

## Effect of temperature on early life history in weatherfish, *Misgurnus fossilis* (L. 1758)

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Received February 26, 2009 / Reçu le 26 février 2009

Revised May 14, 2009 / Révisé le 14 mai 2009

Accepted May 15, 2009 / Accepté le 15 mai 2009

### Abstract

**Key-words:**  
*weatherfish,*  
*ontogeny,*  
*temperature,*  
*mortality*

Effect of incubation temperature (range: 9–36 °C; interval: 3 °C) on artificially propagated weatherfish (*Misgurnus fossilis*) early ontogeny (during interval from egg fertilization to the finish of hatching) was investigated. Both, the amplitude of the incubation period (evaluated in four crucial moments), the total hatching period duration was inversely proportional to the incubation temperature and ranged from 17.5 days at 9 °C to 1.8 days at 24 °C (expressed at  $H_{50}$ ) or from 137 hours at 9 °C to 9 hours at 24 °C, respectively. There were no influence of rising temperature on the total length of newly hatched larvae ( $T_L = 4.23\text{--}4.67$  mm), in contrast to negative correlation with developmental stage (9–18 °C: stage 37; 21–24 °C: stage 36), *i.e.* the length might determine the age at hatching, rather than the age at hatching determines the hatching length. The thermal tolerance range in term of survival lies between 9 and 24 °C (the thermal optimum 15–24 °C, *i.e.* weatherfish is a warm-mesothermic species). Temperatures above 24 °C (in our study 27–36 °C) are considered the lethal temperatures already during embryonic period. It is highly recommended to distinguish an impact of suboptimal temperatures 9–12 °C on development during explored interval only, in contrast to possible other effect of these lower temperatures in context of the whole early ontogeny.

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### RÉSUMÉ

Effets de la température sur les premiers stades de vie de la loche d'étang, *Misgurnus fossilis* (L. 1758)

**Mots-clés :**  
*loche d'étang,*  
*ontogénie,*  
*température,*  
*mortalité*

L'effet de la température d'incubation (gamme : 9–36 °C, intervalles : 3 °C) sur les premiers stades ontogéniques de loches d'étang (*Misgurnus fossilis*) (de la fécondation à la fin de l'éclosion) a été étudié. La durée de la période d'incubation (évaluée à quatre moments clés) et la durée de la période d'éclosion des œufs d'un lot sont inversement proportionnelles à la température d'incubation et s'étalent de 17,5 jours à 9 °C à 1,8 jour à 24 °C (pour l'indice  $H_{50}$ ) et de 137 heures à 9 °C à 9 heures à 24 °C, respectivement. Il n'y a pas d'influence d'une élévation de température sur la longueur totale des larves à l'éclosion ( $T_L = 4,23\text{--}4,67$  mm), alors qu'il y a une corrélation négative avec le stade de développement (9–18 °C : stade 37 ; 21–24 °C : stade 36), *i.e.* la longueur semble déterminer l'âge à l'éclosion, plutôt que l'âge à l'éclosion déterminerait la longueur à l'éclosion. La gamme

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de tolérance thermique en terme de survie va de 9 à 24 °C (l'optimum thermique est entre 15–24 °C, *i.e.* la loche est une espèce mésothermique chaude). Les températures supérieures à 24 °C (dans notre étude 27–36 °C) sont considérées comme températures létales dès la période embryonnaire. Il est vivement conseillé de distinguer l'impact des températures suboptimales 9–12 °C sur le développement pendant l'expérimentation d'un autre effet possible de ces températures basses dans le contexte de tout le développement ontogénique précoce.

## INTRODUCTION

Weatherfish, *Misgurnus fossilis* (L. 1758), is a small freshwater fish inhabiting slowly flowing or stagnant freshwater habitats with heavy water vegetation over-grown and muddy bottom (mixed with detritus and dead vegetation) in the inundation area (especially the secondary arms, isolated backwaters and pools in the floodplain) (Meyer and Hinrichs, 2000; Pekarik *et al.*, 2008). It is spread through the whole Europe from the North France to the Western Russia (including the Donau and Volga river basin) except for Scandinavia, Mediterranean and the British Isles (Kottelat and Freyhof, 2007). In nature, weatherfish is spawning from April to June (Grieb, 1937; Kryzanovskij, 1949; Kotlyarevskaja, 1967) in dependence on water temperature (Kryzanovskij (1949) and Kotlyarevskaja (1967) states temperature 13–14 °C).

However, this species possesses the unique anatomical, morphological (outer filamentous gills in larvae – Grieb, 1937; Kryzanovskij, 1949; Kostomarova, 1975) and physiological adaptations (intestinal and skin breath – Park and Kim (1999) – *M. anguillicaudatus* (Cantor, 1842)), its density decreased rapidly through the whole Europe in the second part of the 20th century (Meyer and Hinrichs, 2000) due to the degraded water quality (impact of industry and agriculture), direct destruction and drying natural habitats (impact of water engineering), (leading to deficiencies in ecological integrity; Karr (1991)) just like in other freshwater species (Kamler *et al.*, 1998; Schiemer *et al.*, 2003). Nowadays its status in Europe is considered least concern (Kottelat and Freyhof, 2007), this species is listed under Annex II of the Council Directive 92/43/EEC (involved into Natura 2000 network) and in many Red lists of endangered fishes in the Europe.

Weatherfish belongs to the commercial unremarkable fish species, but also to the fishes crucial for explanation of floodplain areas importance (Ward, 1998) in respect to fish spawning, early ontogenetic stages distribution and dynamics (fishes are a very important indicator group for river integrity estimation – Karr (1991)).

Therefore a successful reproduction and a high level of survival of early ontogenetic stages are the key events for fish recruitment and fish population dynamics (Kamler, 1992; Schiemer *et al.*, 2003). Fish early life history can be affected by many biotic or abiotic factors such as temperature, concentration of dissolved oxygen (Kotlyarevskaja, 1967; Keckeis *et al.*, 1996; Bohlen, 2003), pH (Prokes *et al.*, 1998), water current (Schiemer *et al.*, 2003) or salinity (Bohlen, 1999a).

Temperature is considered one of the most important ones, affecting development and growth (Penaz *et al.*, 1983; Kamler *et al.*, 1998; Green and Fisher, 2004), morphometric features plasticity (Stouracova *et al.*, 1988; Penaz *et al.*, 1989), maximum swimming speed (Green and Fisher, 2004) or sex determination (Conover and Kynard, 1981). Many of the recent studies focused on early development of fishes arised to predict population dynamics and life requests of the various species for the intents of fisheries management (Keckeis *et al.*, 1996; Kamler *et al.*, 1998; Klimogianni *et al.*, 2004; Jordaan *et al.*, 2006). Therefore a comprehension of temperature effect on the early fish ontogeny is crucial in process of understanding of fish egg and early ontogenetic stages distribution, dynamics and mechanisms of adaptation (Klimogianni *et al.*, 2004) to the fluctuating environmental conditions in the floodplain area (Ward, 1998; Pekarik *et al.*, 2008).

