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AGE AND GROWTH OF THE EUROPEAN BITTERLING, *RHODEUS AMARUS* (CYPRINIDAE, ACTINOPTERYGII), IN THE UDAY AND PEREVOD RIVERS (DNIPRO BASIN, UKRAINE)

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Age and Growth of the European Bitterling, *Rhodeus amarus* (Cyprinidae, Actinopterygii), in the Uday and Perevod Rivers (Dnipro Basin, Ukraine). Podobailo, A., Shukh, A., Kutsokon, Yu. — European bitterling age and growth were examined in 2017, 2018, and 2019 in the Uday and Perevod rivers in Pyriatynsky National Park (Poltava Region, Ukraine). The population was represented by five age groups (0+ to 4+); specimen age was determined by counting annuli on scales. The annuli were visible and often displayed as irregularities in circuli in the centrolateral part of the scales. Standard specimen length varied from 14 to 51 mm; weight varied from 0.09 to 2.5 g. The length-weight relationship was $W = 0.00005*SL^{2.78}$; $W♂ = 0.00048*SL^{2.18}$; $W♀ = 0.00028*SL^{2.32}$ for both sexes, males and females, respectively. The sex ratio was 1 : 1.26. The most abundant group in the samples from the Uday were young-of-the-years, and 1+ fish in the samples from the Perevod. As for mature specimens, 3+ fish predominated in both samples. The fastest relative growth was during the second year of life.
Key words: bitterling, scale, length-weight relationship.

Introduction

Bitterling is a fish of the Cyprinidae family, which is the only shell-dwelling species in the overall ichthyofauna of Ukraine, including the Uday River (Koshovyy & Podobaylo, 2017). Females lay eggs in the mantle cavity of bivalves (*Anodonta anatina* (L.) and *A. cygnea* (L.), *Unio pictorum* (L.), *U. tumidus* (Philipsson) (Reichard et al., 2006) and *U. crassus* (Philipsson) (Tatoj et al., 2017) using an ovipositor. The lifespan of bitterling is five years, but the majority doesn't survive past the first year (Kottelat and Freyhof, 2007). Previously, studies on bitterling growth were conducted in Poland (Przybylski and Garsia-Berthou, 2004), Greece (Koutrakis et al., 2003), and Turkey (Tarkan et al., 2005). The population consists of five age groups (the oldest age group is 4+). There is also data on the populations of this species in the Tym and Elbe rivers (Zhul'kov & Nikiforov, 1987; Holčík, 1960) where the maximum age was 6+ and 8+, respectively. Bitterling reach a length of 40 % or 50 % of their maximum length during their first year of life (Przybylski and Garsia-Berthou, 2004).

The European bitterling is found mainly in the additional system of rivers, floodplains, lakes, and ponds. It prefers habitats with slow-flowing or stagnant water, small depths, and sandy, rocky or muck bottoms. However, it can also occur in rapids (Przybylski, Ziêba, 2000; Reichard et al., 2002). Bitterling schools are usually found among macrophytes near the bottom and rarely go out into open water.

Bitterlings inhabit European freshwater basins of the Black and Caspian seas. In Ukraine, they can be found in all large rivers, except for Crimean waters (Movchan & Smirnov, 1983).

Bitterling is the most abundant species in the water bodies of Pyriatynsky National Park (Glotova et al., 2012; Koshovyy et al., 2018). It is listed in the Berne Convention on the Conservation of European Wildlife and Natural Habitats (1979) and is protected by Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora.

In 2016, Pyriatynsky National Park has become a part of the Emerald Network (UA0000077). Research of key species pecularities, including those of bitterling, is actively performed for preservation.

The aim of this study is to describe the age, growth, and population structure of bitterling in the protected water system of the Uday and Perevod rivers in Pyriatynsky National Park, in order to contribute to an understanding of the environmental and geographical variation on bitterling life history characteristics, and to fill the gaps in the management of this species.

