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INFLUENCE OF FEEDING NATURAL AND FORMULATED DIETS ON CHOSEN RHEOPHILIC CYPRINID LARVAE

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ABSTRACT. The aim of the work was to determine the impact of commercial feed used as an alternative diet for larval fish of the genera *Leuciscus* on growth and survival during rearing under controlled conditions. Larvae of chub, *Leuciscus cephalus* (L.), ide, *Leuciscus idus* (L.), and orfe (its yellow pigmented form), and dace, *Leuciscus leuciscus* (L.), were obtained from breeding under controlled conditions and reared for 21 days in a closed recirculating system. The control group was comprised of larvae fed Artemia nauplii. The experimental group was fed Artemia for the first seven days of rearing and then this was partially replaced with formulated feed. The experimental group was fed from day 14 exclusively with formulated feed. The results obtained indicated that the feeding treatment was highly effective. While the body sizes of the larval dace from the control group were similar to those of the experimental group, the larvae of the other species that received the mixed diet attained greater weights (chub, ide) or greater weights and lengths (orfe) by the end of the experiment.

Key words: *LEUCISCUS*, REARING, ARTEMIA, MIXED DIET, FORMULATED FEED, LARVAL GROWTH

INTRODUCTION

The decline in populations of rheophilic fish species in Poland, including cyprinids, has been noted by many authors (Witkowski 1992, Sych 1996, and Penczak et al. 1998). Halting the decline in the abundance of rheophilic cyprinid species or restoring them can be achieved through intense stocking, among other measures. This is why researchers have focused on improving techniques for rearing stocking material of this group of fish for nearly two decades. Attaining adequate survival rates of larval vimba, *Vimba vimba* (L.), barbel, *Barbus barbus* (L.), ide, *Leuciscus idus* (L.), or asp, *Aspius aspius* (L.) in

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earthen ponds without prior rearing under controlled conditions is impossible (Wolnicki and Myszkowski 1999). The larvae of most cyprinids need natural food during the fist few days of exogenous feeding (Wolnicki and Górny 1995a, Kujawa 2004, Wolnicki 2005). This is linked to the alimentary canal that is not fully formed at this stage which relies on exogenous enzymes for digestion that are consumed along with the prey (Dabrowski 1984a, b). Attempts to rear larvae on starters alone ended in most cases with 100% stock mortality that stemmed from loss of appetite, arrested growth, and a significant decrease in disease resistance (Grudniewski 1980, Kujawa 2004).

Thus, it is justified to perform research aimed at determining the moment when it is safe to begin feeding these fish formulated feed without the risk of increased mortality. This is also an economic issue since Artemia nauplii are more expensive than some commercial feeds. In the majority of studies, larval rheophilic cyprinid fish were fed live or comercial feeds (Kujawa 2004), and the transition from one feed to the next was usually sudden. Thus, there is a lack of adequate information regarding the use of a transition period between the two feed types, which is also known as a mixed feeding period, for larval rheophilic fish reared under controlled conditions. The aim of the current study was to determine the impact formulated feed used as an alternative diet for larval fish of the genus *Leuciscus* had on growth and survival during rearing under controlled conditions.

MATERIALS AND METHODS

SOURCE OF FISH LARVAE AND EXPERIMENTAL DESIGN

Larvae of chub, *Leuciscus cephalus* (L.), dace, *Leuciscus leuciscus* (L.), ide and its yellow pigmented form, orfe, were obtained during controlled reproduction conducted at the hatchery of the Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn (Kucharczyk 2002, Krejszeff et al. 2008). Ide and chub spawners came from the broodstock at the Knieja Hatchery near Częstochowa (southern Poland), while orfe spawners came from the stock at the pond rearing facility of the State Fish Farm in Oleśnica (southwestern Poland). Dace spawners were caught in the rivers of the Warmia and Mazury region (northeastern Poland). At beginning of the experiment, the chub larvae were 5 days post hatch, orfe and ide – 7, and dace – 10. The larvae were reared for another 21 days in a closed recirculating system fitted with devices for temperature regulation (± 0.5°C), photoperiod, and aeration. The water temperature during rearing was set at 25°C. The rearing tanks had a volume of 50 dm^3 and were illuminated with fluorescent light for a photoperiod of 12L:12D. Water was delivered separately to each aquarium through a sprinkling system at a water flow rate of 2 dm^3 min^{-1}.

