Age and growth of European bitterling 
(Rhodeus sericeus) in the Wieprz-Krzna Canal, Poland

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Abstract
The age and growth of bitterling, a small Eurasian cyprinid, were studied in the Wieprz-Krzna Canal, a poorly structured irrigation canal located in eastern Poland. Its population was represented by five age groups (0+ to 4+). Scale annuli were clearly visible and often displayed as irregularities in circuli in the centrolateral part of the scales. The number of annuli detected on scales corresponded to the number of bands seen in the opercular bone; however, the first annulus was difficult to observe in reflected light. The Bhattacharya method followed by modal class progression analysis was useful to validate age. Several normal components were clearly necessary to explain the length-frequency distribution and the most likely solution consisted of 5 age groups with separation index values greater than 2. A comparison of observed and back-calculated lengths showed good agreement. There were no significant differences between mean back-calculated lengths of different sexes. The von Bertalanffy growth function was fitted to length-at-age data and displayed variation in growth rates between sexes. The asymptotic length estimates were suitable for females, for which maximum observed length was 74 mm (weight = 5.46 g) but clearly underestimated for males (for which maximum size was 75 mm and 5.73 g). There were no significant differences between sexes in the slope of the length-weight relationship and the common slope for both sexes (b = 3.64) was significantly greater than 3 indicating an allometric growth pattern of bitterling. A spline regression estimated a shift in the weight-length relationship at average maturity size.

Key words: Bitterling, age, growth, scale, weight-length relationship, von Bertalanffy model.

1. Introduction
The European bitterling Rhodeus sericeus (Pallas 1776) is a small cyprinid that spawns in unionid mussels, this being an example of a unique mode of reproduction (Breder, Rosen 1966). Due to its interaction with mussels, bitterling is a valuable model species in behavioural and evolutionary ecology (Mills, Reynolds 2003). The scientific interest in this species is mostly concentrated on its behavioural aspects of reproduction and the large body of literature con-
cerning them has recently been reviewed by Smith et al. (2004). Apart from these aspects, several studies on the feeding (Przybylski 1996), systematics and morphology of bitterling (e.g. Holčík 1959; Holčík, Jedlicka 1994; Reichard 1998) have been made. Basic population parameters have been collected only for Czech and Slovak (Holčík 1960), German (Schaumburg 1989) and recently Greek (Koutrakis et al. 2003) populations. However, a review of its biology (Holčík 1999) revealed how little is known about bitterling environmental biology in general.

Bitterling is widely distributed throughout Europe and the north-eastern part of Asia (Holčík 1999) but due to its unusual style of reproduction (Wiepkema 1961; Breder, Rosen 1966) its occurrence is determined mainly by the presence of unionid mussels. Although reported as a limnophilic species (Schiemer, Waidbacher 1992), it also occurs in lentic parts of river systems (Copp 1989; Przybylski, Zięba 2000). Bitterling is a species listed under Appendix 3 of the Bern Convention, is relatively rare and endangered in Poland (Witkowski et al. 1999), and since 1995 has been protected by law.

The present study examines the age and growth of a population occurring in a poorly structured irrigation canal. This study aims to assist the management of this fish species and contribute to an understanding of the environmental and geographical variation on its life history characteristics.

2. Materials and methods

The study was conducted in the Wieprz-Krzna Canal, located in eastern Poland. This 140 km long canal was constructed in the late 1950s as a main part of an irrigation system draining swamps of the Łęczynsko-Włodawskie lake area. The canal is a typical straightened watercourse, with trapezoidal-shaped cross section and relatively uniform width (5-10 m) and depth (0.4-1.0 m). Numerous weirs and dams regulate its flow, dividing the whole course into numerous sections. Piles and flagstones restrain the canal banks and the substratum consists mainly of mud. During the growing season, the canal is covered with patches of vascular plants (Potamogeton sp. and Glyceria sp.) that occasionally cover up to 80% of the canal surface.