Up to now, there is only limited knowledge concerning the temperature limits of weatherfish during early ontogeny (Kostomarova, 1975; Alexeeva and Ozernyuk, 1987; Zdanovich *et al.*,

2001). Therefore, the present study experimentally evaluates the thermal sensitivity of embryonic and larval development up to the finish of hatching in artificially propagated weatherfish in a wide temperature range.

## MATERIAL AND METHODS

### > BROODSTOCK AND EGG COLLECTION

Weatherfish (*M. fossilis*) broodstock (4 females: standard length = 223–241 mm, weight = 48–65 g; 3 males: standard length = 167–190 mm, weight = 20–30 g) were collected in April 2007 from a floodplain area of the Lužnice River (Czech Republic, South Bohemia). These fish were held in aquaria (volume = 30 L, temperature = 16–18 °C). In order to synchronize the spawning, the spermiation and ovulation were stimulated with carp pituitary (gonadotropin) injected intramuscularly at one dose of 5.00 mg·kg<sup>-1</sup> b.w. (body weight) for males, at two doses of 0.50 and 4.50 mg·kg<sup>-1</sup> b.w. (interval: 12 hours) for females. Ovulated oocytes were collected by hand-stripping and then fertilized with pooled milt (dry method) on 23rd April 2007. The broodfish were first anesthetized (clove oil, 0.07 mL·L<sup>-1</sup>, 10 min) for safety manipulation during injection and hand-stripping. After spawning, all fish were released to the wild.

### > EGGS INCUBATION AND REARING CONDITIONS

Fertilized and unsticked eggs (stickiness was removed using half-fat milk diluted with water at a ratio 1:5 during 15 min) were incubated at 10 temperatures (range: 9–36 °C; interval: 3 °C). Temperatures were sustained by use of temperature-controlled refrigeration (or heating) system and measured by use of data loggers (RT-F53, Qi Analytical, Prague, Czech Republic) every 30 min in the range c. ± 0.5 °C. Eggs were put into the incubators (type I: glass beakers, volume = 250 mL; type II: transparent plastic boxes, volume = 2000 mL) placed in bath units (glass aquaria, water volume = 25 L) connected to temperature-controlling system. The temperature-acclimated reservoirs (volume = 2000 mL) served as a source for culture water replacement (oxygen saturated water) in each temperature unit.

In all units, eggs were divided in density of 50 eggs (incubator type I) and 1000 eggs (incubator type II). For each water temperature, two types of incubators were used – type I was used for estimation of mortality in triplicates, and type II for developmental sampling in duplicate.

Dissolved oxygen concentrations and pH were measured twice a day by handheld oxygen-pH meter (Oxi 315i, WTW GmbH) with additional regular monitoring of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, chemical oxygen demand (COD<sub>Mn</sub>) at 3-day intervals (in chemical laboratory). Photoperiod was maintained the same (12L:12D) in all temperatures (light intensity of 50–100 lx at the water surface). Summary of the main environmental parameters measured throughout the whole experiment is given in [Table I](#). When it was possible, the experiments lasted for 19 days until hatching was finished, in all temperatures.

### > MORTALITY EVALUATION

At each water temperature (incubators type I), mortality of embryos (eggs) was recorded daily. White opaque eggs were identified as dead eggs and were siphoned off. A total of 105 observations for temperature 9–24 °C (interval: 0.5–6 hours), 6 observations for temperature 27–36 °C (interval: 0.5 hour) were made.

### > SAMPLING

Samples of at least 100 pieces of stripped unfertilized fresh eggs from each female for evaluation female fecundity features and egg characteristics (egg diameter and weight) were collected.

**Table 1**  
**Main physical-chemical water condition measured during *M. fossilis* egg incubation and free stages cultivation.**  
 **$cO_2$ , concentration of dissolved oxygen (expressed in  $mg\ O_2 \cdot L_{H_2O}^{-1}$  /percent of oxygen saturation);  $t_{water}$  water temperature ( $^{\circ}C$ ).**

Temperature	9 °C			12 °C			15 °C			18 °C			21 °C		
	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$
<b>Minimum</b>	7.76	10.40/90.05	8.50	7.38	7.65/71.16	11.50	7.80	8.05/80.26	14.50	7.68	6.85/72.87	17.50	7.40	6.60/74.66	20.50
<b>Maximum</b>	8.20	12.50/108.23	9.50	8.19	11.05/102.79	12.50	8.17	10.35/103.19	15.50	8.52	9.45/100.53	18.50	8.31	8.70/98.42	21.50
<b>Mean</b>	7.98	11.29/97.75	8.90	7.91	9.51/88.46	12.10	8.00	9.12/90.93	14.90	8.01	8.40/89.36	17.85	8.00	7.60/85.97	21.20
<b>S.D.</b>	0.10	0.58/5.06	0.40	0.20	1.12/10.75	0.20	0.10	0.42/4.07	0.20	0.17	0.59/5.04	0.40	0.22	1.02/9.87	0.30
Temperature	24 °C			27 °C			30 °C			33 °C			36 °C		
Parameter	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$	pH	$cO_2$	$t_{water}$
<b>Minimum</b>	6.35	4.40/52.82	23.50	8.00	6.90/87.66	26.50	7.97	6.00/80.65	29.50	8.18	6.30/89.49	32.50	8.34	6.70/101.51	36.50
<b>Maximum</b>	8.29	8.55/102.64	24.50	8.26	7.60/96.57	27.50	8.36	7.20/96.78	30.50	8.42	6.90/98.01	33.00	8.41	6.70/101.51	37.00
<b>Mean</b>	8.00	7.10/85.23	23.90	8.10	7.23/91.87	26.80	8.16	6.50/87.37	29.80	8.30	6.60/93.75	32.80	8.38	6.70/101.51	36.00
<b>S.D.</b>	0.22	0.75/7.12	0.40	0.11	0.29/2.12	0.20	0.20	0.70/6.91	0.20	0.12	0.30/2.74	0.30	0.04	0.00/0.00	0.20

Tableau 1

Principaux paramètres physico-chimiques mesurés pendant l'incubation des œufs de *M. fossilis*, et les jeunes stades larvaires.

$cO_2$ , concentration en oxygène dissous (exprimé en  $mg\ O_2 \cdot L_{H_2O}^{-1}$  /pourcentage de saturation en oxygène) ;  $t_{water}$ , température de l'eau ( $^{\circ}C$ ).

During early development, fish samples (usually 5–10, in the crucial points of hatching at least 30 eggs or free stages) were taken at regular 30 min (from egg fertilization (0 hour post-fertilization (hPF)) to 1 day post-fertilization (1 dPF), 2 hours (period: 2–3 dPF), 6 hours (period: 4–8 dPF), 12 hours (period : 9–19 dPF). Incubated eggs and newly hatched larvae (in total of at least 200 eggs or larvae per temperature) were preserved in 4% PFA (phosphate buffered paraformaldehyde solution) for 10–50 days. This period is recommended for next observation by Lusk and Pokorny (1964), when the changes of length (decrease 1–2%) and weight (increase 5–8%) characteristics reach the minimal values compared to situation in fresh unpreserved individuals.

