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EFFECT OF STOCKING DENSITY IN RELATIONSHIP TO BOTTOM AREAS ON THE GROWTH AND SURVIVAL OF COMMON BARBEL *BARBUS BARBUS* (L.) LARVAE*

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K e y w o r d s: common barbel, *Barbus barbus*, larviculture, controlled rearing, stocking density, larval development.

A b s t r a c t

An experiment was carried out concerning the effect of the initial stocking density per area unit on the growth and survival of the common barbel, *Barbus barbus,* larvae under controlled conditions. During the 21 days of rearing, the effect of fish stocking density between 2500 and 6250 individuals m^{-2} was determined. Water temperature was 25°C throughout the experimental period. Fish were fed on live *Artemia* sp. nauplii. During the experiment, the mean total length (TL) of larvae as well as their weight (*W*) and survival were measured. On the basis of the obtained data, specific growth rate (SGR), Fulton condition coefficient (*K*) and fish biomass per area unit were calculated. The developmental stage of larvae was also determined. This study proved that initial stocking density of larvae does not affect survival, developmental stage or growth parameters. In all experimental treatments survival rate exceeded 98%. On the last day of rearing, all fish reached juvenile stage and TL of 28.5–30.2 mm. SGR ranged from 14.2 to 15% day^{-1}. The analysis of variance did not reveal statistical differences between the groups ($P>0.05$) for each measured parameter. The results obtained in this study indicate the possibility of a significant increase in the production intensity of fry-stocking material of the common barbel under controlled conditions, which should positively affect the economic effectiveness of rearing.

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WPŁYW ZAGĘSZCZENIA OBSADY NA JEDNOSTKĘ POWIERZCHNI NA WZROST I PRZEŻYWALNOŚĆ LARW BRZANY, *BARBUS BARBUS* (L.)

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S ł o w a k l u c z o w e: brzana *Barbus barbus* (L.), larwikultura, podchów kontrolowany, zagęszczenie, rozwój larwalny.

A b s t r a k t

Przeprowadzono eksperyment dotyczący wpływu początkowego zagęszczenia larw w przeliczeniu na jednostkę powierzchni na wzrost i przeżywalność brzany *Barbus barbus* w warunkach kontrolowanych. Badano zagęszczenie ryb od 2500 do 6250 osobn. m^{-2}. Doświadczenie trwało 21 dni. Podchów prowadzono w temperaturze 25°C. Pokarm ryb stanowiły żywe naupliusy *Artemii* sp. W czasie trwania eksperymentu notowano średnią długość całkowitą larw (TL), masę (*W*) oraz przeżywalność. Z uzyskanych danych wyliczono specyficzne tempo wzrostu (SGR), współczynnik kondycji Fultona (*K*) oraz biomasę ryb uzyskaną z jednostki powierzchni. Stopień zaawansowania rozwojowego larw określono na podstawie zgromadzonej dokumentacji. Dowiedziono, że początkowe zagęszczenie larw nie wpływa na przeżywalność, stopień zaawansowania rozwojowego oraz parametry podchowu. We wszystkich wariantach eksperymentalnych przeżywalność przekroczyła 98%. W ostatnim dniu podchowu wszystkie ryby osiągnęły stadium juwenilne dla średniej TL 28.45–30.20 mm. SGR wynosił 14.15–14.99% $dzień^{-1}$. W analizie wariancji nie wykazano różnic statystycznych między grupami ($P>0.05$). Uzyskane wyniki wskazują na możliwość znacznego zwiększenia intensywności produkcji materiału zarybieniowego brzany w warunkach kontrolowanych. Powinno to pozytywnie wpłynąć na ekonomiczną efektywność podchowu.