Material and methods

Pyriatynsky National Park is a nature protection territory in the Pyriatyn District of Poltava Region, located in the valleys of Uday, Perevod, and Ruda rivers. Uday is a left bank second-order tributary of the Dnipro. It is 327 km long, with 62 km flowing through the territory of the national park. Perevod is the largest right bank tributary of the Uday. It is 68 km long, of which 27 km flow through the national park.

Sampling was done during daytime at two permanent hydrobiological monitoring stations (table 1) using a 6 m long beach seine with a 5 mm mesh.

The first station was located in Perevod near the village of Sasynivka close to a railway bridge (50°18.487 N, 32°26.032 E). The second station was located on a beach of the Uday in the village of Keibalivka (50°18.345 N, 32°30.093 E) (fig. 1).

Table 1. Investigated bitterling specimens from the Uday and Perevod rivers

River	Date of sampling	Immature	Males	Females	Total
Uday	1.08.18	73	20	17	110
	27.07.19	2	20	20	42
	total	75	40	37	152
Perevod River	22.07.17	0	15	7	22
	31.07.18	7	12	9	28
	26.07.19	53	5	4	62
	total	60	32	20	112

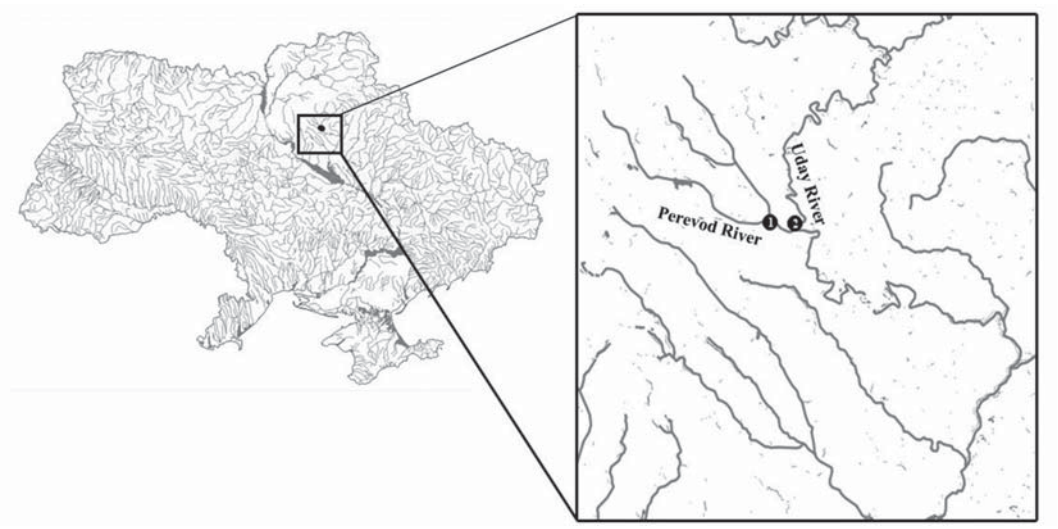

Fig. 1. Study locations: 1 — Sasynivka; 2 — Keibalivka.

The scales were prepared for age determination according to the classical method (Chugunova, 1959): ten scales from above the lateral line under the dorsal fin were taken from each fish; specimen standard length (SL) was measured with a sliding gauge to the nearest 0.1 mm; weight was determined to the nearest 0.01 g using electronic scales; sex was determined visually by the appearance of gonads and presence of the ovipositor in females if it presents

Slides with scales for microscopy were prepared in the laboratory. The scales were soaked in a diluted ammonia solution (3 %), and each scale was separated with needles. They were cleaned of epithelial residues and placed between two slides pressed tightly together. ID, weight, and length of each specimen were indicated on a label. Afterward, the slides were left to dry out (Chugunova, 1959) and later examined using a stereomicroscope МБС-10 and photographed using a digital camera SIGETA UCMOS05100KPA-U-NA-N-C-SQ-NA and a lens adapter for the microscope FMA050. The total radius of the scales and radii of the annuli were measured with an accuracy of 0.01 px using a ToupTek ToupView 3.7.2270. Measurements were performed from the scale centre to the outer edge of the annuli in the place with the largest diameter.