Water quality is generally poor but variable. The following ranges of limnological variables have been observed (Janiec 1993): dissolved oxygen, from 1.0 to 9.6 mg dm⁻³; BOD₅, 1.6-4.8 mg O₂ dm⁻³; suspended matter, 4.0-62.0 mg dm⁻³; ammonium, 0.44-2.42 mg dm⁻³; orthophosphate, 0.15-0.40 mg dm⁻³; and conductivity, 248.0-406.0 µS cm⁻¹. The Wieprz-Krzna Canal is subjected to regular maintenance, such as weed cutting and bank clearance.

The sampling site was located in the middle part of the canal, near the Moœciska Village (51°33′47″N, 23°06′57″E). Here the canal was 5 m wide and the maximum depth was 0.8 m. The European bitterling was the most abundant fish and it co-occurred with three-spined stickleback (Gasterosteus aculeatus L.), gudgeon (Gobio gobio (L.)), roach (Rutilus rutilus (L.)), perch (Perca fluviatilis L.), and a few specimens of mud loach (Misgurnus fossilis (L.)). In the substratum, numerous specimens of mussels, Anodonta sp., were observed.

Bitterling were sampled on 19 April 1995 using a seine net 5m long and 1.5m deep, with a stretched mesh size of 2mm. A sample of 233 bitterling was anaesthetised and preserved in 5% formalin. In the laboratory, these fish were measured (standard and total length) to the nearest 0.1 mm and weighed to the nearest 0.01g. Sex was recognised by external features, e.g. the ovipositor in females or by macroscopic examinations of gonads (young fish or uncertain specimens).

Three to six scales from the left side of the broad part of the body were removed, cleaned and mounted dry between two slides for age estimation by stereomicroscope study.

Fig. 1. Stereomicroscope photography of bitterling scales and the operculum bone of individuals captured on 19 April 1995; (A) immature specimens of age 0+, TL = 36 mm, Wt = 0.53 g; (B) female of age 2+, TL = 47 mm, Wt = 1.08 g; (C) male of age 4+, TL = 67 mm, Wt = 4.11.
For several randomly selected specimens, age was validated from the operculum bone examined under a stereomicroscope. Age validation was also attempted using modal class progression analysis, as available in the Compleat ELEFAN software (Gayanilo et al. 1989). This is the only validation method available in the package for a single sampling date. First, size groups presumed to represent age classes in the sample were identified by the method of Bhattacharya (1967) and this was followed by modal class progression analysis, obtaining a separation index that must be greater than 2 to provide meaningful separations (Gayanilo et al. 1989). The same methodology was successfully applied by García-Berthou, Moreno-Amich (1992).

The previous growth of bitterling was determined by back-calculation from the scale measurements. Due to the lack of a clear focus on the scales (Holčík 1960), measurements were made from the outside edges of each annulus in the longest diameter of the elliptic scale and the scale radius was obtained by dividing these measures in two. To estimate previous growth history, the total length-at-age was back-calculated for each fish using these equations and adjusted according to the ratios between observed and estimated length (Francis 1990). Instantaneous rate of increase (Ricker 1975) was calculated as:

\[ IGR = \ln L_{t+1} - \ln L_t \]

We attempted the fitting of growth data to the von Bertalanffy function (Ricker 1975):

\[ L_t = L_{\text{inf}} (1 - \exp(-k(t - t_0))), \]

where: \( L_t \) is the length at age \( t \) (in years), and \( L_{\text{inf}}, k \) and \( t_0 \) are the three parameters to be estimated; and to the Gompertz function:

\[ W_t = W_0 \exp(G (1 - \exp(-gt))), \]

where: \( W_t \) is the weight (g), \( W_0 \) is weight at the conventional time \( t_0 \), \( G \) is the instantaneous growth rate at time \( t_0 \), and \( g \) describes the rate of decrease of \( G \). Both models were estimated by non-linear regression using the Levenberg-Marquardt algorithm (Marquardt 1963).

