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Endangered weatherfish (*Misgurnus fossilis*) age and growth is affected by the size of the watercourses

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Abstract. The age and growth of weatherfish (*Misgurnus fossilis*), an endangered and protected freshwater fish with a poorly known life history, was studied in two watercourses (the River Ner and the Nowy Rów canal, Poland). The weight, length and sagittal otoliths of 166 specimens collected in April 2015 were measured for weight-length relationships, ageing and back-calculation of length at age. At both sites sex ratio did not differ from 1:1. Weatherfish otoliths were small, elliptic (1.85 mm longer axes of the largest otolith) and the annuli were clearly visible. Female lifespan was six years but the oldest males were four and five years. In both sites populations were dominated by 2+ (the River Ner) and 3+ (the Nowy Rów canal) specimens. In general, weatherfish grows isometrically ($b = 3$) and the intercept of the weight-length relationship differ between study sites but not between sexes. Its total length (TL) was predicted by an interaction between sex and age, as well as capture site and age. Back-calculated estimates of TL fitted a von Bertalanffy growth function, though Taylor's criterion showed that the asymptotic length were overestimated. Multiple comparisons of the von Bertalanffy growth function parameter revealed difference between sexes and sites.

Key words: otolith, von Bertalanffy growth function, weight-length relationship, GLMM, mudloach, threatened cobitid

Introduction

Body size is the most important attribute of an organism, imposing limitations on many features of structure and function. In teleost fishes, body size is a key characteristic (Moyle & Cech 2000) and many life history traits, such as mortality rate and reproductive success, show strong correlations with this parameter (Wootton 1992, Froese 2005). Understanding growth, in consequence, is a crucial step in describing the selective forces that shape teleost life-history evolution (Wootton 1998).

Somatic growth is the product of the input and output of energy resources (Weatherley & Gill

1987). In fish, in contrast to poikilotherms, growth is indeterminate and shows enormous plasticity in response to the environment (Wootton 1998). Although the biology of most of European fishes is well characterised and described (for summary see: Kottelat & Freyhof 2007) some features of the life-history of widely distributed fish are still poorly understood. One such species is the weatherfish (*Misgurnus fossilis*) (L. 1758), the largest freshwater cobitid, naturally distributed through Central and Eastern Europe (Kottelat & Freyhof 2007). Weatherfish inhabit warm, shallow waterbodies with substrates covered with a thick layer of organic matter, often overgrown by dense vegetation. Typical habitats for weatherfish are

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slow-moving rivers, canals and drainage ditches, oxbow lakes and fishponds (Meyer & Hinrichs 2000, Pekárik et al. 2008, Mazurkiewicz 2012). This bottom-dwelling species burrows into soft substrates during dry periods or frosts (Boroń et al. 2002, Kottelat & Freyhof 2007).

In many European countries the weatherfish is classed as endangered due to habitat loss. As a consequence it was listed in European Fauna-Flora-Habitat and Natura 2000 directives (Annex II of the Council Directive 92/43/EEC), representing a species of European Community interest (European Union 1992). It is also included in many national red lists of endangered and protected fish species (Drozd et al. 2009, Hartvich et al. 2010). In Europe the weatherfish has been classified as a species of low concern (LC) (Freyhof & Brooks 2011), but its genetic diversity is the lowest reported for any European freshwater fish (Bohlen et al. 2007). The weatherfish is also regarded as a species at high risk from pesticides (Ibrahim et al. 2013), and proposals have been made to raise its level of threat. Despite these concerns, basic information on weatherfish life-history traits, essential for its conservation, are lacking (Boroń et al. 2002, Kottelat & Freyhof 2007). The aim of present study was to address this shortfall in information and to provide detailed information on the age structure, growth pattern and weight-length relationships of weatherfish from two watercourses in Central and Western Poland. This information is intended to inform decision-makers responsible for conservation actions that might affect the species, such as desilting, and will provide life-history data for the species at the centre of its European range as a baseline for further research.