WATER QUALITY

The aquaria were cleaned daily. Water temperature, oxygen concentration, water pH, and nitrogen and ammonia concentrations were measured with electronic devices. Dissolved oxygen content did not fall below 5 mg dm^{-3}, ammonia nitrogen did not exceed 0.2 mg dm^{-3}, and nitrogen nitrite did not exceed 0.04 mg dm^{-3}. Water pH ranged between 7.5 and 8.0.

FOOD SOURCE, MEASUREMENTS AND ANALYSIS

The food was freshly hatched *Artemia* sp. nauplii and Perla commercial feed (Skretting, Norway). According to manufacturer data, the proximate composition of the commercial feed was protein 62%, fat 11%, carbohydrates 0.8%, phosphorus 1.1%, and ash 10%. The control group (K) larvae were fed Artemia throughout the experimental period. Experimental group (D) was fed Artemia for the first 7 days, and then for the next 7 days with Artemia nauplii and Perla feed. The quantity of Artemia was 50% of the ration fed the fish from the control group. During the last week of the experiment, the fish from the experimental groups were fed only formulated feed. In the mixed feed treatments, the fish were fed formulated feed and then natural feed after 30 minutes. All of the feeding treatment groups were fed three times daily at five hour intervals. The stocking density of larval chub, ide, and orfe was 100 indiv. dm^{-3}, while that of dace was 70 indiv. dm^{-3}. Each treatment was performed in two replicates.

Control measurements were taken after the fish had been anesthetized in a solution of 2-phenoxyethanol (Sigma-Aldrich, Germany). Measurements were taken when the fish were stocked into the tanks and then at intervals of 7 days. Total length (±0.01 mm) measurements of 30 individuals chosen at random were taken under a stereo microscope coupled with the ProgRes® Capture Pro 2.5 image analysis program (Jenoptic, Germany). After the measurements, the larvae were released back into the same aquarium from which they had been taken. The mean individual weight (± 0.1 mg) was determined at the beginning and end of the experiment.

Based on the data compiled, the specific growth rate (SGR) (% d^{-1}) and the specific biomass growth rate (SBR) (% d^{-1}) were calculated using the formula (Brown 1957):

$$SGR = 100 \frac{\ln W_2 - \ln W_1}{\Delta t} \tag{1}$$

and,

$$SBR = 100 \frac{\ln(N_2 W_2) - \ln(N_1 W_1)}{\Delta t} \tag{2}$$

where: W1 and W2 – mean initial and final individual weight (mg), N1 and N2 – stocking density (indiv.) at the beginning and end of rearing, Δt – rearing period (days). These were used to calculate the relative individual growth rate in weight (RGRw) (% d^{-1}) and the relative biomass growth rate (RBR) (Myszkowski 1997):

$$RGR_W = 100\left(e^{\frac{SGR}{100}} - 1\right) \tag{3}$$

and,

$$RBR = 100\left(e^{\frac{SBR}{100}} - 1\right) \tag{4}$$

The relative growth rate in total length (RGR_L) was calculated in the same way. Additionally, the index for increment in total length growth in a time unit ITL (mm d^{-1}) was calculated using the formula (Peňáz et al. 1989):

$$ITL = \frac{TL_2 - TL_1}{\Delta t} \tag{5}$$

where: TL_1 and TL_2 – mean individual length (*longitudo totalis*) at the beginning and end of rearing, Δt – rearing period (days).

The mean lengths and weights of the larval control and experimental groups of the different species were compared with Student's t-test ($P < 0.05$).

RESULTS

CHUB

Initially, the change in the larval chub diet has a significant impact on body size (Fig. 1a). The mean total length of the larvae from control group K was 0.4 mm longer in

TABLE 1

Comparison of indexes of growth in increments of total length (ITL), relative growth rate in weight (RGR_W), relative growth rate in length (RGR_L), relative biomass growth rate (RBR), survival and initial and final characteristics of larval fish of the genus *Leuciscus* fed natural food and formulated feeds

