ACTA ICHTHYOLOGICA ET PISCATORIA (2004) 34 (1): 51–72

MORPHOPHYSIOLOGICAL ASPECTS OF THE EMBRYONIC DEVELOPMENT OF RUFFE, *GYMNOCEPHALUS CERNUUS* (L.) UNDER DIFFERENT THERMAL CONDITIONS

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Bonisławska M., Korzelecka-Orkisz A., Winnicki A., Formicki K., Szaniawska D., 2004. Morphophysiological aspects of the embryonic development of ruffe, *Gymnocephalus cernuus* (L.) under different thermal conditions. Acta Ichthyol. Piscat. 34 (1): 51–72.

Background. Ruffe, *Gymnocephalus cernuus* is a small fish inhabiting vast areas of Europe, Asia, and also North America. Its substantial geographical dispersal range in different climatic zones, in waters of different parameters indicates that it is a particularly plastic species. Recent implementation of new fish-processing machinery has increased dramatically the economical importance of ruffe, which is now treated as a gourmet food. In the wake of the increased interest in this fish we decided to study aspects of its developmental biology.

Methods. Fertilised eggs and the developing embryos of ruffe were incubated under five different temperature regimes. They were monitored in two planes, horizontal and vertical, using a light microscope fitted with a digital camera and connected to a computer with a monitor and a VCR. The data collected were processed using MultiScan software.

Results. Perivitelline space in ruffe constitutes as much 65% of the egg volume. This facilitates movements of an embryo and enhances processes of gas exchange through mixing of perivitelline fluid. It finally contributes to the distribution of the hatching enzyme on the inner surface of the egg shell. Similarly as in the other percid fish, the lack of integration between the "lipid raft" with the embryonic disc, causes the fall of the disc on the lateral side of the vitelline sphere. This lateral position persists throughout all sequential stages of the embryogenesis. The diversified thermal regimes of the development affect the timing of the embryogenesis, as well as the condition, weight, and the size of newly-hatched ruffe larvae. The highest rate of specimens hatched in good condition—marked by the highest weight and body length—was observed at the optimal temperature range of 16–18°C. Newly-hatched larva is small

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(5 mm body length, 0.10 mm^3 of yolk sac volume), active, and fully prepared for independent life in the water. Under the optimal temperature range, determined in the present study, the duration of embryonic development of ruffe (from fertilisation to hatching) was 2122 dh (accumulated degree-hours)

Conclusion. The present study constitutes a contribution to the knowledge of the embryonic development of ruffe and its biological sense, emphasizing the morpho-physiological differences between this species—living under, specific, diversified ecological conditions—and other fish.

Key words: fish, ruffe, *Gymnocephalus cernuus*, reproduction, egg, embryogenesis, embryonic motorics, temperature.

INTRODUCTION

Ruffe, *Gymnocephalus cernuus* (L.) is a fresh-water fish inhabiting central- and eastern Europe, rivers of the entire Siberia, with the exception of the Amur River drainage basin (Berg 1949), drainage basins of the northern shores of the Black- and Caspian Seas, and even the Aral Sea (Nikolski 1970). It migrated also to the waters of the moderate climate zone of the North America (Fairchild and McCormick 1996) and can be found in the estuary areas of rivers and in the brackish waters of the Baltic Sea.

A substantial geographical dispersal range of this fish in different climatic zones, in waters of different parameters such as salinity, pH, nutrient content, and temperature indicate that this is a particularly plastic species. The above conclusion was emphasised by the mentioned earlier authors and many other researchers (Kryžanovskij et al. 1953, Szubińska 1961, Kokurewicz 1971, Pęczalska 1971, Kováč 1993, Čihař 1995, Terlecki 2000). In most cases those studies were limited, describing selected problems of morphology and biology of adult specimens, living in various bodies of water and they were less oriented on the early ontogeny of this, otherwise interesting, species.

Until recently, such relatively low interest, resulted rather from low economic importance of this fish, because of its small size (in the majority of areas) and the difficulties in its efficient processing.

Recently, however, special filleting machines have been implemented for processing ruffe, which resulted in a rapid growth of interest in this species. The final product—filet—has been treated as a gourmet food and its market price far exceeds the prices of many valuable fish species.

All the above, and particularly the reasons related to the reproductive biology of this species, its dispersal, and the ability to adjust to diversified environmental conditions, have prompted us to undertake an attempt of a more detailed study on its ontogeny, with a focus on its crucial phase—the embryonic period. It seems that a complex (not fractional) studying of morphomechanic phenomena of embryogenesis and the associated physiological processes may reveal defined relationships, conditions, and regularities occurring between its specific morpho-mechano-

physiological processes and highly diversified chemical-physical parameters of the environment where the natural reproduction takes place.

MATERIAL AND METHODS

The present study was carried out in spring in the laboratory of the Division of Fish Anatomy and Embryology, Agricultural University of Szczecin.

The study material were the gametes (eggs and sperms) of ruffe, *Gymnocephalus cernuus* (L.) acquired from mature specimens captured at Krzemień Lake. The gametes were delivered to the laboratory where the eggs were inseminated applying the "dry method". The sperms were activated by filtered water of 16–18°C—the optimal temperature for this fish species.

Fertilised eggs were acclimated and subsequently placed into plastic containers. The containers were put in aquaria with a hydromechanical setup responsible for maintaining constant water temperature of 14, 16, 18, 20, and 22°C (Bonisławska 2001).

The temperature values were constantly recorded using a four-way electronic temperature recorder (AKO 1570) enabling its constant registration at 4 sites. The diel temperature fluctuations amounted to 0.1°C.

Live observation of embryonic development of ruffe under five different temperature regimes was carried out with the aid of experimental set-ups previously used at the Division of Fish Anatomy and Embryology. Those setups enabled fish egg observations at two planes—vertical and horizontal (Winnicki and Korzelecka 1997). The sets, consisting of a microscope, digital camera, VCR screen, and a computer enabled subsequent image analysis of pictures recorded on videotape. Images of the developing eggs and newly-hatched larvae were subjected to accurate measurements and statistical analyses (Multiscan 6.08 and Statistica 5.0). Both diameters were measured—the egg and the egg cell (vitelline sphere) located inside. The volume ($V = 4/3\Pi r^3$) and the surface area ($S = 4\Pi r^2$) were calculated, as well as the S/V coefficient (surface : volume ratio) of eggs and egg cells.

Thirty specimens of newly-hatched larvae were collected from each temperature regime. Their total lengths (TL) were measured as well as the length (l) and height (h) of the yolk sac. Approximated volumes of the yolk sacs were calculated from the formula for the volume of elongated ellipsoid (Blaxter and Hemple 1963, Rechulicz 2000).

$$Ve = \Pi/6 \cdot l \cdot h^2$$

Embryonic motorics. All motional activities at individual stages of embryonic development were recorded and subsequently analysed in the developing eggs of ruffe, incubated at four temperature levels. The number of heart contractions of embryo inside the egg, and the heart-beat rate in newly-hatched larvae were counted within one minute, while the number of somatic movements of the body were calculated within 10 min.

