Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus *Leuciscus* (Cyprinidae, Teleostei)

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ABSTRACT: The aim of this study was to determine the effects of variable and constant thermal conditions on embryonic development of three endangered fish species from the genus *Leuciscus*. Wild living spawners of dace *Leuciscus leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) were obtained from rivers belonging to the Pasleka River basin (northeastern Poland) and next transported to a hatchery for the purpose of conducting artificial reproduction. The obtained eggs were fertilized and next incubated under a variety of thermal protocols: slow gradual heating of water (M1), rapid increase in temperature at the end of incubation (M2), fluctuating temperature (M3) and two constant temperatures (optimum and sublethal). Variable thermal changes of water were also continued after hatching, up to the yolk sac resorption by larvae. During the study it was shown that relative to incubation at optimal temperatures (12.3°C for dace, 15.7°C for ide and 19.0°C for chub), thermal modifications had no clear influence on a decrease in survival rates (differences among regimes did not exceed 5%) and increase in developmental deformations (differences below 1.0%) observed among the hatched embryos. The duration of egg incubation and developmental rate increased with increasing temperature. In the systems with modified temperature the embryonic development of dace (from fertilisation to commencement of exogenous food intake) took from 9.5 to 22.5 days, for ide from 6.1 to 12 days and for chub from 5.0 to 10.5 days. The yolk sac resorption stage in the particular species occurred from 11.7 to 23 days for dace, 7.5 to 13.2 days for ide and 5.5 to 12.8 days for chub. Different time of hatching was also reflected in the level of ontogeny of hatched embryos. Among the fish hatched at modified temperatures the largest sizes, similar to those characteristic of fish incubated at optimum temperatures, were observed in individuals originating from variants where the temperature fluctuated. The developmental disproportions among the embryos of studied species originating from different thermal regimes observed at the time of leaving the egg shells were definitely larger than during the following development stages. This study also confirmed that the embryos of studied species can adapt to increasing water temperature due to global warming up to 19.0 (dace), 23.0°C (ide) and 27.5°C (chub). The obtained results are very important not only for practical purposes but also from physiological and ecological aspects.

Keywords: variable temperatures; incubation time; hatching rate; abnormalities; embryonic development; *Leuciscus*

In recent years, despite a certain deceleration of the process, evident decrease or even extinction of many local populations of the majority of rheophilic fish species including dace *Leuciscus leuciscus* (L.), ide *L. idus* (L.), and chub *L. cephalus* (L.) – the only representatives of the genus *Leuciscus* in Polish ichthyofauna – has still been observed. Invariably, the major reasons for that situation are

Supported by the State Committee for Scientific Research (Project No. 2PO4F07727).

the pollution of waters, transformation of habitats and strong pressure from anglers (Saunders et al., 2002; Penczak et al., 2004; Witkowski et al., 2004; Bolland et al., 2008). Production of stocking material based on reproduction, incubation and rearing under controlled conditions together with improvement of environmental conditions gives a possibility of protection of endangered fish species from river ecosystems (Kujawa et al., 1997; Shiri Harzevili et al., 2003, 2004; Krejszeff et al., 2008, 2009; Kucharczyk et al., 2008a,b; Targońska et al., 2008a,b; Żarski et al., 2008a,b; Wolnicki et al., 2009). The rearing of young fish under controlled conditions is the more effective, the more precisely the influence of environmental conditions on the body – relatively sensitive at that time – is determined (Wolnicki, 2005). Temperature is the environmental factor with the largest influence on the development of fish (Herzig and Winkler, 1986; Kamler, 1992, 2002; Kupren et al., 2008a). Its influence on the incubation of dace, ide and chub eggs was described by numerous authors in their papers (Kennedy, 1969; Penaz and Sterba, 1969; Florez, 1972; Mills, 1980; Economou et al., 1991; Calta, 2000; Rechulicz et al., 2002). Kupren et al. (2008a,b, 2010) investigated various aspects of embryogenesis of all those three species under constant thermal conditions. The papers cited above are the source of numerous interesting observations concerning the biology of those species, in the majority of cases, however, made under a wide range of constant thermal conditions. The influence of variable temperatures on the developing fish body was discussed more frequently in the literature devoted to species which have larger economic significance and which encounter large fluctuations in temperatures in the area of their presence during the period of reproduction (Kokurewicz et al., 1988; Wiegand et al., 1989; Dostatni and Łuczyński, 1991; Korwin-Kossakowski, 2008). Data concerning the thermal preferences of the different fish species is also very important in the context of overall warming of the water due to climatic changes. Understanding the effects of rising temperature (especially spring warming) on plant and animal populations, community structure and ecosystem functioning is one of the challenges of modern ecology (Daufresne and Boët, 2007; Moran et al., 2009).