Parameter	Chub		Ide		Orfe		Dace	
	Group K	Group D	Group K	Group D	Group K	Group D	Group K	Group D
Initial stocking density (indiv.)	5000	5000	5000	5000	5000	5000	3200	3200
Mean initial weight (mg)	$1.3±0.3^a$	$1.3±0.3^a$	$1.6±0.2^a$	$1.6 ± 0.2^a$	$1.1 ± 0.15^a$	$1.1 ± 0.15^a$	$3.0 ± 0.4^a$	$3.0 ± 0.4^a$
Mean final weight (mg)	$49.80 ± 23.52^b$	$57.59 ± 14.36^a$	$35.71 ± 11.84^b$	$48.15 ± 12.76^a$	$30.15 ± 9.29^b$	$59.29 ± 15.10^a$	$49.12 ± 12.84^a$	$49.59 ± 21.00^a$
Mean initial length (mm)	$7.86 ± 0.27^a$	$7.86 ± 0.27^a$	$8.10 ± 0.24^a$	$8.10 ± 0.24^a$	$8.57 ± 0.27^a$	$8.57 ± 0.27^a$	$9.12 ± 0.40^a$	$9.12 ± 0.40^a$
Mean final length (mm)	$18.10 ± 2.71^a$	$18.49 ± 1.33^a$	$17.80 ± 1.58^a$	$18.90 ± 1.32^a$	$15.90 ± 1.34^b$	$19.13 ± 1.40^a$	$17.93± 1.60^a$	$17.58 ± 1.94^a$
Intitial biomass (g dm^{-3})	0.13	0.13	0.16	0.16	0.11	0.11	0.19	0.19
Final biomass (g dm^{-3})	3.96	4.90	3.54	4.57	2.98	5.69	3.28	3.26
ITL (mm d^{-1})	0.48 ± 0.2	0.50 ± 0.1	0.46 ± 0.1	0.51 ± 0.2	0.34 ± 0.2	0.50 ± 0.2	0.42 ± 0.1	0.40 ± 0.1
RGR_W (% d^{-1})	19.16 ± 0.3	19.78 ± 0.2	15.90 ± 0.2	17.56 ± 0.2	16.97 ± 0.2	20.80 ± 0.3	14.23 ± 0.2	14.29 ± 0.3
RGR_L (% d^{-1})	4.05 ± 0.1	4.15 ± 0.1	3.82 ± 0.2	4.11 ± 0.1	2.98 ± 0.1	3.89 ± 0.2	3.27 ± 0.2	3.17 ± 0.1
RBR (% d^{-1})	17.87 ± 0.3	18.88 ± 0.2	15.85 ± 0.2	17.27 ± 0.2	16.92 ± 0.2	20.57 ± 0.3	13.98 ± 0.2	13.94 ± 0.3
Survival (%)	79.6	85.1	99.1	94.8	99.1	96.1	97.1	96.0

Data in rows (within species) with the same letter index do not differ statistically; ($P < 0.05$). Data presented are means ± SD