Duration of embryonic development was determined using thermal units—accumulated degree hours (dh) and the rate of embryogenesis was studied

based on 5 defined stages (in 60% of the specimens studied):

– cleavage (8–16 blastomeres)
– blastopore closure
– first heart beats
– body pigment
– hatching:
  - first hatchlings
  - 50% hatched
  - hatching completed

Fertilisation rate was determined in individual samples at the stage of blastopore closure.

Survival rate, determined as percentage of hatched larvae after the completing of hatching, was based on a group of some 200 eggs.

The following statistical methods were used for processing of the result data:

– linear regression function

$$V_{50\%} = a + b \cdot T$$

for relationship between the rate of embryonic development and the incubation temperature (T). The rate of embryonic development is defined as $V = (\tau_{50\%w})^{-1}$ (where V, rate of embryonic development; $\tau_{50\%w}$, time to 50% of hatching) (Kamler 1992). This function of linear regression helped to determine the temperature of biological zero ($t_o = -a \cdot b^{-1}$, when the embryonic development theoretically ceases and this is also the crossing point of the regression line and the x-axis). Also the effective degree-days $D_{eff} = 1 \cdot b^{-1}$ (Vinberg 1987, Kamler 1992, Weltzien et al. 1999).

– single-classification analysis of variance (ANOVA) for the length and volume of the yolk-sac of newly-hatched ruffe larvae incubated under diversified thermal conditions.
– Tukey test to compare mean values of size parameters of larvae hatched from eggs incubated under different thermal conditions.
– W Shapiro–Wilk normality test—to prepare histograms showing size diversity of ruffe eggs representing a mixture of eggs from several females (Stanisz 1998)

In all experimental treatments, on the beginning of embryogenesis and shortly before hatching, the physical and chemical parameters of water were examined. It is evident from Table 1 that in all treatments, despite obvious differences in O_2 contents, all other parameters fit into ranges characteristic for the first-class (sporadically the second-) of the water purity scale (Kubiak et al. 1999). Therefore those parameters might be additional factors that might have influenced the embryogenesis and inflicts its disturbances.

Table 1

Physical and chemical parameters of water under different thermal conditions

Temperature	O_2 [mg · dm^{-3}]	pH	Alkalinity [mval · dm^{-3}]	Hardness [mval · dm^{-3}]	Cl [mg · dm^{-3}]	SO_4^{-2} [mg · dm^{-3}]	PO_4 [mg · dm^{-3}]	Total P [mg · dm^{-3}]	NH_4 [mgN · dm^{-3}]	NO_2 [mgN · dm^{-3}]	NO_3 [mgN · dm^{-3}]
14°C	7.04–9.89	7.6–8.3	2.9–4.3 2.9	5.7–7.0	39.5–60.35	24.16–32.15	0.011–0.106	0.03–0.01	0.03–0.23	0.001–0.02	0.07–0.09
16°C	7.02–9.83										
18°C	6.89–9.49										
20°C	6.44–9.21										
22°C	6.35–8.85										

RESULTS

The results obtained during the incubation of ruffe eggs under different temperature regimes indicate that the optimal temperature range for development this fish species is 16–18°C (Table 2). It has been confirmed not only by the highest fertilisation- and egg survival rates, but also by the highest rate of viable larvae hatched from eggs under such thermal conditions (Table 2). Consequently, the principal- and detailed description of individual phases of embryogenesis of ruffe will be focused on the phenomena occurring in the egg at 16°C (bold-type column in Table 2) and it will constitute a reference point for the parameters of the embryogenesis at other temperatures of incubation.

Size of eggs, egg cell, and egg structures after fertilisation

Eggs of ruffe sampled from 6 females were, at the end of hydration process, very diversified in their size and their diameter ranged from 0.86 to 1.00 mm, exhibiting a normal distribution (Fig. 1).

The egg volume ranged from 0.30 to 0.52 mm^3, on the average Ø = 0.44 ± 0.04 mm^3 ($\bar{x}$ ± s) (Fig. 2). Also the size of the egg cells inside the eggs were diversified (diameter: 0.55–0.80 mm) and their mean volume was 0.18 ± 0.03 ($\bar{x}$ ± s) mm^3 (Fig. 2, Table 3). Other parameters, such as the volume (V), surface area (S), and the S/V ratio, calculated based on the measurements collected, are shown in Table 3.

Structural fat in the egg of ruffe is present in the form of a single sphere of substantial size, located in the vitelline sphere Ø = 0.36 mm ± 0.01 ($\bar{x}$ ± s) and a number (up to 20) smaller droplets ranging in diameter from 0.01 to 0.09 mm. Lipid droplets are never integrated with the embryonic disc. The total volume of fat amounted

to 0.027 mm^3, which constitutes 15% of the egg cell volume and 6.1% of the entire egg volume. As the development progresses, the structural lipids tends to merge into a single sphere and in such form the fat is also present in newly-hatched specimens.

Table 2

Duration of individual phases of embryogenesis and its other major parameters in ruffe, *Gymnocephalus cernuus* (L.) at different temperature regimes

Temperature Phases of development [dh]	14°C	**16°C**	18°C	20°C	22°C
8-16 blastomeres	82	**76**	66	61	56
Blastopore closure	397	**376**	367	360	353
Slow heart beat	1197	**1136**	1096	982	—
Pigment of body	1340	**1228**	1168	1002	—
Hatching of the larvae					
First hatching	2265	**1962**	1738	1412	—
50% of hatch	2322	**2032**	1805	1434	—
Last hatching	2391	**2122**	1954	1452	—
Number of hours of incubation	161.8–170.8	**122.6–132.6**	69.6–108.6	70.6–72.6	—
% Fertilisation	42	**54**	53	40	—
% Survival	13	**15**	10	7	—

