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RESEARCH ARTICLE

Impact of replacing natural food with commercial feed on the growth and survival of blue bream larvae (*Ballerus ballerus*) under controlled conditions

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Abstract. The effect of replacing natural food with commercial feed on the growth and survival of blue bream, *Ballerus ballerus* (L.), larvae reared under controlled conditions was investigated. Live food, brine shrimp, *Artemia* sp. (A), nauplii, was replaced after four (A4P17), eight (A8P13), and 12 (A12P9) days with commercial feed (P). The control groups were larvae fed nauplii (A21) or feed (P21) exclusively for throughout the experiment. The density of larvae was 40 ind. dm^{-3}, and the rearing temperature was 25 ± 0.5°C. The highest blue bream larval growth rate in the groups fed the commercial feed tested was in group A12P9, in which larvae reached an average body weight of 56.1 mg with an average total length of 21.1 mm. These values were only slightly lower than those obtained in the A21 control sample. Final larval survival in each of the feeding groups ranged from 67% (A4P17) to 93% (A12P9). The larval lowest survival and growth parameters were recorded in control group P21, in which larvae received only commercial feed; this indicated the necessity of using natural food when rearing blue bream larvae.

Keywords. artemia, food replacement, blue bream, survival rate, growth rate, controlled conditions

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Introduction

Blue bream, *Ballerus ballerus* (L.), is one of the two representatives of the genus *Ballerus* inhabiting European inland waters and coastal lagoons. Its silhouette resembles that of bream, *Abramis brama* (L.), although the blue bream body is more elongated with a less steep dorsal profile. Its anal fin is much longer than that of bream (Szlachciak 2005). Blue bream is most often confused with white-eye bream, *Ballerus sapa* (Pall.), but unlike this species, blue bream has an upturned mouth and not an inferior one. Its distribution range is quite vast expanding over the systems of large rivers in the basin of the Caspian Sea (Ural and Volga rivers), the Baltic Sea (from the Oder to the Neva rivers), and the Black Sea (from the Kuban to the Danube rivers) (Tadajewska 2000). Recently, this range of occurrence has been shrinking dramatically, especially in Europe. This stems from a series of factors that have been affecting the biodiversity of aquatic ecosystems for many years. These include the persistently exploiting fish resources, anthropogenic transformations, polluting of aquatic environments, and introducing new species. Currently, relatively large, stable blue bream populations in Poland occur exclusively in the Szczecin Lagoon

and Lake Dąbie, although the number of specimens fluctuates considerably (Szlachciak 2005, Tadajewska 2000). In other waters, this species is seen as a faunistic peculiarity. Implementation of passive protection (size and protective periods) or simple active protection measures (protection of natural spawning areas, construction of artificial spawning sites) is successful only in slightly modified, slightly polluted water bodies.

A chance to prevent the blue bream from vanishing from Polish waters lies in aquaculture, which no longer functions as just a source of commercial fish for consumption, but which is playing an increasingly important role in saving endangered species (Kujawa and Piech 2021) and even those threatened with extinction (Kujawa et al. 2022). Stocking material obtained under controlled conditions is a form of the active protection of fishes (Zakęś and Demska-Zakęś 2011, Kujawa et al. 2019). Before launching efforts to culture blue bream stocking material, a method for its reproduction under controlled conditions was developed (Kujawa and Piech 2021). Afterward, the focus shifted to rearing blue bream larvae under controlled conditions. The rationale for this research was the lack of any data in the literature pertaining to the culture of blue bream larvae in artificial conditions. The development and then optimization of these stages of fish rearing can provide a safeguard in the event blue bream natural populations collapse and must be restored to sustain the biological diversity of waters. The aim of the current study was to determine how natural feed substituted with commercial diets affected the growth rates and survival of larval blue bream (*Ballerus ballerus*) under controlled conditions.

