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THE INFLUENCE OF WATER TEMPERATURE ON LABORATORY-REARED LAKE MINNOW *EUPALLASELLA PERENURUS* (PALLAS) LARVAE AND JUVENILES

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ABSTRACT. The growth, condition and survival of live food-fed larvae and starter-fed early juveniles of the critically endangered cyprinid *Eupallasella perenurus* (Pallas) were evaluated in two laboratory experiments at 19, 22, 25, and 28°C. Temperature significantly ($P \leq 0.05$) influenced the growth of larvae, with the maximum final values recorded at 25°C (25.8 mm mean total length, TL; 239 mg mean body weight, BW). No significant differences in the final juvenile fish size were found within the temperature range of 19-25°C (37.6-38.4 mm TL; 901-968 mg BW), whereas those at 28°C grew slower (34.6 mm TL; 819 mg BW). The final condition coefficient values tended to significantly increase along with water temperature. Final survival rates were 96.5-100%, except for juveniles at 28°C (28.3%). All of the present results indicate that the most advantageous temperature conditions for *E. perenurus* controlled rearing would be 25 and 22°C for larvae and juveniles, respectively.

Key words: LAKE MINNOW (*EUPALLASELLA PERENURUS*), LARVAE, JUVENILES, TEMPERATURE, GROWTH, SURVIVAL

INTRODUCTION

The lake minnow *Eupallasella perenurus* (Pallas), until recently classified to the genus Phoxinus (see Kottelat 1997), is a small cyprinid fish that can reach a maximum total length of merely 13 cm (Kusznierz 1998). This species inhabits stagnant waters with dense vegetation, always small and shallow, mostly natural small dystrophic lakes or man-made peat-hags and occurs solely or accompanied by the dwarf form of the crucian carp *Carassius carassius* (L.) and/or the tench *Tinca tinca* (L.) (Kusznierz 1998, 2001). Its western distribution border crosses Poland, where it can be found in only a few isolated regions (Witkowski 1992). In Poland, at present, this species is considered to be in critical danger of extinction, so it is included in the Red Data Book

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of Polish Animals (Kusznierz 2001). The rapid disappearance of *E. perenurus* habitats, which has been observed in recent decades, is mainly due to swamp draining, amelioration, deforestation, and filling up field water bodies (Witkowski 1992, Kusznierz 1998, 2001). Active protection of this species, including restoration of its populations in the wild, must be based on good knowledge of its biology; however, this remains highly limited (Kusznierz 1998, Kusznierz et al. 2002). In particular, nothing is known of the effect of water temperature on this species in the first months of life.

The objective of the present study was to elucidate the influence of water temperature on the growth, condition, and survival of *E. perenurus* larvae and early juveniles in order to find the most favorable conditions for their intensive rearing in a controlled environment. Such data are of key importance for developing optimum techniques either for the long-term culture of this fish under controlled conditions or for the indoor mass-production of its stocking material, both for conservation purposes.

MATERIALS AND METHODS

FISH AND REARING CONDITIONS

Ripe, wild breeders of *E. perenurus* were angled in a small, nameless field pool in the vicinity of Gdańsk, northern Poland, shortly before spawning. Immediately afterwards, the fish were transferred to the laboratory and put into a large shadowed tank filled with well-aerated water. On the next day, the standard propagation technique with the use of GnRH analogue (Horváth et al. 1997) was applied to female fish only to obtain eggs. No treatment to eliminate egg stickiness was used, so after fertilization they were incubated in single layers on the aquaria bottom under continuous water flow at 22°C (range ± 0.5°C). Mass hatching took place after approximately 75°D.

The pooled offspring of 9 females and 5 males, 2400 larval fish, were used in experiment A. Horizontally swimming larvae with completely resorbed yolk-sacs aged 8 days post-hatch were stocked at a density of 60 per dm^3 into eight flow-through aquaria with a water volume of 5 dm^3 each. The initial larval total length was 6.06 ± 0.27 mm (mean ± SD), and their mean wet body weight was 1.0 mg. Water temperature while stocking was about 22°C. The larvae were reared for 40 days at stabilized water temperatures of about 19, 22, 25, and 28°C (Table 1), all in duplicate. All target temperature levels were attained within about 10 hours after stocking. From then onwards, water temperature was measured twice a day throughout the experiment.