## > DATA ANALYSES

Developmental stages were determined using criteria published in Kryzanovskij (1949) with more precise concept set out in Kostomarova (1975) and Fujimoto *et al.* (2006) (early ontogenetic stages in related species *M. anguillicaudatus*). Hatching of free swimming stages is considered the most important developmental threshold and the true onset of larval period (according to Kamler *et al.*, 1998). Within incubation period (time from egg fertilization to hatching of larvae) four serious moments (sample points) were distinguished : (a) start of hatching (*i.e.* the point of hatching of 5% individuals), (b)  $H_{50}$  (*i.e.* the point of hatching of 50% individuals), (c)  $H_{75}$  (*i.e.* the point of hatching of 75% individuals) and (d) finish of hatching (*i.e.* the point of hatching of 95% individuals). Time scale used for the development is presented as days post-fertilization (dPF).

Female fecundity features – the absolute stripping fecundity (total number of stripped eggs per female), the relative stripping fecundity (number of eggs per kg of female body mass (b. m.)) and the relative weight of stripped eggs (weight of stripped eggs/female weight prior to stripping) – were estimated using gravimetric method based on average wet weight of one egg (the last mentioned value was counted from the weight of mixed sample of 100 stripped unfertilized unpreserved eggs per one female). Average diameter of one egg was determined using the optical image analysis method (see below) of 200 stripped unfertilized unpreserved eggs (mixed sample coming from all females).

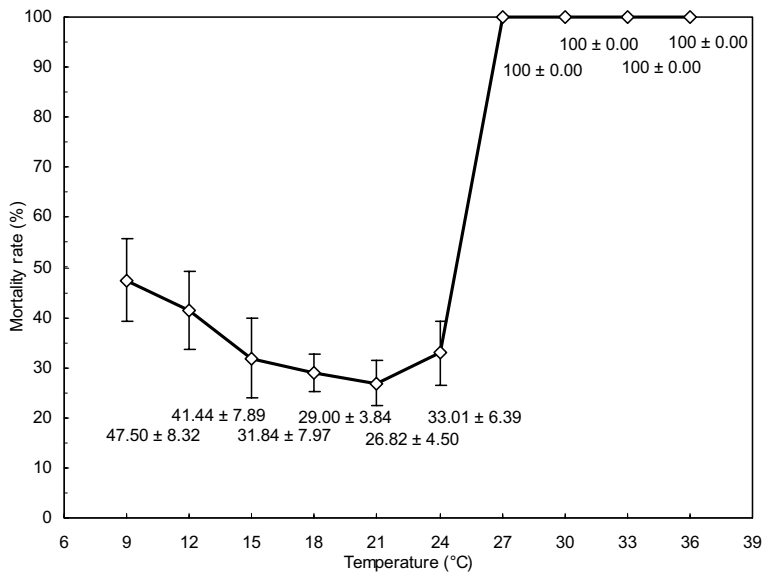
Cumulative mortality rate (from egg fertilization to finish of hatching) was calculated from the difference between initial numbers of eggs after fertilization and numbers of survived larvae after hatching, when possible (measurements at temperatures 27–34 °C finished earlier because of dying of all eggs before start of hatching).

Fixed individuals were examined under a binocular microscope (Olympus SZ 40, SZX9) and photographed using binocular microscope, fitted with a phototube and digital camera (Sony Progressive 3CCD and Olympus Camedia C5060UW). Digital images were then analysed (by video image analysis software – Microlmage version 3.0.1 for Windows) for total length ( $L_T$ ) determination.

Data for the amplitude of the incubation period (assessed in four crucial points) and the total hatching period duration, were analysed by multiple regression method (test criterion F, correlation coefficient R, *P* value) to determination and graphic visualization (2D graphs method was used) of the relationship between observed parameters and temperature. One-way ANOVA (including Tukey HSD test in next step) was used for evaluation of possible significant difference in  $L_T$  (measured at  $H_{50}$ ) among constituent temperature groups. Programme Statistica 7.0 (StatSoft, Inc.) was used for data analysis. However, water temperatures 27, 30, 33 and 36 °C were excluded from further statistical analyses due to fatal effects in term of larvae survival (complete mortality) already during early embryonic period.

## RESULTS

Artificial stripping was successful in 100 percent hormonally stimulated females. The interval of latency (*i.e.* the period after second pituitary dose injection to egg release) varied



**Figure 1**

Cumulative mortality rate (mean ± S.D.; expressed in percents) in *M. fossilis* between egg fertilization and finish of hatching (i.e. the point of hatching of 95% individuals) in relation to temperature ( $n = 50$  for each temperature).

Figure 1

Taux cumulé de mortalité (moyenne ± S.D. ; exprimés en pourcentage) chez *M. fossilis* (de la fertilisation des œufs à la fin de l'éclosion (i.e. point d'éclosion de 95 % des individus) en relation avec la température ( $n = 50$  pour chaque température).

from 16.00 to 17.50 hours ( $16.50 \pm 0.50$ , mean ± S.D.), in contrast to 33 percent achievement in hypophised males. Total number of stripped eggs (the absolute stripping fecundity) ranged between 5800–7900 ( $6900 \pm 830$ ) eggs per female. The relative stripping fecundity (number of eggs/kg b. m.) varied from 88 to 135 ( $121.60 \pm 19.50$ ) thousand eggs per female. The relative weight of stripped eggs formed 8.40–13.00 ( $10.70 \pm 1.70$ ) percent of female weight prior to stripping. The egg wet weight ranged between 0.76–0.96 mg ( $0.88 \pm 0.08$ ;  $N = 400$ ), the egg diameter varied from 1.37 to 1.66 mm ( $1.42 \pm 0.11$ ;  $N = 200$ ).

The accumulated mortality rate (mean ± S.D.) between egg fertilization and finish of hatching (i.e. the point of hatching of 95% individuals) reached value  $47.50 \pm 8.32\%$  (at temperature 9 °C),  $41.44 \pm 7.89\%$  (12 °C),  $31.84 \pm 7.97\%$  (15 °C),  $29.00 \pm 3.84\%$  (18 °C),  $26.82 \pm 4.50\%$  (21 °C),  $33.01 \pm 6.39\%$  (24 °C). At temperature 27–36 °C mortality increased up to 100% dead eggs, when all incubated weatherfish eggs were dying after 8.50 (27 °C), 8.00 (30 °C), 3.75 (33 °C) and 1.00 (36 °C) hours post-fertilization (Figure 1).