Materials and Methods

The blue bream larvae used in the present study were obtained from eggs incubated in Weiss jars at the hatchery of the Centre of Aquaculture and Ecological Engineering, at the University of Warmia and Mazury in Olsztyn. The blue bream spawners originated from Lake Dąbie (Poland). Sex products were obtained under controlled conditions following hormonal stimulation (Piech and Kujawa 2021). The water temperature during egg incubation was 19°C ± 0.5. During the experiment, after the yolk sacs were resorbed and the posterior chambers of swim bladders were filled, which occurred in water at a temperature of 20°C, blue bream larvae started swimming actively in search of food. When blue bream larvae started foraging actively, which occurred at five days post hatching (5 DPH), they were placed in 15 tanks with working capacities of 25 dm^3 each (three tanks for each experimental variant). The tanks were part of a recirculating aquaculture system (RAS) with a water temperature of 25°C (± 0.5). Each of the tanks was stocked with 1,000 blue bream larvae. The RAS was fitted with an efficient water filtration system composed of a biological bed and an external EHEIM Classic 1500 XL canister filter coupled with an EHEIM Universal 1262 pump with a maximum capacity of 3400 l h^{-1} (EHEIM GmbH & Co.KG, Deizisau, Germany). The water flow rate through the tanks was 0.20 dm^3 min^{-1}. Water was supplied to the tanks by sprinklers. The photoperiod was 12 h daylight and 12 h darkness. The first food fed to the larvae was live brine shrimp nauplii (*Artemia* sp.) (A) (Ocean Nutrition Europe, Essen, Belgium). This natural food was replaced after a set time with Perla Larva Proactive feed (P) (Skretting AI & Central Operations, Stavanger, Norway). This commercial feed is specifically formulated for fish larvae and has a particle size of 0.1–0.3 mm. Its ingredients include fish meal, shrimp meal, fish oil, wheat, yeast, lecithin, micronutrients, and the Protect® supplement. It has a very high protein content of the highest digestibility, unsaturated fatty acids, and phospholipids. Perla is a very stable feed that has excellent physical parameters. The food, both live brine shrimp nauplii and Perla Larvae Proactive feed, were supplied manually three times daily at 08:00, 12:00, and 16:00. Feed rations were adjusted to fish biomass the day after each sampling (days 6, 10, 14, 18). Fish in the different feeding variants were fed ad libitum.

The experiment comprised five feed variants: group A21 – only natural food; group A4P17 – natural food for four days followed by commercial feed for 17 days; group A8P13 – natural food for eight days followed by commercial feed for 13 days; group A12P9 – natural food for 12 days followed by commercial feed for nine days; group P21 – only commercial feed throughout the rearing period. The natural food was prepared according to the procedure described by Sorgeloos (Sorgeloos et al. 1977). The size of brine shrimp nauplii (instar I) was 430 microns. Every day, prior to the first feeding, unconsumed food and other debris were removed to maintain appropriate sanitary conditions. Dead larvae were also removed and counted to calculate mortality rates. The experiment ran for 21 days. The dissolved oxygen and the content of nitrogen compounds in the water were measured daily throughout the rearing period. The water oxygen content was measured with a YSI multiparameter meter (YSI Incorporated Brannum Lane Yellow Springs, Ohio, USA) and was within 7.1–7.4 mg O_2 dm^{-3}, the water pH ranged was 7.1–7.3. The ammonia and nitrite contents were measured in the morning (Nowosad et al. 2013) with a HANNA INS HI 83200 with reagents (Hanna Instruments, Woonsocket, RI, USA), and neither was detected in the water during the experiment.