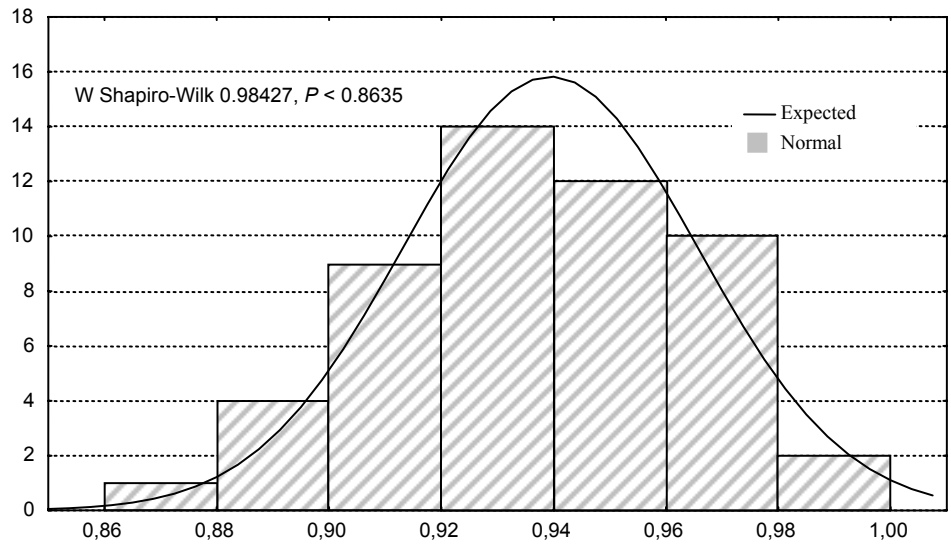

Fig. 1. Distribution of diameters of eggs of ruffe, *Gymnocephalus cernuus* (L.)

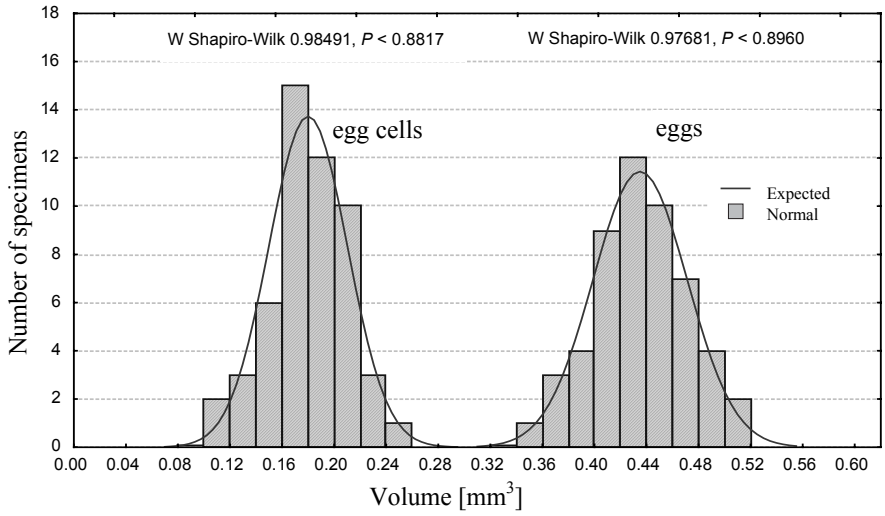

Fig. 2. Volume distribution of eggs and egg cells of ruffe, *Gymnocephalus cernuus* (L.)

Table 3

Size parameters of eggs and egg cells of ruffe, *Gymnocephalus cernuus* (L.) constituting a mixture of gametes collected from 6 females ($\bar{x} \pm s$)

Number of eggs (n)	Diameter [mm]		Volume V [mm^3]		Surface area S [mm^2]		S/V ratio	
	Eggs	Egg cells	Eggs	Egg cells	Eggs	Egg cells	Eggs	Egg cells
50	0.94 ± 0.03	0.70 ± 0.04	0.44 ± 0.04	0.18 ± 0.03	2.77 ± 0.15	1.54 ± 0.17	6.39 ± 0.18	8.62 ± 0.52

The course of embryogenesis at 16°C

Water uptake by an activated ruffe egg lasts some 90 min. At this time a reception mound is formed as a consequence of ectoplasm transfer towards the animal pole. At the same time a large perivitelline space, constituting 59% of the entire egg volume, is formed.

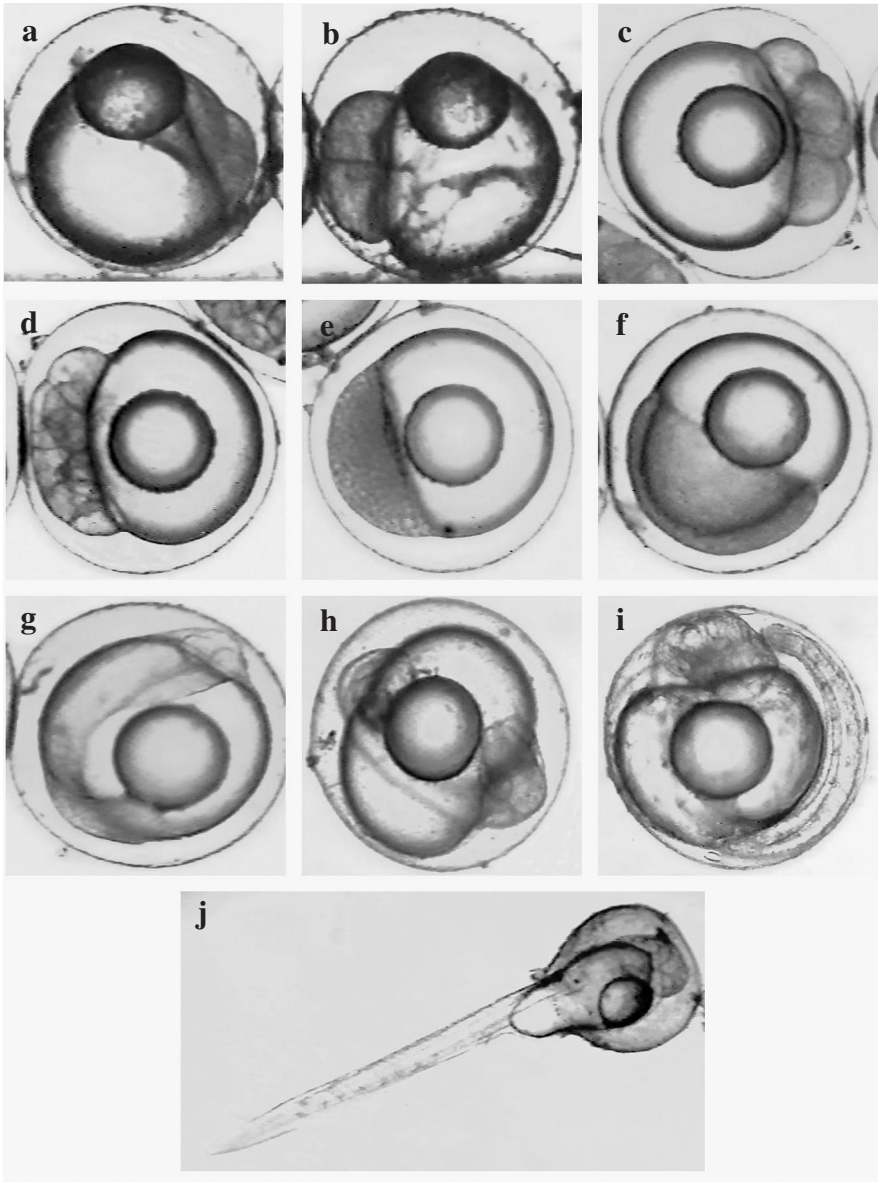

Fig. 3. Stages of embryonic development of ruffe: a) formation of the embryonic disc, lateral view; b) two-blastomere stage, lateral view; c) eight-blastomere stage, view from top; d) coarse morula, view from top; e) fin-grain morula, beginning of gastrulation, view from top; f) epibolia of some ½, view from top; g) outline of an embryo with visible eyes promordia, view from top; h) embryo, with 3 cerebral vesicles (brain vesicles), view from top; i) embryo, shortly before hatching; j) embryo emerging from the egg shell

