

Impact of supplementing natural feed with dry diets on the growth and survival of larval asp, *Aspius aspius* (L.), and nase, *Chondrostoma nasus* (L.)

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Roman Kujawa, Dariusz Kucharczyk, Andrzej Mamcarz, Marta Jamróz, Maciej Kwiatkowski, Katarzyna Targońska, Daniel Żarski

Abstract. The aim of the study was to determine how natural feed supplemented with dry diets affects the growth rates and survival of larval asp, *Aspius aspius* (L.), and nase, *Chondrostoma nasus* (L.). The fish were reared for 21 days. Initially, the larvae were fed live *Artemia* sp. nauplii, and a commercial feed SGP 493 (Aller Aqua) was added after 6 (A6), 8 (A8), 10 (A10), and 12 (A12) days. Depending on the feeding treatment, dietary supplements were then added to the natural feed at intervals of 1, 2, or 3 days. No statistically significant differences were noted in the growth of larval asp from groups A6 or A12, and groups A8 or A10. The relative growth rates (RGR) ranged from 18.16 (A8) to 18.61% d⁻¹ (A12). No statistically significant differences were noted among the experimental groups of larval nase. RGR values ranged from 12.74 (A6) to 12.91% d⁻¹ (A12). The results obtained indicate that supplementing with dry diets, which was done as early as in the first week of larval exogenous feeding, has a positive influence on the fish. This indicates that greater flexibility in feeding can be applied during the initial rearing of the larval stages of the species tested.

Keywords: cyprinids, rheophilic fish, larvae, controlled rearing, natural food, formulated diets

Introduction

Reintroducing endangered rheophilic fish species was initiated by investigating and describing their reproductive biology, which was then followed by the development of methods for artificial reproduction using spawners obtained from the natural environment (Cejko et al. 2008, 2009, Jamróz et al. 2008, Krejszef et al. 2008, 2009, Targońska et al. 2008a, 2008b, Żarski et al. 2008a, 2008b, 2009). The subsequent stage was to develop rearing technologies that can be used under controlled conditions to produce stocking material (Sych 1996, Kujawa et al. 1998a, 1998b, Kujawa 2004, Wolnicki 2005, Kwiatkowski et al. 2008, Żarski et al. 2008c). The cryopreservation of semen (Babiak et al. 1998, Lahnsteiner et al. 2003, 2004) and genome manipulation (Kucharczyk 1999, 2001, Kucharczyk et al. 2008a, 2008b, 2008c) were also studied, and both can be useful in the development of plans to reintroduce endangered species of rheophilic cyprinid fish. The larvae of these fish species are reared in carp ponds (Śliwiński 2009), in lake cages using light at night to attract zooplankton (Kujawa et al. 1998c, Skrzypczak et al. 1998), and in closed recirculation systems (Kujawa 2004, Wolnicki 2005, Żarski et al. 2008d). In the beginning, the initial rearing of rheophilic cyprinid species larvae was done using freshwater zooplankton collected directly from

R. Kujawa [✉], D. Kucharczyk, A. Mamcarz, M. Jamróz, M. Kwiatkowski, K. Targońska, D. Żarski
Department of Lake and River Fisheries
University of Warmia and Mazury in Olsztyn
Oczapowskiego 5, 10-718 Olsztyn, Poland
Tel. +48 89 5233436, e-mail: reofish@uwm.edu.pl