During the rearing period, the blue bream larvae were measured and weighed six times. The first, the zero measurement, was immediately after the tanks were stocked for every feeding variant, and subsequent measurements were performed on days 5, 9, 13, and 17 and at the end of the experiment on day 21. Thirty individuals from each tank were caught in the morning (before feeding). Before the measurements the larvae were sedated in a solution of MS-222 (tricaine methanesulphonate) (50 mg l^{-1} of water) and then weighed on an KERN ALJ 220-5 DNM analytical balance to the nearest 0.1 mg (KERN & Sohn GmbH, Ballingen, Germany). The larvae, which were still anaesthetised, were then placed on Petri plates with some water and observed under a LEICA MZ16Z stereoscopic microscope (Leica Microsystems GmbH, Wetzlar, Germany). Photographic documentation of larvae was taken with a DFC 420 microscope camera (Leica Microsystems GmbH, Wetzlar, Germany). Larval size was analyzed with LAS V 3.1.0 software (Leica Microsystems GmbH, Wetzlar, Germany). Total length was measured to the nearest 0.1 mm. Following manipulations, the larvae were placed in a container with well-oxygenated water for recovery. After they had recovered from the anaesthesia and had started to swim actively, the larvae were returned to the tanks from which they had been taken. Using the data obtained, the coefficient of total length increase per time unit (TLI) was computed with the following formula (Peňáz et al. 1989):

$$TLI = \frac{TL(n_2) - TL(n_1)}{\Delta t}$$

where: TL – mean length of an individual (*longitudo totalis*), n_1 – start of the period, n_2 – end of the period, Δt – duration of rearing (in days).

The specific growth rate (SGR) for body weight and relative biomass growth rate (SBR) from the beginning of feeding until the end of the experiment were determined with the following formulas (Brown 1957):

$$SGR = 100 \times \frac{\ln W_2 - \ln W_1}{\Delta t} \textit{ and, } SBR = 100$$

Relative growth rate for body weight (RGR) and relative growth rate for biomass (RBR) from the beginning of feeding until the end of the experiment were determined with the following formulas (Myszkowski 1997):

$$RGR = 100 \times \left(e^{\frac{SGR}{100}} - 1 \right), and\ RBR = 100 \times \left(e^{\frac{SBR}{100}} - 1 \right)$$

where: W_1 – mean initial weight of an individual (mg) during the rearing period, W_2 – mean final weight of an individual (mg) during the rearing period, n_1 – number of stocked fish (individuals) at the beginning of the rearing period, n_2 – number of stocked fish (individuals) at the end of the rearing period, Δt – duration of rearing (days).

The RGR for body length was calculated analogously. The biomass of the fish in each tank was determined as the product of the average individual

weight and the number of live individuals. The resulting value was divided by the capacity of the tank. The biomass calculated was expressed in g dm^{-3}.

Data analysis

The number of dead fish recorded every day served to plot cumulative mortality curves for each fish feeding variant. To compare the results, the relative final mean length (RFL), the relative final mean weight (RFW), and the relative final mean biomass (RFB) of the experimental fish were calculated, assuming that the length, weight, and biomass of the control fish A21 (21 day feeding regime with live brine shrimp nauplii) at the end of the experiment was 100%. To determine whether the differences in the mean fish length and the mean fish weight in the experimental groups were significant, Duncan's multiple range test ($P \leq 0.05$) was used (Duncan 1955). Survival percentages were normalized (angular transformation) and differences were considered significant at $P \leq 0.05$ (Sokal and Rohl 1981). The results were processed statistically in Excel 16.0 and Statistica 13.0 for Windows.

Results

The first statistically significant differences in average larval weights and lengths from the different feeding variants emerged on day 13 of the rearing period and persisted until the end of the experiment (Figs. 1 and 2). The mean individual weights of blue bream larvae from the variant in which only natural food was supplied (A21) was 62.1 mg. This increase was 1.6 and two-fold higher than that in the two variants that were the worst in this respect, namely larvae fed nauplii for the first four days (A4P17) and those fed only Perla feed (P21). In comparison with the other variants, this increase was 1.3-fold higher than that of larvae from variant A8P13, which were fed live brine shrimp nauplii for eight days, and 1.1-fold higher than that noted for larvae from variant A12P9, which were fed nauplii for the first 12 days of life. The highest growth rate of blue bream larvae from the food substitute variants tested was recorded in the A12P9 group, where larvae reached an average body weight of 56.1 mg with an average total length of 21.1 mm. These values were only slightly worse than those obtained in the A21 control sample. Final survival of larvae in each feeding group ranged from 67% (A4P17) to 93% (A12P9). Likewise, the RGR for