Growth was back calculated using Lea's method (Chugunova, 1959):

$$Sn/S = Ln/L,$$

where S is the total radius of the scale (px), Sn is the radius of the annuli n, L is total fish length (mm), and Bertalanffy's method (Ricker, 1975):

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

where Lt is fish length at age t (years), Linf, k, t0 are coefficients.

Growth rate was calculated using the formula of instantaneous growth rate (IGR) (Ricker, 1975). IGR was back-calculated according to Lea's method:

$$IGR = \lg L_{t-1} - \lg L_t,$$

where L_{t-1} is fish length corresponding to the length of the scales at the end of the last year of life, L_t is fish length corresponding to the length of the scales at the beginning of the last year of life.

Length-weight relationship (Ricker, 1975):

$$W = a \cdot SL^b,$$

where W is weight (g), SL — standart length (mm), a and b — coefficients.

The Shapiro-Wilk test was used to check the distribution for normality. Student's t-test, χ^2 test, ANOVA, ANCOVA were used for statistical processing of the obtained results. Student's test and ANOVA, ANCOVA were used to compare morphological features. The χ^2 test was used to assess the accuracy of the sex ratio (Lakin, 1990).

Statistical processing was performed in STATISTICA 7.

Results

The Sasynivka station was covered with dense beds of higher aquatic vegetation including *Lemna minor* L., *Spirodela polyrhiza* (L.), *Sagittaria sagittifolia* L., *Elodea canadensis* Michx. The river bottom at the sampling point was composed of gravel. The Keibalivka station was also covered with dense beds of higher aquatic vegetation represented by *Lemma minor, Spirodela polyrhiza, Ceratophyllum demersum* L., *Nuphar lutea* L. (Smith). The river bottom at the sampling site was sandy. The beach was a popular local recreation area. It was also actively grazed by geese.

The studied bitterling population in the Uday and Perevod rivers consisted of five age groups (0+ to 4+). A total of 264 specimens was investigated. Annuli were best seen on the back and side parts of the scales. The shape of the scales changed with age: in juvenile bitterlings it was more rounded, and acquired the shape of an ellipse as the fish grew (fig. 2). Due to these changes, the measured radius did not have a constant place but moved along the caudal part of the scales with age.

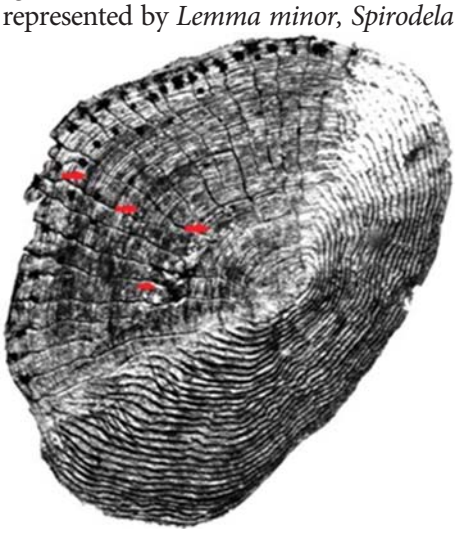

Fig. 2. Bitterling scales. The arrows show the annuli.