In view of existence of numerous ambiguities presented above, this study aimed at determining the effect of constant and variable thermal conditions on the result of eggs incubation, embryonic development rates and changes in length and volumes of yolk sacs of the hatched embryos and larvae of three *Leuciscus* species.

MATERIAL AND METHODS

Origin of spawners and their reproduction

Spawners of dace, ide and chub were obtained from rivers belonging to the Pasleka River drainage basin (northeastern Poland) at different times before natural spawning (February–June). After collection the fishes were transported to a hatchery of the Department of Lake and River Fishery of the University of Warmia and Mazury and placed in 1000 dm^3 separate tanks with the possibility of thermal regulation, aeration and controlled photoperiod (Kujawa et al., 1999). For the purpose of spawning synchronization, the females and males of each species (dace: 13 females of body weight 240–295 g and 10 males 80–280 g, ide: 12 females of 780–1150 g and 14 males of 300–650 g and chub: 14 females of body weight 300–390 g, and 12 males of body weight 160–350 g) were subjected to hormonal stimulation. In the case of all the species Ovopel (Unic-Trade, Hungary) was used in two doses of 0.2 and 1.0 granules per kg as the preparation stimulating maturation. The interval between the injections was 24 h for dace and ide and 12 h for chub. Following the second hormonal injection the water temperature in the tanks with spawners was increased to 12.0°C for dace, 14.5°C for ide and 18.0°C for chub. These temperatures often occur during spawning (Mann, 1996) and are recommended for reproduction conducted under controlled conditions (Kucharczyk et al., 2008a; Kupren et al., 2008a, 2010). Before manipulations, spawners were anaesthetised in a solution of 2-phenoxyethanol (0.5 mg/dm^3) (Sigma-Aldrich, Steinheim, Germany). Milt was collected with plastic syringes and kept at 4°C before further treatment. Females were checked every three hours between the 20^{th} and 48^{th} h after resolving injections. Eggs were collected to plastic vessels and were next fertilised using the dry method with pooled sperm collected from at least a few males.

Conditions of incubation and rearing of free swimming embryos and larvae

Fertilised eggs of the three studied species were incubated at three modified (variable) and two

Table 1. Characteristics and marking of thermal modifications applied during eggs incubation and rearing of hatched embryos and larvae of three fish species of genus *Leuciscus*

Thermal variant	Method of conducting the thermal modification
Constant optimum temperature (C1)	Eggs and free swimming embryos are maintained all the time in constant optimum temperature (dace – 12.3; ide – 15.7; chub – 19.0°C)
Fluctuating temperature (M1)	Eggs and free swimming embryos as of 24 h after fertilization are subjected to periodic thermal fluctuation, i.e. every 24 h alternately increase and decrease of temperature by 2°C from the optimum temperature (C1)
Rapid change of temperature just before hatching (M2)	Until eye pigmentation the eggs are incubated at optimum constant temperature. At reaching that stage it is subject to rapid temperature increase by 2°C each day up to reaching the temperature lower by 1°C than the sublethal (C2)
Radual, slow temperature increase (M3)	Eggs and free swimming embryos as of 24 h after fertilization is subjected to water temperature increase by 1°C each day up to reaching the temperature lower by 1°C than the sublethal (C2)
Constant upper tolerated (sublethal) temperature (C2)	Eggs and free swimming embryos are maintained all the time in constant sublethal temperature (dace – 19.0; ide – 23.0; chub – 27.5°C)

constant temperatures (optimum and sublethal). Individual thermal variants were set on the basis of incubation progress one year earlier at a wide range of constant temperatures (Kupren et al., 2008a) (Table 1). The optimum temperature ensured the highest survival rate until hatching and the embryos that left the egg shells were the best developed. The upper tolerated temperature (sublethal temperature) was characterised by an evident decrease in egg survival rate (< 50%) and the hatched embryos were the poorest developed (Kupren et al., 2008a, 2010). The time of thermal adaptation to the given constant temperature was 1.5°C/h.

Each experimental variant consisted of two lighted and aerated 40-litre aquaria equipped with thermal control and submerged in the 1000 dm^3 tank with water. In each of the aquaria the eggs were incubated on two Petri dishes (150–180 eggs/dish) and after separation using a talk solution in a basket of fine nylon mesh (approx. 800 eggs). Eggs incubated on dishes allowed to precisely determine survival, deformation rate and time of incubation. Eggs and specimens in baskets were used to identify developmental rate.