Material and Methods

A total of 166 weatherfish were collected in April 2015 by electrofishing (EFGI 650, BSE Bretschneider Specialelektronik, Germany) from two watercourses: medium-sized lowland River Ner and a small drainage ditch the Nowy Rów. The River Ner (52°08'83.76" N, 18°87'70.17" E) has a straight, regulated and wide (20-25 m) corridor with steep banks. Current velocity was 10 m³ s⁻¹ and water depth varied between 0.5 and 1.0 m. The substrate was dominated by fine sand with submerged vegetation covering less than 1%. The Nowy Rów (51°12'38.29" N, 16°43'17.34" E) is a drainage canal with a uniform width (2-3 m) and steep banks. Current velocity was < 0.1 m³ s⁻¹ while water depth varied between 0.5 and 1.0 m. The

entire length of the watercourse was overgrown with submerged and emergent vegetation, covering approximately 70% of the water surface. The bottom substrate was dominated by sand covered with a thick layer of organic matter (> 0.5 m). Both sites are located in Natura 2000 protected areas in the Bzura-Ner glacial valley (PLH100006) and Łęgi Odrzańskie (PLB020008), respectively. A total of 84 specimens were caught in the River Ner and 82 from Nowy Rów. In both cases the local weatherfish populations were sufficiently large to permit fish collection without posing any conservation threat. In contrast to the study sites, weatherfish typically occur at low densities (Pyrzanowski et al. 2015). Samples were obtained from short canal stretches. The presence of weatherfish at high dominance in the entire River Ner drainage, despite low water quality, was demonstrated in previous studies (Kostrzewa & Penczak 2002, Penczak et al. 2010).

After capture, fish were immediately euthanized with an overdose of clove oil (Javahery et al. 2012). They were subsequently frozen, stored and thawed before processing. In the laboratory all specimens were measured for total length (TL) and standard length (SL) to the nearest 1 mm and weighted (W) to the nearest 0.1 g. For analysis TL was used and was related to SL by the function: $TL = 2.23 + 1.125 SL$, $r^2 = 0.998$; $n = 166$ (major reduced axis, Smith 2009). After measurement, fish were dissected and sex was determined by inspection of the gonads. For each sampling site the sex ratio was tested for deviations from parity using a binomial test (Wilson & Hardy 2002). The χ^2 goodness of fit test was used to determine whether size frequency distributions differed significantly between sexes and sites following subdivided contingency table (i.e. a partitioned χ^2 analysis) (Zar 2010).

From each specimen the sagittal otoliths were extracted from the vestibular apparatus (cranial cavity), cleaned, stored dry and used for age determination. One otolith from each individual was attached to a slide using cyanoacrylate glue, then polished using emery paper with grit sizes of 30 μ m and 3 μ m. Prepared otoliths were immersed in glycerine and photographed under a stereomicroscope with transmitted and reflected light (Nikon SMZ1000, Japan). Blind readings of age (annuli) was conducted by three independent experimenters on images of otoliths.

In the event of a disagreement between experimenters over annuli counts, the disputed

otoliths were later re-examined by all experimenters until a consensus was reached. The age of a specimen (the total number of true annuli, taking the first winter ring as a reference) was determined when two or more readings agreed. Otoliths with ambiguous age readings (less than 5%) were discarded. Following ageing, the otoliths were measured to allow lengths at age to be derived by back-calculation based on the assumption of a constant proportional deviation from mean body size (Francis 1990). To do this the distance from the nucleus to the outside edge (total radius – OR) and to each annuli were measured along the longest axis of the elliptic otolith using LUCIA 4.61. Back-calculations were made by substituting otolith radii of respective annuli to a linear regression of fish total length (TL) on total radius (OR), with a different function fitted for each sex and site. Back-calculated length-at-age for each specimen was adjusted by proportion between observed and estimated length (Francis 1990). Individual age-at-length and back-calculated length data were used to construct length-age keys for each sex and site.

Fish size was modelled as a function of sex, age and sampling site. Because multiple size-at-age data were available for each individual weatherfish, estimated from length-age keys, data were modelled using a general linear mixed model (GLMM) with fish sex (*fSex*), age (*age*) and capture site (*fSite*) as covariates (Zuur et al. 2009). A random intercept for fish identity was included in the model to introduce a correlation structure between length estimates for the same individual. Assuming estimates of fish length (*TL*) for fish *i* measured on sampling occasion *j* followed a Gaussian distribution with mean μ_{ij} the model was specified as:

$$TL_{ij} = N(\mu_{ij}, \sigma^2)$$

$$E(TL_{ij}) = \mu_{ij} \quad \text{and} \quad \text{var}(TL_{ij}) = \sigma^2$$

$$\mu_{ij} = \text{Intercept} + fSex_{ij} \times age_{ij} + fSite_{ij} \times age_{ij} + a_j$$

$$a_j \sim N(0, \sigma^2_{\text{sample}})$$

The model was fitted using R (version 3.5.2; R Development Core Team 2018). To describe the growth of weatherfish over its lifespan we used the von Bertalanffy growth function (VBGF) defined as (von Bertalanffy 1957, Ricker 1975):