Standard length varied from 14 to 51 mm, weight from 0.09 to 2.5 g. Length-weight

Table 2. The average length (SL) of bitterling depending on sex

River	Immature			Males			Females		
	mean	SD	range	Mean	SD	range	Mean	SD	range
Uday	19.83	2.86	14–26	39.26	4.22	32–51	39.74	3.51	33–47
Perevod	24.56	2.53	19–29	38.73	4.65	30–48.5	38.1	4.78	29–47.2

relationship was $W = 0.00005*SL^{2.78}$; $W♂ = 0.00048*SL^{2.18}$; $W♀ = 0.00028*SL2^{.32}$ ($r^2 = 0.94$, $df = 262$, $t = -5.08$, $r^2 = 0.63$, $df = 70$, $t = -4.07$, $r^2 = 0.68$, $df = 55$, $t = -3.18$ for both sexes, males, and females, respectively).

The length-weight relationship is said to be isometric, there is cubic relationship between length and weight. Sex ratio in both samples was 57 females and 72 males or 1 : 1.26. No statistically significant deviation from the ratio 1: 1 was detected ($\chi^2 = 0.013$, $p = 0.05$).

ANCOVA showed no statistically significant difference between the length of males and females from different rivers ($F = 0.7$, $p = 0.05$, $F = 2.1$, $p = 0.05$ for males and females respectively). However, this difference was present in immature specimens: bitterlings from the Perevod were larger than specimens from the Uday ($F = 18.8$, $p = 0.05$) (table 2). This may be because the samples were taken in different years.

Annual ichthyofauna monitoring in the waters of the Pyriatynsky National Park, revealed that bitterling was the most common and abundant species in the Uday and its tributaries (Koshovyy et al., 2015). The share of bitterling in ichthyological samples varied from 18.8 % in 2016 to 73.9 % in 2015. Only in the catches of 2016 the relative number of bitterling was inferior to the *Rutilus rutilus* (L.). In 2016, there was an abrupt decline in the number of bitterling; almost no juvenile specimens were observed in samples. In 2017, a large number of juveniles appeared, and almost no age-1+ were observed in samples. The generation gap gradually narrowed in 2018–19 and almost disappeared in 2019 (fig. 3). -