Water temperature during incubation was measured to the nearest 0.1°C four times a day. Changes in the temperature during the experiment rarely exceeded 0.3°C. In order to ensure good and stable conditions of incubation (oxygen saturation > 80%, ammonia and nitrite < 0.1 and 0.05, respectively) the water in the aquaria was changed daily (min 50% of the volume).

Hatching success in individual experimental groups was expressed as the ratio of hatched, normally developed embryos to the number of incubated eggs. The percentage of hatched, abnormal embryos (abnormalities) was also recorded. The duration of hatching was calculated as the difference between the moment of hatching of the last individuals (95% hatched) and appearance of the first individuals (5% hatched) (Kamler et al., 1998).

At the eyed-egg stage, the dishes with incubated eggs were placed in baskets of fine mesh where they stayed until the end of the experiment, i.e. resorption of yolk sacs by the fish. Constant incubation temperatures remained unchanged while in the case of modified temperatures the changes in water thermal conditions characteristic of a given experimental variant were continued. Until head straightening the larvae were fed live *Artemia* sp. nauplii.

Monitoring of embryonic development

For the purpose of determining and comparing the development of dace, ide and chub during the earliest stages of ontogenesis, 13 relatively easily recognizable development stages covering the time between fertilization and yolk sack resorption were selected and identified by numbers from 0 to 13 (Kupren et al., 2008a).

Sampling of minimally 7 individuals was done at regular, gradually increasing time intervals. During the first day eggs samples were collected every 2–4 h. Until the time of pigment appear-

ance in embryos' eyes that intensity was 6 times a day. From that moment to the resorption of yolk sacks by the fish, samples were collected every 6 hours. The embryos collected were preserved in 4% formaldehyde solution (Takizawa et al., 1994) and placed in separate tubes. The observation of embryonic development was carried out under a stereoscopic microscope and the time taken for 50% of the embryos to reach the given stage of development was recorded (Luczyński and Kirklewska, 1984; Gadomski and Cadell, 1996).

Morphological measurements after hatching

At the moment of hatching, at the beginning of exogenous feeding and at the resorption of yolk sacks 30 individuals were sampled from each replication for morphological measurements. Fish were scanned using DP-Soft software from SZ CPV Olympus stereoscopic microscope mounted with Olympus DP 12 digital camera connected to a computer. The total length, height and length of the yolk sack were measured to the nearest 0.01 mm. The measurements of the yolk sack were used for determination of its volume (Blaxter and Hempel, 1963).

Statistical analysis

Differences between groups in the embryonic and larval total length and volume of yolk sac were analysed by analysis of variance (ANOVA) and Duncan's *post hoc* test ($\alpha = 0.05$). The percentage of survival and abnormalities was normalised using arcsine transformation (Sokal and Rohlf, 1969). The differences were regarded as significant at $P \leq 0.05$.

RESULTS

Hatching success and abnormalities

In the incubation variants where constant temperatures and thermal modifications were applied, the survival of dace eggs was from 52.2% to 55.8% and the percentage of abnormal embryos ranged from 2.0% to 2.9% (Table 2). The hatching rates for ide were 60.7–86.8% (Table 2). The percentage of developmental deformations among the embryos of that species was from 1.3% to 10.7% (Table 2). In the case of chub those ranges were 31.6–92.8% and 2.9–28.4%, respectively (Table 2). The survival rates obtained after incubation in systems where

Table 2. Effect of incubation temperature on the survival to hatch (hatching success) and abnormalities of dace (*L. leuciscus*), ide (*L. idus*) and chub (*L. cephalus*) eggs conducted at constant and modified temperatures

Species	Thermal variant	Hatching succcess (%)	Abnormalities (%)
L. leuciscus	C1	54.5 ± 2.1[a]	2.1 ± 0.2[a]
	M1	54.0 ± 6.6[a]	2.1 ± 0.2[a]
	M2	55.8 ± 2.4[a]	2.9 ± 0.3[a]
	M3	52.2 ± 2.5[a]	2.5 ± 0.3[a]
	C2	55.5 ± 5.8[a]	2.0 ± 0.1[a]
L. idus	C1	86.8 ± 4.8[a]	1.3 ± 0.2[b]
	M1	82.4 ± 2.8[ab]	1.3 ± 0.3[b]
	M2	81.9 ± 1.7[b]	1.9 ± 0.3[b]
	M3	83.5 ± 3.0[ab]	1.4 ± 0.1[b]
	C2	60.7 ± 7.3[c]	10.7 ± 1.7[a]
L. cephalus	C1	92.8 ± 0.7[a]	2.9 ± 0.2[b]
	M1	89.5 ± 1.0[a]	3.1 ± 0.2[b]
	M2	89.4 ± 1.9[a]	3.9 ± 1.0[b]
	M3	90.4 ± 1.5[a]	3.0 ± 0.2[b]
	C2	31.6 ± 3.8[b]	28.4 ± 2.7[a]

