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THE EFFECT OF STOCKING DENSITY ON THE GROWTH AND SURVIVAL OF LARVAL ASP, *ASPIUS ASPIUS* (L.), AND EUROPEAN CHUB, *LEUCISCUS CEPHALUS* (L.), DURING REARING UNDER CONTROLLED CONDITIONS

Daniel Żarski, Dariusz Kucharczyk, Maciej Kwiatkowski, Katarzyna Targońska, Krzysztof Kupren, Sławomir Krejszeff, Marta Jamróz, Anna Hakuć-Błażowska, Roman Kujawa, Andrzej Mamcarz

Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. The aim of the study was to determine the effect of increased stocking density on the growth and survival of larval asp, *Aspius aspius* (L.), and European chub, *Leuciscus cephalus* (L.), during mass rearing under controlled conditions. Fish larvae were obtained during artificial spawning at a hatchery. The larvae were reared for 21 days in a semi-closed recirculating system at a water temperature of 25°C. They were stocked at four densities (50, 100, 150, and 200 indiv. dm^{-3}) and were fed freshly hatched *Artemia* sp. nauplii. The fastest growth rate for both species was obtained at the smallest stocking density (ITL = 0.67 and 0.58 mm d^{-1} for asp and chub, respectively). The highest mortality of larval asp was recorded at the density of 200 indiv. dm^{-3} (11.68%), and for chub at the density of 50 indiv. dm^{-3} (40.4%). The results obtained indicated that the stocking densities applied do not have an impact on larval growth or survival in the first week of rearing.

Key words: RHEOPHILIC CYPRINIDS, LARVICULTURE, GROWTH RATE, STOCKING DENSITY

INTRODUCTION

In addition to its primary function for food production, aquaculture is also a tool that can be used for restoring threatened populations of fish through controlled larval production and rearing (Philippart 1995, Kucharczyk 2002). One of the most important aspects taken into consideration during the development of biotechnologies for the production of stocking material is the profitability of the method implemented (Turkowski 2002). High economic effectiveness can be achieved in the rearing of cyprinid fish in recirculating systems that permit high stocking densities (Kujawa 2004, Wolnicki 2005).

CORRESPONDING AUTHOR: Daniel Żarski, University of Warmia and Mazury in Olsztyn, Department of Lake and River Fisheries, Oczapowskiego 5, 10-957 Olsztyn, Poland. Tel./Fax: +48 895234436, +48 895233969; e-mail: danielzarski@poczta.interia.pl

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Asp, *Aspius aspius* (L.), is predatory fish inhabiting the flowing waters of central and eastern Europe, southern Scandinavia, and catchment basins of the Black and Caspian seas in Asia (Mamcarz 2000). They occur abundantly in dam reservoirs and in lakes that are linked to rivers (Kirsipuu and Pihu 1996). This species is currently classified as endangered in the European Union. Although European chub, *Leucisus cephalus* (L.), is a species of little economic value, it is a significant element of freshwater ichthyofauna in the flowing waters of Europe, Asia Minor, and South Caucasus. This species is under considerable recreational fishing pressure in Poland, similarly to asp, and this has led to decreased abundance in many local populations (Błachuta 1998, Penczak et al. 1998).

It is widely known that the stocking density of larvae during rearing significantly influences growth rates and final larval survival. Despite this and the analysis of works by many authors, it was impossible to determine unequivocally the impact larval stocking density in a volume unit had on the growth rate or mortality of rheophilic larval cyprinid fish. This was also rendered impossible by the various feeding regimes used (feed type, quantity, frequency) water temperature, and photoperiod (Wolnicki 2005). The aim of the current work was to determine the effect stocking density had on larval asp and chub growth rates and survival during mass rearing under controlled conditions.

MATERIALS AND METHODS

The study materials were larval asp and chub obtained from artificial reproduction conducted at the hatchery of the Department of River and Lake Fisheries at University of Warmia and Mazury in Olsztyn. Asp spawners were caught in the Pierzchalskie Dam Reservoir (northeastern Poland) in fall 2007, and were held until the following spawning season in earthen ponds at the Czarci Jar Fish Farm near Olsztynek (northeastern Poland). The cultivated brood stock of European chub came from the Knieja Fish Farm near Częstochowa (southern Poland), and artificial spawning was conducted according to methods described by Krejszeff et al. (2008). Ovopel (Unic-trade, Hungary) was used to stimulate gamete maturation in both species.