The amplitude of the incubation period (time from egg fertilization to hatch of larvae) decreased according to rising temperature. Mean time to reach all four sample points (see Material and Methods) was inversely proportional to temperature, i.e. ranged from c. 13 days at 9 °C to 1.60 days at 24 °C (start of hatching), from c. 17.50 days at 9 °C to 1.80 days at 24 °C ( $H_{50}$ ), from c. 18 days at 9 °C to 1.90 days at 24 °C ( $H_{75}$ ) and from c. 18.50 days at 9 °C to 2 days at 24 °C (finish of hatching) (Table II). Values of the start of hatching showed the negative exponential relationship with incubation temperature (Figure 2) (evaluated by multiple regression method; 2D graphs) described by formula:  $y = 40.1354 * e^{(-0.1402 * x)}$  ( $F(1, 178) = 148.89$ ,  $P < 0.01$ ,  $R = 0.92$ ) as well as  $H_{50}$  ( $y = 50.7341 * e^{(-0.1541 * x)}$ ;  $F(1, 178) = 129.10$ ,  $P < 0.01$ ,  $R = 0.90$ ),  $H_{75}$  ( $y = 57.3162 * e^{(-0.1508 * x)}$ ;  $F(1, 178) = 130.38$ ,  $P < 0.01$ ,  $R = 0.91$ ), the finish of hatching ( $y = 57.2396 * e^{(-0.1474 * x)}$ ;  $F(1, 178) = 130.21$ ,  $P < 0.01$ ,  $R = 0.91$ ).

Temporal ontogeny prolongation in dependence on temperature is also appreciable in the total hatching period duration (i.e. interval from start to finish of hatching), which is decreasing with rising temperature, from c. 137.00 hours at 9 °C to 9.00 hours at 24 °C (Table II),

**Table II**

The amplitude of the incubation period (time from egg fertilization to hatch of larvae) evaluated in four critical points and total hatching period duration (i.e. interval from start to finish of hatching) (mean  $\pm$  S.D.) in *M. fossilis*.

T, temperature; hPF, hourpost-fertilization (first line); dPF, day post-fertilization (second line); h, hour; N, number of observations; start, start of hatching (i.e. the point of hatching of 5% individuals); H<sub>50</sub>, point of hatching of 50% individuals; H<sub>75</sub>, point of hatching of 75% individuals; finish, finish of hatching (i.e. the point of hatching of 95% individuals).

Tableau II

Amplitude de la période d'incubation (de la fertilisation à l'éclosion des larves) évaluée à quatre moments critiques et durée totale de la période d'incubation (i.e. intervalle du début à la fin des éclosions) (moyenne  $\pm$  S.D.) chez *M. fossilis*.

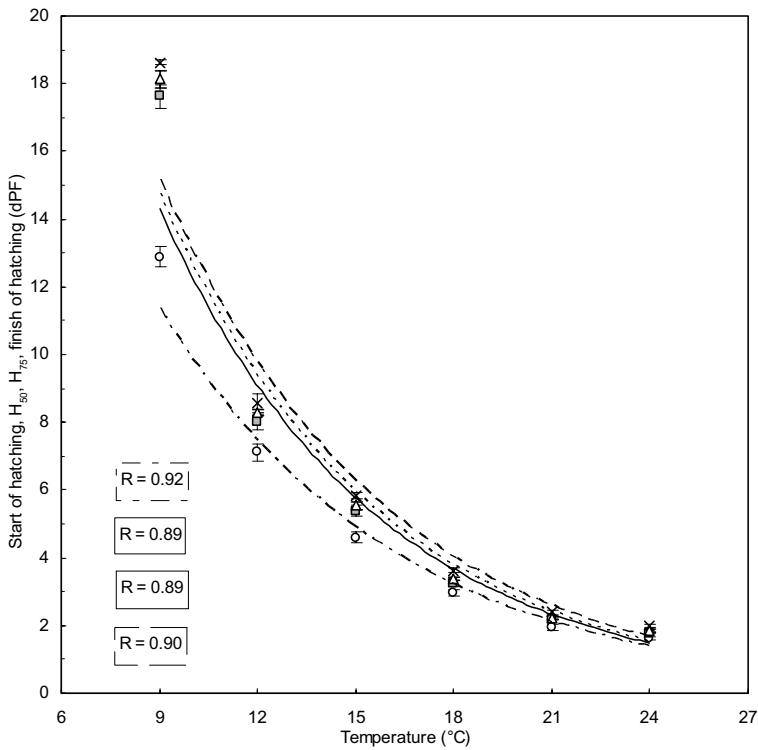
T, température ; hPF, heures post-fertilisation (première ligne) ; dPF, jours post-fertilisation (seconde ligne) ; h, heure ; N, nombre d'observations ; start, début d'éclosion (i.e. le point d'éclosion de 5 % des individus) ; H<sub>50</sub>, point d'éclosion de 50 % des individus ; H<sub>75</sub>, point d'éclosion de 75 % des individus ; finish, fin de l'éclosion (i.e. le point d'éclosion de 95 % des individus).

T	Hatching (hPF/dPF)									Hatching period		
	N	start		H <sub>50</sub>		H <sub>75</sub>		finish		N	duration (h)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		Mean	S.D.
9 °C	30	309.15	7.17	422.95	7.98	435.15	6.41	446.75	1.64	30	136.80	5.67
		12.88	0.30	17.62	0.33	18.13	0.27	18.61	0.07			
12 °C	30	170.65	6.13	191.95	5.41	198.75	2.28	205.70	6.37	30	34.25	3.23
		7.11	0.26	8.00	0.23	8.28	0.10	8.57	0.27			
15 °C	30	110.55	3.82	129.15	3.92	132.95	2.77	140.05	2.35	30	29.10	1.27
		4.61	0.16	5.38	0.16	5.54	0.12	5.84	0.10			
18 °C	30	71.35	2.33	77.55	2.29	81.15	1.46	87.15	1.86	30	16.80	1.60
		2.97	0.10	3.23	0.10	3.38	0.06	3.63	0.08			
21 °C	30	46.75	2.59	52.60	0.86	53.75	1.05	57.65	1.59	30	10.90	0.80
		1.95	0.11	2.19	0.04	2.24	0.04	2.40	0.07			
24 °C	30	38.65	0.86	42.60	0.62	44.35	0.72	47.65	0.78	30	9.00	0.69
		1.61	0.04	1.78	0.03	1.85	0.03	1.99	0.03			

is followed by negative exponential relationship with temperature (evaluated by multiple regression method; 2D graphs) described by formula:  $y = 390.9970 * e^{(-0.1677 * x)}$  (F (1, 178) = 86.73,  $P < 0.01$ , R = 0.81) (Figure 3).

The total length of newly hatched larvae (evaluated during H<sub>50</sub>) varied from  $4.23 \pm 0.24$  mm to  $4.67 \pm 0.22$  mm (mean  $\pm$  S.D.) (Table III). The significant difference among temperatures (ANOVA F(5,75) = 6.24,  $P < 0.01$ ) was found. The total length did not significantly differ in larvae incubated at 9, 15, 18, 21, 24 °C compared to situation at 12 °C (Tukey HSD test,  $P < 0.05$ ) (Table III).