TABLE 1

Final growth and survival data of *E. perenurus* larvae reared at different water temperatures in experiment A

Group	A19	A22	A25	A28
Water temperature (°C)	19.1 ± 0.1	22.0 ± 0.2	25.1 ± 0.4	27.9 ± 0.3
Total length (mm)	22.6 ± 1.2^{c}	25.3 ± 1.8^{b}	25.8 ± 1.7^{a}	23.0 ± 1.7^{c}
Body weight (mg)	137.8 ± 32.8^{d}	200.5 ± 56.9^{b}	239.0 ± 46.6^{a}	187.0 ± 48.6^{c}
Condition coefficient (K)	1.18 ± 0.17^{d}	1.21 ± 0.09^{c}	1.37 ± 0.13^{b}	1.49 ± 0.13^{a}
Survival rate (%)	98.3^{a}	98.2^{a}	97.2^{a}	96.5^{b}

Temperature and growth data are presented as mean ± standard deviation, n = 80 and 100, respectively. The data in rows which are followed by different superscripts are significantly different at $P \leq 0.05$.

In the 90-day experiment B, eight 20 dm^3 flow-through aquaria were used and each was stocked with 120 *E. perenurus* individuals of initially 20.6 ± 1.5 mm total length and 97.7 ± 24.1 mg wet body weight (mean ± SD). The fish, progeny of one female and two males, were just at the transformation stage from the larval to juvenile period of life (Kamiński et al., unpublished data). Before experiment B commenced, they were reared at 22°C (range ± 0.5°C), from first feeding (day 8 post-hatch), in a single 20 dm^3 aquarium for 40 days. Just as in experiment A, four water temperatures were employed in experiment B (Table 1 and 2, respectively) all in duplicate. The target temperature levels were achieved within a period of three hours after stocking.

TABLE 2

Final growth and survival data of *E. perenurus* juveniles reared at different water temperatures in experiment B

Group	B19	B22	B25	B28
Water temperature (°C)	19.1 ± 0.2	22.0 ± 0.2	25.0 ± 0.2	27.9 ± 0.3
Total length (mm)	37.6 ± 3.3^{a}	38.4 ± 2.6^{a}	38.0 ± 3.5^{a}	34.6 ± 3.9^{b}
Body weight (mg)	901 ± 275^{ab}	919 ± 187^{ab}	968 ± 269^{a}	819 ± 317^{b}
Condition coefficient (K)	1.65 ± 0.21^{bc}	1.60 ± 0.11^{c}	1.72 ± 0.10^{b}	1.89 ± 0.18^{a}
Survival rate (%)	98.8^{b}	100.0^{a}	99.6^{ab}	28.3^{c}

Temperature and growth data are presented as mean ± standard deviation, n = 180 and 60, respectively. The data in rows which are followed by different superscripts are significantly different at $P \leq 0.05$.

WATER PROPERTIES

Water supplied to the experimental aquaria originated from four separate, identical rearing units (total water volume of approximately 1 m^3 each) where it was electrically heated, aerated, and stirred. Each unit was equipped with a diatomite gravel biofilter. In both experiments, water was delivered to the aquaria at a constant rate of 10 dm^3 per hour. The dissolved oxygen content in the aquaria was measured twice a day and was maintained at a minimum of 70% of saturation. Other water quality properties in the aquaria, tested once a week, were as follows: ammonia – maximum 0.1 mg dm^{-3}; nitrites and $Fe^{+2/+3}$ – both below 0.02 mg dm^{-3}; pH – between 7.5 and 8.0.

DIETS AND FEEDING

In experiment A, as well as while preparing larvae for experiment B, live, freshly hatched *Artemia* spp. nauplii (EG grade; INVE Aquaculture N.V.) were used. The juvenile fish in experiment B were delivered the Polish non-commercial dry feed ASTA exclusively (crude protein 52.4%, crude fat 9.8%, gross energy 18.9 MJ kg^{-1}), which is eagerly consumed by the juvenile stages of some cyprinid fish species such as the crucian carp (Myszkowski et al. 2002). Live and dry diets were supplied manually every two hours between 08:00 and 20:00, i.e. seven times daily. At the same time (08:00-20:00), the aquaria were artificially illuminated with fluorescent tubes that provided a light intensity of about 1000 lux at the water surface. The nauplii were always fed in noticeable excess, which was considered ad libitum. Throughout experiment B, the fish were supplied dry feed at a constant amount of 2 g per aquarium daily. It constituted about 17% of the fish total biomass on day 1 and 2% or more on day 90.