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

where L_t is total length (TL in mm), L_{inf} is the asymptotic standard length (mm), k is the rate at which the asymptotic length is approached, t_0 is the origin of the growth curve and t is age (in years). To judge the accuracy of the VBGR we used Taylor's criterion (Taylor 1962), which states that the asymptotic length is satisfactorily estimated when the maximum observed length represents approximately 95% of L_{inf} .

Because the parameters L_{inf} and k are inversely correlated (Moreau et al. 1985) the index of growth performance ϕ' (Munro & Pauly 1983) was calculated as:

$$\phi' = \log_{10}(k) + 2\log_{10}(L_{inf})$$

Based on the back-calculated total length-at-age, parameters of the VBGF and their standard errors for each sample, split by sex and site, were estimated by non-linear regression implemented in FiSAT (Gayaniilo et al. 1994). Multiple comparisons of the VBGF parameters between sexes and water bodies were made using t-tests with sequential Bonferroni correction. To assess the pattern of

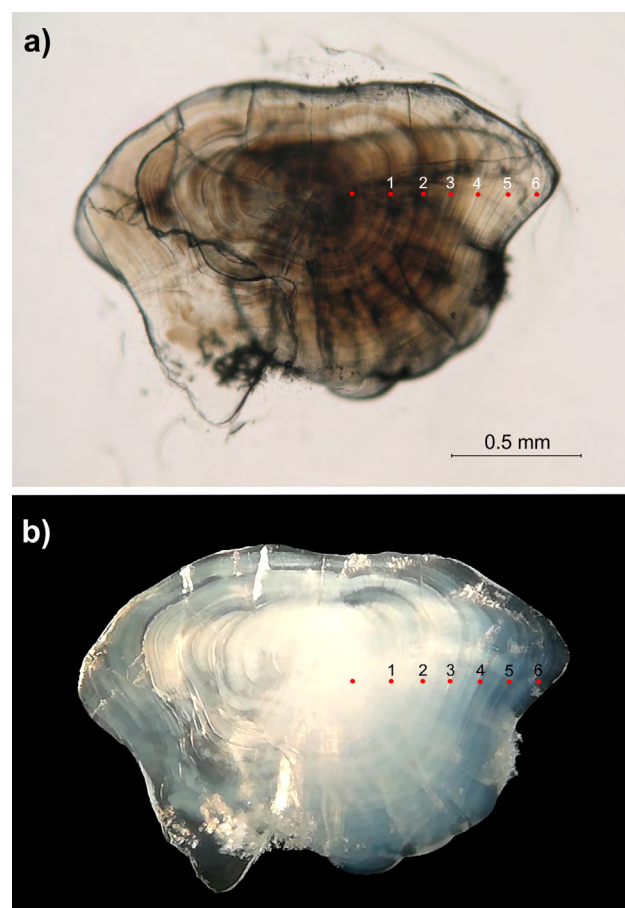


Fig. 1. Otolith of weatherfish in transient light (a) and in reflected light (b).



growth; either isometric or allometric (Ricker 1975), the weight-length relationship (Le Cren 1951) was determined by linear regression (log-transformed data). The slopes (b-values) for sexes and sites were tested against isometry; i.e. $b = 3$, with Bailey's t-test. Differences between regressions were also estimated using analysis of covariance (ANCOVA) (Zar 2010).

Somatic condition factor (CF) was calculated using Fulton's coefficient (Le Cren 1951):

$$CF = 100 (W/TL^3)$$

where W is weight in g, and TL is total length in cm. Two-way ANOVA was used to test differences in CF between sexes and sites (Zar 2010). Diagnostic plots of residuals against fitted values and a QQ plot of residuals were used to assess the normality of residuals. Homoscedasticity was tested using Levene's test. If ANOVA showed a significant effect

of any factor, the Honestly Significant Difference (HSD) Tukey post-hoc test was used to identify which factors differed. Analyses were performed using STATISTICA 10 (StatSoft 2011).