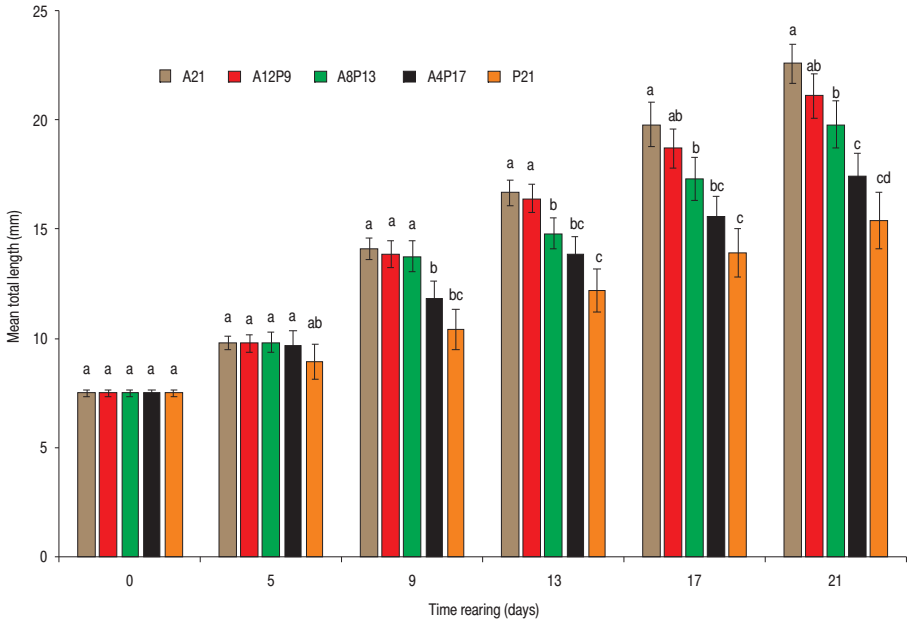

Figure 1. Increase in the total length of blue bream (*B. ballerus*) larvae reared on natural (A) and commercial food (P). The change to commercial food was after four, eight, or 12 days.