MEASUREMENTS AND DATA ANALYSIS

The initial fish samples in experiments A and B numbered 40 and 30 individuals, respectively. The final samples were comprised of 50 or 30 individuals per aquarium, respectively. All the sampled fish were individually measured to the nearest 0.1 mm (total length, TL), and their wet body weight (BW; 0.1 mg) was determined. On the final day of both experiments, all the fish remaining in the aquaria were counted, and the final survival rates were calculated. Daily increments in the total length (ITL) of the fish were computed according to the following formula: $ITL = (TL_n - TL_0)\ n^{-1}$, where TL_n and TL_0 were, respectively, the final and initial mean total length in mm, and n was the duration of rearing in days. On the basis of the final TL and BW data, the fish condition, expressed by the K coefficient, was determined. To calculate K, the following formula

was used: $K = 100\ BW\ TL^{-3}$, where BW was the body weight in mg, and TL was the total length in mm. To determine whether the differences in the final fish TL, BW and K between the experimental groups were significant, Duncan's multiple range test ($P \leq 0.05$) was used. Survival percentages were normalized (angular transformation, Sokal and Rohlf 1969), and differences were considered significant at $P \leq 0.05$.

RESULTS

In experiment A, significantly ($P \leq 0.05$) the largest final size of the fish was recorded at a temperature of 25°C, where they reached a mean total length of 25.8 mm and a mean body weight of 239 mg (group A25, Table 1). No difference in larval growth in the total length was found between temperatures of 19 and 28°C (groups A19 and A28, respectively). However, at both these temperatures, larvae grew significantly slower than at 22°C in group A22. In terms of the final condition, all differences between the experimental groups were significant. The final condition coefficients increased with water temperature, and the maximum K value of 1.49 was found at 28°C. The final survival rates of larvae were very high at all temperatures, but the value of 96.5%, recorded for the fish reared at 28°C, was significantly lower in comparison with all the remaining fish groups.

In experiment B, no significant ($P \leq 0.05$) differences were found between groups B19, B22 and B25 in terms of fish growth in either length or weight (Table 2). However, in group B28, the fish final size was significantly smaller in comparison with group B25. The initial fish condition coefficient value was 1.11. The highest final K value of 1.89 was found for the fish reared at the highest temperature, whereas that recorded in group B25 was the second highest (K = 1.72, Table 2). Final survival rates of at least 98.8% were recorded in all groups except B28, with the maximum value of 100% at 22°C. In group B28, from the beginning of the second month of the experiment, consistent losses were recorded throughout the trial. Losses were preceded by anorexia and also a decline in swimming activity. Either external deformities (spinal curvature) or internal abnormalities (e.g. large fat deposits surrounding the alimentary tract, liver discoloration) were observed in the motionless, dying specimens.

DISCUSSION

According to available information, the experiments presented here were the first ever attempts to intensively rear *E. perenurus* larvae and juveniles under controlled conditions. In the present study, rearing techniques used previously for the youngest stages of other cyprinid species were followed, including such basic parameters as fish diets, daily duration of feeding, stocking densities and water temperatures (e.g. Wolnicki and Górny 1995a, b, Wolnicki and Myszkowski 1999a, b).

The earliest larval stages of some tiny cyprinid species, such as the European minnow *Phoxinus phoxinus* L., are known to be unable to feed on live freshly hatched Artemia nauplii from the first feeding (Stalmans and Kestemont 1991). It is therefore noteworthy that common nauplii of the commercial strain of Artemia were an excellent start diet for *E. perenurus* larvae reared in our study.