Means (± SD) in the same column with different letters are significantly different ($P < 0.05$)

variable water thermal conditions were applied (M1, M2, M3) did not differ significantly from the values recorded for optimum temperatures (12.3°C for dace, 15.7°C for ide and 19.0°C for chub) (Table 2). During the incubation of ide and chub eggs at sublethal temperatures (for those species 23.0 and 27.5°C, respectively) the definitely lower survival rates were recorded than in the variants with modified temperatures. In the case of dace the values obtained from individual treatments were not significantly different from each other (Table 2).

Duration of incubations, hatching time and rate of embryonic development

The hatching of 50% of dace individuals occurred between day 7 (group C2) and day 19 (group C1) of incubation and took from 3 (C2) to 5.2 days (M1) (Figure 1a). Ide hatched between day 3 (C2) and day 7 (C1) (Figure 1b). The duration of hatching period in the case of this species was from 1.0 (M3) to 2.7 days (M1) (Figure 1b). In the case of chub a half of the hatched embryos could be observed after 1.5 days (C2) at the earliest, and after 4.5 days since fertilization (C1) at the latest (Figure 1c). Duration of hatching was from 0.5 day (M2) to 1.2 days (C1) (Figure 1c).

In the systems with modified temperature the embryonic development of dace (from fertilization to commencement of exogenous food intake) took from 9.5 to 22.5 days, for ide from 6.1 to 12 days and for chub from 5.0 to 10.5 days. The yolk sack resorption stage in the case of individual species occurred from 11.7 to 23 days for dace, 7.5 to 13.2 days for ide and 5.5 to 12.8 days for chub (Figure 2).

In the case of all species, the incubation times until hatching and reaching individual developmental stages were the shortest at constant sublethal temperature (C2) and the longest at the controlled optimum temperature (C1). In systems where temperature variations were applied, both hatching (50% hatched) and completing the embryonic period (beginning of exogenous feeding) occurred the fastest in the system where the water temperature was increased by one degree per day (M3), next in the variant where after eye pigmentation the temperature increased by two degrees per day (M2), and the longest in the system with fluctuating temperature (M1) (Figures 1 and 2).