Embryonic disc is formed after activation. Initially, because of, as it seems, quasiperistaltic movements, ectoplasm builds up at the apical egg pole, transforming soon into the embryonic disc. Because of the disruption of the vitelline sphere balance the shield slides on the side. The reception mound and consequently also cleavage disc and the developing embryo remain for the entire period of embryogenesis on the side of the vitelline sphere (Fig. 3a, b)

Appearance of the first furrow, signalling the beginning of the cleavage phase occurs some 2 h from the moment of egg fertilisation (Fig. 3b). The subsequent divisions occur after 4 h (8 blastomeres, Fig. 3c), 6.5–7 h (16–32 blastomeres, Fig 3d). After some 8 h a fine cell morula is formed (Fig. 3e). Subsequent divisions produce large cell morula, which is attained in 11 h after fertilisation.

Gastrulation begins after 200 accumulated degree-hours (Fig. 3f) and after 300 dh the eggs are have their yolk in 3/4 overgrown. This process ends about 4.5 h later (blastopore closure) and the body outline of the developing embryo is visible inside (Fig. 3g).

Organogenesis. After 28 h from fertilisation the embryo, lying flat on the vitelline sphere, is visible. Its anterior part enlarges and the outline of prime brain vesicles and eye primordia occur (452 dh)(Fig. 3h). Twelve hours later (644 dh) three prime brain vesicles and lenses in eyecups (vesicles) are distinctly visible. Somites appear in thickening main-body part.

After three days of embryonic development (800 dh) further changes in the brain can be recorded, consisting in differentiation of brain vesicles into five sections and in further enlargement and development of eyecups and lenses. Olfactory fossae appear. Distinct metameres are visible on the body.

By the end of the third day of incubation, the embryo is well developer. Its body is distinctly elongate (end of tail reaches the head) and first somatic movements (1–2 per 10 min) can be recorded (Fig. 4). At the same time the heart primordia slowly start to work (20 contractions per min) (Fig. 5). The first body movements are of small amplitude. In time, their frequency grows up to 30 movements per 10 min (1100 dh) and their extent is larger (whole body movements).

Subsequently (about 1200 dh) the heart works faster and more rhythmically (70–80 contractions per min) (Fig. 5). After some 65–70 dd (accumulated degree-days) (1560–1860 dh) additional eye movements appear and at 1824 dh another type of movement can be observed — “trembling of embryo”. It consists of series of short (4–5 s) movements appearing 3 to 4 times per 10 minutes (Fig. 6).

Distinct pigmentation on the dorsal side of head and main body and the tremors of the entire embryo announce that the time of leaving the egg shells is near (Fig. 3i).

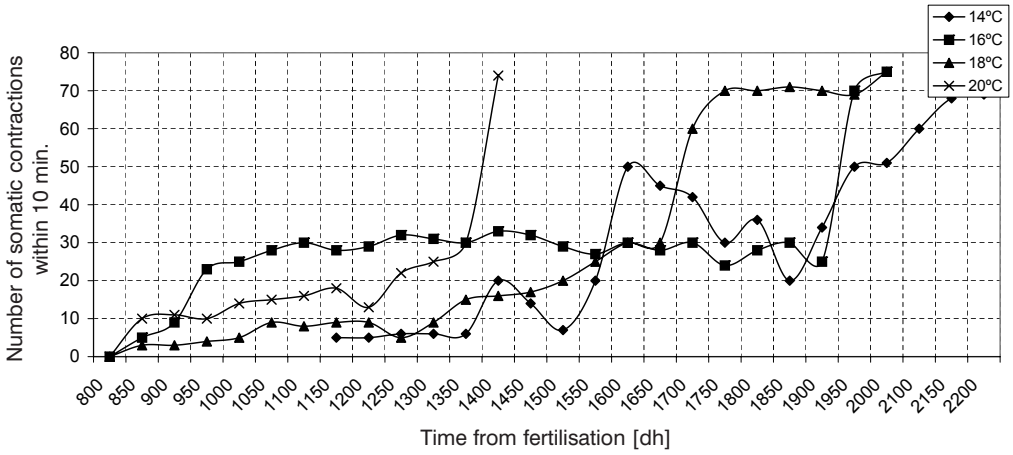

Fig. 4. Somatic motorics of ruffe at different temperatures

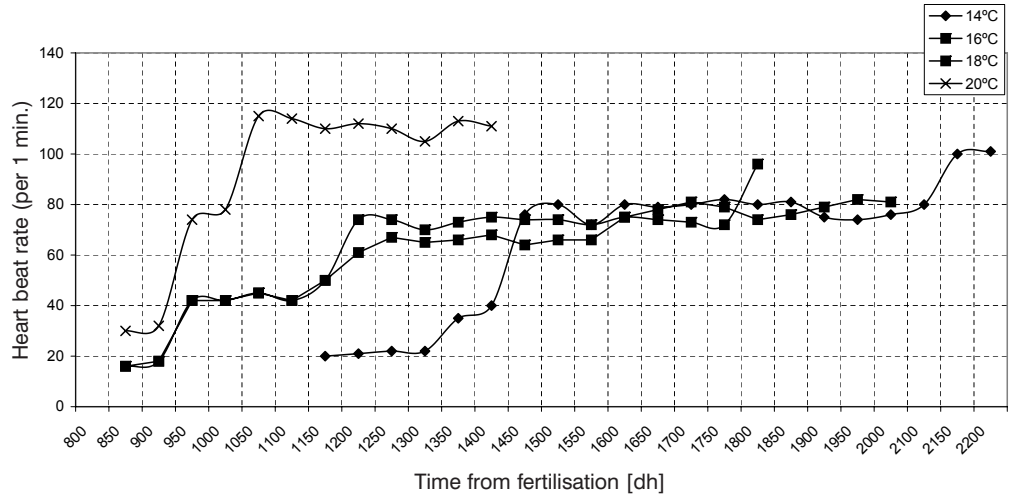

Fig. 5. Heart beat of ruffe embryos at different temperatures

Hatching. The first egg hatched at hour 123 of the embryonic development (1962 dh) and 50% of specimens left the egg shells 4 h later (2032 dh). The last larvae hatched within additional 5 h (2122 dh) (Table 2). The embryos leaving the egg shells emerged either tail first (Fig. 3j) or head first.