To assess the presence of an allometric growth pattern (Ricker 1975) the weight-length relationship was determined by linear regression. Differences between sexes were tested with test of homogeneity of slopes and the hypothesis of isometric growth was tested with a t-test (Zar 1984). Regression lines were compared using ANCOVA, following García-Berthou and Moreno-Amich (1993). All statistical analyses (except those of ELEFAN) were computed with SPSS 11.5.

### 3. Results

Bitterling total length (\( TL \) in mm) was significantly related to its standard length (\( SL \) in mm) and the estimated relationship was:

\[ TL = 1.811 + 1.176 SL \]

The population of bitterling in the Wieprz-Krzná Canal was represented by five age groups, i.e. from 0+ to 4+. The scale annuli of bitterling were clearly visible in all parts of the scales. In the centrolateral part of the scale, the annulus was frequently marked by irregularities in circuli (Fig. 1). The number of annuli detected on scales corresponded to the number of bands seen in the operculum bone (Fig. 1). Although the first annulus was difficult to see in reflected light on the operculum, scales and opercular bones provided the same age estimation. The shape of the bitterling scale changed with fish size. Younger specimens have scales more nearly circular than older fish, where the scale becomes more elliptic (Fig. 1). Due to this change of scale shape, the measurement radius does not occupy a constant position on the scale but shifts towards the caudal part of the scale with fish size. Moreover, in the anterior part of scales annuli were crowded near the margin but in the lateral parts annuli had no fixed position producing some difficulties in radial measurement of the annuli.

### Table I. Decomposition of length-frequency data of bitterling total length of the Wieprz-Krzná Canal population using the method of Bhattacharya (1967) followed by modal class progression analysis. To provide a meaningfully separated group, the value of the separation index must be greater than 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total length (mm)</th>
<th>n</th>
<th>Index of separation</th>
<th>Estimated age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37.1</td>
<td>3.9</td>
<td>285.83</td>
<td>0+</td>
</tr>
<tr>
<td>2</td>
<td>43.5</td>
<td>1.8</td>
<td>357.35</td>
<td>1+</td>
</tr>
<tr>
<td>3</td>
<td>50.6</td>
<td>1.0</td>
<td>12.87</td>
<td>2+</td>
</tr>
<tr>
<td>4</td>
<td>56.8</td>
<td>2.3</td>
<td>27.25</td>
<td>3+</td>
</tr>
<tr>
<td>5</td>
<td>64.4</td>
<td>1.1</td>
<td>9.71</td>
<td>4+</td>
</tr>
</tbody>
</table>
The Bhattacharya method was useful to validate age (Table I) and several normal components were clearly necessary to explain the length-frequency distribution (Fig. 2). The most likely solution consisted of 5 age groups with separation index values greater than 2 (Table I). The explained distribution was significantly different from the observed distribution ($\chi^2 = 66.3$, df = 5, $P < 0.001$) but tests with df less than 10 are unreliable (Gayanilo et al. 1989).

Comparison of total length estimated with the Bhattacharya method (Table I) and scale reading (Table II) showed small but significant differences only for age 2+ ($P = 0.030$) and 3+ ($P = 0.025$).

Due to the changes of scale shape with fish size the measurements of scale radius for back-calculation caused some difficulties. However, significant linear relationships were found between scale radius (SR in arbitrary ocular units) and fish length for males and females separately. The equations found were:

Males:

$$TL = 22.70 + 1.545 \text{SR},$$

Females:

$$TL = 26.75 + 1.370 \text{SR}.$$  

Although those relationships explained only 84% and 74% of length variation for males and females respectively, they were used for back-calculation. The comparison of observed and back-calculated lengths showed a good agreement (Table II) and there were no significant differences between mean back-calculated lengths of different sexes ($P = 0.147$). Mean annual increments of the bitterling length varied but the highest increments were noted between the second and the third year for both sexes. Nevertheless, the instantaneous growth rate decreased progressively for females, while males mirrored annual increment (Table II).