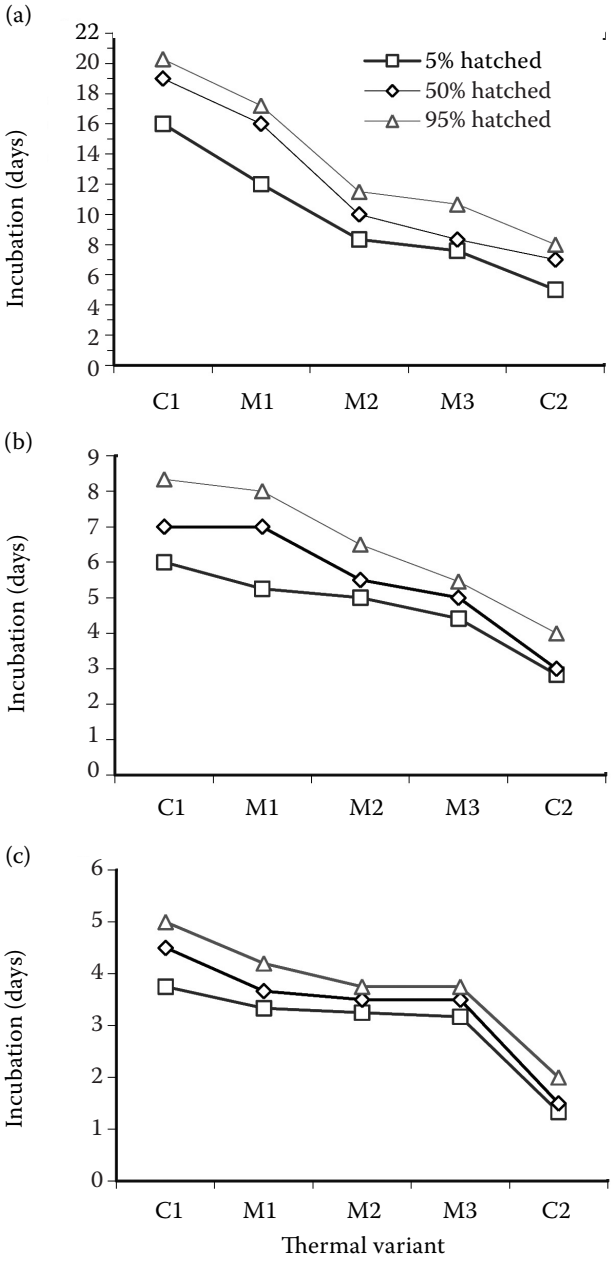

Figure 1. Time (in days) to hatch 5, 50, and 95% of dace (*L. leuciscus*) (a), ide (*L. idus*) (b) and chub (*L. cephalus*) (c) embryos at different thermal regimes

Changes in total length and yolk sac volume of fish after hatching

The average length of hatched dace embryos ranged between 6.63 and 8.30 mm. In ide embryos it ranged from 6.18 to 7.13 mm, and in chub from 4.20 to 5.90 mm (Table 3). In all three studied species the length of hatched embryos was directly proportional to the time of incubation. As a consequence, the longest embryos were obtained following incu-