After the hatching the larvae were 3.85 ± 0.08 ($\bar{x} \pm s$) mm long (TL) and their yolk sac was small 0.10 ± 0.01 ($\bar{x} \pm s$) mm^3. The larvae easily stayed in the water column and were fully prepared for independent living in the external environment. The heart beat rate in a newly-hatched specimen was about 80 contractions per min.

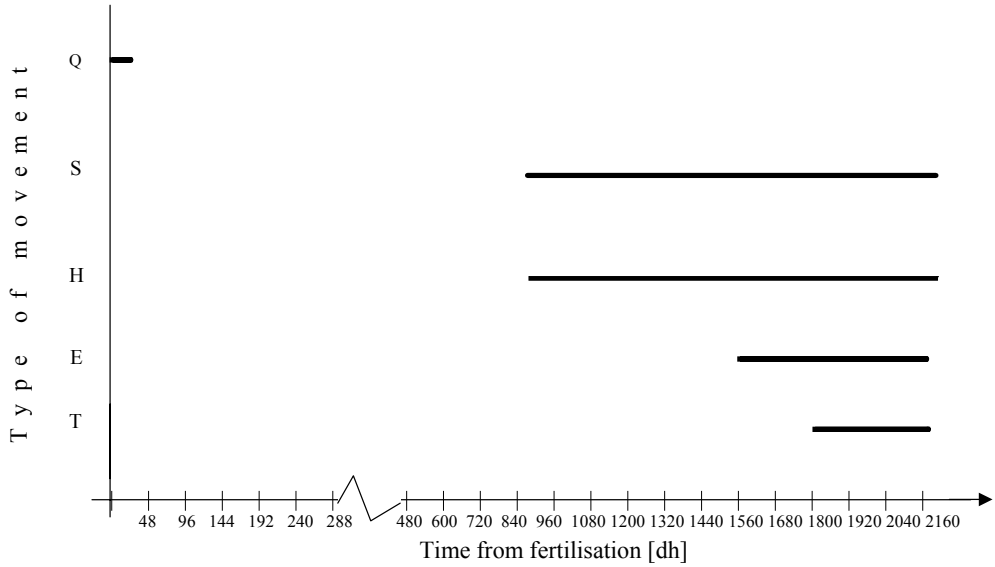

Fig. 6. Appearance sequence and the duration of different movements in ruffe embryos incubated at 16°C; Q, quasiperivitelline ectoplasm movements (the shield is formed; S, somatic movements; H, heart beats; E, eye ball movements; T, "trembling" of embryo

Effect of temperature on the duration of embryonic development of ruffe

The duration of embryonic development (from activation to hatching), measured in thermal units (as evident from Fig. 7) was not uniform and according to expectations is the longest at the lowest (14°C) temperature. It is moderate at medium temperatures and the shortest at 20°C. The regime of 22°C turned out to be too high and the embryonic development ceased at the stage of blastopore closure (Table 2).

Diversified water temperature affect the time of individual stages and the most distinct differences in the duration of individual stages of embryogenesis are visible when the heart of embryo starts to work (Fig. 7).

The course and the rate of the embryonic development in ruffe eggs incubated at different temperatures

The correlation between the rate of the embryonic development (calculated for 50% hatch, $V_{50\%}$) and the temperature of incubation (T) is very close, as evidenced by a high correlation coefficient (r) (Fig. 8). The temperature of "biological zero" and effective degree days (D_{eff}) calculated based on the parameters of the regression function are: $t_0 = 9.68$°C and $D_{\text{eff}} = 32.2$.

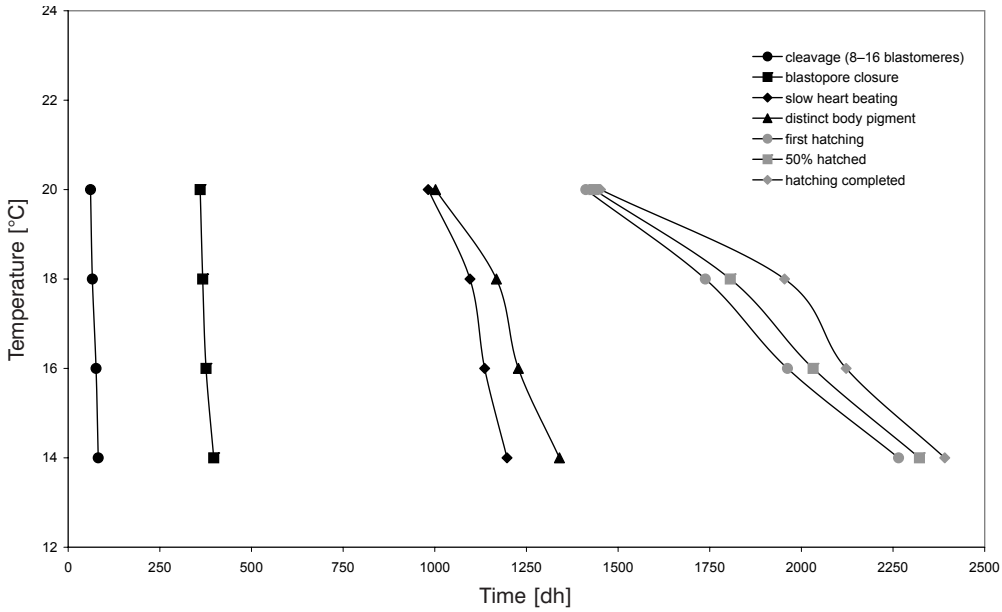

Fig. 7. The course of embryogenesis of ruffe (stages)

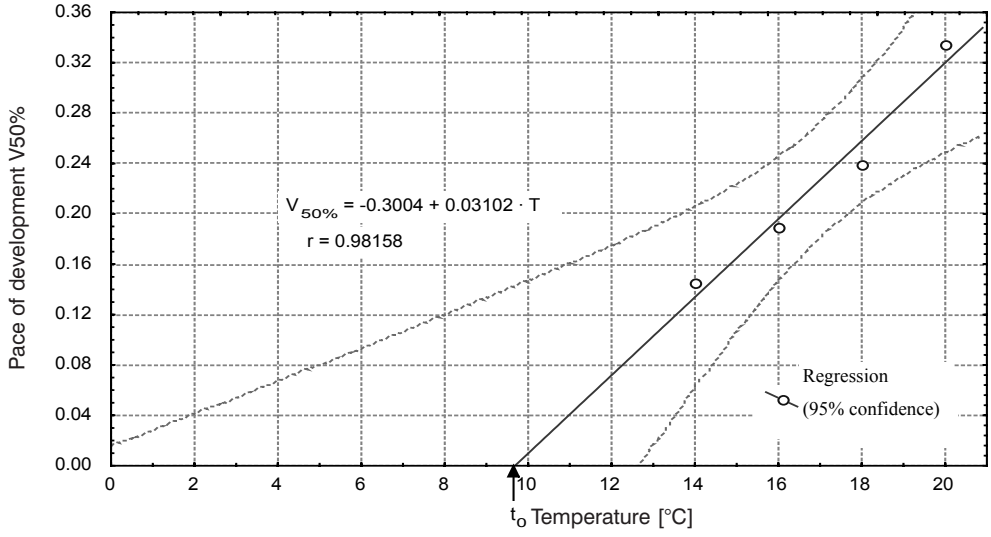

Fig. 8. Relationship between the rate of embryonic development and the incubation temperature of ruffe eggs