Introduction

In recent years, the level of global fish biodiversity has been characterized by a decreasing trend (ROSS 2008). It is estimated that many natural ecosystems will disappear, resulting in a decrease in the number of existing species. River regulations and pollutions have a direct threat to populations in running waters (KECKEIS et al. 2001). An additional aspect which has had an impact on a decrease stocks of many species is overfishing (POLICAR et al. 2007). This largely concerns rheophilic cyprinids which form the basis of river ichthyofauna (MANN 1996).

A dramatic decrease in the population of many species of fish has become a reason for undertaking actions aimed at restitution or protection of endangered populations of fish. In the active protection of endangered species, sustainable aquaculture plays a very important role (e.g., ROSS 2008, ŻARSKI et

al. 2011b), where the biotechnology of larval rearing under controlled conditions is one of the most effective methods of producing fry-stocking material required for restocking purposes (SHIRI HARZEVILI et al. 2003, HAMACKOVA et al. 2009). Improvement of fry-stocking material production, particularly at the stage of intensive larviculture, can have a significant effect on the quantity and the quality of the fry-stocking material produced, which can determine restocking effectiveness (COWX 1994). On the other hand, production carried out under controlled conditions makes it possible to constantly monitor both the quantity and quality, as well as the growth rate of fish. However, effective procedures of larvae and juveniles rearing of many species of fish are lacking, and data concerning the effect of individual factors on the efficiency of rearing of many fish species are fragmentary.

The common barbel, *Barbus barbus* (L.), is a typical representative of cyprinid rheophilic fish, usually populating the upper and the central course of clean rivers with hard, stony and gravel substrates. An important morphological feature of this species is its adaptation to bottom life (MANN 1996, BRYLIŃSKA 2000). In recent decades, a decrease in the size of population of many species of rheophilic fish, including the common barbel (POLICAR et al. 2007), has been recorded in Europe. In many countries of central Europe, the observed decrease in the common barbel stock has been so serious that this species has become endangered (e.g., PROKES et al. 2006, POLICAR 2007). Consequently, restocking of open waters with the material obtained as a result of controlled reproduction and rearing can significantly support the natural recruitment (COWX 1994, PHILIPPART et al. 1995). In the case of the common barbel, the high price of fry-stocking material, resulting from its lower fecundity and efficiency of artificial reproduction in comparison to other rheophilic cyprinids (KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2011), significantly limits restocking attempts on a large scale. At the same time, production of the common barbel may be one of the most profitable among freshwater aquaculture activities (HAKUĆ-BŁAŻOWSKA et al. 2010). Undertaking all possible actions aiming at increasing rearing intensity can significantly reduce the cost of production and at the same time increase the production. Published data concerning common barbel larviculture concern early ontogenesis (CALTA 1998) and determination of optimum nutritional conditions and the effect of stocking density per volume unit (KUJAWA 2004, WOLNICKI 2005, ŻARSKI et al. 2011a). However, there are no data concerning the effect of larval density per area unit, which in case of bottom-dwelling fish significantly condition the efficiency of rearing (SCHRAM et al. 2006, AKSUNGUR et al. 2007). A negative effect of stocking density on fish growth was reported for many fish species. In many cases, the influence of social interactions, cannibalism and changes in physicochemical parameters of the water affected by application of high

stocking densities indirectly affected fish metabolism and, in consequence, growth rate (e.g. KING et al. 2000, ALVARES-GONZALES et al. 2001, ŻARSKI et al. 2011a,b). However, the mechanism still remains unclear.

The aim of this study was to determine the effect of initial stocking density of barbel larvae per area unit on the growth and survival of fish under controlled conditions.

Materials and Methods

Common barbel larvae originated from controlled reproduction of cultured stock reared and reproduced at the Czarci Jar Fish Farm (North-Eastern Poland). Hormonal stimulation was carried out according to the method described by TARGOŃSKA et al. (2011). The eggs from three females were fertilized using the "dry" method, with a mixture of sperm obtained from five males. Fertilized eggs were incubated in Weiss jars, at a constant temperature of 20°C. Freshly hatched larvae were transferred to a 150 L collective tank, operating in a closed recirculating system. From the moment when all larvae hatched, water temperature was increased by 1°C each day up to 25°C. Larvae were kept under such conditions until the moment they started to swim actively and inflated the posterior chamber of their swim bladder (5 days post hatch [DPH]), after which they were placed in an experimental rearing system.