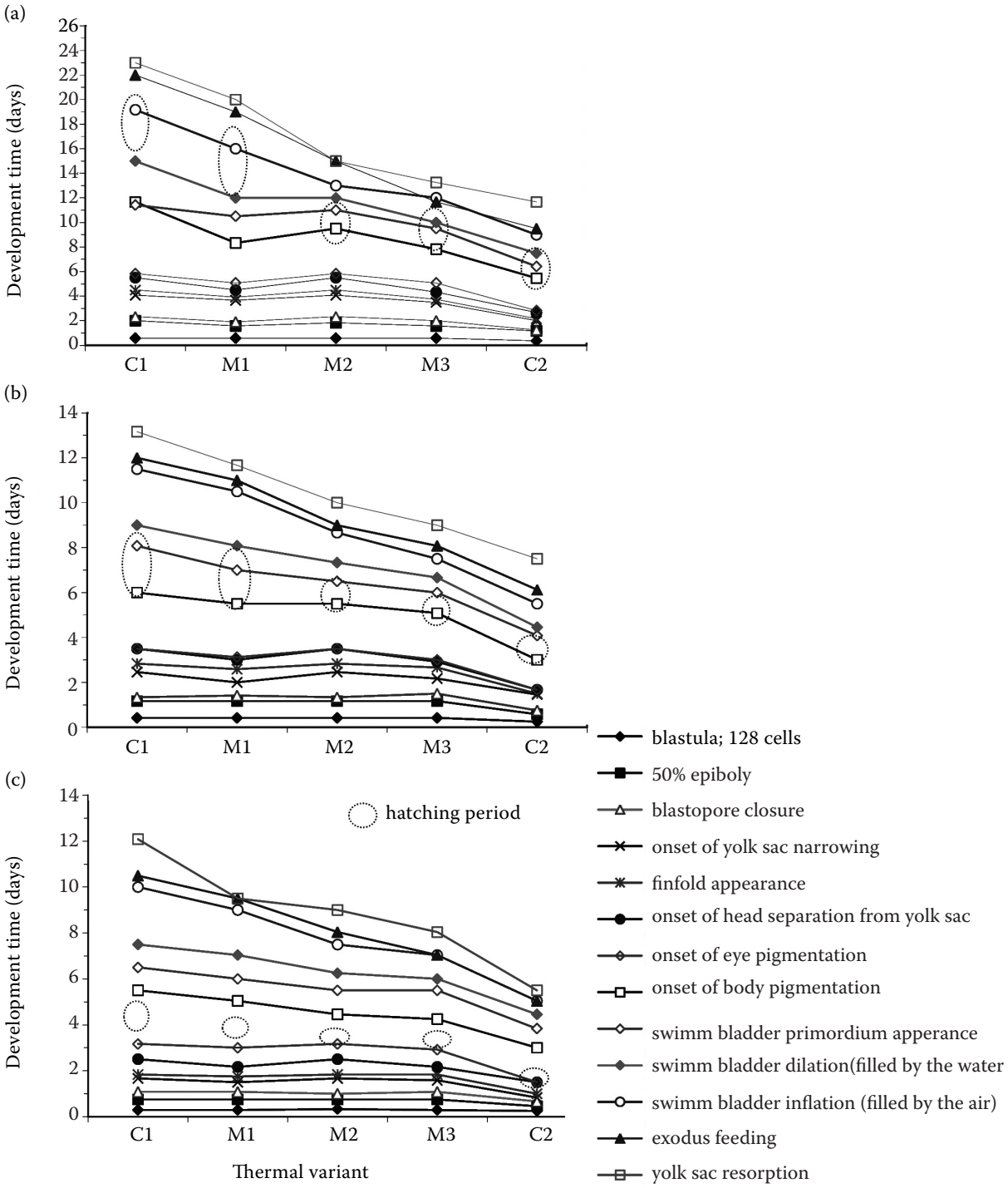

Figure 2. Time (in days) for attaining different ontogenic stages of dace (*L. leuciscus*) (a), ide (*L. idus*) (b) and chub (*L. cephalus*) (c) embryos and larvae (until yolk resorption) at different thermal variants

bation in group C1 and the shortest in group C2. In the groups with variable thermal conditions, the longest embryos originated from the system with fluctuating temperature (M1). Total length of dace and ide embryos hatched from that system did not differ significantly from the length that characterized individuals obtained at optimum temperature (C1). Fish hatched in the thermal variant in which the temperature was changed by one degree per day after hatching (M3) and in the system where after eye pigmentation the temperature was increased by two degrees per day (M2) were slightly shorter.

Table 3. Mean total length and volume of the yolk sacs dace (*L. leuciscus*), ide (*L. idus*) and chub (*L. cephalus*) embryos and larvae at the moment of hatching, exogenous feeding and resorption of the yolk sac of 50% of individuals

Species	Thermal variant	Hatching		Exogenous feeding		Yolk sac resorption total lenght (mm)
		total lenght (mm)	yolk sac volume (mm^3)	total lenght (mm)	yolk sac volume (mm^3)	
L. leuciscus	C1	8.30 ± 0.18^{a}	0.57 ± 0.15^{b}	8.67 ± 0.27^{a}	0.00 ± 0.01^{c}	8.72 ± 0.23^{a}
	M1	8.03 ± 0.09^{a}	0.28 ± 0.11^{c}	8.42 ± 0.28^{ab}	0.13 ± 0.11^{b}	8.66 ± 0.24^{a}
	M2	6.54 ± 0.10^{c}	1.29 ± 0.36^{a}	8.19 ± 0.37^{b}	0.07 ± 0.06^{b}	8.18 ± 0.28^{b}
	M3	7.01 ± 0.11^{b}	1.28 ± 0.24^{a}	8.18 ± 0.28^{b}	0.08 ± 0.13^{b}	8.54 ± 0.33^{a}
	C2	6.63 ± 0.10^{c}	1.04 ± 0.19^{a}	7.74 ± 0.32^{c}	0.24 ± 0.11^{a}	8.22 ± 0.33^{b}
L. idus	C1	7.13 ± 0.09^{a}	1.23 ± 0.25^{c}	8.41 ± 0.24^{a}	0.18 ± 0.06^{b}	8.65 ± 0.20^{a}
	M1	7.10 ± 0.04^{a}	1.14 ± 0.18^{c}	8.38 ± 0.29^{ab}	0.14 ± 0.12^{b}	8.78 ± 0.22^{a}
	M2	6.34 ± 0.0b^{c}	1.60 ± 0.14^{ab}	8.12 ± 0.24^{b}	0.10 ± 0.06^{b}	8.73 ± 0.20^{a}
	M3	6.46 ± 0.05^{b}	1.47 ± 0.22^{b}	8.29 ± 0.17^{ab}	0.12 ± 0.10^{b}	8.57 ± 0.26^{a}
	C2	6.18 ± 0.07^{c}	1.70 ± 0.17^{a}	7.71 ± 0.25^{c}	0.28 ± 0.16^{a}	8.21 ± 0.49^{b}
L. cephalus	C1	5.90 ± 0.07^{a}	1.03 ± 0.11^{b}	7.85 ± 0.16^{ab}	0.07 ± 0.05^{ab}	8.15 ± 0.24^{a}
	M1	5.63 ± 0.11^{b}	1.10 ± 0.08^{b}	7.94 ± 0.12^{a}	0.00 ± 0.00^{b}	7.94 ± 0.12^{ab}
	M2	5.3 ± 0.05^{c}	1.37 ± 0.10^{a}	7.62 ± 0.08^{b}	0.06 ± 0.04^{ab}	7.80 ± 0.11^{b}
	M3	5.53 ± 0.06^{bc}	1.19 ± 0.28^{ab}	7.63 ± 0.2^{b}	0.01 ± 0.01^{b}	7.92 ± 0.09^{ab}
	C2	4.20 ± 0.03^{d}	1.37 ± 0.24^{a}	7.42 ± 0.15^{c}	0.12 ± 0.10^{a}	7.50 ± 0.08^{c}

Means (± SD) in the same column with different letters are significantly (Duncan test $P < 0.05$) different

Only dace embryos originating from the two systems described above differed significantly from each other in total length (Table 3). In the three variants where the water temperatures reached the highest values (C2, M2, M3), the volumes of yolk sac of hatching embryos did not differ significantly. The fish obtained from the other treatments had evidently smaller yolk sacs. These differences were significant (Table 3).