the natural environment. However, because this method is labor intensive and is a likely vector for the transmission of disease and disease-causing microbes *Artemia* sp. nauplii were used instead. Since prices for *Artemia* cysts are very high and fluctuate annually, larval rheophilic cyprinid fish are often fed commercially available dry starter diets for carp, *Cyprinus carpio* (L.), or trout, *Oncorhynchus mykiss* (Walbaum) or also larval marine fish (Kamler et al. 1987, Foresti 2000, Shiri Harzevili et al. 2003, Kujawa 2004, Wolnicki 2005, Kupren et al. 2008, Kwiatkowski et al. 2008). These solutions are not suitable, since, as has been demonstrated with other larval cyprinid fish, digestive enzymes in the initial stage of life are very weak, and dry starter diets are not recommended at this stage of life (Dabrowski 1984a, 1984b). In the initial stage of life, digestion is aided by the enzymes that are contained in the zooplankton consumed (Dabrowski 1984a, 1984b). Depending on the species, larvae are not able to digest starter diets effectively until after a certain period. To date, no suitable starter diet has been developed for larval rheophilic cyprinid fish, which is why many scientific institutions are currently researching the impact feeding commercially available diets has on the results of rearing larvae (Kujawa 2004, Wolnicki 2005, Kamler and Wolnicki 2006, Kwiatkowski et al. 2008). Research is also being conducted to determine the suitability of different types of feeds, and the possibilities of introducing them following a designated period of feeding with *Artemia* nauplii. Larval rheophilic cyprinid fish require natural food for a period that ranges from several to more than a dozen days, and if this period is too short, survival and growth rates are affected negatively (Kujawa 2004, Wolnicki 2005, Kwiatkowski et al. 2008). However, it is not always feasible in daily fisheries practice to apply prescribed feeding procedures. Additionally, no studies to date have investigated the impact alternating live food and dry starter diets has on larval rheophilic cyprinid fish.

The aim of the current study was to determine how natural feed substituted with formulated diets affected the growth rates and survival of larval asp, *Aspius aspius* (L.), and nase, *Chondrostoma nasus* (L.).

Materials and Methods

Obtaining larvae for the experiments

Asp and nase larvae were obtained through artificial reproduction under controlled conditions. Spawners had been caught in the wild, they were transported to a hatchery and rearing facility and released into tanks with a volume of 1000 dm³ and fitted with an environmental control system (Kujawa et al. 1999). Eggs and milt were obtained after the fish had been stimulated with the hormone preparation Ovopel (Unic-trade, Hungary), which comprises an active GnRH analogue and metoclopramide (Horváth et al. 1997). Two doses were injected intraperitoneally beneath the ventral fin at 24-hour intervals. The Ovopel doses were 0.2 and 1.0 pellet kg⁻¹ of spawner body weight. Detailed descriptions of the spawning methods for the two species were published in Targońska et al. (2008b) and Żarski et al. (2008a, 2008c).

Eggs were obtained from nase 24 hours and from asp 36 hours following the second injection. After fertilization, the adhesiveness was removed from the eggs. The eggs were incubated for 10 days in Weiss jars in water at a temperature of 14°C. After hatching, the larvae resorbed their yolk sacs in water at a temperature of 20°C. The rate of water temperature change was below 1°C h⁻¹.

Experimental setup and larval feeding procedure

At five days post hatch, the larvae, which had partially resorbed yolk sacs and inflated swim bladders, were placed in glass tanks (aquariums) with volumes of 25 dm³. The aquariums were placed in a closed rearing rearing water bath system (Kujawa et al. 2000). The flow of water into the individual aquariums caused little water movement, thanks to which the feed supplied to the tanks was evenly distributed throughout the tank water where it remained suspended in the water column for a substantial period of time.

During rearing, water oxygen saturation ranged from 6.5 to 7.3 mg O₂ dm⁻³, while pH values ranged from 7.3 to 7.6. No ammonia nitrogen was noted in the water during the experiment. The daily light cycle was constant at 12L:12D. Water temperature during rearing was 25°C (±0.5), which is the optimum temperature for rearing larvae asp and nase (Wolnicki and Myszkowski 1998, Kujawa 2004). The larvae density in the tanks was 40 indiv. dm⁻³. The fish were fed four times daily at 08:00, 11:00, 14:00, and 17:00. The quantity of feed supplied was increased proportionally to larval growth. The feed remained suspended in the aquariums for an hour after it had been delivered. Initially, the larvae were fed live *Artemia* sp., this was followed by a starter diet SGP 493 (Aller Aqua). It contained 53% protein, 15% fat, 12% carbohydrates. The starter was begun on days 6 (A6), 8 (A8), 10 (A10), and 12 (A12), and then, depending on the treatment, natural feed was supplied alternately with the starter at intervals of 1 (A6), 2 (A8), or 3 (A10) days. The larvae that were fed natural feed for 12 days (A12) received starter until the end of the experiment. The period during which the larvae in all of the feeding treatments received natural food was constant at 12 days. The order in which natural food and starter were supplied is presented in the feeding scheme in Fig. 1. The duration of each of the experiments was three weeks. The tanks were cleaned of leftover food, excrement, and dead larvae every morning prior to the first feed of the day. The experiments were repeated in two replicates.