Larvae were reared in a closed recirculating system in glass tanks (aquaria), with the possibility of water level adjustment (as described by KUJAWA et al. 2000). The surface of each tank was 0.16 m^2. Depending on the experimental group, the water in aquaria was increased to the volume of: 20, 30, 40 and 50 L. The larval density in each of the experimental treatments was 20 individuals L^{-1}. In this way, a stocking density per area unit amounting to 2500, 3750, 5000 and 6250 fish per m^2 was obtained. The experiment was carried out in three replications. Water was supplied to the tanks in the form of an upper inflow. At the beginning of the experiment, water flow was adjusted in such a way so that its exchange took place five times an hour in each of the experimental tanks. Water flow was gradually increased to obtain ten-time water exchange at the end of the experiment. Larvae were reared for 21 days at 25°C (±0.1) under a 14h light photoperiod (14L:10D). The fish were fed three times (at 08.00, 12.00, 16.00) a day *ad libitum* with freshly-hatched *Artemia* sp. nauplii in such amounts that the nauplii were present in water from the moment of the first feeding until the end of the light day. Before the first feeding, remains of food, faeces and dead fish (the number of which was recorded) were removed from the rearing tanks. Twice a week,

ammonia and nitrate content were measured in each of the rearing tanks (photometer Slandi, Poland) along with oxygen content in water (HI 9828, Hanna Instruments, Italy).

Fish for total length measurements were sampled on the day when the experiment began, and then on day 7, 14 and 21 of the experiment. During the analysis, the total length of fish was determined (TL, ± 0.1 mm). The mean weight of fish (± 0.1 mg) was determined at the beginning and at the end of the experiment. Measurements were carried out on 30 randomly-selected specimens from each experimental tank (90 specimens per each experimental group were measured). Before taking measurements, the fish were anesthetized in a 2-phenoxyethanol solution (Sigma-Aldrich, Germany) (0.4 mL L^{-1}). After performing measurements, the fish returned to the same tanks from which they were caught.

On the basis of the data obtained, specific growth rate (SGR [% day^{-1}]) and Fulton condition coefficient (K) were calculated, according to the following formulas:

$$SGR = ((\ln W_T - \ln W_t)100)(T - t)^{-1}$$

where:

W_T, W_t – mean body weight at the beginning and at the end of rearing, respectively [mg],

T – t – number of days between measurements.

$$K = 100\ (W\ TL^{-3})$$

where:

W – fish body weight [mg],

TL – total length of the fish body [mm].

On the basis of the obtained data, the developmental stage was determined for each experimental group (according to the method described by ŻARSKI et al. 2011a). Additionally, fish biomass obtained from an area unit was calculated (total weight of fish obtained from area unit). Data expressed in percentage before the analysis were subject to arc-sine transformation. An analysis of variance (ANOVA) was applied to establish significant statistical differences between the groups at the level of $\alpha = 0.05$. Linear regression analysis was performed for biomass of fish obtained in individual experimental groups. Statistical analyses were carried out using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) and MS Excel for Windows.

Results

During the experiment, the level of oxygen in all rearing tanks did not drop below 85% saturation. Ammonia and nitrates content ranged from 0.05 to 0.1 mg L^{-1}.