At the commencement of exogenous feeding the range of the lengths of free dace embryos reared at modified temperatures was from 7.74 to 8.67 mm, ide from 7.71 to 8.41 mm and chub from 7.42 to 7.94 mm. As it concerns the yolk sac volume, those ranges were 0.00–0.24, 0.10–0.28 and 0.00 to 0.12 mm^3, respectively (Table 3). The length of individuals of all the three species, similarly like the size at hatching, was the largest at the optimum (C1) and fluctuating (M1) temperatures. The lengths of dace and ide larvae originating from the remaining treatments (M2, M3), although significantly lower than those of embryos kept at optimum conditions, did not differ from those obtained at fluctuating temperatures (M1). Chub embryos in all variants involving thermal changes (M1, M2, M3) did not differ significantly in length from the individuals obtained at the optimum temperature (C1). In all species, at the commencement of exogenous food intake, the smallest larvae with the largest yolk sacs originated from the constant limit temperatures (C2). Individuals from the other treatments had similar yolk volumes (Table 3).

At the moment of yolk resorption, the range of the lengths of dace larvae originating from constant and modified temperatures was 8.18–8.72 mm, ide 8.21 to 8.78 mm and chub 7.50–8.15 mm (Table 3). At the time of yolk sac resorption, the so far observed clear differences in total length achieved by larvae originating from different systems were not so evident any more (Table 3). Only the lengths of fish from the upper tolerated temperature (C2) differed significantly from those observed for individuals originating from the other studied variants (Table 3).

In general at corresponding systems of modified temperatures dace embryos were the longest at hatching while the chub embryos were the shortest. Dace had the smallest yolk sac at that time. These values were similar in ide and chub. At the commencement of exogenous food intake and at the resorption of yolk sac the embryos and larvae of ide

were characterized by very similar size compared to the size of young dace individuals. Their length was clearly larger than that of chub (Table 3).

DISCUSSION

The results of studies conducted on a relatively large group of species and devoted to the influence of constant thermal conditions on various aspects related to the embryonic development of fish indicate first of all that the water temperature influences the survival of incubated eggs directly (Jungwirth and Winkler, 1984; Herzig and Winkler, 1986; Kucharczyk et al., 1997; Kujawa et al., 1997; Kamler, 1998, 2002; Rechulicz et al., 2002). The range of temperatures favourable for embryonic development is usually linked tightly to the reproduction temperature of a given species or population. The survival of eggs until hatching depends on the water temperature in quite a characteristic way. The highest survival rate is observed within certain ranges of temperatures (optimum temperatures) while above and below them it decreases rapidly (Kokurewicz, 1969, 1997; Herzig and Winkler, 1986; Kucharczyk et al., 1997; Bermudes and Ritar, 1999). Embryonic mortality of fish eggs is particularly high until the blastopore is closed. The earliest ontogenetic stages (cell cleavage, epiboly) are sensitive morphogenetic periods due to forces involved in the cell migration process. High mortality is caused not only by a temperature but also by a low oxygen level or physical shock (Blaxter, 1969; Bermudes and Ritar, 1999). Our results and data from other studies (Florez, 1972; Kupren et al., 2008a, 2010) indicate that all the studied species, similarly like other rheophilic cyprinids e.g. *Vimba vimba* or *Chondrostoma nasus* (Kamler, 2002), have a very similar range of tolerated incubation temperatures. This range is about 6–8°C above and below optimal thermal conditions. A wider range of tolerated temperatures is generally characteristic of fish that inhabit enclosed freshwater bodies (e.g. bream *Abramis brama* or tench *Tinca tinca*) and encounter larger fluctuations in temperatures during the period of reproduction (Kucharczyk et al., 1997; Kamler, 2002; Moran et al., 2009). The range of tolerated temperatures for the embryonic period may differ in particular populations and might reflect adaptations of different fish populations to specific local environmental conditions (Kucharczyk et al., 1997). A good example of this thesis is dace. Our results show the significantly lower sensitivity of dace embryos to a high temperature than reported by Mills (1980) for fish from English rivers.

