-wise comparisons.

The percent agreement (PA) was defined as the proportion of fish that were assigned the same age by two readers. The readability (reader confidence) of each structure was calculated as the percentages of structures which were deemed to have 'excellent' or 'very good' readability. The data were analysed by means of the Microsoft Excel 2010 and GraphPad Prism 8 software.

RESULTS

The age composition of the sampled specimens of the Peipsi whitefish, *Coregonus maraenoides*, based on different structures exhibited variation in their age estimates (Fig. 2). The fish were aged as representing 1–6 years with scales and otoliths and as 1–7 years with fin rays. The differences in mean consensus age estimates from the three aging structures were significant ($F = 7.159$, $P = 0.001$). The mean age based on the scales (mean = 2 ± 0.1 SE) was significantly lower than the mean ages estimated from the pectoral fin rays (mean = 2.7 ± 0.13 SE; $P < 0.001$) and otoliths (mean = 2.6 ± 0.14 SE; $P < 0.05$). The mean ages of pectoral fin rays and otoliths did not differ from each other ($P > 0.05$). With regards to the age distribution predicted by the different structures, more fish were assigned to younger age classes based on the scale analysis, while the fin ray and otolith age estimation did not identify any 1-year-old fish (Fig. 2). In general, the scales underestimated the age when compared to otoliths and pectoral fin rays. The mean body length (TL) at age based on the otoliths and fin rays was lower than the mean length at age based on the scales (Fig. 3).

Reader bias and precision. The age bias plots did not reveal significant bias between the readers regarding the scales (β_1 , $t = 0.1641$; $P = 0.79$; β_0 , $t = 0.281$; $P = 0.79$; $df = 4$), fin rays (β_1 , $t = 1.4037$; $P = 0.255$; β_0 , $t = 0.171$; $P = 0.704$; $df = 3$) or otoliths (β_1 , $t = 1.739$, $P = 0.46$), except the intercept for otoliths which was significantly different from zero (β_0 , $t = 0.004$; $P = 0.05$; $df = 4$) (Fig. 4). The ANOVA indicated differences among structures in the mean CV of age estimates across the readers ($F = 7.468$, $P < 0.001$). The PA of age readings between two independent readers was the lowest and the CV was the highest for otoliths (PA = 32.2%, CV = 27.3%) (Table 1). The CV value was significantly higher than CVs for scales (14.12%, $P < 0.05$) and for fin rays (14.02%, $P < 0.05$). No significant differences between CVs for scales and fin rays ($P = 0.9$). PA was the highest for pectoral fin rays (57.4%) followed by scales (46%) (Table 1).

Structure bias and precision. The structure-related bias was not evident between age estimates from scales and fin rays (β_1 , $t = 0.456$; $P = 0.54$; β_0 , $t = 1.379$; $P = 0.239$; $df = 4$), scales and otoliths (β_1 , $t = 0.089$; $P = 0.78$; β_0 , $t = 1.09$; $P =$

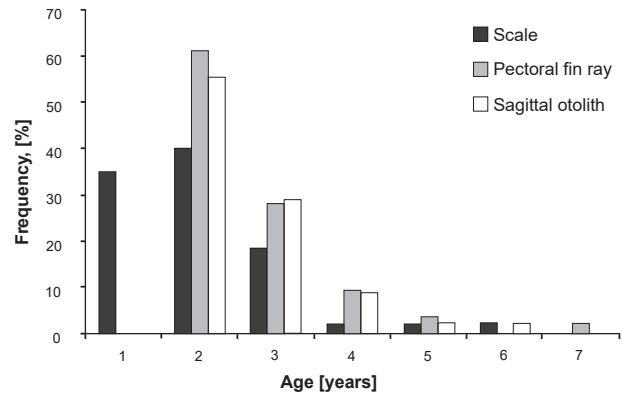


Fig. 2. Age-frequency distribution based on scale, pectoral fin, and sagittal otolith of Peipsi whitefish, *Coregonus maraenoides*, collected from the Iskar Reservoir, Bulgaria