Fitting the length-at-age data to the von Bertalanffy and Gompertz models displayed further variation in growth rates between sexes (Table III). High values of the coefficients of determination show that both functions fit well to all data. According to Taylor (1962), the von Bertalanffy equation provides a good description of the growth pattern if the maximum observed length is

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**Table II. Mean observed and back-calculated total lengths (mm) obtained from measurement of scales.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Observed length (mm) average (SD)</th>
<th>No of fish</th>
<th>Length-at-age (years)</th>
<th>Instantaneous rate of increase IGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>0+</td>
<td>34.8 (2.1)</td>
<td>17</td>
<td>35.6</td>
<td>0.167</td>
</tr>
<tr>
<td>1+</td>
<td>40.9 (4.5)</td>
<td>16</td>
<td>36.2</td>
<td>0.146</td>
</tr>
<tr>
<td>2+</td>
<td>47.0 (3.4)</td>
<td>19</td>
<td>38.4</td>
<td>0.109</td>
</tr>
<tr>
<td>3+</td>
<td>56.9 (3.7)</td>
<td>41</td>
<td>37.8</td>
<td></td>
</tr>
<tr>
<td>4+</td>
<td>63.0 (4.5)</td>
<td>30</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Mean (95% C.L.)</td>
<td>37.4 (0.7)</td>
<td>44.2 (0.9)</td>
<td>51.4 (0.9)</td>
<td>57.5 (1.6)</td>
</tr>
<tr>
<td>Annual increment</td>
<td>6.8</td>
<td>7.2</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Instantaneous rate of increase IGR</td>
<td>0.167</td>
<td>0.146</td>
<td>0.109</td>
<td></td>
</tr>
</tbody>
</table>

|           |                                  |            | 2                     |                                   |
| 1+        | 42.1 (3.1)                       | 30         | 37.1                  |                                   |
| 2+        | 45.1 (3.6)                       | 47         | 36.4                  |                                   |
| 3+        | 58.1 (4.5)                       | 20         | 43.4                  |                                   |
| 4+        | 63.4 (3.9)                       | 15         | 40.7                  |                                   |
| Mean (95% C.L.) | 38.3 (0.8) | 44.0 (1.1) | 54.3 (1.5) | 58.9 (2.4) |
| Annual increment | 5.7 | 10.3 | 4.6 |
| Instantaneous rate of increase IGR | 0.139 | 0.210 | 0.081 |

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approximately 95% of the asymptotic length. This would suggest poor descriptions for both sexes, but slightly better for females (75%) than males (71%).

Estimates for the Gompertz growth function show the lack of differences between sexes. The slopes of the weight-length relationship (Fig. 3) differed significantly between immature and mature fish (P < 0.0005), but there were no significant differences in slope (P = 0.33) or intercepts (P = 0.74) between males and females. The common slope for both sexes ($b_c = 3.643; s_c = 0.0548$) was significantly greater than 3 (P < 0.0001) and showed that bitterling grow allometrically. A spline regression model estimated by non-linear regression was:

$$\log \text{Weight} = -1.042 + 0.472 \log 10 \text{TL} + 3.102 (\log \text{TL} - 1.567) \quad (\text{if } \log \text{TL} > 1.567)$$

$$\log \text{Weight} = -1.042 + 0.472 \log 10 \text{TL} \quad (\text{if } \log \text{TL} \leq 1.567),$$

and

$$\log \text{Weight} = -5.9028 + 3.574 \log \text{TL} \quad (\text{if } \log \text{TL} > 1.567).$$

As also shown in Fig. 3, this spline regression estimates a shift in the weight-length relationship at $\text{TL} = \log 1.567 = 36.9$, which corresponds to average maturity size. Therefore, the weight-length relationship increases its slope at maturity but there is no significant difference in condition between males and females.