Rearing was conducted for 21 days in a semi-closed recirculating system equipped with regulation devices for water temperature (±0.1°C), photoperiod, and aeration.

Water temperature during rearing was 25°C. The daily water exchange rate in the system was 30% of its capacity. The rearing tanks (aquaria with a volume of 50 dm^3) were illuminated with fluorescent lights for a photoperiod of 12L:12D. The light intensity was not measured. Rain water was directed into each tank separately. The larvae were stocked in the tanks at the moment they began active swimming, and they were fed three times daily *ad libitum*. The feed was comprised of freshly hatched *Artemia* sp. nauplii. Rearing was conducted at four stocking densities: 50, 100, 150, 200 indiv. dm^{-3} (asp at A50, A100, A150, A200 and chub at L50, L100, L150, L200). The experiment with asp was conducted in three replicates, while that with chub in two, which was because of an insufficient quantity of larvae. The concentration of ammonia, nitrites, and nitrates was monitored with an LF250 photometer (Slanøi, Poland), and the dissolved oxygen content and pH were measured with an HI 9828 multi-parameter instrument (Hanna Instruments, Italy).

The total length (± 0.1 mm) of 30 individuals from each experimental variant were measured at intervals of 7 days with the ProgRes® Capture Pro 2.5 (Jenoptik, Germany) image analysis system. Prior to the measurements, the fish were anesthetized in a solution of 2-phenoxyethanol (0.4 cm^3 dm^{-3}; Sigma-Aldrich, Germany), and then released into the aquarium from which they were caught. The results permitted calculating the relative growth rates of weight (RGR_W) and length (RGR_L) (Myszkowski 1997) according to the formula:

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

where: SGR = 100 (ln W_1 – ln W_2) Δt^{-1}, W_1 – initial weight/length, W_2 – final weight/length, Δt – fish growth period (days).

The relative biomass rate (RBR) was calculated according to the formula:

$$RBR = 100\left(e^{\frac{SBR}{100}} - 1\right) \tag{2}$$

where: SBR = 100 SGR = 100 (ln (N_2 W_2) – ln (N_1 W_1)) Δt^{-1}, N_1 – stock number at the start of the experiment, N_2 – stock number at the end of the experiment.

The index of increments of total length growth in a time unit (ITL) was calculated according to the formula (Peňáz et al. 1989):

$$ITL = \frac{TL_2 - TL_1}{\Delta t} \tag{3}$$

where: TL_1 – mean individual length (*longitudo totalis*) at the start of the experiment, TL_2 – mean individual length (*longitudo totalis*) at the end of rearing.

Statistical differences between groups regarding larval length and weight were analyzed with analysis of variance (ANOVA) and Tukey's post hoc test ($P < 0.05$).

RESULTS

The highest asp survival was noted in the A100 group, while the lowest was in the A200 group (Table 1). The highest mortality was noted in larval asp during the first week of rearing, and in group A200 until day 10 of the experiment (Fig. 1a). The fish from variant A50 had the highest body length growth rate (Fig. 2a). Until day 14 of rearing, no significant differences were noted in the growth rate of the larvae at the other stocking densities. After another 7 days of rearing, the length of the larvae in tanks with the highest density (group A200) differed significantly from that of the other groups ($P < 0.05$). The highest value of the ITL index was confirmed in group A50 (0.67), while the lowest was noted in group A200 (0.55). The most rapid growth in asp body length was confirmed in group A50, and the slowest in group A200 (Table 1).