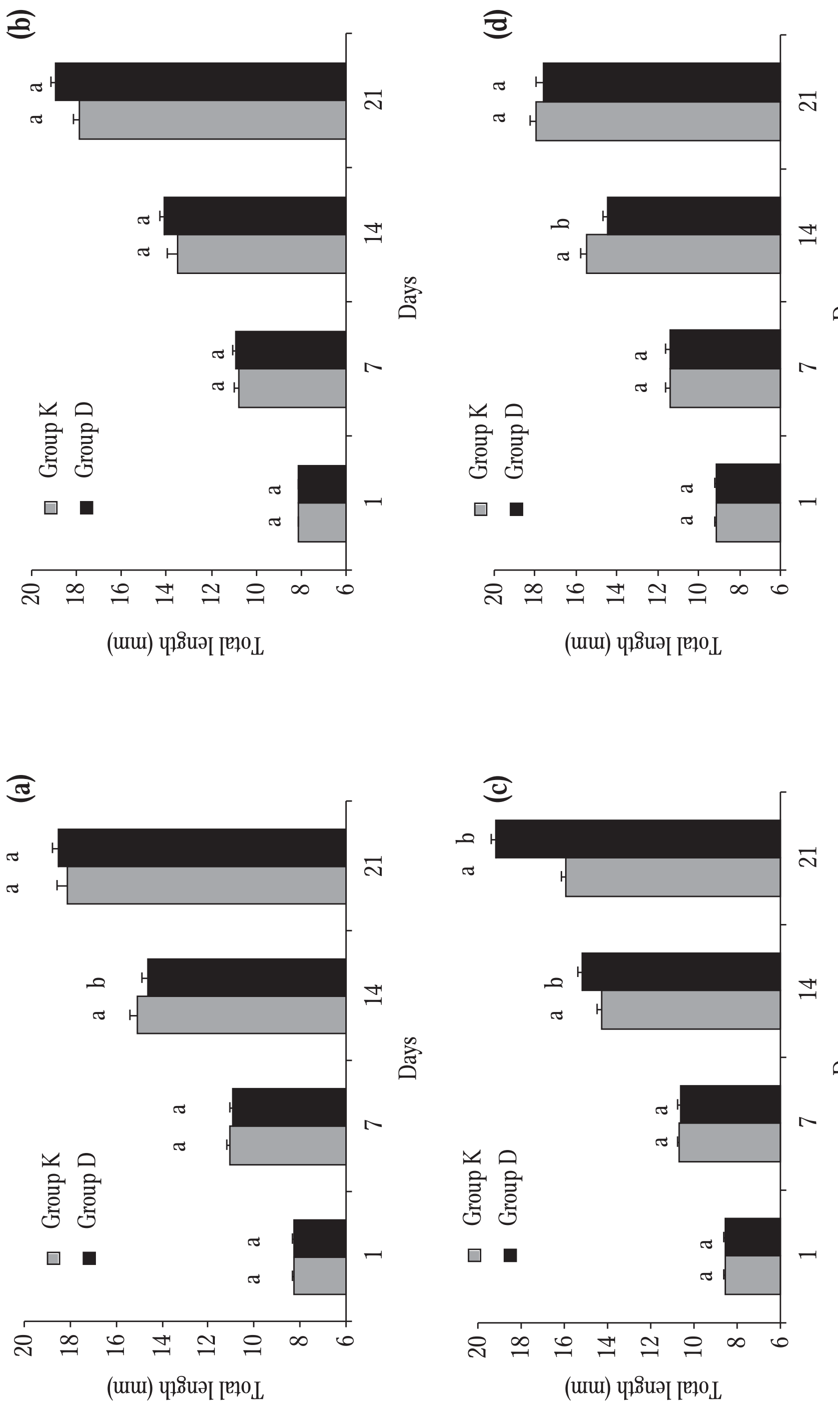

Fig. 1. Mean (±SE) length of larval chub (a), ide (b), orfe (c), dace (d) during rearing.

comparison with the larvae from group D on day 14 of rearing. At the end of the experiment, the mean body length of larvae from groups K and D did not differ significantly statistically (Table 1; $P < 0.05$). On the last day of rearing, the mean body weight of group K (49.9 mg) was significantly lower in comparison to group D (57.59 mg). The ITL of larval chub was similar in the control and experimental groups (0.48 and 0.50 mm d^{-1}, respectively). Larval chub from the control group had a mean RGR_L that was smaller by 0.1 % d^{-1} in comparison with group D. The RGR_W in the larvae from group D was 0.62 % d^{-1} higher than in the group fed only Artemia, while the mean RBR of group D was higher by 1.01 % d^{-1} in comparison to group K (Table 1). The survival of larval chub in the group fed Artemia was 79%, and in the experimental group it was 85%. Larval mortality ran a similar course in both groups (Fig. 2a) and was the highest in the first 9 days of the experiment.

IDE

The type of feed was not a significant impact on the total length (Fig. 1b). The mean total length of larvae in group K was 17.80 mm, while in group D it was 18.90 mm (Table 1). The mean body weight of individuals in group K (35.71 mg) was statistically significantly lower in comparison with group D (48.15 mg). The ITL of larvae from the control group was 0.46 mm d^{-1}, and in group D it was 0.51 mm d^{-1}. The larval ide from group D had an RGR_L that was 0.29% d^{-1} higher in comparison to that of group K. The RGR_W of the larvae from group D was 1.66% d^{-1} higher than that of group K, while the mean RBR of group D was higher by 1.42% d^{-1} in comparison to the larvae fed only Artemia (Table 1). Ide survival in group K was higher at 99%, while in group D it was 94%. An increase in the mortality of ide fed a mixed diet was noted between days 14 and 17 of the experiment (Fig. 2b).