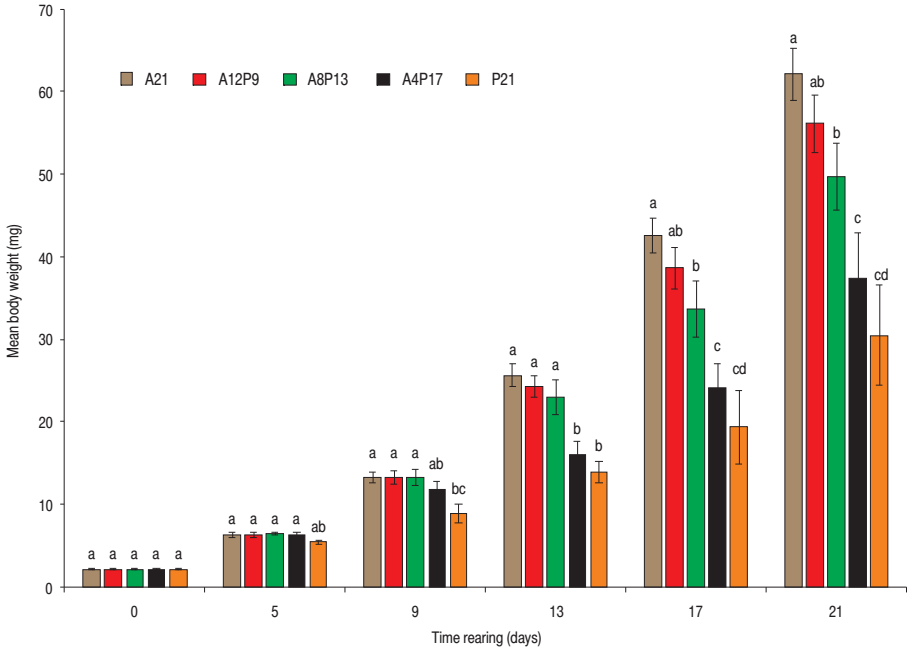

Figure 2. Increase in body weights of blue bream (*B. ballerus*) larvae reared on natural (A) and commercial food (P). The change to commercial food was after four, eight, or 12 days.

the weight of individual larvae was the highest in variant A21 (17.5% d^{-1}) (Table 1). Larvae from variant P21 had a daily relative growth of individual weight of 13.6% d^{-1}. The analysis of the growth in the length of larvae proved that the longest larvae (22.6 mm) were from the variant where the diet was composed of only live brine shrimp nauplii (A21). For comparison, larvae from group P21 had an average body length of 15.4 mm. The TLI for larvae from variant A21 was 0.72 mm d^{-1}. The lowest TLI was noted

Table 1
Results of rearing blue bream (*B. ballerus*) larvae fed with natural food (A) that was changed to Perla commercial feed after four (A4P17), eight (A8P13), or 12 days (A12P9). Mean value ± SD. Results in rows with the same letter index are not statistically significantly different (α = 0.05)

	Experimental groups				
Parameter	A21	A12P9	A8P13	A4P17	P21
Initial mean body length (mm)	7.50 ± 0.15^{a}	7.50 ± 0.15^{a}	7.50 ± 0.15^{a}	7.50 ± 0.15^{a}	7.50 ± 0.15^{a}
Initial mean body weight (mg)	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}
Final mean body length (mm)	22.60 ± 0.9^{a}	21.10 ± 1.0^{ab}	19.80 ± 1.1^{b}	17.40 ± 1.1^{c}	15.40 ± 1.3^{cd}
Final mean body weight (mg)	62.10 ± 3.2^{a}	56.10 ± 3.5^{ab}	49.70 ± 4.1^{b}	37.40 ± 5.5^{c}	30.50 ± 6.1^{cd}
Initial stock (ind.)	1000.00	1000.00	1000.00	1000.00	1000.00
Final stock (ind.)	960.00 ± 17^{a}	930.00 ± 25^{ab}	887.00 ± 36^{b}	670.00 ± 60^{c}	380.00 ± 74^{d}
Survival (%)	96.00 ± 1.7^{a}	93.00 ± 2.5^{ab}	88.70 ± 3.6^{b}	67.00 ± 6.0^{c}	38.00 ± 7.4^{d}
Total length increase (TLI) (mm d^{-1})	0.72 ± 0.04^{a}	0.65 ± 0.04^{ab}	0.59 ± 0.05^{b}	0.47 ± 0.05^{c}	0.38 ± 0.06^{cd}
Relative growth rate (RGR) for weight (% d^{-1})	17.50 ± 0.29^{a}	16.93 ± 0.51^{ab}	16.26 ± 0.49^{b}	14.70 ± 0.73^{c}	13.59 ± 1.11^{cd}
Relative growth rate (RGR) for length (% d^{-1})	5.39 ± 0.19^{a}	5.05 ± 0.24^{ab}	4.73 ± 0.28^{b}	4.09 ± 0.31^{c}	3.49 ± 0.42^{cd}
Relative growth rate (RGR) for biomass (% d^{-1})	17.27 ± 0.28^{a}	16.53 ± 0.48^{ab}	15.60 ± 0.49^{b}	12.53 ± 0.71^{c}	8.47 ± 1.05^{d}
Biomass (g)	59.62 ± 3.8^{a}	52.17 ± 4.2^{ab}	44.08 ± 3.9^{b}	25.06 ± 3.32^{c}	11.59 ± 2.3^{d}
Biomass (g dm^{-3})	2.38 ± 0.08^{a}	2.09 ± 0.19^{ab}	1.76 ± 0.16^{b}	1.00 ± 0.13^{c}	0.46 ± 0.09^{d}