Results

Sample size, fish length, weight and Fulton's condition index are presented in Table 1. In both sites, the sex ratio expressed as the proportion of males in the sample (f_M) did not differ from parity; $f_M = 0.476$ in the River Ner ($p = 0.375$) and $f_M = 0.451$ in the Nowy Rów canal ($p = 0.220$). Length-frequency differed significantly between sites and sexes (partitioned $\chi^2 = 57.83$, $df = 12$, $p < 0.001$). Two-way ANOVA revealed that the Fulton coefficient differed significantly between sites ($F_{1,162} = 9.39$, $p = 0.003$), but not between sexes ($F_{1,162} = 3.82$, $p = 0.052$), with no significant interaction between sex and site ($F_{1,162} = 0.429$, $p = 0.513$). A post-hoc HSD Tukey test showed that only females from the River Ner (F_{Ner})

Table 1. Summary of sample size (n), total length (TL), body weight and Fulton's condition factor (CF) of weatherfish split by site of collection (site) and sex (sex). The same letters in superscripts denote groups that did not statistically differ (HSD Tukey post-hoc test).

site		River Ner		Nowy Rów canal	
sex		F	M	F	M
total length (mm)	n	44	40	45	37
	mean	143.5	129.7	144.2	143.4
	sd	38.2	25.5	29.1	21.9
	min	90	96	114	110
weight (g)	max	233	173	247	200
	mean	12.51	8.86	13.78	12.94
	sd	9.73	4.94	13.69	6.51
	min	2.75	3.06	6.12	5.51
CF	max	42.93	18.52	65.13	28.95
	mean	3.51 ^a	3.70 ^{ab}	3.86 ^{ab}	4.24 ^b
	sd	0.50	0.62	0.49	1.71

Table 2. Length-weight regression parameters and their standard errors (SE) (\log_{10} -transformed data) for weatherfish collected from the River Ner and Nowy Rów canal.

site	sex	a	SE a	b	SE b	r ²	n	p
River Ner	Females (A)	-5.402	0.172	2.974	0.080	0.970	44	< 0.0001
	Males (B)	-5.236	0.276	2.904	0.131	0.928	40	< 0.0001
Nowy Rów canal	Females (C)	-6.008	0.215	3.275	0.100	0.961	45	< 0.0001
	Males (D)	-5.194	0.319	2.902	0.148	0.919	36	< 0.0001
slope comparisons				$F_{3,157} = 2.1697$		$p = 0.119$		
common regression slope (b _c)				3.031	0.055			
intercept comparisons				$F_{3,163} = 5.0436$		$p = 0.003$		
Tukey's post hoc test				<u>D C</u> <u>B A</u>				



Table 3. Back-calculated length-age keys for female and male weatherfish from the River Ner and the Nowy Rów canal.

TL (mm)	River Ner								Nowy Rów													
	Females				Males				Females				Males									
	1	2	3	4	5	6	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	
> 50	4																1					
51-60	13																19					
61-70	13	8					3				3						15	7				
71-80	7	6					14				26						24					
81-90	4	7					4	12			7	15					6	17				
91-100		3					4	5			3	13					1	13				
101-110		5	2				9	4			2	2	1						4	1		
111-120		4	1				2	7	1			3						1	4			
121-130		7	3	1				9	1			2	1							2		
131-140			6	2				5	1				2							1	2	
141-150		1	5	6					11				1	1								1
151-160				3					2	5			1	3								
161-170				3						1						1						
171-180			1		3												1					
181-190				1												1						
191-200						1																
201-210					1																	
211-220						1																

Table 4. Summary of Gaussian generalized linear mixed model (GLMM) for modelling weatherfish total length (TL) as a function of sex (*fSex*), age (*age*) and capture site (*fSite*) as main effects with fish identity included as a random term in the model.