Measurements (TL ± 0.1 mm; body weight ± 1 mg) were taken every four days using 30 individuals chosen at random from each of the rearing tanks. Before the fish were measured, they were anesthetized in a solution of Propiscin (Kazuń and Siwicki 2001).

Initially, the concentration used was 0.5 ml dm⁻³, but as the experiment continued, the dose was reduced to 0.15 ml dm⁻³. After the measurements, all of the fish were returned to the same tanks from which they had been removed.

Data analysis

The biomass of the fish from each tank was determined by multiplying the mean weight of the individuals and the number of live individuals, and then dividing this by the volume of the tank (g dm⁻³). The relative growth rate (RGR) of individual weight gain from the beginning of exogenous feeding until the end of the experiment was calculated with the formula by Ricker (1975):

$$RGR = 100 (e^G - 1)$$

$$G = (\ln BW_f - \ln BW_i) n^{-1}$$

where:

G – relative growth rate; BW_i – mean initial individual weight (mg) during rearing; BW_f – mean final individual weight (mg) during rearing; n – rearing period (days).

The index of increase in total length within a time unit ITL (mm d⁻¹) was calculated using the following formula from Peñáz et al. (1989):

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

where :

TL – mean individual length (*longitudo totalis*);

n₁ – beginning of time unit;

n₂ – end of time unit;

Δt – rearing period (days).

Feeding procedure/Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A 6																					
A 8																					
A 10																					
A 12																					

 - nauplii *Artemia* sp.
 - formulated diet SGP 493 (Aller Aqua)

Figure 1. The larvae feeding regime.

Table 1

Results of rearing asp (*Aspius aspius*) larvae. Natural feed was supplied first for 6 (A6), 8 (A8), 10 (A10), and 12 (A12) days (depending on the feeding treatment), and then this was substituted alternately with a formulated diet at intervals of 1, 2, and 3 days. Means \pm SD. The results in rows with the same letter index do not differ significantly statistically ($P \leq 0.05$)

Parameter	A6	A8	A10	A12
Mean initial weight (mg)	3.00 ^a \pm 0.2	3.00 ^a \pm 0.2	3.00 ^a \pm 0.2	3.00 ^a \pm 0.2
Mean final weight (mg)	106.5 ^b \pm 14.0	99.8 ^a \pm 13.0	100.7 ^a \pm 10.0	108.0 ^b \pm 15.4
Mean initial length (mm)	9.1 ^a \pm 0.2	9.1 ^a \pm 0.2	9.1 ^a \pm 0.2	9.1 ^a \pm 0.2
Mean final length (mm)	23.8 ^b \pm 0.86	22.8 ^a \pm 0.85	22.9 ^a \pm 1.1	24.1 ^b \pm 1.0
Initial stocking density (indiv.)	1000	1000	1000	1000
Mean final stocking density (indiv.)	979.5 ^a \pm 2.1	989.5 ^a \pm 0.7	986.0 ^a \pm 0.7	996.5 ^b \pm 0.0
Index of increase in total length (ITL) (mm d ⁻¹)	0.70 ^a \pm 0.13	0.65 ^a \pm 0.09	0.66 ^a \pm 0.08	0.71 ^a \pm 0.12
Relative growth rate (RGR) (% d ⁻¹)	18.53 ^b \pm 0.07	18.16 ^a \pm 0.09	18.21 ^a \pm 0.1	18.61 ^b \pm 0.10
Biomass (g dm ⁻³)	4.17 ^b \pm 0.05	3.95 ^a \pm 0.08	3.97 ^a \pm 0.08	4.30 ^b \pm 0.10
Survival (%)	97.95 ^a \pm 0.2	98.95 ^{ab} \pm 0.07	98.60 ^a \pm 0.07	99.65 ^b \pm 0.0

Table 2

Results of rearing nase (*Chondrostoma nasus*) larvae. Natural feed was supplied first for 6 (A6), 8 (A8), 10 (A10), and 12 (A12) days (depending on the feeding treatment), and then this was substituted alternately with a formulated diet at intervals of 1, 2, and 3 days. Means \pm SD. The results in rows do not differ significantly statistically ($P \leq 0.05$)