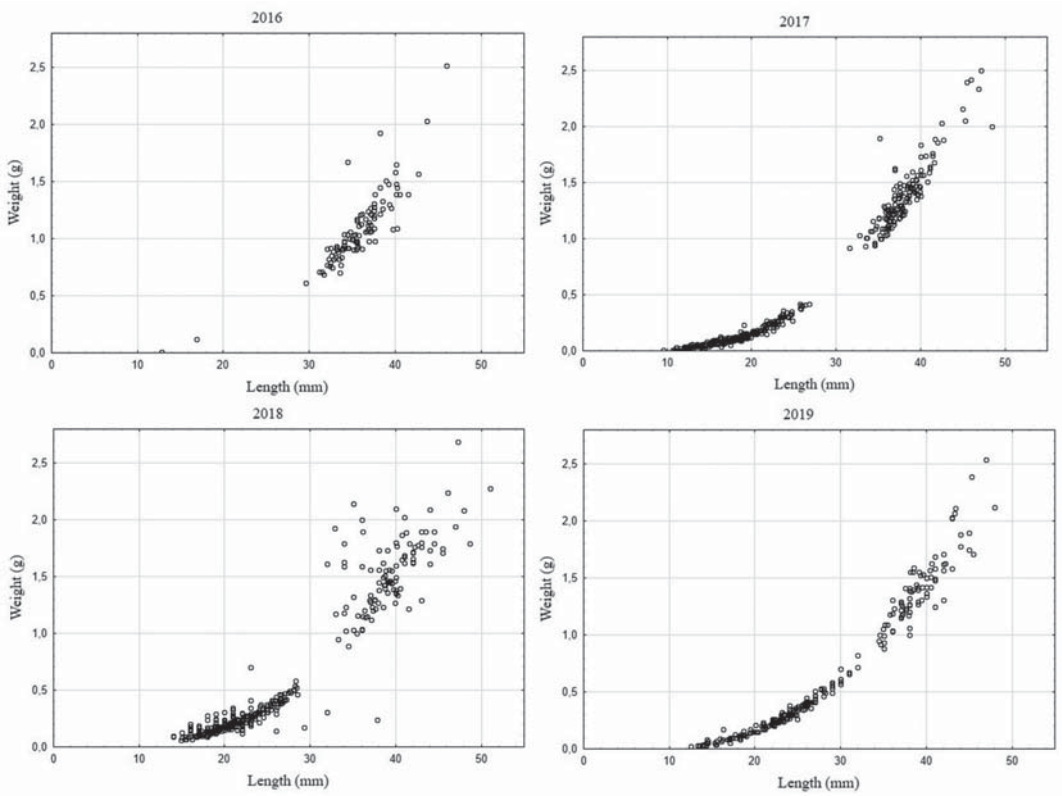

Fig. 3. Length weight ratio of bitterling in samples from the rivers Uday and Perevod in 2016–2019.

Table 3. Mean observed and back–calculated total lengths (mm) obtained from measurement of scales in rivers Uday and Perevod combined data

Age group	Standard length	Number of sp.	Length at the age of, mm			
			1+	2+	3+	4+
0+	20.16 ± 2.61	93				
1+	25.86 ± 2.01	42				
			Males			
2+	35.00 ± 3.04	25	16.07	34.02		
3+	39.33 ± 1.95	30	13.23	30.24	38.75	
4+	44.41 ± 2.93	17	14.65	32.13	40.16	43.94
			Females			
2+	34.87 ± 2.66	18	19.94	36.08		
3+	39.40 ± 1.64	24	16.14	31.80	39.40	
4+	43.98 ± 1.87	15	18.99	35.13	41.30	45.10

All lengths and age groups were represented in the specimens selected for the study because samples were collected during 2017–2019. The most represented group from the Uday samples was 0+, while 1+ dominated in samples from the Perevod. Among sexually mature specimens, 3+ predominated in both samples (table 3). The average specimen length in each age group was less than the back calculated length. However, empirical average lengths of males and females at age 3+ and 4+ did not differ from each other), but in the age 2+ females are larger than males (ANOVA post-hoc test, 6 groups, $p = 0.002$). The parameters of the von Bertalanffy growth equation showed further variation in growth rates between sexes (table 4). The difference between sexes can be explained by using the largest fish in group as asymptotic SL. The growth rate of bitterling slowed down with age (table 5). During the second year of life, the relative length gain of fish was highest for both sexes. During the second and third years of life, bitterling from the Perevod and Uday rivers grew at the same rate, but during the fourth year of life, both males and females from the Uday grew slower ($p = 0.0027$ for the relative length gain and $p = 0.0024$ for instantaneous growth rate).

Ranking of fish by weight and length depending on age was performed for each age group. The minimum and maximum weights and lengths were determined for each age; for immature

Table 4. Estimates of L_{inf}, k and t_0 of female and male bitterling in the Uday and Perevod rivers

Parameters	Females	Males	Sex combined
n	57	72	129
L_{inf}	48.5	51	51
k	0.636	0.439	0.449
t_0	1.01	0.58	0.54

Table 5. Relative length gains and instantaneous growth rate of bitterling in the Uday and Perevod rivers

Parameters	Ages		
	1–2	2–3	3–4
	Uday River		
Relative length gain, %	33.71 ± 6.40	24.48 ± 4.82	16.85 ± 4.52
Instantaneous growth rate	0.18 ± 0.04	0.12 ± 0.03	0.08 ± 0.02
	Perevod River		
Relative length gain, %	33.52 ± 5.55	25.43 ± 4.93	22.05 ± 4.38
Instantaneous growth rate	0.18 ± 0.4	0.13 ± 0.03	0.11 ± 0.02