The ontogenetic stage at hatching tended to decrease with rising temperature. Eggs incubated at temperatures between 21 and 24 °C hatched at Kostomárova's (1975) stage 36 (i.e. stage with first visible otholits in othic capsule, head slightly separated from the yolk sac, 10–13 caudal somites, still curved caudal part), (or the 44-somite stage of *M. anguillicaudatus* – Fujimoto et al. (2006)) and those incubated at 9–18 °C, showed the traits of the stage 37 (i.e. stage with first visible germ of pectoral fin, head noticeably separated from yolk sac, 17 caudal somites, straight caudal part), (or the 50-somite stage of *M. anguillicaudatus* – Fujimoto et al. (2006)) at hatching.



**Figure 2**

Relation of time until start of hatching (i.e. the point of hatching of 5% individuals),  $H_{50}$  (i.e. the point of hatching of 50% individuals),  $H_{75}$  (i.e. the point of hatching of 75% individuals), finish of hatching (i.e. the point of hatching of 95% individuals) and temperature (mean  $\pm$  S.D.) in *M. fossilis* (without lethal temperature 27, 30, 33, 36 °C) (fitted by exponential function).

dPF, days post-fertilization;  $-\circ-\circ-$ , start of hatching;  $-\square-$ ,  $H_{50}$ ;  $-\triangle-\triangle-$ ,  $H_{75}$ ;  $-\times-\times-$ , finish of hatching; R denotes correlation coefficient.

Figure 2

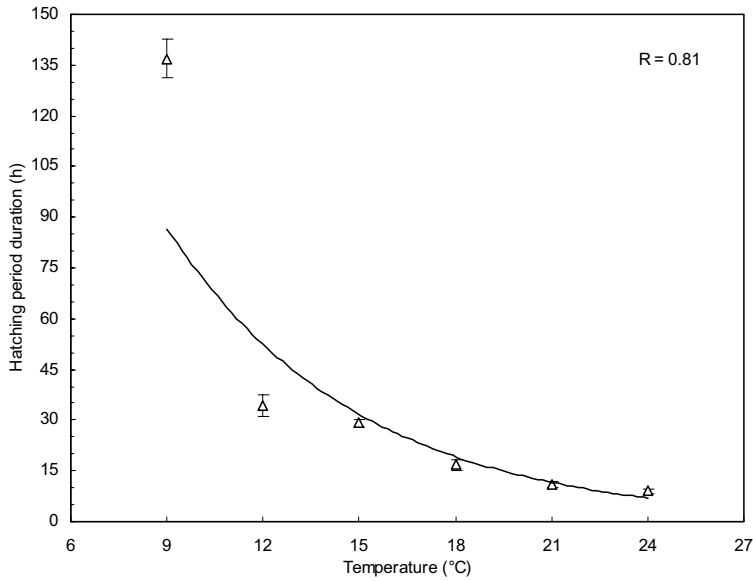
Relation entre la durée d'incubation jusqu'au début de l'éclosion (i.e. point d'éclosion de 5 % des individus),  $H_{50}$  (i.e. point d'éclosion de 50 % des individus),  $H_{75}$  (i.e. point d'éclosion de 75 % des individus), et la durée d'incubation jusqu'à la fin de l'éclosion (i.e. point d'éclosion de 95 % des individus) et la température (moyenne  $\pm$  S.D.) chez *M. fossilis* (sans les températures létales 27, 30, 33, 36 °C) (ajustement à une courbe exponentielle).

dPF, jours post-fertilisation;  $-\circ-\circ-$ , début d'éclosion;  $-\square-$ ,  $H_{50}$ ;  $-\triangle-\triangle-$ ,  $H_{75}$ ;  $-\times-\times-$ , fin d'éclosion; R est le coefficient de corrélation.

## DISCUSSION

The absolute stripping fecundity (approximately 6.90 thousand eggs on average) of weatherfish female evaluated in our study reached the same figure as Bohl (1993), but 20% or 50% or even 60% lower value compared to Geldhauser (1992), Kouril et al. (1996) or Adamkova-Stibranyiova et al. (1999) respectively. The conformable situation as in previous parameter was recorded in the relative stripping fecundity (121.60 thousand eggs per kg b. m. on average) and the relative weight of stripped eggs (10.70% on average) too, when our data averaged the half value compared to Geldhauser (1992), Kouril et al. (1996) and Adamkova-Stibranyiova et al. (1999). The reason for this discrepancy might be an origin of the fish. In our study, we used the adults from the wild (they spent only 14 days before stripping in captivity). In comparison to the authors cited above who used the broodstock reared the whole season in condition of intensive (or extensive) pond culture, i.e. in condition





**Figure 3**

Effect of temperature on the hatching period duration (i.e. interval from start to finish of hatching) (mean  $\pm$  S.D.) in *M. fossilis* (without lethal temperature 27, 30, 33, 36 °C) (fitted by exponential function).

*h*, hours; *R* denotes correlation coefficient.

Figure 3

Effet de la température sur la durée de la période d'éclosion (i.e. intervalle de temps entre le début et la fin de l'éclosion) (moyenne  $\pm$  S.D.) chez *M. fossilis* (sans les températures létales 27, 30, 33, 36 °C) (ajustement à une courbe exponentielle).

*h*, heures ; *R* est le coefficient de corrélation.

**Table III**

Total length of *M. fossilis* larvae (mean  $\pm$  S.D.) after hatching (evaluated in  $H_{50}$ ). Groups with the same superscript (a, b) do not significantly differ (Tukey HSD test,  $P < 0.05$ ).

$L_T$ , total body length; *N*, number of observations;  $H_{50}$ , point of hatching of 50% individuals.

Tableau III

Longueur totale des larves de *M. fossilis* (moyenne  $\pm$  S.D.) à l'éclosion (évaluée à  $H_{50}$ ). Les groupes avec la même lettre (a, b) ne diffèrent pas significativement (Tukey HSD test,  $P < 0.05$ ).

$L_T$ , longueur totale ; *N*, nombre d'observations ;  $H_{50}$ , point d'éclosion de 50 % des individus.

Temperature	$L_T$ (mm) [ $H_{50}$ ]		
	<i>N</i>	Mean	S.D.
9 °C	30	4.31 <sup>a</sup>	0.15
12 °C	30	4.67 <sup>b</sup>	0.24
15 °C	30	4.29 <sup>a</sup>	0.23
18 °C	30	4.29 <sup>a</sup>	0.24
21 °C	30	4.23 <sup>a</sup>	0.24
24 °C	30	4.30 <sup>a</sup>	0.18

with relative sufficiency of food resources and stable water temperature and chemistry. In fact, we could obtain just one portion of eggs (based on information in related species *M. anguillicaudatus* reached by Suzuki (1983) under laboratory condition – data about fraction spawning in *M. fossilis* still absent). Fish species inhabiting the environment with often unfavourable conditions (high ammonium, sulphate concentration), frequent temperature, pH and water level disturbances (weatherfish is a typical representative) are often the multiple spawners (in order to extend reproductive period and to raise a probability of reproduction success), as described Bohlen (1999b) in the related species – spined loach (*Cobitis taenia* – *sensu* L. 1758) under laboratory conditions too.