Parameter	A6	A8	A10	A12
Mean initial weight (mg)	10.00 \pm 0.6	10.00 \pm 0.6	10.00 \pm 0.6	10.00 \pm 0.6
Mean final weight (mg)	124.0 \pm 14.0	125.2 \pm 16.0	126.5 \pm 12.3	128.0 \pm 8.5
Mean initial length (mm)	11.6 \pm 0.3	11.6 \pm 0.3	11.6 \pm 0.3	11.6 \pm 0.3
Mean final length (mm)	24.1 \pm 0.9	23.9 \pm 1.4	23.9 \pm 1.1	24.4 \pm 0.5
Initial stocking density (indiv.)	1000	1000	1000	1000
Mean final stocking density (indiv.)	979.0 \pm 1.4	979.5 \pm 4.9	978.5 \pm 3.5	972.5 \pm 2.1
Index of increase in total length (ITL) (mm d ⁻¹)	0.59 \pm 0.02	0.57 \pm 0.01	0.59 \pm 0.03	0.61 \pm 0.02
Relative growth rate (RGR) (% d ⁻¹)	12.74 \pm 0.06	12.79 \pm 0.1	12.84 \pm 0.14	12.91 \pm 0.09
Biomass (g dm ⁻³)	4.86 \pm 0.06	4.91 \pm 0.08	4.95 \pm 0.12	4.98 \pm 0.08
Survival (%)	97.90 \pm 0.14	97.95 \pm 0.49	97.85 \pm 0.35	97.25 \pm 0.21

Data expressed in percentages were subjected to arcsin transformation prior to statistical analysis. Statistical differences among groups were analyzed after ANOVA analysis of variance and then Tukey's post-hoc test had been performed ($P < 0.05$).

Results

Asp larvae growth was similar in groups A6 and A12 and in A8 and A10 (Fig. 2). On the final day of rearing the mean larval asp body weight from treatment

A12 was 108.0 mg at a mean length of 24.1 mm, which was slightly higher than in treatment A10 (100.7 mg and 22.9 mm, respectively). The indexes of increase in total length per unit of time (ITL) in larval asp in groups A6 and A12 and A8 and A10 were very similar (Table 1). Larval mortality during rearing was low (Fig. 2c). Increased mortality in groups A6 and A10 (at 0.9 and 1.05%, respectively) was noted from day 12 of rearing. On the final day of the experiment, survival in these groups was significantly lower than in treatment A12, in which the survival rate was 99.65%.