At the moment when the experiment started, all larvae were feeding, although some energy originated from incompletely resorbed yolk sacs. Inflation of the anterior chamber of the swim bladder (second in turn) occurred in about 5% of larvae. The fin fold revealed a distinct narrowing in the dorsal part. The dorsal fin and the caudal fin had visible rays. After 7 days of the experiment, the fin fold in the larvae was significantly reduced and its remaining parts were visible near the pelvic areas of the body and in the caudal part. The size of pelvic fins differed in individual fish and in most of them they extended beyond the edge of the fin fold. On day 14 of rearing, in each experimental treatment, the fin fold was visible between the pelvic fins and the anus only in less than 5% of fish. The fin fold completely disappeared on day 21 of rearing, which proves that all fish reached the juvenile stage.

Fish survival, regardless of the experimental group, was very high and amounted to about 99% (Table 1). A high growth rate was recorded for the entire experiment. The analysis of variance did not reveal any statistical differences between the groups ($P>0.05$). During the study, the highest values of the mean fish body length (30.2 mm) and the mean fish body weight (204.5 mg) were recorded on the last day of rearing in the group where the density amounted to 3750 ind. m^{-2}. The value of SGR coefficient ranged from 14.15 to 14.99% day^{-1}. In case of K coefficient, the highest value (0.78) was recorded

Table 1

Characteristics of the common barbel (*Barbus barbus*) over 21 days at different stocking densities under controlled conditions (SGR – specific growth rate; K – Fulton's condition coefficient)

	Stocking density [ind. m^{-2}]			
	2500	3750	5000	6250
Total body length [mm] at:				
day 0	12.20 ± 0.41	12.20 ± 0.41	12.20 ± 0.41	12.20 ± 0.41
day 7	17.34 ± 0.50	17.42 ± 0.15	17.36 ± 0.45	17.19 ± 0.37
day 14	23.17 ± 0.10	23.47 ± 0.44	22.56 ± 0.28	23.09 ± 0.76
day 21	28.60 ± 1.69	30.20 ± 0.28	28.45 ± 1.28	29.07 ± 0.52
Wet body weight (mg) at:				
day 0	8.7 ± 0.9	8.7 ± 0.9	8.7 ± 0.9	8.7 ± 0.9
day 21	188.70 ± 16.96	204.55 ± 8.07	178.30 ± 20.85	180.21 ± 13.71
Survival rate [%]	99.00	99.06	98.38	99.43
SGR [% day^{-1}]	14.57 ± 0.46	14.99 ± 0.17	14.15 ± 0.67	14.38 ± 0.37
K	0.78 ± 0.03	0.74 ± 0.01	0.75 ± 0.01	0.73 ± 0.02

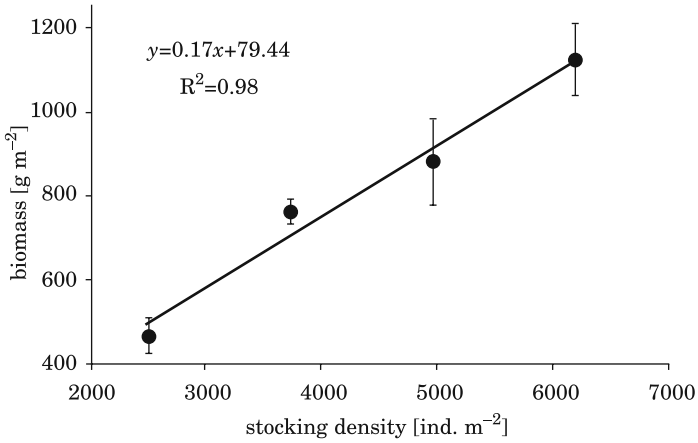

Fig. 1. Correlation between stocking density and mean fish biomass ($n=3$ for each stocking density) after 21 days controlled rearing of common barbel (*Barbus barbus*)

in the group where fish density was 2500 ind. m^{-2}. A regression analysis revealed a significant linear relation between density and biomass of fish obtained per area unit (Figure 1).