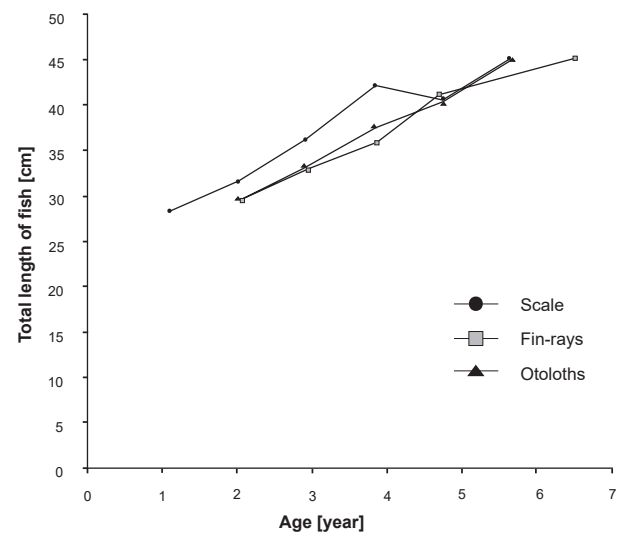


Fig. 3. Mean total length of Peipsi whitefish, *Coregonus maraenoides*, from the Iskar Reservoir, Bulgaria, versus age estimated using scales, otoliths, and fin rays

0.336; $df = 4$), fin rays and otoliths (β_1 , $t = 1.324$; $P = 0.33$; β_0 , $t = 1.473$; $P = 0.23$; $df = 4$) (Fig. 5). Comparison of age estimates from the different structures revealed the highest PA and lowest CV values between otoliths and pectoral fin rays (PA = 62.6%; CV = 10.03%), while the lowest PA and highest CV (PA = 35%, CV = 25.03) were observed between age estimates from fin rays and scales (CV, $P > 0.005$). The otolith and scale ages agreed for 46.7% and CV was 21.5 (Table 1). The scales were considered by both readers with the highest level of readability (88.9%) than the sectioned pectoral fin rays (70.4%) and sectioned otoliths (50%). Nine otoliths were removed as totally illegible and consensus regarding the age of these specimens was not reached.

DISCUSSION

The maximum estimated age of Peipsi whitefish, *Coregonus maraenoides*, naturalized in the Iskar Reservoir was 7 years. The maximum recorded age