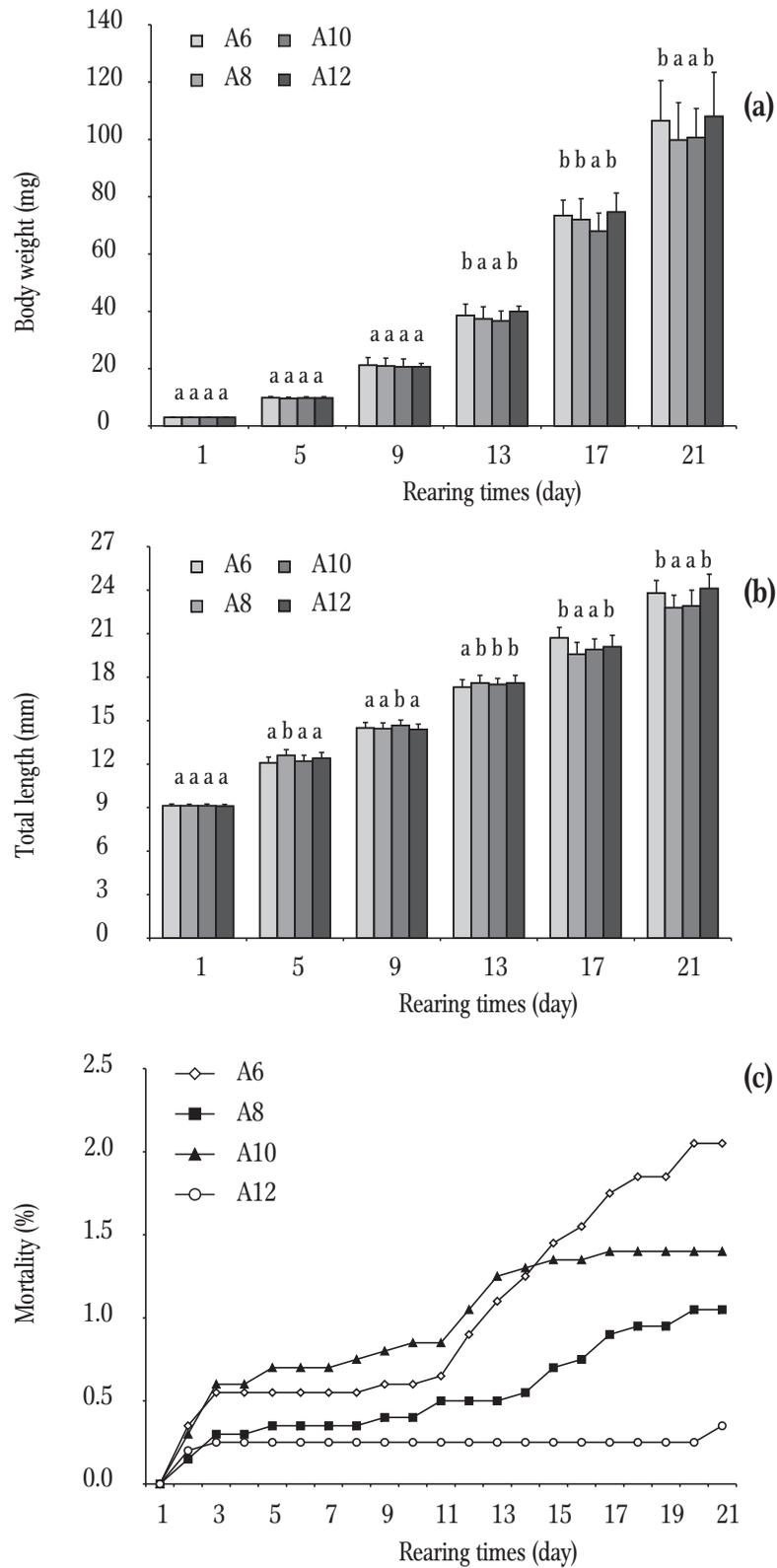


Figure 2. Growth in body weight (a) and total length (b) and cumulative mortality (c) in asp larvae fed initially with *Artemia* sp. and then a formulated diet. Feed was supplied after 6 (A6), 8 (A8), 10 (A10), or 12 (A12) days. Body weight and total length values are presented as means (\pm SD). Measurement values from subsequent days with the same letter index do not differ significantly statistically ($P > 0.05$).