ORFE

Mean total length depended significantly on dietary composition (Fig. 1c). The larvae fed Artemia attained a mean length of 15.90 mm by the end of the experiment, while the mean length of the larvae fed a mixed diet was 19.13 mm (Table 1). Larval orfe fed a mixed diet of Artemia nauplii and formulated feed had a faster total length growth rate ($P < 0.05$). The mean individual weight of group D larvae was nearly twofold greater than that of the control group (Table 1). The ITL of larval orfe from the con-

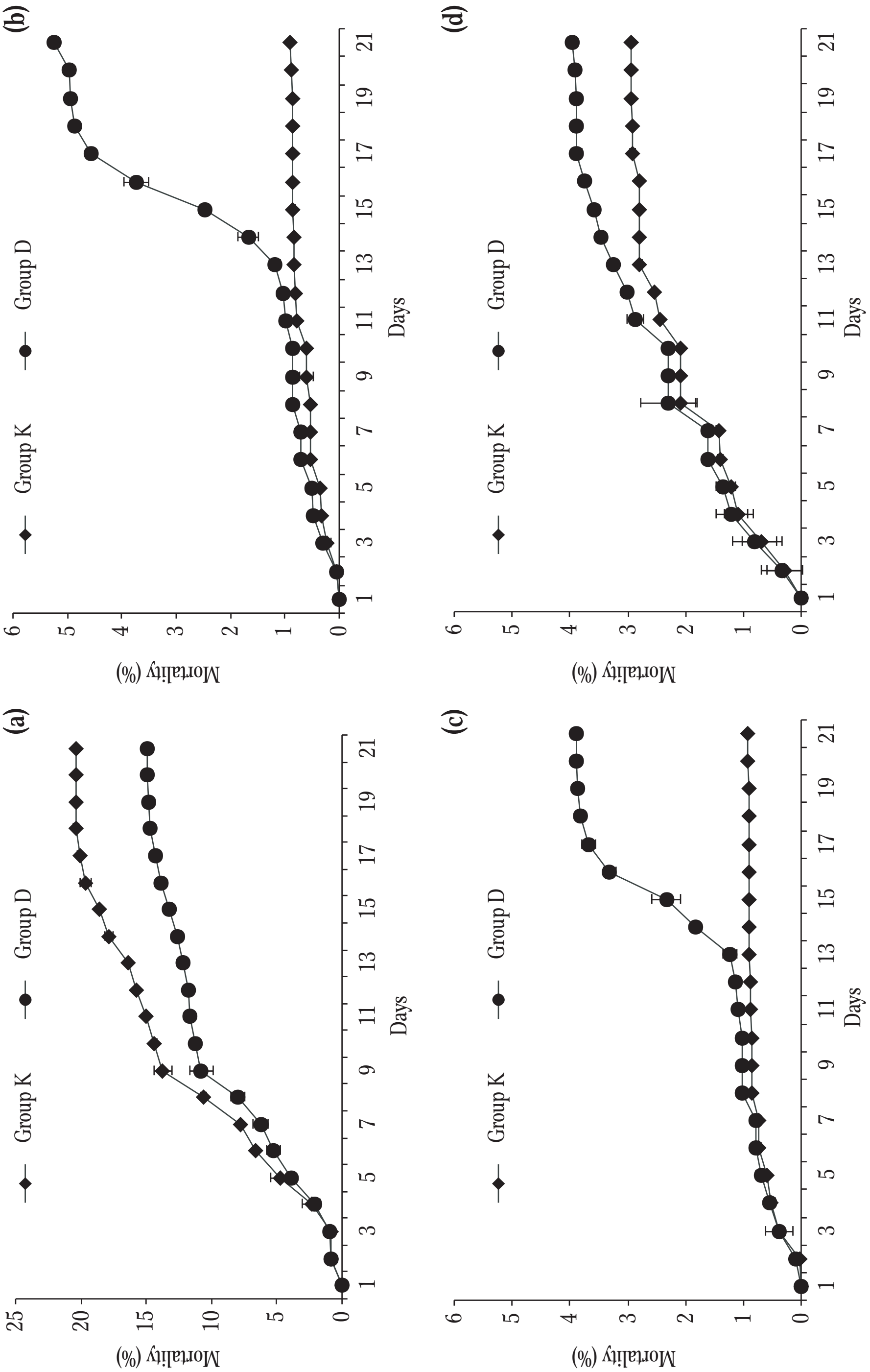

Fig. 2. Cumulative mortality of larval chub (a), ide (b), orfe (c), dace (d) during rearing.