Table 2
Final relative mean lengths, weights, and biomasses of blue bream (*B. ballerus*) larvae reared on natural food (A) and commercial feed (P) (water temperature 25.0 ± 0.5°C (± SD), 21 day rearing period). The relative mean final weights (RFW), relative mean final lengths (RFL), and relative mean final biomasses (RFB) of the experimental fish were calculated assuming that the final lengths, weights, and biomasses of the control fish (fed *Artemia* nauplii for 21 days – A21) were 100%

Feeding period with commercial feed (days)	RFW (%)	RFL (%)	RFB (%)
0–21 (P21)	77.66	64.75	49.04
5–21 (A4P17)	84.00	75.88	72.55
9–21 (A8P13)	92.91	87.76	90.33
13–21 (A12P9)	96.74	93.69	95.72

in larvae from variant P21 at 0.38 mm d^{-1}. No statistically significant differences were noted among individuals from the same feeding variants.

The biomass of the blue bream larvae reared on natural food (A21) was 2.38 g dm^{-3} and was nearly 2.4-fold higher than that of the group of larvae fed brine shrimp nauplii for only four days (A4P17) and over five-fold higher than that of the group of larvae fed only Perla feed (P21). The highest larval survival rate was in the group fed only live natural food (A21) at 96%, which was much higher than in the other groups. The group with the lowest survival rate (38%) at the end of the rearing period was in group P21 that was fed only Perla commercial feed. The average cumulative mortality of bream larvae from each feeding variant during rearing is presented in Fig. 3. No statistically significant differences were observed in larval mortality within any of the feeding variants tested.

Similar results were also obtained when natural food was replaced on day 13 with Perla feed (A12P9), which was confirmed by the mean relative final lengths, weights, and biomasses (Table 2). Considering the mean relative final weight (RFW), this index for the larvae fed with Perla feed from day 13 was 96.74% relative to the weight of larvae from the control group (A21). The mean relative final length (RFL) calculated for larvae receiving Perla feed from