Model parameter	Estimate	SE	<i>p</i>
Intercept	53.09	1.94	< 0.001
<i>fSex</i> _(male)	1.50	2.28	0.511
<i>age</i>	21.96	0.36	< 0.001
<i>fSite</i> _(NowyRow)	-10.00	2.28	< 0.001
<i>fSex</i> _(male) × <i>age</i>	-3.59	0.43	< 0.001
<i>fSite</i> _(NowyRow) × <i>age</i>	-3.53	0.43	< 0.001

had a lower condition than males from the Nowy Rów canal (M_{canal}) while other groups did not differ significantly (Table 1).

The relationship between TL and W for each site and sex are presented in Table 2. The slope (*b*) coefficients indicated isometric growth for females in the River Ner ($b = 2.97$, $t = 0.33$, $df = 42$, $p = 0.375$) and for males from both: the River Ner ($b = 2.90$, $t = 0.73$, $df = 38$, $p = 0.301$) and the Nowy Rów ($b = 2.90$, $t = 0.66$, $df = 34$, $p = 0.317$). However, females in the Nowy Rów ($b = 3.28$) had a regression coefficient significantly greater than $b = 3$ ($t = 2.75$,

$df = 43$, $p < 0.001$). ANCOVA demonstrated a lack of differences in slopes between sites (Table 2). Estimated intercepts for the regression between TL and W did not differ between males and females but there was a significant difference between sites (Table 2).

Of 166 otoliths that were examined, 157 (95.1%) were readable, with 80 collected from fish from the River Ner and 77 from Nowy Rów used to age weatherfish. Otoliths of weatherfish are small and have an irregular elliptical shape with numerous insets. The largest otolith measured 1.85 mm (long axis) and 0.93 mm (short axis). They are characterised by high transparency with annuli unambiguous and with no false annuli. Difficulties in determining the most representative radius were associated with accurately measuring distances between individual annuli (Fig. 1). In the case of larger and older individuals, annual rings at the central region of the otolith were more visible in transient light (Fig. 1a) while growth checks at the margins were more pronounced in reflected light (Fig. 1b).

Determination of age based on otoliths for weatherfish from the River Ner indicated five age classes (2+ to 6+) for females, and four (2+ to

Table 5. Estimation of von Bertalanffy growth function (VBGF) parameters and their asymptotic standard errors (SE) for weatherfish in the River Ner and the Nowy Rów canal.

	Female				Male			
	River Ner		Nowy Rów canal		River Ner		Nowy Rów canal	
	SE	SE	SE	SE	SE	SE	SE	
L_{inf}	304.5	178.3	264.0	147.7	196.5	233.0	207.0	124.9
k	0.150	0.157	0.143	0.138	0.203	0.485	0.162	0.178
t_0	-0.917	0.888	-0.801	0.745	-1.216	2.183	-1.102	0.939
ϕ'	4.142	0.821	4.000	0.691	3.893	1.471	3.841	0.199
r^2	0.836		0.866		0.784		0.812	
ad r^2	0.805		0.841		0.654		0.807	
n	116		136		100		177	
max TL_{obs}	223		230		168		200.5	
lifespan	6		6		4		5	
Taylor's (%)	73.2		87.1		85.5		96.9	

Taylor's (%) – the criterion for the fit of the von Bertalanffy growth function (VBGF) to the data; i.e. the maximum observed length (TL_{obs}) as the percentage of asymptotic length (L_{inf}).

5+) for males. Similarly, in the Nowy Rów canal population, females comprised five age classes (2+ to 6+), while males were represented by three (2+ to 4+). At both sites fish younger than 2+ were not observed. Thus weatherfish appeared to differ in longevity in relation to sex and sites; in the River Ner the oldest females were 6+ years and males were 5+, while in the Nowy Rów, the oldest females were 6+ and males 4+ years.

The relationship between otolith radius (OR) and fish length (TL) (data pooled for females and males; separately for both capture sites) were: River Ner, $TL = 1.60 + 279.15 \times OR$ ($r^2 = 0.42$; $n = 80$); Nowy Rów $TL = 40.05 + 180.81 \times OR$ ($r^2 = 0.71$; $n = 77$). These functions were used for back-calculation of total lengths-at-age (Table 3). Weatherfish total length was predicted by an interaction between sex and age, as well as capture site and age (Table 4). Females tended to be larger than males at a given age, irrespective of site of collection, while fish from the River Ner tended to be larger than those from Nowy Rów canal, irrespective of sex.