TABLE 1

Initial and final characteristics of the rearing of larval asp, *Aspius aspius,* (mean ± SD)

	Stocking density (indiv. dm^{-3})			
Parameter	50	100	150	200
Mean initial weight (mg)*	3.2 ± 0.2^a	3.2 ± 0.2^a	3.2 ± 0.2^a	3.2 ± 0.2^a
Mean final weight (mg)*	88.4 ± 17.31^c	74.2 ± 21.72^b	72.0 ± 14.30^b	60.5 ± 16.76^a
Mean initial length (mm)*	9.55 ± 0.4^a	9.55 ± 0.4^a	9.55 ± 0.4^a	9.55 ± 0.4^a
Mean final length (mm)*	23.55 ± 1.49^c	22.71 ± 1.67^b	22.30 ± 1.58^b	21.19 ± 1.78^a
Survival (%)	92.75 ± 1.72	94.83 ± 2.65	94.11 ± 1.76	88.32 ± 1.96
Index of incremental total length (ITL) (mm d^{-1})	0.67 ± 0.0	0.63 ± 0.1	0.61 ± 0.0	0.55 ± 0.1
Relative growth rate in weight (RGR_W) (% d^{-1})	17.19 ± 0.2	16.22 ± 0.3	16.04 ± 0.1	15.09 ± 0.2
Relative growth rate in length (RGR_L) (% d^{-1})	4.39 ± 0.1	4.21 ± 0.2	4.12 ± 0.1	3.87 ± 0.1
Relative biomass rate (RBR) (% d^{-1})	16.77 ± 0.2	15.92 ± 0.3	15.71 ± 0.1	14.41 ± 0.2

**Results in the same row with different letter indices differ significantly statistically ($P \leq 0.05$)*

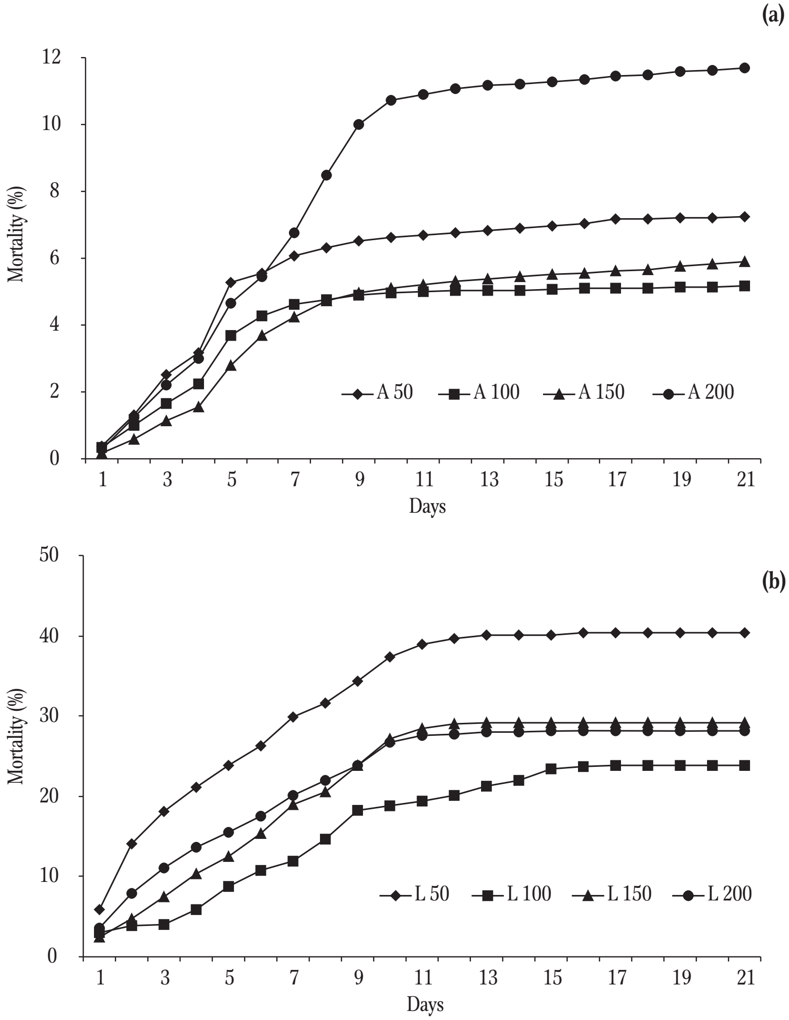

Fig. 1. Cumulative mortality of larval asp, *Aspius aspius* (a) and chub, *Leuciscus cephalus* (b), during rearing under controlled conditions.

The RGR_W in group A50 was 17.19% d^{-1}, and in group A200 it was 15.09% d^{-1}. The final body weight of the fish in group A50 differed significantly statistically from the other variants ($P < 0.05$). The fastest relative increase in biomass was noted in group A50, and the slowest in group A200 (Table 1).