Hatching and characteristics of larvae hatched from eggs incubated under different thermal conditions

The duration of the hatching process depends on the water temperature and at 14°C it lasts some 9 hours, while at 20°C—only 2 h (Fig. 9). The present study demonstrated that in the egg mixture, sampled from 6 ruffe females, incubated at different temperatures, the hatched specimens differ in their meristic and mensural parameters, because they leave the egg shells within different time periods (measured in thermal units) of incubation (Table 4).

Larvae hatched at 16°C had the fewest malformations and defects, although the overall survival rate in all experimental treatments was low (Table 2). The highest percentage of the abnormalities occurred at maximal and minimal temperatures. The majority of malformations were expressed as spinal curvatures and "Siamese twins" (at 20°C, Fig. 10a, b).

Table 4

Characteristics of larvae of ruffe, *Gymnocephalus cernuus* (L.) hatched from eggs incubated at different temperature conditions ($\bar{x} \pm s$)

Temperature / Larvae characteristics	14°C	16°C	18°C	20°C
Total length (mm) (ANOVA $P < 0.01$)	3.70 ± 0.09^{b}	3.85 ± 0.08^{c}	3.67 ± 0.19^{b}	3.52 ± 0.18^{a}
Volume of yolk sac (mm^3) (ANOVA $P < 0.01$)	0.10 ± 0.01^{bd}	0.10 ± 0.01^{acd}	0.09 ± 0.01^{a}	0.11 ± 0.01^{bc}
[n] Number of hatched larvae (of ca. 200 fertilised eggs)	60	67	41	31
% of malformed larvae	11	9	14	40

The mean values with the same superscripts do not show high statistically significant differences ($P < 0.01$, Tukey test).

The bodies of all specimens hatched from eggs incubated at 14, 16, 18, and 20°C were pigmented, although the intensity of pigmentation varied. It was the darkest at 16 and 18°C. The eye pigment visible in larvae inside egg shells, was observed only in the specimens incubated at 20°C (Fig. 11a, b).

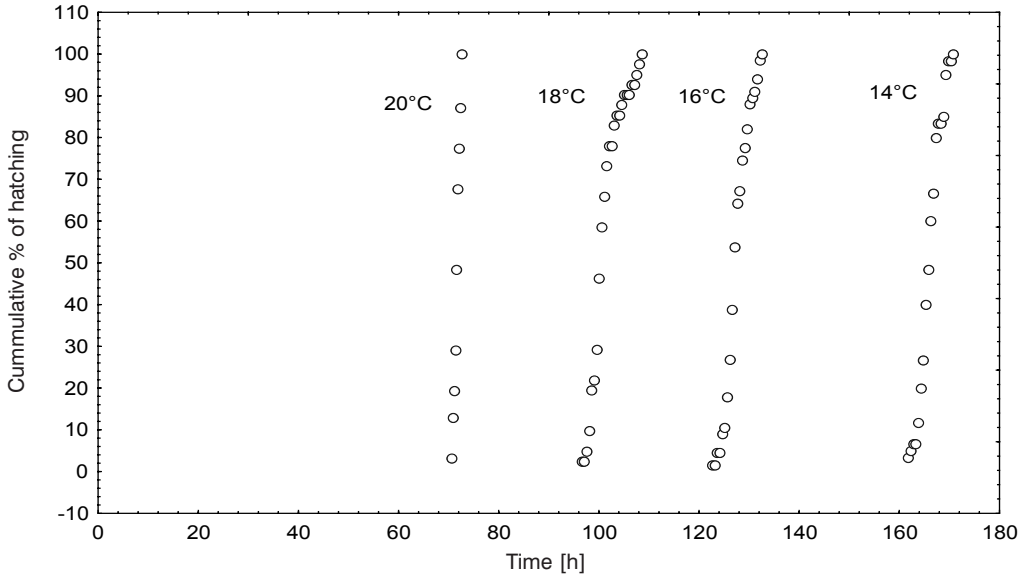

Fig. 9. Duration of the hatching process of ruffe eggs incubated under different temperatures

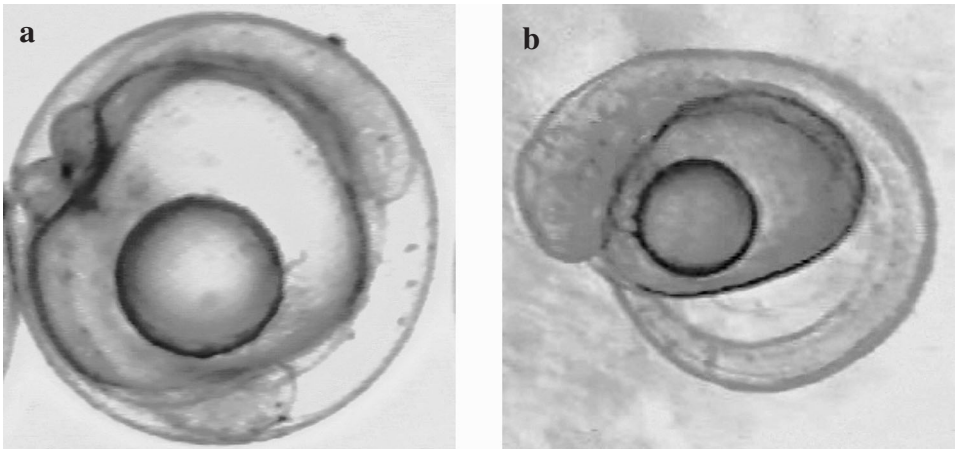

Fig. 10. Abnormalities in the structure of ruffe
a) twin forms of embryos; b) deformation of a larva