In general, the absolute stripping fecundity (and the other fish female fecundity features derived from this parameter) should be considered the approximate parameter only (because of inaccuracies), highly dependent on fish attributes (age, size, health condition) reviewed by Kamler (2005), natural and artificial influence during pre-spawning (temperature, application of hormonal preparation and their dose) and spawning period (including stripping experience) (summarized by Kamler, 1992). However, in the endangered fish species such as weatherfish (the endangered and protected species in the whole Europe – see Introduction) this method is only one possible way to estimation of these data (dealing with fecundity) without need to kill the adult fish.

Both, the value of average wet egg weight (0.88 mg on average) and diameter (1.42 mm on average) obtained in our study, were significantly lower compared to the figures showed by Kouril *et al.* (1996), Adamkova-Stibranyiova *et al.* (1999) (our data are c. 10–15% lower) in case of egg wet weight and by Kryzanovskij (1949), Kostomarova (1975) (our data are c. 13–23% lower) in case of egg diameter. Nevertheless, the value of average egg diameter (e.d.) in weatherfish (reached during our experiment) is higher compared to the related “loach” species – *Misgurnus anguillicaudatus* (e.d. = 0.72–0.84 mm; Zheng, 1985), *M. mizolepis* (Günther, 1888) (e.d. = 1.10 mm; Kim *et al.*, 1987), *Cobitis taenia* or *C. bilineata* (e.d. = 1.14 ± 0.07 mm or 1.21 ± 0.09 mm respectively; Bohlen, 1998).

However, we have to bear in mind a fact, that the average egg diameter and weight seems to be considerably influenced by yolk mass volume and dependent on the female attributes (Pepin *et al.*, 1997; Marteinsdottir and Steinarsson, 1998), vary interseasonally (as well as during one individual season) and intraspecifically (between population), (Kamler, 1992; Marteinsdottir and Steinarsson, 1998) too. Therefore it is very difficult to make a serious comparison or deep analysis between our data and the cited ones, due to the absence of data regarding female condition (especially age, size, spawning experience and fecundity) in cited literature.

Both, the amplitude of the incubation period (evaluated in all four crucial moments mentioned above – see chapter Material and Methods) and the total hatching period duration were inversely proportional to the incubation temperature, in accordance to results of Penaz *et al.* (1983) (in common carp), Ojanguren and Braña (2003) (in brown trout), Klimogianni *et al.* (2004) (in common pandora) or Kamiński *et al.* (2006) (in lake minnow), *i.e.* the amplitude of the incubation period (or the total hatching period duration) at minimal temperature generally reached approximately nine times (or fifteen times, respectively) the value at maximal temperature.

Values of the incubation period amplitude (in temperature range 18–24 °C) evaluated in our study, reached the similar level (time thermal-dependent range) presented by Kouril *et al.* (1996) (at 18 °C), Kostomarova (1975) (at 21.50 °C) in weatherfish or Suzuki (1953) (19–21 °C), Watanabe *et al.* (1948) (20–21 °C), Fujimoto *et al.* (2006) (20 °C) in related species, oriental weatherfish (*M. anguillicaudatus*).

In contrast, Grieb (1937), Geldhauser (1992) in weatherfish or Zheng (1985) in oriental weatherfish present noticeably different figures compared to our findings. We probably have to look for a reason of this discrepancy either in an unknown origin of incubated eggs (Grieb, 1937) or in unstable water conditions during incubation (Kryzanovskij, 1949; Zheng, 1985; Geldhauser, 1992). Grieb (1937) used for his observation fertilized eggs from the wild (unknown age), subsequently incubated at temperature 15 °C in laboratory (but value

**Figure 4**  
 Comparison of the amplitude of the hatching period (in hours post-fertilization) reached in our study (evaluated in points: start of hatching,  $H_{50}$ , finish of hatching) with data known from literature in relation to temperature.  
 White rectangle black framed, our data (in *Misgurnus fossilis*); grey rectangle, data from literature (in *Misgurnus fossilis*); white rectangle with grey letters, data from literature (in *Misgurnus anguillicaudatus*); start (of hatching), point of hatching of 50% individuals;  $H_{50}$ , point of hatching of 50% individuals; finish (of hatching), point of hatching of 95% individuals.