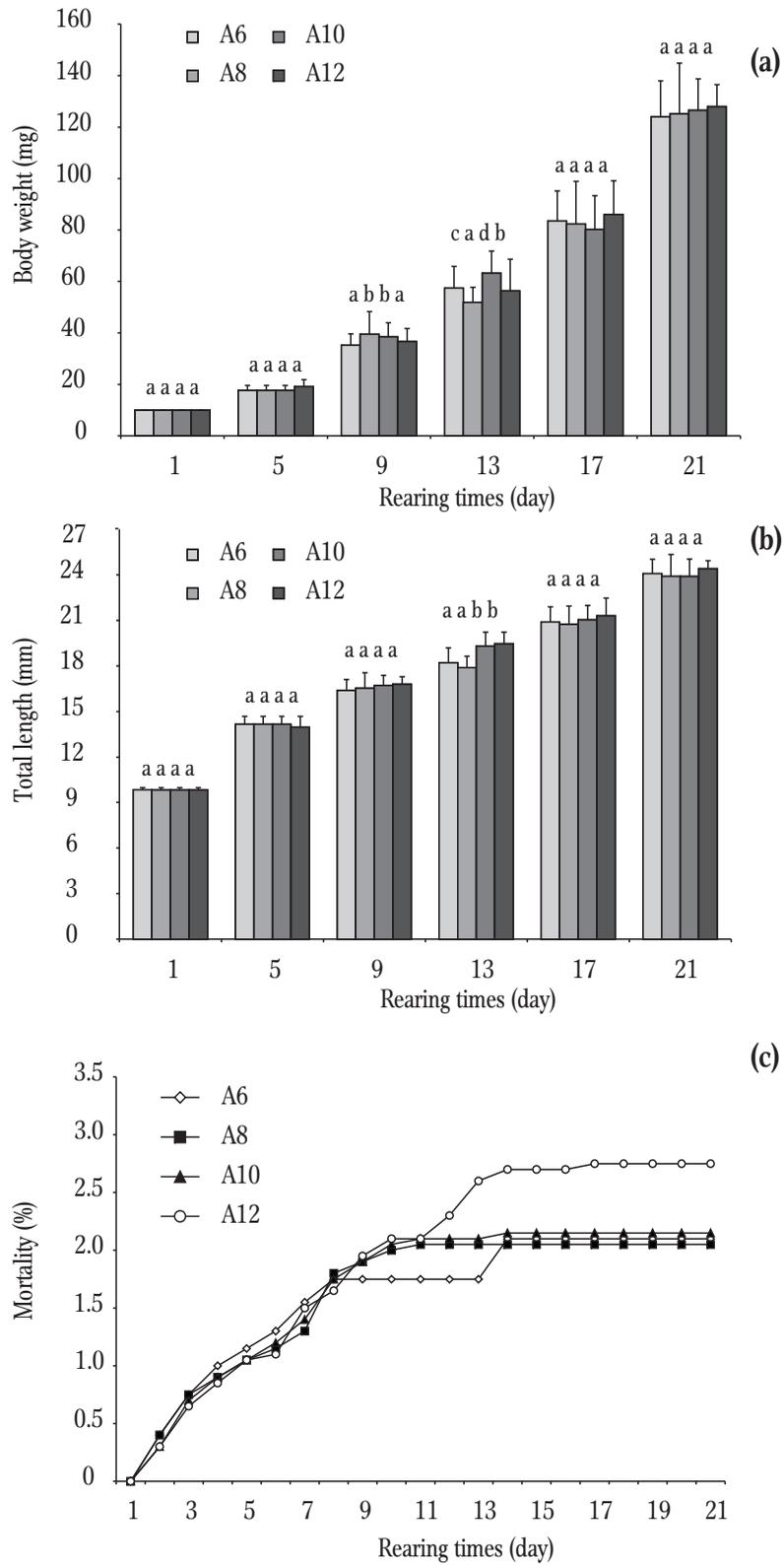


Figure 3. Growth in body weight (a) and total length (b) and cumulative mortality (c) in nase larvae fed initially with *Artemia* sp. and then a formulated diet. Feed was supplied after 6 (A6), 8 (A8), 10 (A10), or 12 (A12) days. Body weight and total length values are presented as means (\pm SD). Measurement values from subsequent days with the same letter index do not differ significantly statistically ($P > 0.05$).

Nase larvae weight and length growth did not differ significantly statistically among any of the treatment groups (Fig. 3). By the end of rearing, the nase larvae from group A12 had attained a weight of 128.0 mg at a length of 24.4 mm, while the values for larvae from group A6 were 124.0 mg and 24.1 mm (Table 2). Deaths of individuals were noted successively for the first two weeks of rearing; however, the cumulative mortality for larval nase in all the feeding treatment groups was very low and did not exceed 3% (Fig. 3c).

The fish studied grew at rates of 0.7 mm d^{-1} (asp) and 0.6 mm d^{-1} (nase). The relative growth rate in weight for asp larvae ranged from $18.2\% \text{ d}^{-1}$ (A8) to $18.6\% \text{ d}^{-1}$ (A12), while the RGR calculated for nase larvae from all of the treatments studied oscillated around $12.8\% \text{ d}^{-1}$. The final larval biomass was approximately 4.0 g dm^{-3} for asp and 4.9 g dm^{-3} for nase.

Discussion

Deteriorating habitat conditions for rheophilic fish have resulted in declining populations of rheophilic cyprinid fish (Backiel 1985, Penczak et al. 1998, Bolland et al. 2008), and even to their extinction in some areas (Marszał and Przybylski 1996, Witkowski 1996a, 1996b). In order to either stop or decelerate this process, work has been undertaken with the aim of saving endangered populations. One method that can contribute to increasing the numbers of threatened species is the implementation of appropriate regulations that protect both the species and their habitats (Witkowski et al. 1999, Kamler and Wolnicki 2006). However, passive protection alone can be insufficient if, in seriously deteriorated aquatic habitats, fish only spawn sporadically and the eggs deposited under such conditions do not survive for long. This is why work has been undertaken to develop reproduction and rearing technologies for rheophilic cyprinid fish under controlled conditions (Wolnicki 2005, Hamačková et al. 2007, 2009, Bolland et al. 2008, Cejko et al. 2009, Krejszeff et al.