Back-calculated TL showed a good fit to the von Bertalanffy growth model (Table 5). However, according to Taylor's criterion the von Bertalanffy growth function can be considered to describe growth well if the maximum observed length is approximately 95% of L_{inf} . In the study populations the maximum observed lengths varied from 73 to

97% of L_{inf} (Table 5), suggesting that asymptotic lengths were overestimated. Only males sampled in the Nowy Rów canal met Taylor's criterion (Table 5). Multiple comparisons of the von Bertalanffy growth function parameter presented in Table 4 revealed differences between sexes and sites. Female asymptotic length in the River Ner was higher than for females from the Nowy Rów canal ($t = 2.24$, $df = 248$, $p = 0.033$) and differed significantly from that for males from both sites (River Ner: $t = 5.60$, $df = 231$, $p < 0.001$; Nowy Rów, $t = 5.40$, $df = 214$, $p < 0.001$). However there were no differences in this parameter for males between sites ($t = 0.56$, $df = 215$, $p = 0.339$). Females from the River Ner and Nowy Rów differed in asymptotic length from males originating from both sites: females from the River Ner and males from the River Ner ($t = 5.60$, $df = 232$, $p < 0.001$); females from the River Ner and males from Nowy Rów ($t = 5.39$, $df = 215$, $p < 0.001$); females from the Nowy Rów and males from the Nowy Rów ($t = 3.50$, $p < 0.001$); females from the Nowy Rów and males from the River Ner ($t = 3.45$, $df = 251$, $p = 0.001$).

Discussion

The weatherfish is the largest loach species in Europe (Kottelat & Freyhof 2007). This fish has been reported to grow to a TL of 300 mm (Muus & Dahlström 1968), but with variation in its maximum observed size from 265 mm (SL) (Oliva



& Chittravadivelu 1973) to 350 mm (TL) (Berg 1949). Kottelat & Freyhof (2007) state that the maximum length (TL) is 270 mm. The largest weatherfish individual from the Rivers Odra and Vistula had a maximum of only 235 mm (SL) (Kotusz 1996). Weatherfish maximum body size is sexually dimorphic, with females expressing a larger size-at-age than males (Boroń 2000, Boroń et al. 2002, Kottelat & Freyhof 2007). In a drainage canal in the lower Havel flats, Saxony-Anhalt (Germany) the largest females were 279 mm (TL), while males only achieved a maximum size of 220 mm (Meyer & Hinrichs 2000). Weatherfish collected by Drozd et al. (2009) in April 2007 from a floodplain area of the River Lužnice (Czech Republic) were even smaller; the largest female measured 241 mm (SL) and males 190 mm (SL). Thus fish examined in the present study were of the same size range as those from other European water bodies. The largest female did not exceed 250 mm (TL), while the largest male measured only 200 mm (TL), both from the Nowy Rów canal. A strong correlation between female fecundity and body size is often cited as the reason for larger female body size in teleosts (e.g. Wootton 1998, Froese 2005). This pattern is often observed in small fishes, and is also a feature of other cobitid species; e.g. *Cobitis paludica* (Przybylski & Valladolid 2000), *Cobitis elongatoides* (Erös 2000), *Cobitis narentana* (Schneider et al. 2000, Zanella et al. 2003), *Cobitis taenia* (Slavík & Ráb 1995, Kostrzewa et al. 2003) and *Cobitis calderoni* (Valladolid & Przybylski 2008).

Variability in traits associated with fish body length (e.g. maximal length at maximal age) may be explained by latitude, which has proven reliable in explaining size differences in multiple European freshwater stream fishes (Blanck & Lamouroux 2007). However, for populations in close proximity; such as the River Ner and Nowy Rów canal, observed differences in life-history traits more likely result from local scale among-habitat variability in abiotic and biotic factors. Large rivers may secure long-time persistence of local fish populations, and consequently positively influence their growth rate, owing to lower susceptibility to changes in abiotic conditions and higher diversity of microhabitats available for particular fish species and their prey. However, this outcome may not be the case for small, human-maintained habitats, like Nowy Rów, where not only the changes in abiotic factors (e.g. water level depletion, drop in water oxygenation, rise of water temperature) are more severe, but also higher conspecific densities may

lead to depensatory growth, resulting in an increase in relative fish size differences, thereby influencing fish survival and fecundity (Wootton 1998).