The highest survival in chub was noted in group L100, and the lowest in group L50 (Table 2). Intense mortality in chub larvae was noted after day 11 of rearing (Fig. 1b).

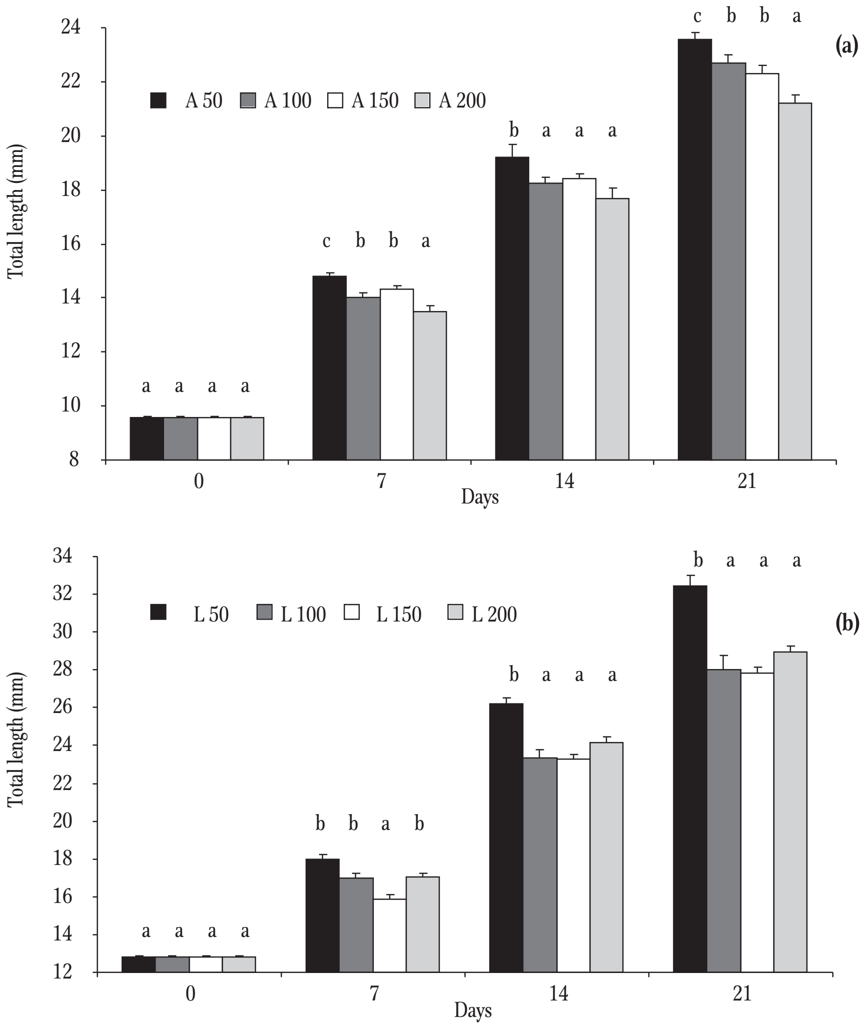

Fig. 2. Mean (± SE) length of larval asp, *Aspius aspius* (a) and chub, *Leuciscus cephalus* (b) during rearing. Data with the same letter index do not differ significantly statistically ($P > 0.05$).

The fish from group L50 had the largest body measurements at the end of the experiment (Fig. 2b; $P < 0.05$). RGR_W in group L50 was 21.61% d^{-1}, while the slowest growth was noted in group L150 at 18.01% d^{-1}. The RGR_L values for the fastest (L50) and slowest (L150) growth in body length were 4.54 and 3.77% d^{-1}, respectively. The highest ITL index recorded during chub rearing was noted in group L50, while the lowest was in group L150 (0.58 and 0.44, respectively). The fastest relative biomass rate

(RBR) was confirmed in variant L50 (20.31% d^{-1}), while the slowest was in group L150 (17.12% d^{-1}).