2008, 2009, Targońska et al. 2008b, Źarski et al. 2009).

One of the main problems that occurs during the rearing of larvae under controlled conditions is ensuring that the feed is appropriate in terms of both quality and quantity. Rheophilic larvae cyprinid fish begin exogenous feeding in the final stages of yolk sac resorption after the swim bladder has inflated. During this critical stage of larval development, their feeding is dependent on the density of food, its sizes, its ability to move, and the time it is suspended in the water (Dabrowski 1976, Hartmann 1983, Wieser et al. 1988, Kujawa 2004). This is also why, at this stage of larval life, natural food is decidedly more suitable than commercial feed. Additionally, the ingestion of exogenous digestive enzymes along with live food results in decidedly better digestion and absorption by the fish (Dabrowski 1984a, 1984b, Wiggins et al. 1986). Many studies of cyprinid fish fed formulated diets confirm that their introduction must be preceded by a period of feeding with live natural food (Kujawa et al. 1998a, 1998b, Wolnicki and Myszkowski 1999a, 1999b, Kujawa 2004, Wolnicki 2005). Some authors suggest that larvae cyprinids can be fed commercial feeds once they have achieved a suitable body weight. Bryant and Matty (1981) report that the mean weight for carp is between 5 to 15 mg, while Stanny (1984) reports that it is from 10 to 12 mg. Other authors suggest, however, that larvae require live food for a period ranging from several to more than a dozen days. Natural food is most frequently supplied at the beginning of rearing, and only later is a dry diet supplied; this is sometimes done through the application of transition periods when live food and starters are mixed (Kujawa 2004, Wolnicki 2005, Kwiatkowski et al. 2008).

Data from the literature indicates that nase larvae can be reared from the beginning on formulated diets (Kujawa et al. 1998b, Wolnicki and Myszkowski 1998); however, mortality rates during the initial rearing period are higher. The growth rates of nase larvae fed formulated diets exclusively were also slower in comparison to those noted when mixed feed or live food was used (Kujawa et al. 1998b, Wolnicki and Myszkowski 1999a, 1999b,

Kujawa 2004). Feeding asp larvae dry diets from the beginning of exogenous feeding is not recommended because the results of rearing are considerably worse (lower body weight gain and very high mortality). Satisfactory results were achieved, however, when feed was substituted for *Artemia* after 12 days of rearing (Kujawa 2004) or when mixed feeding was used for a period of seven days following rearing on natural feed (Kwiatkowski et al. 2008). In the current study, supplying asp and nase larvae natural feed and dry starter did not impact either growth rates or survival significantly, and, despite significant differences with regard to asp, the results were very satisfactory and comparable with those reported by other authors (Kujawa et al. 1998a, 1998b, Kujawa 2004, Wolnicki 2005, Kwiatkowski et al. 2008, Źarski et al. 2008b). This indicates that during the first three weeks of rearing if a 12-day period of feeding with natural food is maintained, regardless of the feeding treatment applied, asp and nase larvae can be fed both natural food and formulated diets. Additionally, it must be underscored that a strict diet of natural food is only required for six days, which, in comparison with the results of a study by Kujawa (2004) during which natural food was supplied for 12 days, is an excellent result. This finding is especially valuable for those who produce stocking material of these two species under controlled conditions.