trol group was 0.16 mm d^{-1} lower in comparison to group D. The larval orfe from the group fed mixed feed had an RGR_L that was higher by 0.91% d^{-1} in comparison with group K. RGR_W in larval orfe from group D was 3.83% d^{-1} higher than in group K, while the mean RBR of group D was higher by 3.65% d^{-1} in comparison with larvae fed only Artemia (Table 1). The survival of larval orfe from group K was higher at 99%, while in group D it was 96%. An increase in the the mortality of larval orfe fed the mixed diet was noted between days 14 and 17 of the experiment (Fig. 2c).

DACE

The type of feed was not found to have a significant impact on the growth of body weight or length of larval dace (Table 1). A significant difference was noted in total length growth between the control and experimental groups only on 14 day of rearing (Fig. 1d). However, at the end of the experiment the mean total length of larval dace did not differ significantly statistically between the groups ($P < 0.05$). The indexes of growth in length, weight, and biomass were similar for the two groups (Table 1). Survival in the control group was 97%, and in the experimental group it was 96%. The highest mortality was observed between days 7 and 10 of rearing (Fig. 2d).

DISCUSSION

During the rearing of larval fish, it is essential that the food they receive is in adequate supply and of the appropriate quality (Kujawa 2004). Before fish have fully developed digestive canal live food in their diet with the exogenous digestive enzymes that permit proper development. Inadequate quantities or the lack of food results in decline growth rates and impaired disease resistance which result in massive death (not infrequently of the whole stock) (Grudniewski 1980). Larval cyprinids should be fed natural food during the first few days of exogenous feeding. Not until later can they effectively assimilate the substances contained in formulated feeds (Dabrowski 1984a, Dabrowski and Poczyczyński 1988). It is significant that the contact time between food and digestive enzymes in such young fish is very short (Kaushik 1986, Szlamińska 1987, Wolnicki 2005). Additionally, according to the classification by Dabrowski (1984b), larval cyprinid fish belong to the third group of species that is characterized by a short, weakly developed digestive tract with a poor enzyme composition.

The onset of exogenous feeding is one of the most critical moments in the lives of larvae (Vladimirov 1970, Dąbrowski 1975). Kujawa et al. (1998) observed that chub and asp fed for at least eight days with Artemia nauplii had significantly higher survival rates (97%) than did those which were fed only starters (50%). Wolnicki and Górny (1995a, b), Wolnicki (1996), and Kujawa et al. (1998) reported that it is possible to use starter feeds from the beginning of feeding with only two species of rheophilic cyprinid species: barbel and nase, *Chondrostoma nasus* (L.), but they also reported slower larval growth and the occurrence of higher mortality. With rheophilic cyprinid species such as chub, ide, and dace, Kujawa (2004) suggested that the period natural diets are fed should last from 8 to 12 days. Only after this, was it possible to use commercial feed without any negative impact on larval mortality. In the current study, the period during which only natural food was supplied was shorter at 7 days. The rather long transition period of feeding larvae mixed feed was aimed at limiting the negative impact commercial feed had on the studied rearing parameters. Similar techniques have been proved to be excellent during the rearing of many marine fish species (Hart and Purser 1996, Pousao-Ferreira et al. 2003).

The larval growth in the current experiment indicates the feed used is highly suitable for rearing fish from the genus *Leuciscus*. There was no statistically significant difference between the lengths achieved by larval chub, ide, or dace (in either groups K or D). The results were not confirmed by Kujawa (2004), who did not begin giving the larvae formulated feed until after day 13 of rearing, and subsequently noted significantly higher growth rates in the larvae that had received natural feed. Contradictory results were obtained during the rearing of larval orfe, in which the fastest growth rate was obtained with a mixed diet. In study of rainbow trout, *Oncorhynchus mykiss* (Walbaum), Tymchuk and Devlin (2005) noted that cultured individuals grow decidedly more quickly and are in better condition than those from wild populations. In turn, Brummett et al. (2004) reported that this is impacted by the degree of domestication (number of generations in captivity), and their inbred degree. The orfe reared on the mixed diet treatment (Group D) has the fastest relative growth rate in length in comparison to all four of the species. Similar dependencies were not identified in either chub or ide (or in those from the broodstock). This is probably related to the degree of domestication, which, in comparison to that of ide and chub (respective generations F5 and F2, S. Piszczała, personal communication), is substantially more advanced. Significantly greater differences were noted in the analysis of the mean weight attained by larval chub, ide, and orfe, which was significantly greater in experimental groups D than in the control groups. Such differences were not noted by Kujawa (2004) in ide or dace. However, the same author did not apply a transition period (7 day period of feeding equal measures of Artemia and feed), which could have influenced the better results achieved in the current experiment. Kujawa (2004) had better rearing results with natural feed. This is probably linked to the scale of the experiment and the initial larval stocking density of 40 indiv. dm^{-3}.