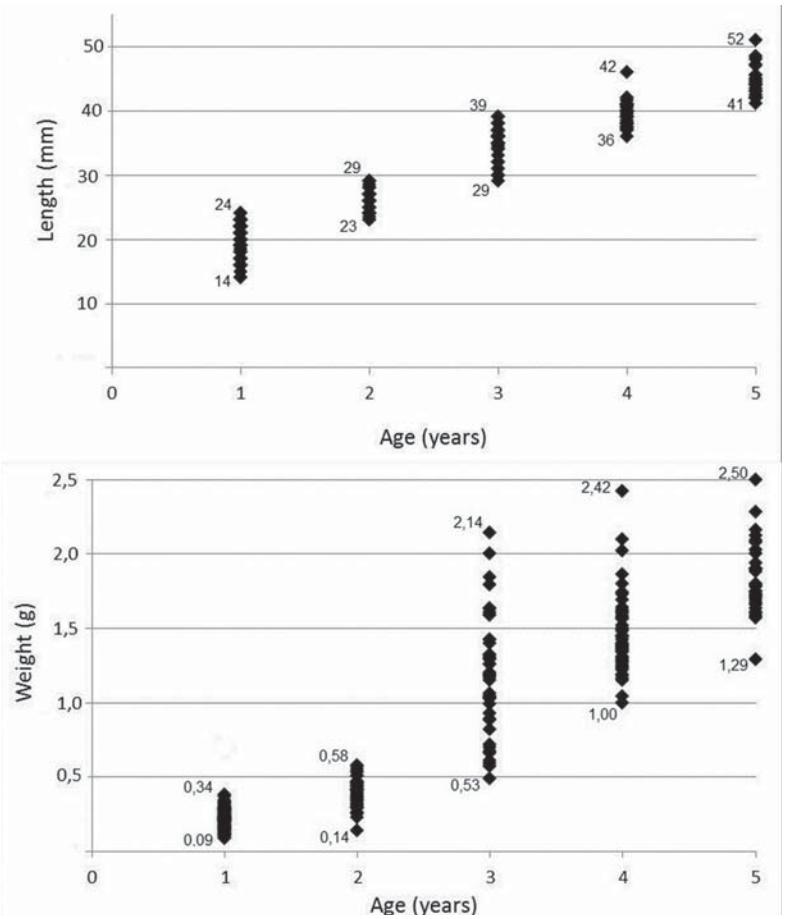

Fig. 4. Length and weight of bitterling depending on age.

0+ and 1+ fish, the lengths almost did not overlap, while weights overlapped almost completely. When bitterlings became mature (age-1+), their growth rate accelerated as well as weight gain, so a clear boundary of 30 mm between 1+ and 2+ was observed for both sexes (fig. 4).

The linear growth of mature bitterling was quite slow. Fish length ranges partially overlapped. In contrast to linear growth, bitterling weight varied greatly within one age group, so it is impossible to determine the age group of fish belongs according to its weight.

Discussion

Determination of bitterling age has been the subject of several studies (Holčík, 1999; Tarkan, 2005; Koutrakis, 2003), but only Przybylski (2004) confirmed the age determined by scale reading using operculum. Annual rings on the operculum were clearly visible, and the number of annual rings on scales and operculum coincided. Determination of bitterling age using operculum is as accurate as using scales.

Bitterlings from the Uday and Perevod rivers were characterized by a short life cycle. We found five age groups (the oldest was 4+). This coincided with the general pattern observed in other studies (Holčík, 1960; Koutrakis, 2003; Przybylski and Garsia-Berthou, 2004; Tarkan, 2005). On the other hand, the results were very different from the results from the Elbe River (Zhul'kov & Nikiforov, 1987; Wagler, 1949 cited by Holčík, 1999), where the oldest age group was group 8+.

Smith et al. (2000) determined that the sex ratio in the bitterling population in oxbow lakes in the Czech Republic was always 1 : 1.

In the Rihios River (Greece) (Koutrakis, 2003), males outnumbered females. The sex ratios may differ in different populations and in different years.