In experiment A, satisfactory fish growth was found throughout the temperature range of 19-28°C, with the maxima recorded at 25°C (Table 1). Since all differences in the final fish size were significant ($P \leq 0.05$), one can conclude that the temperature of 25°C is closest to the optimum for *E. perenurus* growth in the larval period of life. This temperature is known to be advantageous for the growth of live food-fed larvae of many common cyprinid species, such as bream, *Abramis brama* (L.) (Kucharczyk et al. 1997), asp, *Aspius aspius* (L.) (Wolnicki and Myszkowski 1999b), barbel, *Barbus barbus* (L.) (Wolnicki and Górny 1995b, Wolnicki 1997), nase, *Chondrostoma nasus* (L.) (Wolnicki and Myszkowski 1998b, Keckeis et al. 2001), chub, *Leuciscus cephalus* (L.) (Wolnicki and Myszkowski 1999b), ide, *L. idus* (L.) (Wolnicki and Górny 1995a) or vimba, *Vimba vimba* (L.) (Wolnicki 1996). At 25°C, larval *E. perenurus* grew at the daily rate of 0.49 mm total length (Table 3). This is noticeably less than the rate determined for most cyprinids, for which, under comparable rearing conditions, values higher than 0.6 mm d^{-1} ITL were usually found. It is, however, slightly more than in the case of the common cohabitant of *E. perenurus*, *T. tinca*, reared at its temperature optimum of 28°C.

Although all the differences in the final fish size were considerably less distinct in experiment B than those in experiment A, the results of both indicate that the temperature of 28°C is outside of the optimum for *E. perenurus* early growth (Table 2 and 1, respectively). However, it seems unlikely that this temperature alone was accountable for poor fish survival in group B28. It is known from many studies that temperatures as high as 28°C usually result in good growth and high survival rates of juvenile

stages of cyprinid fish, for instance *T. tinca* and *V. vimba*, provided that they are fed good quality diets (Wolnicki et al., unpublished data). Therefore, relatively slow growth, different external and internal abnormalities, and high losses observed in juvenile fish at 28°C in experiment B might have been influenced by the inadequate quality of the fish diet rather than by water temperature or any other factors.

It should be stressed that the usefulness of using commercial starters exclusively, especially those with a high level of dietary lipids, is highly limited in the intensive feeding of juvenile cyprinids in a controlled environment (e.g., Wolnicki and Myszkowski 1998a, 1999a, Myszkowski et al. 2002). When used without additives of natural food, such dry diets often result in low survival rates (Quiros and Alvariño 1998) and/or slow fish growth (Wolnicki and Myszkowski 1998a). In some cases, fat-rich dry diets produce mostly abnormal individuals of extremely high condition coefficients (e.g., Myszkowski et al. 2002). This phenomenon was observed in experiment B at 28°C, where a considerable increase of the K value from 1.11 on the first day to 1.89 on the final day was noted. It should be mentioned that juvenile *E. perenurus* of the sizes and ages comparable to those in our study exhibit K values of merely 0.9-1.0 when living in their natural environment (Kusznierz 1998).

TABLE 3

Daily increments in total length (ITL) and final survival rates (S) for *E. perenurus* and some other cyprinids reared under comparable controlled conditions. Larvae: diet - Artemia nauplii; stocking density – 30-60 per dm^3; duration of rearing – 20-40 days. Juveniles: diet – dry feed; stocking density – 5-8 per dm^3; duration of rearing – 120-150 days.

Period	Species	Temperature (°C)	ITL (mm d^{-1})	S (%)	Reference
Larval	*Eupallasella perenurus*	25	0.49	97.2	1
	Aspius aspius	25	0.79	99.0	2
	Barbus barbus	25	0.64	99.0	3
	Leuciscus cephalus	25	0.73	93.0	2
	Leuciscus idus	25	0.73	93.0	4
	Tinca tinca	28	0.45	91.0	5
	Vimba vimba	25	0.70	99.0	6
Juvenile	*Eupallasella perenurus*	25	0.20	99.6	1
	Carassius carassius	25	0.31	99.5	7
	Chondrostoma nasus	25	0.26	97.4	8
	Tinca tinca	24	0.19	100.0	9
	Vimba vimba	25	0.30	99.0	5

1 – this paper; 2 – Wolnicki and Myszkowski (1999b); 3 – Wolnicki and Górny (1995b); 4 – Wolnicki and Górny (1995a); 5 – Wolnicki et al. (unpubl. data); 6 – Wolnicki (1996); 7 – Myszkowski et al. (2002); 8 – Wolnicki and Myszkowski (1999a); 9 – Wolnicki and Myszkowski (1998a).