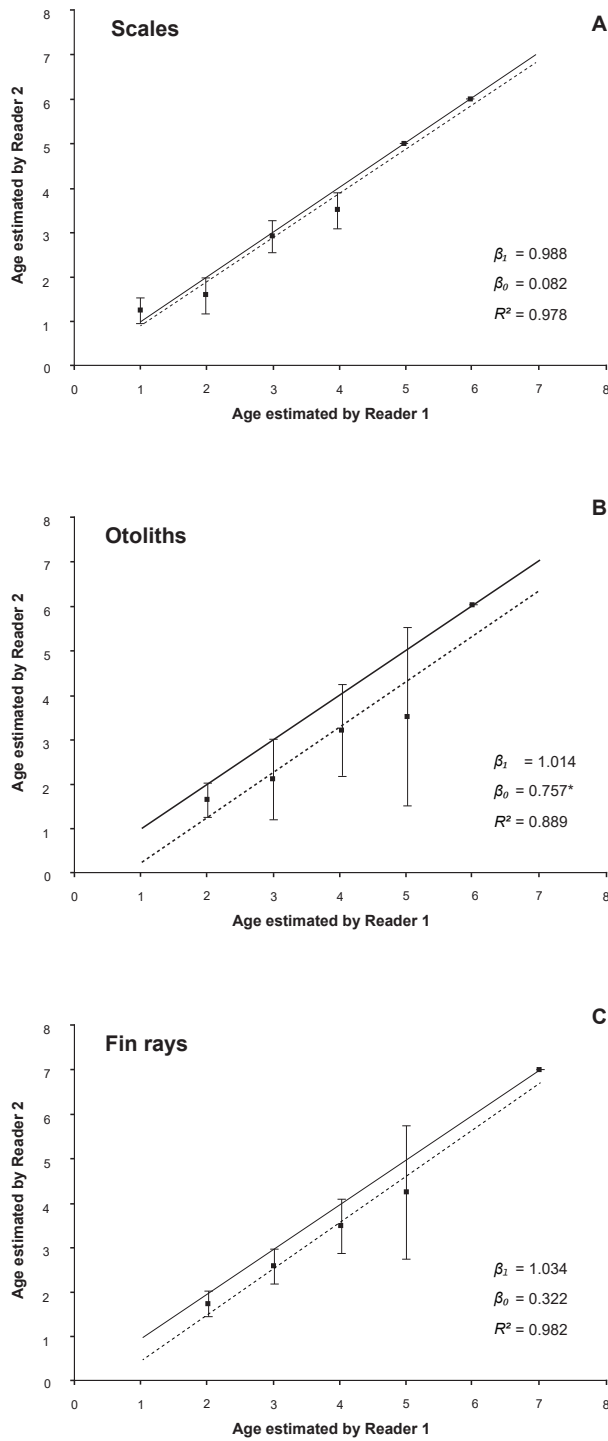


Fig. 4. Age bias graphs between two independent readers (Reader 1 and Reader 2) in age estimates from scales (A), sectioned otoliths (B), and sectioned fin rays (C) of Peipsi whitefish, *Coregonus maraenoides*, from the Iskar Reservoir, Bulgaria; the solid lines represent 1 ÷ 1 agreement in age estimation between the two readers; dashed lines are least-squares regression lines; each error bar represents the 95% confidence interval for the age assigned by Reader 2 to all fish assigned a given age by Reader 1; asterisks indicate a significant difference from zero for the intercept (β_0) or a slope (β_1) that is significantly different from 1