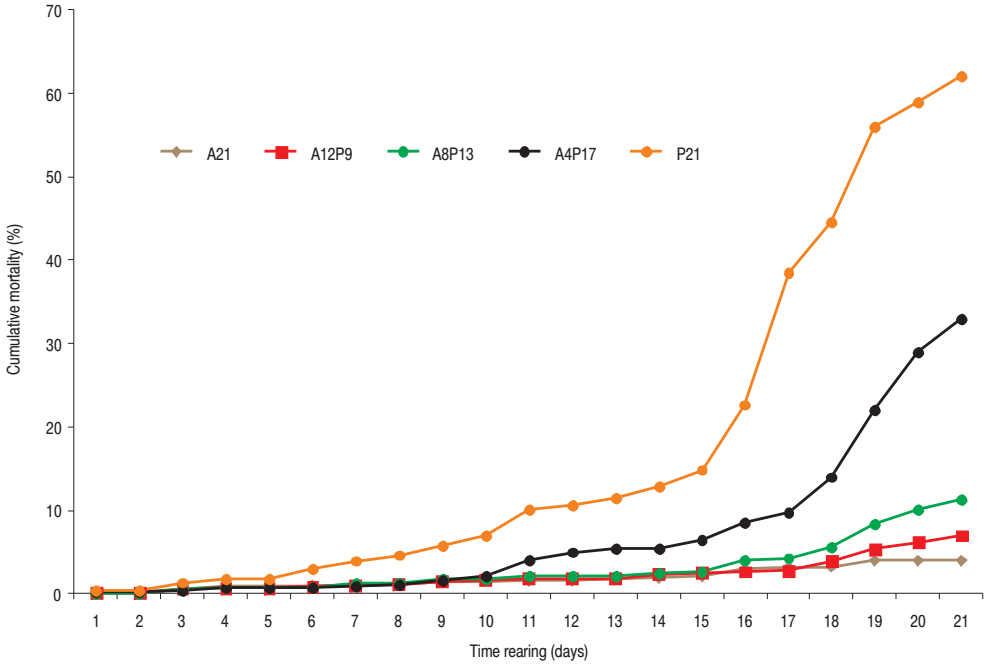

Figure 3. Cumulative mortality of blue bream (*B. ballerus*) larvae fed with natural food (A) that was changed to Perla commercial food after four, eight, or 12 days.

day 13 was 93.69% relative to the length of larvae from the control group. The final values of the relative average biomass (RFB) were analogous to the values given above.

Discussion

One of the greatest challenges of rearing fish larvae under controlled conditions is providing them with the right quality and quantity of food. There is a huge variety of commercial fish larvae rearing feeds suitable for the fish species in question. For most fish species reared for consumption, feeds have already been developed for specific stages of development (Belal 2007, Barbu et al. 2008, Molnar et al. 2010, Şara et al. 2010, Sverinciuc et al. 2017). Appropriate commercial feed compositions ensure rapid growth of larvae and fingerlings while maintaining high survival rates. Aquaculture, however, goes beyond raising commercial fish for consumption; it also produces stocking material of endangered fish species (Kujawa and Piech 2022). These are mostly wild species, not yet domesticated, for which no suitable commercial feeds have been developed. One example is rheophilic cyprinid fishes, the larvae of which begin to take exogenous food at the final stage of yolk sac resorption after the swim bladder has filled (Kupren et al. 2015, Nowosad et al. 2021). The literature available provides information that the first exogenous food can be rotifers, *Brachionus calyciflorus*, which were successfully administered in the first stage of rearing chub, *Squalius cephalus* (L.) (Shiri Harzevili et al. 2003). However, the most frequently used live food, thanks to its ready availability and assimilation by most fishes, is the freshwater zooplankton, or nauplii, of brine shrimp, *Artemia* sp. (Bryant and Matty 1980, Bengtson et al. 2018). Nevertheless, these two types of food have recently become less widely available, and consequently their prices have increased significantly. They also require labor-intensive preparation before being fed to fish larvae.

Compound feeds have become an alternative, but their use requires knowledge of the structure and functioning of the fish larva digestive tract. According to the classification by Dabrowski (1984), 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 contact time between food and digestive enzymes in such young fish is very short (Kaushik 1986, Szlamińska 1987, Wolnicki 2005). Fish larvae compensate for digestive enzyme deficiency during digestive tract development by consuming live food, from which larvae digest and assimilate the nutrients necessary for proper growth and body function (Dabrowski 1984, Wiggins et al. 1986). Determining the right time to replace natural food with feed is a key issue when rearing larvae under controlled conditions.

Scientists investigating this issue suggest that larvae need live food for up to 12 days. Reviewing the literature available on this issue (Hofer and Uddin 1985, Wolnicki and Korwin-Kossakowski 1993, Wolnicki and Górny 1995a, 1995b, 1995c) and the rearing experiment discussed in this paper proved that blue bream larvae, like those of other rheophilous cyprinid fishes, should be fed natural food initially, and only after some time are they able to assimilate effectively the nutrients contained in commercial foods. Some scientists report that feeding cyprinidae larvae commercial feed is clearly related to their individual body weight (Bryant and Matty 1981, Stanny 1984). According to Bryant and Matty (1981), the body weight of an individual fish ranges from 5–15 mg, while according to Stanny (1984) it is 10–12 mg. However, both suggest that live food should be used in the initial feeding stage. This allows the larvae to achieve rapid weight gain and reach subsequent developmental stages. This might explain, at least to some extent, why lower weight and length gains were observed during the initial rearing period of the much lighter blue bream larvae. Similar observations have been made by other researchers who have reared cyprinid fish larvae on starter feeds (Wolnicki 1996, Mookerji and Ramakrishan 1999). This is also confirmed by studies on rearing cyprinid fish larvae using starter feeds (Wolnicki and Myszkowski 1999). These reports confirm that the larvae of many species of cyprinid fishes require several days of feeding with natural

food at the beginning of the rearing period. Only the larvae of rheophilic cyprinid fishes such as barbel, *Barbus barbus* (L.), and common nase, *Chondrostoma nasus* (L.), with average initial body weights of 10 mg, can be fed commercial starter feeds from the beginning of life (Kujawa et al. 2010).