The fastest juvenile *E. perenurus* growth in length was slower than in other dry-feed fed cyprinids in the early juvenile period of life, excluding *T. tinca* whose daily growth rate of about 0.2 mm ITL was almost equal (Table 3). This figure is, however, twice as high when compared with field observations of aged 0+ *E. perenurus* by their first fall (Kusznierz 1998).

In summary, water temperatures within the range of 19-28°C proved to considerably influence the growth of *E. perenurus* under controlled conditions, especially in the larval period, but not their survival (Table 1 and 2). In light of the results presented here, one can conclude that the most favorable temperature for early growth would be 25°C (larval period), whereas 22°C would be optimal later on when they grow fastest at extremely high survival rates and with satisfactory condition coefficients.

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STRESZCZENIE

WPŁYW TEMPERATURY WODY NA PODCHOWYWANE W LABORATORIUM LARWY I MŁODOCIANE STADIA STRZEBLI BŁOTNEJ (*EUPALLASELLA PERENURUS* (PALLAS))

W warunkach laboratoryjnych badano wpływ temperatury (19, 22, 25, 28°C) na wzrost, kondycję i przeżywalność larw oraz wczesnych stadiów młodocianych strzebli błotnej *Eupallasella perenurus* (Pallas), krytycznie zagrożonego w kraju gatunku ryby karpiowatej. Wiedza na ten temat ma kluczowe znaczenie między innymi dla opracowania optymalnych biotechnik produkcji w warunkach kontrolowanych materiału zarybieniowego, przeznaczonego do celów aktywnej ochrony gatunku.

W doświadczeniu A larwy w obsadzie 60 sztuk na dm^3 podchowywano od 8 dnia po wykluciu przez 40 dni, karmiąc żywymi naupliusami solowca. Do doświadczenia B (90 dni) użyto ryb w wieku 48 dni od wyklucia, o średniej długości całkowitej i średniej masie ciała odpowiednio 20,6 mm i 97,7 mg, karmionych naupliusami w temperaturze 22°C przed rozpoczęciem doświadczenia, a w jego trakcie starterem.

Temperatura w badanym zakresie miała istotny ($P \leq 0,05$) wpływ na wzrost larw, z maksimum w 25°C, gdzie zanotowano końcową średnią długość całkowitą i średnią masę ciała odpowiednio 25,8 mm i 239 mg (tab. 1). W doświadczeniu B nie stwierdzono istotnych różnic między temperaturami 19, 22 i 25°C pod względem końcowej średniej wielkości ryb (długość całkowita 37,6-38,4 mm, masa ciała 901-968 mg; tab. 2), podczas gdy w 28°C zanotowano odpowiednio 34,6 mm i 819 mg.

W doświadczeniach A i B końcowe wartości współczynnika kondycji ($K \geq 1,18$; tab. 1 i 2) były znacznie wyższe od notowanych u dziko żyjących osobników strzebli błotnej ($K = 0,9$-$1,0$), o porównywalnych rozmiarach i wieku. Współczynniki kondycji wykazywały tendencję do wzrostu wraz ze wzrostem temperatury. Końcowa przeżywalność ryb była wysoka i wynosiła co najmniej 96,5% (tab. 1 i 2), z wyjątkiem temperatury 28°C w doświadczeniu B (28,3%).

Tempo wzrostu długości larw i młodocianej strzebli, odpowiednio 0,49 i 0,20 mm $dzień^{-1}$, w porównywalnych warunkach termicznych i pokarmowych ustępowało większości innych, z wyjątkiem lina, gatunków ryb karpiowatych (tab. 3).

Wyniki badań wskazują, że najlepsze warunki do podchowu strzebli błotnej w larwalnym okresie życia stwarza temperatura 25°C; temperatura 22°C jest najkorzystniejsza dla wyników podchowu wczesnych stadiów młodocianych.