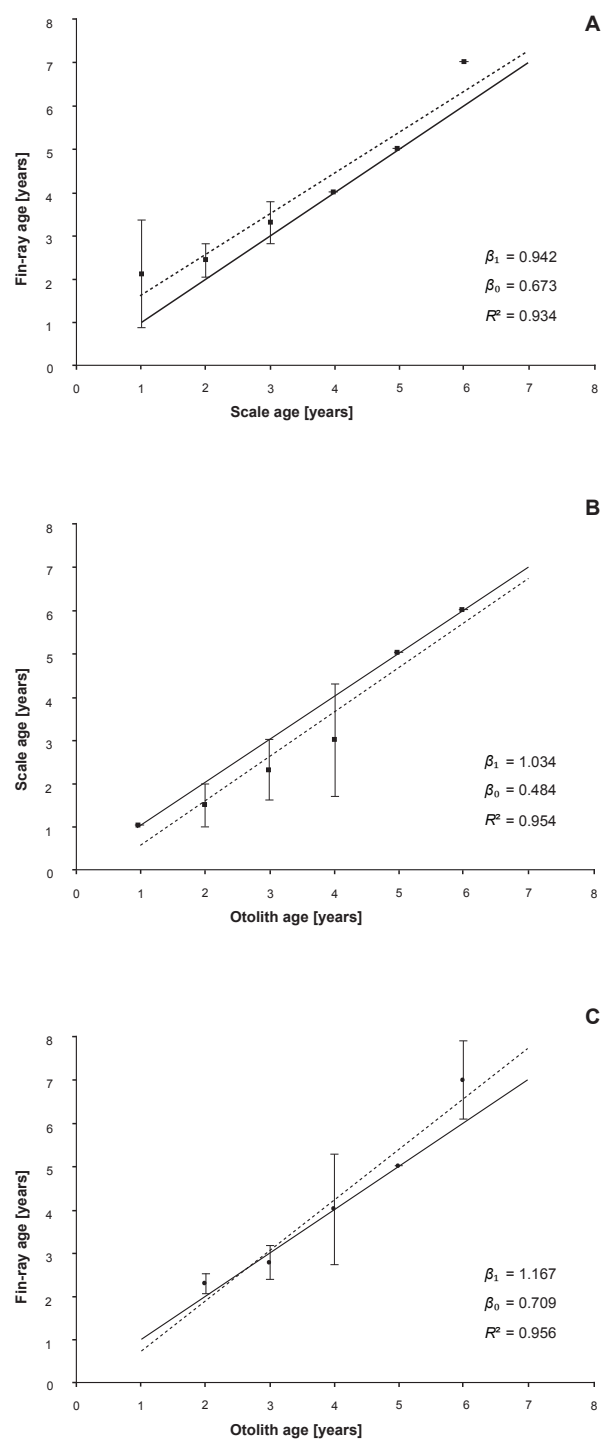


Fig. 5. Age bias graphs for consensus age estimates from fin rays vs. scales (A), scales vs. otoliths (B), and fin rays vs. otoliths (C) of Peipsi whitefish, *Coregonus maraenoides*, from the Iskar Reservoir, Bulgaria; solid lines indicate represents 1 ÷ 1 agreement in age estimation between structures; dashed lines are the least squares regression lines; each error bar represents the 95% confidence interval

Table 1

Statistical analysis of age readings of Peipsi whitefish, *Coregonus maraenoides*, estimated by two independent readers from scales, sectioned pectoral fin rays, and sectioned otoliths

Structure	Mean CV [%]	SD of CV	SE of CV	PA [%]
Between readers				
Rays	14.02	18.61	2.53	57.4
Scales	14.12	20.79	2.83	46.6
Otoliths	27.28	18.85	2.81	32.0
Between structures				
Scales vs rays	25.03	21.8	2.9	35.2
Scales vs otoliths	21.5	22.2	3.3	46.7
Otoliths vs rays	10.03	14.4	2.1	62.6

CV = coefficient of variation, SD = standard deviation, SE = standard error of the mean, PA = percent agreement.

of the Peipsi whitefish native population was 15 years (Krause and Palm 2000), while the maximum age of the populations inhabited water bodies outside of the native range was 9 years (Pereskokov and Rogozin 2001, Ševčenko et al. 2014). In our study, the scales tended to reveal lower ages than those provided by the otoliths and pectoral fin rays. In general, this trend is not surprising because similar results have been reported in numerous studies on the coregonid fishes (Mills and Beamish 1980, Barnes and Power 1984, Skurdal et al. 1985, Muir et al. 2008a, 2008b, Herbst and Marsden 2011). Usually, it has been observed that differences between the age estimates from scales and other structures (e.g., otoliths, fin rays) increased linearly after the age of 4–5 for long-lived and slow-growing salmonid fish (Skurdal et al. 1985, Zymonas and McMahon 2009). More precise age estimates from scales than the other structures have been reported only for coregonids up to 3–4 years of age and for those from fast-growing populations (Mills and Beamish 1980, Muir et al. 2008a). The observed disagreement between the scale and fin ray or otolith ages is probably due to the fact that scale growth is directly linked to somatic growth rate and when somatic growth slows down significantly, the formation of new annual zones on scales may stop (Skurdal et al. 1985, Muir et al. 2008a, 2008b). Kruse et al. (1993) found that precision of age estimated from scales was also influenced by the latitude. Hoxmeier et al. (2001) supposed that the age of fish from populations subject to relatively short, distinct growing seasons can be more precisely estimated with scales than those with longer, indistinct growing seasons. In this study, it was likely that one or more annuli identified on sectioned pectoral fin rays and otoliths of several fish were not counted on the scales. Studies on tagged lake whitefish report that scales fail to form an annulus in the period between marking and re-capture (Mills and Beamish 1980, Mills and Chalanchuk 2004, Mills et al. 2004). In contrast to scales, it has been found out that otoliths grow and new annuli are laid down even when the somatic growth ceases (Beamish and