The reasons behind this variation may be different and numerous: species differences, fish body length, seasonal aspects, feeding and reproductive periods, different growth rates in males and females, earlier maturation of one of the sexes, overall mortality and accidental (due to fishing technique) mortality difference for each sex and perhaps environmental conditions (Peczalska, 1968).

In the Uday river the sex ratio is 1 : 1.08 and in the Perevod river the sex ratio is 1 : 1.6, with the number of males dominating over females but near to 1 : 1 for both rivers. In the population of Lake Ömerli reservoir in Istanbul (Tarkan, 2005), on the contrary, females dominate: the sex ratio is 1 : 2.32.

In bitterling, as in other cyprinids, there is some sexual dimorphism in average length of males and females (Holčík, 1960), but it was absent in the Uday and Perevod rivers. As suggested by Reichard & Jurajda (1999), Tarkan (2005) stated that bitterling reached sexual maturity in both sexes at age-1+ at a length of 30–35 mm. We found that maturity in bitterling occurred at a length of about 30 mm in the third year of life. All specimen with a length of 30 mm (2+) were sexually mature. Among specimens shorter than 30 mm long, there was only one female of 29 mm long (2+), the others were immature 0+ and 1+.

Probably such a difference was because fish in the Uday and Perevod rivers reached a shorter length than in other water bodies. Thus, the maximum specimen length in both the Uday and the Perevod was 51 mm, and the average length ± SD was 37.1 ± 5.9 mm. These values are much lower than those given for fish from other water bodies of Ukraine (e. g., average length for the Dnipro basin was 52.4 ± 0.1 mm, for the Danube basin — 52 ± 0.2 mm (Movchan, 1983)), and than values obtained in other studies.

This may be due to conditions in the Uday and the Perevod. These rivers have a more northern location compared to other studied water. Thus, later maturity was most likely associated with smaller bitterlings.

We noted fluctuations in the number of bitterlings in the catches during 2014–2019. During the summer of 2016, there was an extremely unfavorable oxygen regime in the Uday (Koshovyy & Podobaylo, 2017) that probably led to mass death of the young-of-the-year, which were absent in the catches in 2016.

Bitterling is a slow-growing species. During the first year of life, fish reach only 40 % of their maximum possible length. Similar data was found on bitterling from the Ömerli (Tarkan, 2005). Other studies (reviewed by Holčík 1999; Koutrakis, 2003) indicate that bitterling reached 50 % or more of their maximum length during the first year of life. Although the Wieprz-Krzna population (Przybylski and Garsia-Berthou, 2004) is the northernmost of the studied bitterling populations, fish from there are larger than from the more southern Uday. This difference may be due to the geographical location and related differences in water temperature and food supply, as has been studied for other small fish (Mann et al., 1984).

In addition, we determined the limits of body length of different age groups in the population of bitterling in the Uday and Perevod rivers, which was not done in previous studies. With this, we can approximately determine the age of bitterling according to their length. It is quite difficult to distinguish between the young-of-the-year and age-1+ fish based only on their lengths. This is possible for mature specimen. According to the graph (fig. 4), there is a clear boundary between age-1+ and age-2+ fish, which was 30 mm. Older age groups can be distinguished roughly: most likely, a fish of 30–35 mm long is 2+, while bitterling longer than 40–42 mm is more likely to be 4+. It is most difficult to distinguish age-2+ and age-3+ fish based on length, because their lengths overlap the most.

Bitterling is the most abundant of 32 fish species in the Pyriatynsky National Park (Koshovyy et al., 2018). Our study demonstrated that entire generations could have extremely low abundances under adverse environmental conditions. In such cases, the population age structure can be deformed. The impact of such events remains noticeable for several years and

can pose a danger to short-cycle fish species. Therefore, monitoring of the European bitterling population in Pyriatynsky as an Emerald network object should be carried out continuously.

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