4. Discussion

Many researchers have stressed the need to validate the methods used to determine age (e.g. Beamish, McFarlane 1983, 1987; Carlander 1974, 1987). One validation method is to use different skeletal elements for age determination and to compare the results. Scales are most frequently used for age determination because collection is relatively easy and not lethal. However, opercular bones are often preferred to scales for determining fish age because opercular annuli are easier to recognise. Jearld (1983) reported a statistical approach based on length frequency distribution as a validation method. Although bitterling age determination has been the subject of some studies (Holčik 1999), our study is the first where the age determination has been validated. In several studies of cyprinid age, small differences between opercular and scale reading have been noted (L’Abee-Lund 1985; Sinis et al. 1999), but in our study, the age determined from scales was the same as from the opercular bones. Moreover, the age population structure is the same as was revealed by the Bhattacharya method.

The age groups found for the Wieprz-Krzna canal population are consistent with those in Czech and Slovak populations described by Holčik (1960). On the other hand, the results differ greatly from the Elbe River population where
the oldest fish were 8+ (Wagler 1949, cited by Holčík 1999). Most studies have shown that bitterling commonly only live for two to three years and as there is little reason to suspect that the habitat of the Elbe River differs greatly from other rivers in Central Europe, it suggests that the age of that population might have been overestimated, but the ageing has to remain in doubt in the absence of validation.

Changes in scale shape with the size of bitterling were also shown by Holčík (1960), who also discussed the suitability of several methods for back-calculation. He found a non-linear body-scale relationship. In the Wieprz-Krznka population, linear regressions of fish length on scale radius were a good fit but scale growth may not have been proportional throughout the year.

In our study the mean total length for a particular age did not differ greatly according to whether age was derived from scales or from opercular bones. Growth rate of bitterling in the Wieprz-Krznka Canal was of the same order of magnitude as in Czech and Slovak waters (Holčík 1999).

In the Elbe River system the females were larger than the males (Bauch 1955), whereas Czech and Slovak waters showed the opposite (Holčík 1960). The slope of the weight-length relationship did not differ between the sexes in the Wieprz-Krznka population. Similar results were also noted by Holčík (1960) and Koščo (1988). Allometric growth was noted for several bitterling populations and only the Severka River bitterling seemed to grow isometrically (b = 2.952) (Holčík 1999). For that population the length-at-age was the smallest recorded to date.

Our data gave a good fit to a von Bertalanffy growth function but L1n1 was greater than expected, especially for males. In the Wieprz-Krznka bitterling population, asymptotic lengths calculated for each sex separately as well as for combined sex were much higher than those reported for other populations (Holčík 1999). The highest bitterling asymptote was noted in a typical bitterling habitat i.e. the Karasi Oxbow (Koščo 1988, Holčík 1999) as well as in a site out of its natural distribution i.e. the Karasi Oxbow (Koščo 1988, Holèík 1999) as well as in a site out of its natural distribution i.e. the Karasi Oxbow (Koščo 1988, Holèík 1999).

L1n1 is seen as a capacity for growth (Bagenal, Tesch 1978) and inter-population differences in this parameter correspond to varying environmental conditions. Variation in von Bertalanffy parameters reveals plasticity of fish growth (Wootton 1990). Thus the differences between our results and those cited by Holčík (1999) and Koutrakis et al. (2003) might also result from differences in latitude and associated differences in water temperature and productivity, as previously observed for other small European fish species (Mann et al. 1984). The Wieprz-Krznka population is the most northerly population examined to date. On the other hand, the von Bertalanffy function can be a result of resource allocation between growth and reproduction (Kozłowski 1996). Therefore, inter-sex or inter-population differences in L1n1 could also reflect differences in reproductive effort of the fish. This question would benefit from further study.

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5. References


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