The only published data on weight-length relationships for weatherfish come from Belgium (Verreycken et al. 2011). Although data from Belgium did not include sex and habitat variation, the average non-linearized regression parameters and their 95% CI (Verreycken et al. 2011) can be compared with our pooled data (Table 2). The slope of the regression for the Belgian population differs slightly from that for Polish populations, but still indicates an isometric weight-length relationship. The intercept of the regression for Polish populations (-2.68) is higher than that for data from Belgium (-5.50). This difference in the scaling of the relationship probably arises from the small Belgian dataset ($N = 24$) with a narrow TL range from 126 to 233 mm (Verreycken et al. 2011). The slope of the weight-length relationship is frequently used as a measure of fish condition (Le Cren 1951, Froese 2006), especially when Fulton's coefficient is positively skewed and correlates with fish size (Bolger & Connolly 1989). In this situation, body form and condition are consistent for small and large individuals, and is evident for weatherfish from Polish and Belgian populations.

Weatherfish from the Nowy Rów drainage canal, tended to be heavier at a given length than those from the River Ner, and had a slightly higher condition factor. The population density of weatherfish in the Nowy Rów canal was over 26 times that in the River Ner (2.1 vs. 0.08 indiv. m^{-2} , respectively). While body condition is often negatively density dependent, high population density can also indicate high habitat quality (Van Horne 1983, Wilson et al. 2015). This finding implies that drainage canals might provide a favourable habitat for weatherfish, in this case supporting a population density exceeding one indiv. m^{-2} (Meyer & Hinrichs 2000, Pyrzanowski et al. 2015). Until recently, a high density of weatherfish was also observed in the River Ner, but along with an improvement in water quality and gradual increase in other fish species, potentially leading to an increase in interspecific competition, a decrease in the number of the weatherfish has been observed (Kostrzewa & Penczak 2002, Penczak et al. 2010). The weatherfish often inhabits drainage channel systems that are under high agricultural influence (Stoate et al. 2009) and the fish is known to be highly dependent on sediment quality due to its benthic lifestyle (Meyer & Hinrichs 2000, Kottelat & Freyhof



2007). The weatherfish is also an opportunistic feeder and when the food base is scarce, can switch to a diet comprising large quantities of detritus (Pyrzanowski et al. 2019). Due to high flow variability in the River Ner, detritus deposits are eluted from the main river corridor and limited to a narrow bank zone. In contrast, in the Nowy Rów canal organic matter is in large quantities albeit frequently associated with low dissolved oxygen concentrations and a limited availability of preferred prey types. In drainage canals, weatherfish usually occur alone or with Prussian carp *Carassius gibelio*, which are also tolerant of low dissolved oxygen concentrations (Meyer & Hinrichs 2000, Pyrzanowski et al. 2015). During sampling in the Nowy Rów canal the only other fish species encountered were tench (*Tinca tinca*), which is in accordance with Schreiber et al. (2018), indicating a low number of piscivorous predators in artificial canals. In the River Ner only limited fish assemblage were noted (Kostrzewa & Penczak 2002) and weatherfish co-exist with bream (*Blicca bjoerkna*), roach (*Rutilus rutilus*), gudgeon (*Gobio gobio*), bleak (*Alburnus alburnus*), ruffe (*Gymnocephalus cernua*), perch (*Perca fluviatilis*), pike (*Esox lucius*) and burbot (*Lota lota*). In this fish assemblage, only the presence of pike and burbot may have a negative effect on the survival of small weatherfish.

In cobitids, especially the genera *Cobitis* and *Sabanejewia*, the sex ratio is usually biased to females due to polyploidy of females (Bohlen & Ritterbusch 2000). At both sites in our study the sex ratio did not differ from 1:1, which is typical for weatherfish and was reported by Meyer & Hinrichs (2000) from a drainage canal. The equal sex ratio we observed provides circumstantial evidence that the study populations are dominated by diploid individuals. Within the Cobitidae, the genus *Misgurnus* includes both diploid and tetraploid species (Vasil'ev et al. 1989). For both *M. fossilis*, and the related oriental weatherfish *M. anguillicaudatus*, bisexual diploid individuals are most common (Arai et al. 1993, Boroń 2000, Arai 2003). However, different levels of ploidy (triploids, tetraploids and intermediate aneuploids) may also occur in wild populations (Raicu & Taisescu 1972, Drozd et al. 2010).