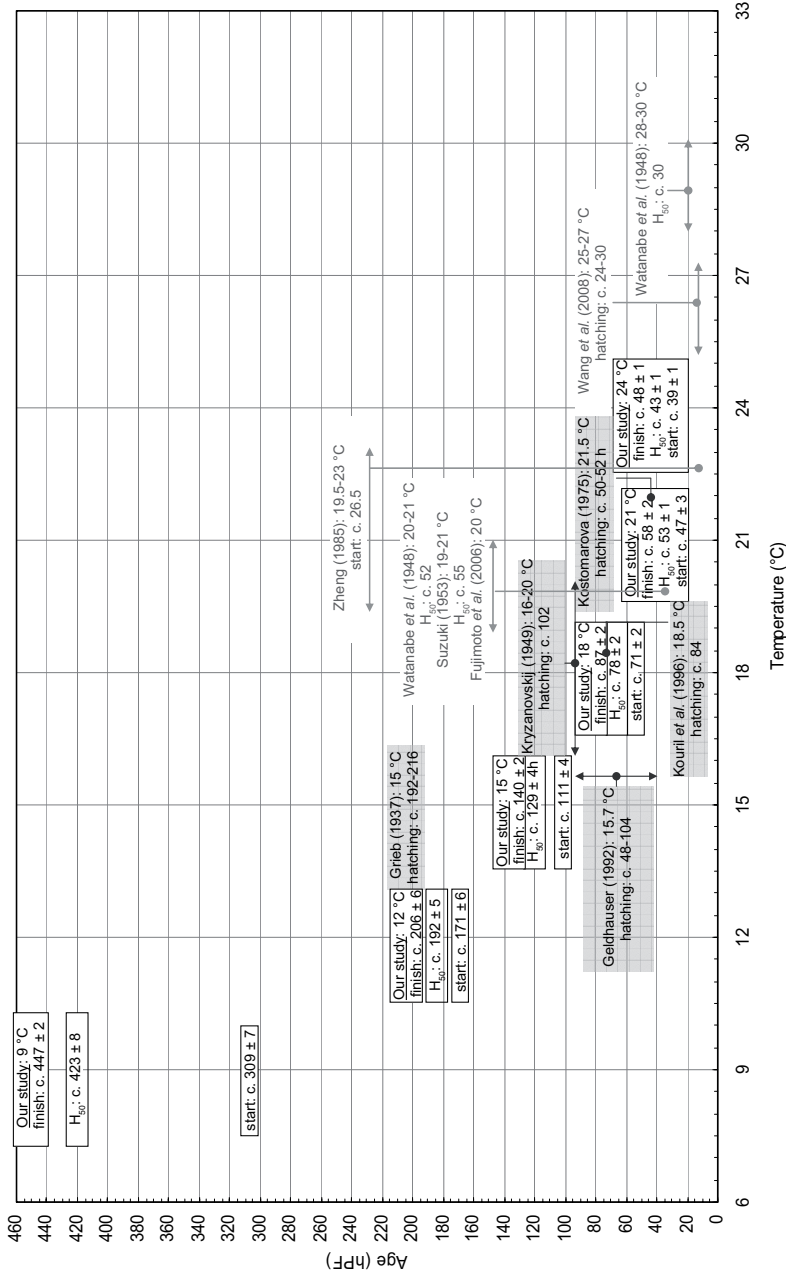


Figure 4

Comparaison de l'amplitude de la période d'éclosion (en heures après la fertilisation) obtenue dans notre étude (évaluée aux points : début d'éclosion,  $H_{50}$ , fin de l'éclosion) avec les données de la littérature en rapport avec la température.  
 Rectangle blanc à bordure noire, nos données (chez *Misgurnus fossilis*) ; rectangle gris, données de la littérature (chez *Misgurnus fossilis*) ; rectangle blanc avec lettres grises, données de la littérature (chez *Misgurnus anguillicaudatus*) ; start (début d'éclosion), point d'éclosion de 5 % des individus ;  $H_{50}$ , point d'éclosion de 50 % des individus ; finish (fin d'éclosion), point d'éclosion de 95 % des individus.

of the incubation period amplitude shown by Grieb (1937) presents a typical situation at 12 °C, in our study). Water temperature fluctuated in range 16–20 °C (Kryzanovskij, 1949), 19.50–23 °C Zheng (1985) or 15–16.90 °C (Geldhauser, 1992). Values of the incubation period amplitude shown by Kryzanovskij (1949), Zheng (1985) or Geldhauser (1992) present situation of fluctuation of water temperature in range 15–18 °C, around 24 °C or 15–21 °C respectively, in our study (value presented by Zheng (1985) probably represents data for hatching of larvae at earlier ontogenetic stage – but the author does not refer a developmental stage of newly hatched larvae). A summarized comparison of the hatching period amplitude reached in our study with data known from literature (in relation to temperature) is given in Figure 4 (values presented by Watanabe *et al.* (1948) and Wang *et al.* (2008) serve as the reference figures for hatching in temperature range 25–30 °C in *M. anguillicaudatus* – we did not observe hatching in weatherfish at temperatures above 24 °C (due to lethal impact – see Discussion below)).

Size of newly hatched larvae seems to be considerably influenced by parental attributes (Wootton, 1990; Panagiotaki and Geffen, 1992; Kamler, 2005), or water conditions (Keckeis *et al.*, 1996; Prokes *et al.*, 1998; Schiemer *et al.*, 2003). We observed a significant decrease of the total length of newly hatched larvae (our data are c. 15% lower on average) compared to the figures presented by Grieb (1937), Kryzanovskij (1949), Kotlyarevskaja (1967) or Kostomarova (1975), probably caused by smaller egg size (observed during stripping – see Discussion above). According to Kotlyarevskaja (1967), a ground of this variance might be found in oxygen level (total length of freshly hatched weatherfish larvae reaches c. 5 mm in treatment with oxygen concentration 6.00–8.50 mg O<sub>2</sub>·L<sup>-1</sup>, or c. 4 mm in treatment with oxygen concentration 2.40–4.00 mg O<sub>2</sub>·L<sup>-1</sup> respectively), but in our study, a concentration of dissolved oxygen overreached on average value 7 mg O<sub>2</sub>·L<sup>-1</sup> (c. 90% of oxygen saturation) over all temperatures. Nevertheless, the size of larvae after hatching in weatherfish (reached in our study) generally represents double of the length presented by Zheng (1985) (c. 1.95–2.40 mm) or Fujimoto *et al.* (2006) (c. 2.60 mm on average) in newly hatched larvae of the related loach, *M. anguillicaudatus*.

Generally, preceding studies dealing with the effect of temperature on hatching size, have offered contradictory conclusions. The most common situation is an inverse correlation of the larval size at hatch and temperature (higher temperature produces smaller larvae; hatching is an age-related rather than size-related event) (Penaz *et al.*, 1983; Ojanguren and Braña, 2003; Jordaan *et al.*, 2006).

In contrast to previous opinions, Alderdice and Forrester (1974) (in flathead sole) or Pepin *et al.* (1997) (in Atlantic cod) described a decrease of newly hatched larvae size with declining temperature (in latter, this situation might be considered the impact of increased metabolic requirements at temperatures close to the lower boundary of its thermal tolerance range).

However, our results do not suggest any correlation (neither positive nor negative) of the size of newly hatched larvae and temperature (in wide temperature range 9–24 °C), *i.e.* the total length of larvae at hatch do not significantly differ in dependence on temperature, according to situation observed by Blaxter and Hempel (1963) in herring or Jordaan *et al.* (2006) in Atlantic cod (batch 2). The significant difference of L<sub>T</sub> at 12 °C compared to other temperatures might be probably affected either by real impact of low temperature leading to bigger larvae, or more probably by collector's mistake, who selectively chose the larvae with bigger size (significant difference ( $P < 0.05$ ) between 12 °C and other temperatures is only c. 0.30 mm on average).

In addition, our results suggested that the length might determine the age at hatching, rather than the age at hatching determines the hatching length. Therefore it has been assumed that embryonic growth (together with temperature) probably determines the time of hatching in *M. fossilis* (and therefore growth within eggs is most likely unequal in this species) at least within the temperature range that we used.

According to Yamagami (1988), the hatching event is only loosely linked to the developmental stage in many fish species. A developmental stage reached by weatherfish

at hatching is negatively correlated with temperature (violation of the equiproportional rule), in accordance with results presented by Penaz *et al.* (1983) (in common carp) or Ojanguren and Braña (2003) (in brown trout). It means, eggs cultivated at temperature 21 and 24 °C hatch at the earlier ontogenetic stage (stage 36 or the 44-somite stage, respectively) compared to the stage 37 (the 50-somite stage, respectively) at temperature 9–18 °C (more detailed ontogenetic staging for finer distinguishing of particular ontogenetic stages, than in Kostomarova (1975) or Fujimoto *et al.* (2006), is not available). Variation of the reached stage at hatching (in our study) should not be caused by oxygen condition (concentration of dissolved oxygen was on average over 7 mg O<sub>2</sub>·L<sup>-1</sup> (c. 90% of oxygen saturation) in all temperatures), compared to Kotlyarevskaja (1967) (stage 37 hatched in oxygen concentration 6.00–8.50 mg O<sub>2</sub>·L<sup>-1</sup>; stage 36 in oxygen concentration 2.40–4.00 mg O<sub>2</sub>·L<sup>-1</sup>, respectively).

However, correlation (positive or negative) of the developmental stage and the larval size at hatch (except at 12 °C – see comments above) was not found, in contrast to suggestion (positive correlation of developmental stage and size) of Penaz *et al.* (1983) (in common carp), Penaz *et al.* (1989) (in tench) or Ojanguren and Braña (2003) (in brown trout).