With the decrease in hatching success at extreme temperatures the incidence of abnormalities increases significantly (Stott and Cross, 1973; Wiegand et al., 1989; Kucharczyk, et al., 1997; Kupren et al., 2010). During the conducted studies it was observed that the percentage of deformations among the fish hatched after incubation at modified temperatures was similar to that observed at optimum temperatures and definitely (even) several times lower than at sublethal temperatures. The dace was an exception. In this species no significant differences were observed in the survival rates of hatched embryos and in the percentage of abnormal embryos depending on the incubation variant. The reason for that situation was probably different thermal tolerance of embryos originating from different parts of Poland. The range assumed for the studied population originating from northern Poland was different from that for the population originating from a river in the centre of the country (the Pilica River basin) studied a year earlier. Then the upper limits up to which the water temperature in variants M2 and M3 was increased were established. In the incubation of eggs originating from the northern population 19.0°C was the extreme temperature at which live embryos were obtained, while for the individuals obtained during the second year it was higher, 23.0°C (Kupren et al., 2008a, 2010). Abnormalities observed in all treatments in this study were usually so severe (pronounced spinal deformity, ballooning of the abdominal cavity) that these fish would not survive in the wild.

Water temperature also influences the moment of embryo hatching and the level of their ontogenetic development. An increase in incubation temperature shortens the time until hatching (Wiegand et al., 1988; Korwin-Kossakowski, 2008; Kupren et al., 2008a). The ontogenic stage of hatched embryos, and as a consequence their body length and volume of yolk sac, depend on water temperature in quite a characteristic way. The best developed fish with the largest body length and smallest yolk sack hatch at temperatures within the optimum range. At other temperatures the individuals that left the egg membranes usually show less advanced ontogenic development (Kokurewicz, 1969, 1970; Kupren et al., 2008a). The reason for faster hatching of embryos at higher temperatures is their increased mobility in warmer water and earlier excretion of

the hatching enzyme. These characteristic behaviours of embryos incubated at higher temperatures were common in numerous fish species (Blaxter, 1969, 1992; Penaz, 1974; Kamler, 1992). Also in this study, after the application of thermal modification during the earliest stages of ontogenesis, developmental rate and time to hatch, hatching period duration and times required to reach a given ontogenic stage decreased as the temperature increased from an optimum to sublethal level. At the moment of hatching, smaller lengths of embryos originating from regimes where thermal water characteristics were changed, as compared to individuals incubated in optimum conditions, were caused by the fact that the development occurred in water at a higher temperature. The differences in average total length between treatments at this moment reached 1.70 mm (dace) (Table 3). The water thermal characteristics also influenced the size of the larvae during the other stages of early ontogenesis, although the differences observed among treatments as well as among individual species were not so evident. The reason for this finding were undoubtedly similar sizes of eggs [hydrated eggs used in this experiment had the mean ± SD diameter of 2.28 ± 0.12 (ide), 2.23 ± 0.12 (dace) and 1.89 ± 0.06 (chub) mm] and the size of the first exogenous food (*Artemia* sp. nauplii). Slightly larger differences among studied treatments in average total length at the moment of food commencement (max 0.93 mm) than observed at the stage of yolk sac resorption (max 0.65 mm) were caused by water temperature (Table 3). In the case of fish rearing in warm water the first feeding occurs very frequently at a relatively lower consumption of stocks of nutrients contained in the yolk sac. On the other hand, the intake of exogenous food usually occurs at the lowest tolerated temperatures only after complete resorption of the yolk sac. This is undoubtedly related to the increased metabolism of fish in warmer water (Kamler, 1992; Kupren, et al., 2008a).

This study confirms that not excessively rapid changes in thermal characteristics of water during embryonic development, occurring additionally within the range of tolerated temperatures, do not have any negative influence on the developing embryos and larvae (Florez, 1972; Kokurewicz et al., 1988; Bestgen and Williams, 1994). This study also confirms that dace, ide and chub embryos from Polish rivers can accommodate to climatic changes due to global warming up to 23°C and 27.5°C, respectively.

Besides ecological aspects, the knowledge of the influence of temperature during embryogenesis is a prerequisite for successful hatchery production. Practical application of thermal modifications shortens the time of keeping the eggs in incubation devices and contributes to the cut of costs of stocking material production. It also allows faster and more synchronized obtaining of larvae of the size similar to that characteristic of individuals incubated at optimum temperatures.

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Received: 2009-11-02
Accepted after corrections: 2010-10-06

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