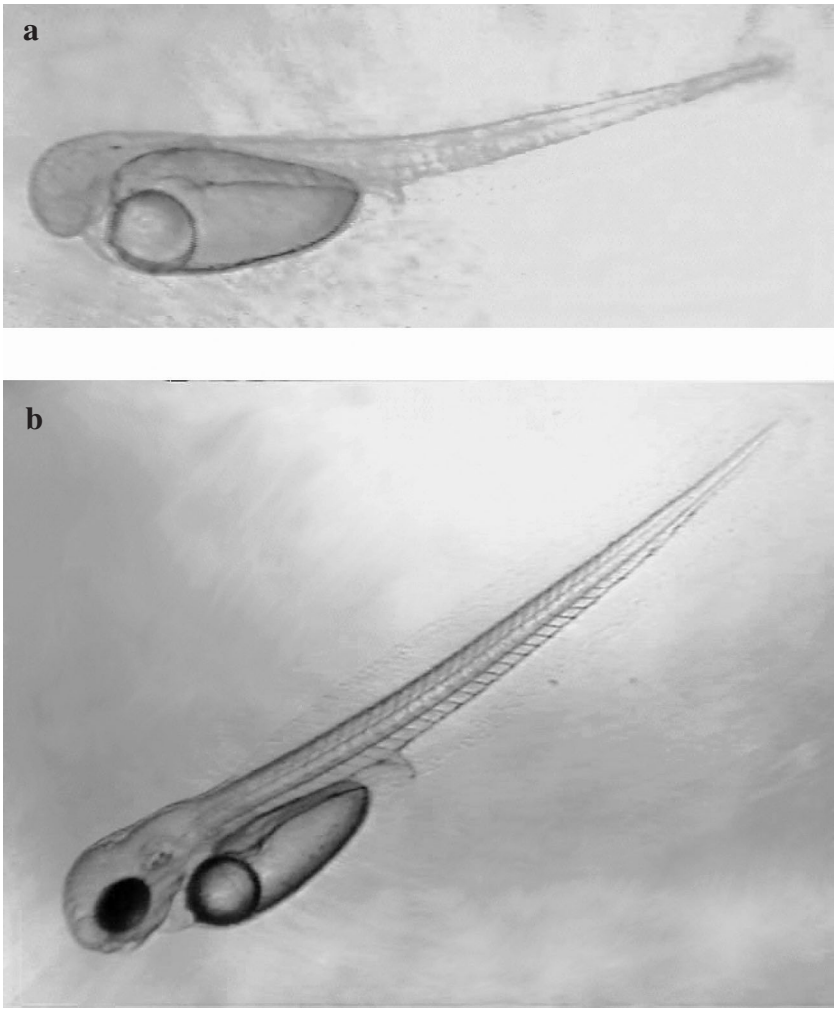

Fig. 11. A larva, shortly after hatching (with small yolk sac)
a) incubated at 14°C, without ocular pigment; b) incubated at 18°C, with ocular pigment

DISCUSSION

The results of this study, along with observations on the embryogenesis of ruffe, effect of diversified thermal conditions on its duration, and also on the hatching process itself and the characteristics of newly-hatched specimens indicate the occurrence of certain curiosities and at the same time regularities during the embryonic development of this commonly occurring fish species.

The results on the embryonic development of ruffe obtained by other researchers (Kryžanovskij et al. 1953, Kováč 1993, Fairchild and McCormick 1996, Saat and

Veersalu 1996) are not always consistent with the results of the present study. The ambiguous points will be discussed below.

The size characteristics of eggs, egg cells, and egg structures after fertilisation

Activated eggs of ruffe, *Gymnocephalus cernuus* (L.) are very small, compared to size of eggs of other local freshwater fishes reproducing within spring and summer seasons. Their volume, on the average 0.44 ± 0.04 ($\bar{x} \pm s$) mm^3, constitutes 48.5% of the egg volume of closely related perch, *Perca fluviatilis* (L.) (cf. Korzelecka et al. 1998) and only 11% of the egg volume of (even close related with the ruffe) the Danube streber, *Zingel streber* (V = 3.82 mm^3) (Kováč 2000). The volume of ruffe eggs constitutes only slightly above 5% of the volume of eggs of sea trout, *Salmo trutta* L. (an autumn spawner) (V = 78.5 ± 6.06 ($\bar{x} \pm s$) mm^3) (Bonisławska and Winnicki 2000).

It is the small size of ruffe eggs and egg cells, translating into low values of *S/V* ratio (the ratio between the surface area of an egg and its volume), that provide the developing egg (at the time of increasing temperature) with the most convenient conditions of oxygen diffusion, which as commonly known is a necessary and indispensable condition of normal development of an embryo (Bonisławska and Winnicki 2000).

Smaller size of ruffe eggs (obtained from the mixture of eggs from many females) obtained in the present study, compared to the results of Kryžanovskij et al. (1953) and Kováč (1993) can be explained by size diversification of eggs from individual females (Bonisławska et al. 2000) and also by size differences in roe deposited by different stocks of ruffe, inhabiting different water bodies of Europe (Table 5).

Very large perivitelline space, constituting in ruffe as much as 60% of the egg volume provides more beneficial oxygen conditions, because it does not restrain movements of the developing embryo. The movements contribute to mixing of the perivitelline liquid enhancing the availability of oxygen by the epithelium, responsible for diffusion of this gas.

Kryžanovskij et al. (1953) presented a similar value of the perivitelline space for ruffe (55.2%), whereas Kováč (1993) determined that the size of the perivitelline space was only 34.3% (Kováč 2000). The latter value is very similar to that of the Danube streber (*Zingel streber*). The above-mentioned diffreences may be caused by the fact that the measurements of fertilised eggs should be carried out when the water intake process is completed. Only such approach ensures accurate data on the size of egg and egg cell. In the paper of Kováč (1993) there is no indication about the details when the measurements were carried out (Table 5).

In the egg of ruffe the fat, in the form of one larger and a few smaller droplets inside the vitelline sphere, is not integrated with the embryonic disc (similarly as in perch), constitutes as much as 15% of the cell volume, which is ca. 6% of the whole egg volume. This fat, despite that it takes up such large volume of yolk is not utilised during the embryogenesis. Only in the later period it constitutes a resource material

for newly-hatched specimens and, on the other hand it helps them to maintain themselves in the water column.

The course of embryogenesis, embryonic motorics

Lateral position of the embryonic disc, observed in ruffe, can also be observed in perch (Korzelecka et al. 1998) and is, as it seems, characteristic for the family Percidae. Such position results from the fact that the structural fat is not integrated with the embryonic disc. As a consequence the shield drops down on the side until it is stopped by the inner side of the egg shell. This may be an evidence for marine origin of this fish, when the shield was located on the bottom of the vitelline sphere and the fat played another role.

Different water temperatures accompanying the embryonic development affect the functioning of (initially) primordia and later on—the fully-formed heart causing acceleration at temperatures exceeding 20°C and deceleration at lower temperatures (14°C) (Fig. 5). In the range of higher temperatures, as commonly known, metabolic processes in the poikilothermal animals go faster (van't Hoff–Arrhenius law stating that the temperature increase by 10°C is accompanied by an increase in reaction speed of 2–4 times) (Embody 1934, Romanenko 1980, Ivleva 1981, Vinberg 1983). In warmer water, however, the amount of available oxygen declines which forces the heart of an embryo to work faster. Inverse situation can be observed when the embryos develop at lower temperatures.