TABLE 2

Initial and final characteristics of the rearing of chub, *Leuciscus cephalus* (mean ± SD)

Parameter	Stocking density (indiv. dm^{-3})			
	50	100	150	200
Mean initial weight (mg)*	1.3 ± 0.3^a	1.3 ± 0.3^a	1.3 ± 0.3^a	1.3 ± 0.3^a
Mean final weight (mg)*	76.4 ± 22.9^c	49.8 ± 23.5^b	40.6 ± 10.5^a	49.6 ± 10.6^b
Mean initial length (mm)*	7.87 ± 0.2^a	7.87 ± 0.2^a	7.87 ± 0.2^a	7.87 ± 0.2^a
Mean final length (mm)*	19.99 ± 1.97^b	17.25 ± 2.71^a	17.12 ± 1.22^a	17.82 ± 1.14^a
Survival (%)	59.60 ± 3.70	76.20 ± 2.94	70.77 ± 2.26	71.89 ± 1.98
Index of incremental total length (ITL) (mm d^{-1})	0.58 ± 0.1	0.45 ± 0.2	0.44 ± 0.1	0.47 ± 0.0
Relative growth rate in weight (RGR_W) (% d^{-1})	21.61 ± 0.2	19.16 ± 0.3	18.01 ± 0.2	19.14 ± 0.1
Relative growth rate in length (RGR_L) (% d^{-1})	4.54 ± 0.1	3.81 ± 0.1	3.77 ± 0.1	3.97 ± 0.1
Relative biomass rate (RBR) (% d^{-1})	20.31 ± 0.2	18.44 ± 0.3	17.12 ± 0.2	18.29 ± 0.1

**Results in the same row with different letter indices differ significantly statistically ($P \leq 0.05$)*

DISCUSSION

The growth rate of cyprinid fish in controlled conditions depends on many factors including water temperature; the type, availability, and quantity of feed; the length of the daily feeding period; the stocking density of the fish; and tank size (Brown 1957, Myszkowski et al. 2002, Kujawa 2004, Wolnicki 2005). Studying the impact of single factors requires maintaining all the others at comparable levels. The relative growth rate in weight (RGR_W) of larval asp at a stocking density of 50 indiv. dm^{-3} (group A50) was lower than that reported by Wolnicki and Myszkowski (1999) and Wolnicki (2005) (21.1 and 19.9, respectively), who applied a slightly higher stocking density (60 indiv. dm^{-3}). In turn, the RGR_W calculated for larvae reared at a stocking density of 25 indiv. dm^{-3} (Kujawa 1998) was the same as that in the current work for a stocking density that was fourfold higher. There is a lack in the literature of information regarding the rearing of asp at stocking densities higher than 100 indiv. dm^{-3}, which makes it impossible to compare the current results with those of other researchers. However, the results obtained do indicate that there is a negative link between density and growth rate and survival, both of which decreased as larval asp stocking density increased.

A similar relationship was noted in other fish species, including the spotted sand bass, *Paralabrax maculatofasciatus* (Steindachner) (Alvarez-Gonzales et al. 2001), turbot, *Scophthalmus maximus* (L.) (Irwin et al. 1999) and catfish, *Clarias batrachus* (L.), (Sahoo et al. 2004). In the instance of asp rearing, this might have been linked with both the higher stocking density applied as well as with the fluctuations in the physicochemical parameters (mainly of oxygen) in the waters of tanks with higher stocking densities. The negative impact of environmental factors on fish growth rate was described previously (Harris 1999, Foss 2003, Biswas et al. 2006). The larval asp survival obtained in the current experiment did not differ from the results reported by other authors that at the end of rearing over 90% of the individuals survived (Kujawa 1998, Kujawa et al. 1998, Wolnicki and Myszkowski 1999, Wolnicki 2005). This refers to groups A50, A100, and A150. The mortality of over 11% in group A200 could have resulted from the excess larval stocking density, which had the greatest impact on the mortality rate in the second week of rearing. The cumulative mortality curves for both species were similar regardless of the stocking density, and were very similar to those noted in earlier works (Kujawa 2004, Wolnicki 2005). This was probably linked to the mortality of larvae that did not feed or those that had developmental abnormalities that always lead to death at similar times (Kujawa 2004).