McFarlane 1993). A significant slowdown in the growth rate is typical for the period beyond sexual maturity in coregonids. In Lake Peipsi, *C. maraenoides* become sexually mature at the age of 4–5 years (Krause and Palm 2000). However, outside of its native range, the sexual maturation of the Peipsi whitefish has been reported to occur earlier than 3 years of age (Pereskokov and Rogozin 2001). It is possible the environmental conditions and food availability in the Iskar Reservoir facilitate the sexual maturation of *C. maraenoides*. The early sexual maturation is accompanied by slowing down the somatic growth and this can explain the observed only one-year ring on the scales of several *C. maraenoides* with a length of > 350 mm and a weight of > 450 g caught in the period after 1 January.

The observed relatively low agreement between readers when otoliths were used for age estimation of Peipsi whitefish indicated that not all annuli were being identified similarly. In the presently reported study, both readers reported difficulties in identifying the annular growth rings on otoliths and only 13 otoliths were classified as ‘excellent’ or ‘very good’ according to their readability. Low agreement between readers and high variance among age estimates for *C. maraenoides* otoliths were in contrast to that has previously been reported for other coregonids (Skurdal et al. 1985, Muir et al. 2008a, Herbst and Marsden 2011, Zhu et al. 2015).

Fin rays are a common structure used for estimating the age of fish (DeVries and Frie 1996). Some studies were found that fin rays are more precise than scales to estimate age of different salmonid fishes (Williamson and Macdonald 1997, Zymonas and McMahon 2009). High predictability of the fin ray method of aging has been found out in a mark-recapture age validation study with the lake whitefish *Coregonus clupeaformis* (Mitchill, 1818) (see Mills and Beamish 1980) and confirmed by a number of other studies (Chilton and Bilton 1986, Mills and Chalanchuk 2004, Muir et al. 2008b). Zymonas and McMahon (2009) report that pelvic fin rays provide more precise age estimates than scales and represent a viable non-lethal alternative to otoliths when estimating the age of bull trout, *Salvelinus confluentus* (Suckley, 1859). Although the mean estimated age from the pectoral fin rays and otoliths in our study were similar, both readers had higher confidence in estimating the age using the pectoral fin rays and noted better clarity of the annuli in the pectoral fin rays compared with otoliths. On the other hand, the fin rays are easier to remove, process, and observe than the otoliths. Scales and fin rays were equally precise, but in both cases, CVs were higher than those suggested by Campana (2001) as an acceptable level of precision.

From the comparison of age estimates from the three ageing structures within the presently reported study revealed that fin rays exhibiting the lowest CV and the highest PA between age readers as compared to other structures and provided the most suitable age estimates in *C. maraenoides*. Sectioned otoliths were less precise and difficult to read structure for age estimation of Peipsi whitefish. The

scales exhibited the highest level of readability, however, underestimated ages when compared to otoliths and pectoral fin rays and it is quite likely that the growth rates have been overestimated and longevity have been underestimated in the studies of the Peipsi whitefish that used scales.

Therefore, at this stage of the research, we recommend pectoral fin rays be use, when possible. Additionally, scales and fin-ray collection should be continued throughout the year in order to assess the temporal variation in annulus formation on these structures and evaluate the precision of age estimation of different structures from small, up to 1 year old and in small *C. maraenoides* up to 1 year old, and also in adults over seven years. Future research should focus on validating the age estimation methodologies of the Peipsi whitefish conducting mark-recapture studies in aquaculture ponds.

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