The last type of embryonic movements is "trembling" of an embryo, occurring when embryos prepare to leave the egg shells. Those short, very intensive contractions possibly squeeze out from the glands the hatching enzyme which is subsequently rubbed in the inner side of the egg shell, causing their digestion and thus facilitating later their fracture.

Effect of temperature on duration of embryonic development

The presently obtained results on duration of embryogenesis, condition, size characteristics, and the survival rate under diversified thermal conditions of the external environment, indicate that the optimal range of temperatures for embryonic development of ruffe is 16–18°C (Table 2).

Saat and Veersalu (1996) studying the rate of embryonic development of ruffe and perch (from fertilisation to the stage of 4 and 16 blastomeres), under different thermal conditions (6–24°C) ended their observation at the stage of cleavage. A wide temperature range suitable for ruffe eggs 7.3–23°C (6 and 24°C lethal temperatures) determined by them seems to be too wide. As it was visible in our own study, the eggs at 22°C attained the cleavage stage and even gastrulation, but they all died out later (Table 2). Therefore, it seems to be justified, to carry out studies on optimal temperatures throughout the entire period of embryogenesis, i.e. from fertilisation to the moment of leaving the egg shell by the embryos.

Fairchild and McCormick (1996) studied the effect of temperature on the duration of embryonic development of ruffe from fertilisation to the stage of free-swimming larvae.

Table 5

Duration of embryonic development of ruffe under different thermal conditions; size characteristic of eggs, egg cells, and fat droplets, and also specimens obtained from eggs incubated at various temperatures (according to different authors)

Author	Diameter [mm]			Incubation temperature [°C]	Duration of development			Length of larvae [mm]	Ocular pigment	Survival rate [%]
	Eggs	Egg cells	Fat droplets		Days	Degree hours	Degree days			
Kryžanovskij et al. 1953	1.07–1.23 $\bar{x}$ = 1.15	0.79–0.97 $\bar{x}$ = 0.88	0.39 – 0.45 $\bar{x}$ = 0.41	15–16	4.5	1620–1728	67.5–72	3.8	Very weak	—
				20–21	5.583	2680–2814	111.7–117.2	4.29	Absent	—
Kováč 1993	0.97–1.07 $\bar{x}$ = 1.02	0.78–0.92 $\bar{x}$ = 0.85	0.40–0.45 $\bar{x}$ = 0.425	(16.2 – 23) $\bar{x}$ = 19.4	5.083	2366.8	98.61	3.65	Absent	—
Fairchild and McCormick 1996	—	—	—	6	27–29	3888–4176	162–174	—	Absent	2.5
				11	12	3168	132	—	Absent	55
				16	5.6	2150.4	89.6	—	Very weak	57.5
				21	4	2016	84	—	Strong	48.8
Present study (2001)	0.86–1.00 $\bar{x}$ = 0.94	0.55–0.80 $\bar{x}$ = 0.70	$\bar{x}$ = 0.36	14	6.91	2322	96.74	3.7	Absent	13
				16	5.29	2032	84.64	3.85	Very weak	15
				18	4.18	1805	75.24	3.67	Mild	10
				20	2.99	1434	59.75	3.52	Absent	7
				22	—	—	—	—	—	0

The study was carried out in the estuary of the St. Louis River in North America and the temperature range was very wide. Despite, however, such diversified thermal conditions they observed very high survival rate exceeding 48%, at temperatures of 11–21°C (Table 5). In the present study the highest survival rate at the stage of hatching was only 15% at 16°C (Table 5). Those differences, concerning not only the wider temperature range suitable for embryonic development, but also increased survival rate of the larvae hatched may be caused by the geographic and climatic differences and in general by the local environmental conditions.

Moreover, such diversified survival ratio of the ruffe larvae can have been influenced by the size and age of the respective female brood-fish as well as by their growth rate, as it was emphasized by Vladimirov (1965, 1975).

The temperature of biological zero, defining the temperature where the embryonic development ceases was determined in the present study as 9.68°C. This value was higher than the bottom lethal temperature determined for ruffe by Saat and Veersalu (1996) and than the lowest temperature reported for development of ruffe (with only 2.5% survival) by Fairchild and McCormick (1996) (Table 5). The above differences may be caused by the fact that we failed to study the embryonic development at temperatures below 14°C.

Observations of Kryžanovskij et al. (1953) indicate that the temperature range for the embryonic development of ruffe in this part of the world is slightly shifted towards higher temperatures as the larvae staying inside egg the longest were incubated at 20–21°C (Table 5).

This may be explained by the variable time of spawning of ruffe at different water bodies of different specific conditions (e.g. the Don River—spawning at the end of April at 11°C; Gluboke Lake near Moscow—spawning in May—July at 16–22°C) (Kryžanovskij et al. 1953).

Effect of the incubation temperature on the condition of newly-hatched larvae

The temperature of egg incubation, as it is evident from the presently described study, as well as from published data, affects in a significant way, the size parameters of the specimens hatched such as: weight, body length, size of the gall bladder. It also affects the survival rate of the specimens hatched (Table 4) (Kaur et al. 1986, Jonassen et al. 1999, Bonisławska 2001, Bonisławska and Winnicki 2002), and, among other things, also development of the myocytes (Pavlov 1985, Pavlov and Sadrin 1998) embryonic motorics (Korzelecka 1999), and bioenergetics of the embryos (Kamler 1992, Kamler et al. 1998).

The temperature also affects the time of pigment occurrence on individual body parts and in eyes (Tables 1, 5). Similarly as in other fish species (Lecyk 1965, Soin 1968, Timošina 1972, Kaur et al. 1986, Bonisławska 2001) the most intensive body pigment and pigment in the eyes appeared in embryos developing at optimal temperatures. It is probably caused by the fact that in excessively high temperatures

the developmental processes in embryos are directed to a quickest attainment of the stage enabling emergence from the egg shells and change of the environment for the one providing more life-supporting oxygen.

Ocular pigmentation, as has been demonstrated, is indispensable for the embryos for vacating their egg and free living in the water column. Too low temperature (14°C) retarding all developmental processes causes the emergence of some kind of "premature larvae" without ocular pigment and with delicate body coloration.

The differences in time of appearance of ocular pigment in larvae developing under different thermal conditions and coming from other climatic zones (Kryžanovski et al. 1953, Fairchild and McCormick 1996) can be probably explained by slightly different temperature ranges optimal for embryonic development of ruffe in different parts of the world.

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Received: 12 November 2004
Accepted: 18 June 2004