Shiri Harzevili et al. (2003), who reared chub at a stocking density of 10 indiv. dm^{-3}, reported similar growth rates to those of group L50 in the current study. However, survival at such a low stocking density was similar to that of group L200. It is worth noting that Shiri Harzevili et al. fed the fish rotifers (*Brachionus calyciflorus*) for five days prior to feeding them brine shrimp nauplii. Kujawa (2004) pointed out that mortality in chub larvae was nearly zero (0.1%) during a three-week rearing experiment at a stocking density of 50 indiv. dm^{-3}. In the current experiment, mortality at the same stocking density was over 40%. Such high larval mortality could have resulted from the use in the current experiment of less developed larvae. However, it is also worth noting the greater differentiation in larval chub length at stocking densities of 50 and 100 indiv. dm^{-3} (groups L50 and L100, respectively) than in groups L150 and L200 as well as the higher survival rates in group L200 than in group L50. Similar dependencies between survival and density were observed with larval cod, *Gadus morhua* (L.), (Baskerville-Bridges and Kling 2000); African catfish, *Heterobranchus longifilis* (Val.), (Imorou Toko et al. 2008); and perch, *Perca fluviatilis* (L.), (Baras et al. 2003). While the negative impact of inappropriate feeding methods was confirmed with larval cod, higher survival in the case of the other two species was influenced by weaker cannibalism at greater stocking densities. Kujawa (2004) reared chub at different densities (25, 50, and 75 indiv. dm^{-3}) and confirmed similar dependencies. This might be linked to the fact that higher stocking densities (about 200 indiv. dm^{-3} or even higher) can result in lesser losses strictly connected to the confirmed phenomenon of cannibalism. While cannibalism was confirmed during larval European chub rearing, it was not noted among larval asp, which is classified as an obligatory predator. Cannibalism in intensive larviculture is not a rare phenomenon, but it is most frequently reported among predatory fish (Kucharczyk et al. 1998, Baras et al. 2003).

The results obtained in the current experiment indicate faster growth rates in both asp and chub at a stocking density of 50 indiv. dm^{-3} in comparison with the other experimental variants. There is a lack of significant differences in growth rate in the first week of larval rearing for both species, thus indicating that it is possible to apply a stocking density in this period as high as 200 indiv. dm^{-3} without the risk of lowering growth rates. It is noteworthy that severalfold higher quantities of stocking material can be obtained from the same volume of water at the highest stocking densities, which can have a significant impact on the final profitability of production at hatcheries. Additionally, the lack of significant differences in the growth rates among the L100, L150, and L200 groups during chub rearing suggests that it may be possible to further intensify the production of stocking material of this species under controlled conditions.

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STRESZCZENIE

WPŁYW ZAGĘSZCZENIA OBSADY NA WZROST I PRZEŻYWALNOŚĆ LARW BOLENIA *ASPIUS ASPIUS* (L.) I KLENIA *LEUCISCUS CEPHALUS* (L.) W TRAKCIE PODCHOWU W WARUNKACH KONTROLOWANYCH

Celem pracy było określenie wpływu zagęszczenia na wzrost oraz przeżywalność larw bolenia *Aspius aspius* (L.) i klenia *Leuciscus cephalus* (L.) w masowym podchowie w warunkach kontrolowanych. Materiał do badań (larwy) pozyskano w trakcie kontrolowanego rozrodu w warunkach wylęgarniczych. Podchów prowadzono przez 21 dni w półzamkniętym obiegu wody o temperaturze 25°C. Larwy karmiono świeżo wyklutymi stadiami nauplialnymi solowca (*Artemia* sp.). Larwy przetrzymywano w czterech zagęszczenia (50, 100, 150 i 200 osobn. dm^{-3}). Podchów prowadzono w zbiornikach o pojemności 50 dm^3 każdy. Najszybsze tempo wzrostu larw obu gatunków uzyskano w najmniejszym zagęszczeniu obsad (ITL = 0,67 i 0,58 mm d^{-1} odpowiednio dla bolenia i klenia). Największa śmiertelność larw bolenia stwierdzono w grupie o zagęszczeniu 200 osobn. dm^{-3} (11,68%), a klenia w zagęszczeniu 50 osobn. dm^{-3} (40,4%). Uzyskane wyniki, z uwagi na brak różnic w tempie wzrostu larw obu gatunków w pierwszym tygodniu podchowu, wskazują na możliwość obniżenia kosztów produkcji poprzez stosowanie większych zagęszczeń w tym okresie.