Using commercial feeds after a short time of feeding larvae with natural food is a common solution that leads to satisfactory outcomes. The prerequisite is to select a commercial feed that is suitable for a given fish species. In our experiment rearing blue bream larvae, brine shrimp nauplii was changed to Perla commercial feed, and this enabled us to determine how long the larvae should receive natural food so that they are able to absorb the nutrients from commercial feed. The research results confirmed the observations of other authors that in the first stage of life, which lasts for different lengths of time depending on the cyprinid species, larvae must receive natural food (Skrzypczak et al. 1998, Mamcarz et al. 1998). These authors demonstrated that from days five to 13 of rearing (depending on the fish species and feeds tested), it was possible to replace natural food with feeds while avoiding any considerable losses of larvae. Nonetheless, the growth rates of larvae that received brine shrimp nauplii initially followed by commercial feed tended to be lower than those of fish larvae that received only natural food.

The analysis of the results of rearing blue bream larvae in our experiment revealed that their growth and survival clearly depended on feeding them natural food. The longer the larvae were fed brine shrimp nauplii (*Artemia* sp.), the better the growth gains were in both average weight and total length. The largest larvae were obtained in the variants in which the natural food period was the longest. Larvae from variant A21 fed only brine shrimp nauplii were more than twice as heavy as the larvae fed only Perla feed (P21). This result clearly indicated that there was a relationship between larva mass and food type. The indices of RGR for length, weight, and biomass calculated for the larvae from these variants were also significantly higher than the others. The largest larvae (after control group A21) were in group A12P9, in which natural food was fed for the longest and feed for the shortest period of time. Larvae in group A12P9 were more than 1.5 times heavier than larvae fed natural food for only four days (A4P17). This result clearly suggests that there is a relationship between the body weight of larvae and the period for which they were fed brine shrimp nauplii. This is also clearly confirmed by the final larval biomass from group A12P9 of 2.09, which was more than two times higher than that of the larvae from group A4P17.

The results of rearing blue bream larvae presented in this paper confirm observations of other species of rheophilic cyprinid fished reported by other authors who have studied replacing natural food with commercial feed (Wolnicki and Górny 1995c, Kujawa et al. 1998). Rearing blue bream larvae under controlled conditions requires providing them with access to live food during the initial nursery period. Blue bream larvae, like other rheophilic cyprinid fishes, can be fed commercial feed only after an initial period of feeding them natural food. Replacing live food with commercial feed too early decreases growth parameters and increases larval mortality. The current study showed that replacing natural food with commercial feed after 12 days (A12P9) resulted in high blue bream larva survival rates and satisfactory weight and length gains. It might be possible to shorten this period if a domesticated population of blue bream larvae was maintained. Similar associations were observed when raising domesticated populations in fish of the genus *Leuciscus* (Kwiatkowski et. al. 2008). Increasing the efficiency of rearing blue bream, *Ballerus ballerus*, larvae, as well as that of other rheophilic fishes of the cyprinid family, will be possible after developing commercial feeds with suitable compositions that are enriched with the appropriate exogenous enzymes.

Author contributions. P.P., R.K. designed the study. P.P. conducted the field investigations and provided environmental data. R.K. managed the database and did some of the analyses. R.K. conducted the statistical analyses. P.P did some of the data analyses and drafted the manuscript. All authors contributed to writing the manuscript and approved the submitted final version of it.

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