The RGR values for asp noted during the current study indicate that the growth rate of these larvae was fast in comparison to those noted by other authors. The worst result was noted in group A8 (18.16% d⁻¹), but this is still significantly higher than the best result reported by either Źarski et al. (2008b) (17.19% d⁻¹) or Kujawa et al. 1998a (15.3% d⁻¹). However, the best result (18.61% d⁻¹) was worse than that noted by Wolnicki (2005) (19.9% d⁻¹). In the case of nase, however, the RGR rates noted (the values of this index ranged from 12.74 to 12.89% d⁻¹) were moderate in comparison with those obtained by other authors. Kujawa (2004) reported a value of 11.02% d⁻¹ when a comparable feeding regime to that of group A8 in the current study was applied. In turn, the best result reported by Spurný et al. (2004) was 8.92% d⁻¹. The value of the ITL index obtained for asp in the current

study was higher than either of those noted by Źarski et al. (2008b) (0.67 mm d⁻¹) or Wolnicki (2005) (0.57 mm d⁻¹). Corresponding dependencies were also noted with regard to nase. Spurný et al. (2004) reported as the best result a value of 0.45 mm d⁻¹, while Kujawa (2004) reported 0.54 mm d⁻¹.

Total larval mortality was low, and did not exceed 3%. The percentage of surviving larvae was high and comparable to that noted by, among others, Kujawa (2004) and Wolnicki (2005). It was also substantially higher than that described by Kwiatkowski et al. (2008) for larvae of the genus *Leuciscus*. However, a comparison of mortality curves indicates that they are similar to those in studies by Kwiatkowski et al. (2008) and Źarski et al. (2008b). Increased mortality was noted from day 12 of rearing. This might be linked to any number of factors, including the deaths of fish that did not begin to feed. In this case, increased mortality occurred at the same time because of the uniform water temperature during rearing (25°C).

Recently, many aspects of feeding rheophilic cyprinid fish larvae reared under controlled have been studied, and feeding techniques have been altered substantially (Kujawa 2004, Spurný et al. 2004, Wolnicki 2005, Kwiatkowski et al. 2008, Źarski et al. 2008b, Hamačková et al. 2009). New feeds are continually becoming available on the market, as are new technologies for obtaining the most suitable of natural foods for use during larval rearing (Targońska 2007), both of which permit obtaining increasingly better production results. However, from a practical point of view it remains very important to define the biological conditions that can influence production effectiveness. The results obtained during the current study indicate that the initial feeding methods for larval rheophilic cyprinid fish are far more flexible than was believed previously.

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Streszczenie

Wpływ suplementacji pokarmu naturalnego paszą granulowaną na wzrost i przeżywalność larw bolenia, *Aspius aspius* (L.) i świnki, *Chondrostoma nasus* (L.)

Celem pracy było określenie wpływu sposobu suplementacji paszy komponowanej pokarmem naturalnym na tempo wzrostu oraz przeżywalność larw bolenia, *Aspius aspius* (L.) oraz świnki, *Chondrostoma nasus* (L.). Podchów prowadzono w zbiornikach o pojemności 25 dm³ umieszczonych w zamkniętym obiegu wody przez 21 dni. Larwy początkowo żywione były żywymi naupliusami solowca (*Artemia* sp.). Podawanie paszy (Aller Aqua – SGP 493) rozpoczęto po 6 (A6), 8 (A8), 10 (A10) i 12 (A12) dniach. Następnie, w zależności od wariantu żywienia, stosowano suplementację diety pokarmem naturalnym, zadawanym w odstępach 1, 2 lub 3 dniowych. W przypadku larw, które otrzymywały początkowo przez 12 dni pokarm naturalny, paszę podawano już do końca podchowu. Nie stwierdzono różnic istotnych statystycznie we

wzroście larw bolenia pochodzących z grup A6 i A12 (relatywny przyrost masy RGR wyniósł odpowiednio, 18,53 i 18,61% d⁻¹) oraz A8 i A10 (RGR odpowiednio 18,16 i 18,21% d⁻¹). W przypadku larw świnki nie odnotowano różnic istotnych statystycznie pomiędzy grupami doświadczalnymi (zakres RGR zawierał się w przedziale 12,74-12,91% d⁻¹). Uzyskane wyniki wskazują na pozytywny wpływ suplementacji paszy komponowanej, którą wprowadzono już w pierwszym tygodniu odżywiania egzogennej larw. Śmiertelność podchowianych larw, zarówno bolenia jak i świnki nie przekroczyła 3%, co w świetle wyników innych autorów jest bardzo dobrym rezultatem. Stwarza to możliwość znacznego uplastycznienia wstępnego podchowu larw badanych gatunków ryb, dotychczas traktowanego bardzo restrykcyjnie.