The most frequently used structures for age determination in teleost fishes, including cobitids, are scales and otoliths. In cobitids, scale readings are imprecise due to the lack of a calcified scale centrum and poorly-defined annual increments (Fedorčák et al. 2017). Furthermore, the scales of weatherfish are small and hidden in the skin,

which is covered with an unusually dense layer of mucus. Age studies based on annual increments of the otoliths have proven effective for temperate fish taxa, including cobitids (Lampart-Kałużniacka et al. 2013), because they provide accurate estimates of age over the broadest age ranges whilst also relatively easy to prepare and interpret (Fowler 2009, Green et al. 2009).

Otolith readings from Nowy Rów and the River Ner indicated rapid growth rates for weatherfish in both studied populations, despite this being considered a slow-growing species. Weatherfish showed a maximum age of six years (Kottelat & Freyhof 2007, Mazurkiewicz 2012), and achieved a TL of approximately 44 mm in their first year, 88 mm in their second year, 137 mm in their third and 175 mm after four years (Table 3). After this initial period of rapid growth, growth rates declined. Five-year-old fish achieved a TL of 192 mm and six-year-old fish 229 mm. Weatherfish reared in carp (*Cyprinus carpio*) ponds can reach a size of 170 mm TL and mass of 21 g within two years. However, this rate of growth is only possible through access to an energy-rich diet and farm conditions (Boroń et al. 2002). In animals with indeterminate growth, such as teleost fishes, the growth trajectory is usually well approximated by the von Bertalanffy equation. In an asymptotic function like this, length represents a measure of growth capacity and derives from the allocation of resources to growth and reproduction (Kozłowski 1996). In older individuals, somatic body weight increase slows down, while relative contribution to reproduction tends to increase (Czarnołęski & Kozłowski 1998). In the present study, weatherfish caught in the Nowy Rów were on average longer in each age class than those from the River Ner, indicating a higher growth rate and reflecting a potentially more optimal habitat, rich in nutrients and free from predators.

Maximum lifespan was greater for females than males. This pattern has been seen in previous work on cobitids, for example in *C. paludica* (Przybylski & Valladolid 2000, Oliva-Paterna et al. 2002) and *C. calderoni* (Valladolid & Przybylski 2008). Strikingly, specimens younger than 2+ were absent at both study sites. A scarcity of some age groups or dominance of a particular sex in the population, especially in case of bottom-dwelling fishes, is not unusual and can arise from a length bias in sampling (Zalewski & Cowx 1990), or a sex-bias in habitat preferences (Băcescu 1962). The lack of juveniles in the 0+ and 1+ age classes among



weatherfish in the River Ner and in Nowy Rów canal may result from the morphology of these sites. As reported by Meyer & Hinrichs (2000), juvenile weatherfish prefer water depths below 0.1 m, which is characteristic of the reed zone and allows offspring to hide among vegetation or coarse detritus, while older individuals avoid areas of extremely shallow water. In contrast, larger fish, prefer deeper microhabitats overgrown by submerged, dense vegetation, such as Canadian waterweed (*Elodea canadensis*), floodplains typically used for spawning (Schreiber et al. 2018). This ontogenetic shift in microhabitat preferences of weatherfish is typical of many freshwater fishes.

In conclusion, this study provides the first data for the age structure and growth parameters of weatherfish based on an analysis of otoliths. We confirmed the deposition of annual increments in the sagittal otoliths, validating its utility in age and growth studies for this species. Otoliths proved useful for age estimation, study of growth rates and for estimating relationships between otolith morphometrics and fish length, in weatherfish and other cobitids. Weatherfish growth proved to be influenced by fish density, which may be a function of the size of sampling site, supporting higher growth in larger rivers, irrespective of

fish sex. Consequently, small human-maintained ditches, as more susceptible and more vulnerable, should be of particular concern for habitat managers when planning conservation measures for weatherfish. A particular focus should be to maintain the spatial continuity of ditches and to avoid excessive declines in water level which may result in a decline in their function as nurseries. A decline in the number of stretches supporting high densities of weatherfish may also result in a reduction in migration into larger rivers, where fish could achieve larger body size and higher reproductive success.

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