The lowest survival during rearing was noted for chub (85.1% in the control group and 79.6% in the experimental group). The change in diet did not show result drastic mortality in experimental groups, which was confirmed in the observations by Wolnicki (2005), who reported that chub can be fed commercial feed successfully as quickly as 5 days after feeding Artemia. However, the 5% higher mortality in group D at the end of the experiment is not without significance. This mortality could have resulted from cannibalism that was noted during rearing (Targońska et al., unpublished data). A similar scale of mortality during chub rearing was noted by Shiri Harzevili et al. (2003) and Żarski et al. (2008). The lack of a dependency between mortality and feeding treatment in the current study was also confirmed in dace (less than 1% difference). Distinct increases in mortality were, however, noted in ide as well as in orfe. This was noted in group D when the larvae were fed formulated feed exclusively (after 14 days of rearing). Again, orfe larvae were slightly better at adapting, and there were about 1% fewer dead individuals in this period.

The results obtained indicate that larval chub and dace are better adapted to ingesting formulated feed at the same stage of life. Additionally, it is apparent that the larvae of domesticated fish populations are better adapted to rearing conditions than larvae obtained from the controlled spawning of individuals from wild populations. The feeding treatment used (with the 7 day transition period) permitted achieving a growth rate with formulated feed that was comparable to the control group, and, in the case of orfe, one that was significantly better. This result differs from those reported by Kujawa (2004). The positive impact of a mixed diet has been observed in other rheophilic cyprinid fish species (Abi-Ayad and Kestemont 1994, Kaiser et al. 2003).

The positive effects of the large-scale rearing of larval fish from the genus *Leuciscus* suggest that the success of the intense stocking material production under controlled conditions is highly likely. The possibility of replacing natural food with commercial feed without compromising larval quantity or quality lays the foundation for more latitude in production, which is often dependent on the production capabilities of hatcheries and their technical facilities.

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STRESZCZENIE

WPŁYW ŻYWIENIA WYBRANYCH GATUNKÓW KARPIOWATYCH RYB REOFILNYCH Z ZASTOSOWANIEM POKARMU ŻYWEGO ORAZ PASZY KOMPONOWANEJ

W niniejszej pracy przeprowadzono masowy wychów larw karpiowatych ryb reofilnych: klenia, *Leuciscus cephalus* (L.), jelca, *Leuciscus leuciscus* (L.), jazia, *Leuciscus idus* (L.) oraz jego ksantorycznej odmiany orfy. Larwy uzyskano w trakcie rozrodu w warunkach kontrolowanych. Podchów prowadzono przez 21 dni w zamkniętym obiegu wody. Grupę kontrolną stanowiły larwy karmione przez całe doświadczenie naupliusami *Artemia* sp., a grupę doświadczalną larwy, którym po pierwszych 7 dniach podchowu solowca częściowo zastąpiono paszą sztuczną. W 14 dniu eksperymentu grupa doświadczalna była karmiona wyłącznie paszą. Uzyskane wyniki wskazują na wysoką skuteczność zastosowanego wariantu żywieniowego. Rozmiary ciała larw jelca z grupy kontrolnej były zbliżone do grupy doświadczalnej, natomiast u pozostałych gatunków zastosowanie pokarmu mieszanego wpłynęło osiągnięciem większej masy ciała (kleń i jaź) bądź masy i długości całkowitej (orfa) na koniec eksperymentu. Końcowa przeżywalność była wysoka (>90%), jedynie w przypadku klenia była znacznie niższa i w grupie kontrolnej wynosiła 79%, a w grupie doświadczalnej 85%.