In term of environmental implications, a ground of hatching of weatherfish at almost the same size and developmental stage, practically non-affected by temperature (based on our data), might be explained as a set of adaptations for survival and successful reproduction in the wild. An outcome represents a functional compromise between ontogenetic and behavioural traits conditioned by endogenous and exogenous (abiotic and biotic) factors leading to a synergistic influence on fish populations and their dynamics.

In energetic aspect, larvae hatching at lower temperatures (at 9 and 12 °C) could not reach higher size due to a longer time spent in egg, leading to hatch of larvae with a low energetic source stored in a yolk sac (larvae hatch close to complete yolk sac depletion followed by starvation conducting to dead, soon) according to Kamler (1992).

Weatherfish inhabits environment with often unfavourable, all the time changing conditions (see Discussion – female fecundity) and puts sticky eggs over underwater vegetation (Grieb, 1937; Kryzanovskij, 1949). Weatherfish larvae after hatching hang fast-stuck to plants by head over several days (Grieb, 1937; Kryzanovskij, 1949; Kostomarova, 1975). Therefore, freshly hatched larvae (larvae incubated at higher temperature – in our study at 15–24 °C) in case of hatch at lower ontogenetic stage (according to a common opinion that higher temperature produces smaller larvae at the lower developmental stage – see above) may risk their soon death due to staying in condition of almost no oxygen, with low temperature and high concentration of ammonium, sulphate or marsh gas (organs of an additional breathing such as outer filamentous gills, segmental blood vessels in fin-fold or intestinal respiration occur even during stage 38, stage 39 or in adult fish, respectively – Kryzanovskij, 1949; Kostomarova, 1975; Park and Kim, 1999: *M. anguillicaudatus*). According to Kotlyarevskaja (1967), weatherfish larvae have to actively seek for suitable environmental conditions and feed by climbing over aquatic vegetation.

Biotic factors (especially interspecific food competition and predation) can be as important as egg size (see Discussion above) or abiotic environmental conditions (see above) in term of governing of size at hatch in weatherfish larvae (and reaching developmental stage too).

Larval size at hatch determines the initial food size by exogenous nutrition start (*i.e.* longer larvae at higher developmental stage have a chance to win in interspecific food competition) and together with egg size defines the predator size which is able to utilize these early ontogenetic stages (*i.e.* an earlier ontogenetic stage with bigger yolk sac might be more vulnerable to predation) (Wootton, 1990). The ability of larvae to react to assumed predation danger, maximum and mean escape speed increase after hatching till complete yolk sac depletion in dependence on temperature (Green and Fisher, 2004). In addition, predation risk may be notably scaled down during several days after hatching due to speedy growth and variation in behaviour related to ontogeny (Eaton and DiDomenico, 1986; Webb and Weihs, 1986).

The accumulated mortality rate between egg fertilization and finish of hatching (*i.e.* the point of hatching of 95% individuals) varied from 26.80 to 100% in dependence on incubation temperature (in addition survival reached value 0.00–73.20% during the same period). Consequently, the thermal tolerance range for the early ontogeny (*i.e.* the period from egg fertilization up to finish of hatching) in *M. fossilis* lies between 9 °C and 24 °C.

Temperatures 15–24 °C are considered the range of the thermal optimum for weatherfish, (survival over 60% of eggs alive is considered the limit for optimum temperature classification by Kostomarova (1975) in weatherfish, Penaz *et al.* (1983) in common carp and Ozernyuk *et al.* (1987) in some cyprinids) in our study. These temperatures are also in accordance to the temperatures observed during spawning of weatherfish in the wild (Grieb, 1937; Kryzanovskij, 1949), as well as to the optimal range obtained under laboratory conditions (Kostomarova (1975) presents range (13)14–(20)24 °C; Alexeeva and Ozernyuk (1987) and Zdanovich *et al.* (2001) range 14–22 °C, respectively). Within the optimal thermal range (concretely at temperature 18 °C), we observed an apparent difference in mortality level (our data reached value 29%) compared to cited data (further mentioned), *i.e.* 10–15% lower value in comparison to Kouril *et al.* (1996) (mortality reached 40–50%), but *c.* 10% higher figure in comparison to Bohl (1993) (mortality at level 80%, presented by Bohl (1993) is probably caused by female over-maturation during end of June).

Nevertheless, we have to bear in mind a necessity to specify a term “optimal temperature”. According to general opinions (summarized in Kamler (1992) or Pavlov (2007)), the optimal temperature range for embryonic/larval stages survival may not be the same, compared to the range for optimal growth or effectiveness of food utilization (which we have not investigated in present study).

Relatively acute boundary in mortality of incubated eggs was observed between temperature 24 and 27 °C (accumulated mortality rate rapidly increased from above-average value (33%) up to 100%), when all weatherfish eggs were dying up to several hours post-fertilization. Consequently, temperatures above 24 °C (in our study temperature in range 27–36 °C) are considered the lethal temperatures due to the live-inhibiting effect already during embryonic period.

Temperatures lying under 15 °C (in our study, range 9–12 °C) should be assumed the suboptimal temperatures (for the period from egg fertilization up to finish of hatching) due to quite low level of accumulated mortality (value less than 50%). Nevertheless, we have no evidence about an accute boundary in term of lethality at low temperatures (boundary between still tolerated and lethal temperature) in range under 9 °C (generally, temperature 9 °C should be sublethal, in accordance to common situation in cyprinids – Wieser (1991)). However, it is highly recommended to distinguish and evaluate an impact of temperatures 9 and 12 °C (temperatures close to the lower boundary of the thermal tolerance range as Pepin *et al.* (1997) in Atlantic cod) on the embryonic and larval period up to finish of hatching only (survival over 50%), in contrast to possible other effect of these lower temperatures in context of the whole early ontogeny (it is a topic for the next possible study).

Generally, according to Wieser’s (1991) classification, weatherfish (*M. fossilis*) as well as the related species, spined loach (*Cobitis taenia* sensu L.) (Bohlen, 2003), belongs to the warm-mesothermic species (thermal optimal range during early ontogeny 15–24 °C), but does not tolerate such a high temperature as a close-related species, oriental weatherfish (*M. anguillicaudatus*). In this Asian loach species, Watanabe *et al.* (1948) described common hatching of larvae at 28–30 °C, Kubota and Matsui (1955) considered the suitable temperatures for early ontogeny in range 20–28 °C (with optimum at 25 °C, or even at 25–27 °C according to Wang *et al.* (2008)).

Further studies (undertaking not only under laboratory conditions, but also in the wild) should be directed to understanding of interactions among early ontogeny, behaviour and exogenous factors influencing early-life history (especially in order to follow an exact role of early-life history characters determining survival), towards the management of the natural population of this hidden living fish species.

## ACKNOWLEDGEMENTS

This investigation was financially supported by the Grant Agency of the University of South Bohemia Ceske Budejovice (86/2006/P-VURH) and the Czech Ministry of Education, Youth and Sports (project USB RIFCH No. MSM6007665809). We would like to thank M. Prokes (Institute of Vertebrate Biology, Academy of Science of the Czech Republic, Brno) for his constructive comments during creation of manuscript.

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