Discussion

The obtained results in this study indicate that the stocking density of barbel larvae per area unit did not have a negative effect on rearing parameters or survival of barbel larvae. WOLNICKI (1997) obtained SGR of 14.82% day^{-1} during 15-day rearing of barbel larvae at 25°C and density of 30 ind. L^{-1}, while WOLNICKI and GÓRNY (1995) recorded 13.8% day^{-1} at a density of 40 ind. L^{-1}. POLICAR et al. (2007) obtained an SGR of 14.5% day^{-1} and a mean LT of 24.3 mm during 21-day rearing of barbel at 21°C. It is worth to mention that POLICAR et al. (2007) started research on 13 DPH. KUJAWA (2004) after 21 days of rearing barbel larvae at 25°C recorded the highest mean total length of 25.6 mm at density of 20 ind. L^{-1}. ŻARSKI et al. (2011a) examined the effect of high density per volume unit reaching up to 200 ind. L^{-1} obtained similar results (mean final weight of 138.17 mg, mean final TL of 27.11 mm) to this obtained in the present study (mean final weight of 187.94 mg, mean TL of 29.08 mm). In addition, the developmental stage of fish at the end of the experiment was similar in this study with the study of ŻARSKI et al. (2011a). Therefore, the obtained results indicate that the growth rate of barbel larvae observed in this study was the highest growth rate reported so far, which confirms the optimal rearing conditions.

The effectiveness of rearing fish larvae under controlled conditions depends on many factors. They include, first of all, temperature, oxygen content, photoperiod, type and amount of food provided and feeding frequency (WOLNICKI et al. 2003, WOLNICKI 2005, POLICAR et al. 2006, VORLIČKOVÁ et al. 2006). These parameters in our study were identical in all experimental groups. Some authors reported that stocking density is also a very important factor affecting the growth rate and survival of fish (KUJAWA 2004, SZKUDLAREK and ZAKĘŚ 2007, CELADA et. al. 2007). KUJAWA (2004) recorded differences in the growth rate between the groups during three-week rearing of barbel larvae at 25°C at the initial stocking density of 25, 50 and 75 ind. L^{-1}. However, ŻARSKI et al. (2011a) did not record any differences between densities reaching 20 and 200 fish L^{-1} which probably resulted from a much smaller scale of the experiment and consequently, the possibility of maintaining very good experimental conditions of rearing and precise amount of food (number of *Artemia* nauplii per capita instead of *ad libitum* method). The possible effect of rearing conditions on the growth rate of fish in various densities has been already reported (KING et al. 2000). It must be emphasized that the scale of the experiment, water temperature, period of rearing, type and amount (*ad libitum*) of supplied food, as well as larval density used in this experiment were the same as applied by KUJAWA (2004) in the best of experimental treatments. On the other hand, a better growth rate in comparison to results obtained by KUJAWA (2004) could be caused by the 2h longer photoperiod applied in this study, the factor that has been proven to affect the growth rate (WOLNICKI et al. 2003). Additionally, these differences could result from the varied origin of the larvae used in the experiments. KUJAWA (2004) obtained larvae from wild fish, while in this study, the larvae originated from cultured stock. Moreover, it cannot be excluded that the results obtained in this study in various densities (no differences in the growth rate) could depend on the origin of larvae. However, an explanation of those possible variables requires further, more detailed research.

There are also assumptions that the value of density expressed per area unit of the bottom, in case of bottom-dwelling fish, also influences growth parameters (SCHRAM et al. 2006, AKSUNGUR et al. 2007). It was assumed that use of large stocks has a negative impact on the growth rate of the body because of stress which may affect elevated cortisol level in blood plasma (e.g., BOLASINA et al. 2006). However, the results obtained in this study, like the results obtained by ŻARSKI et al. (2011a), show that barbel larvae in the early developmental stages are little susceptible to stress, which eliminates the density factor as a potential stressor. This applies both to the stocking density examined as a number of individuals per volume unit (ŻARSKI et al. 2011a) and per bottom area unit (the present study). Similar dependencies were also

reported for other species of rheophilic cyprinids (KUPREN et al. 2011), as well as for the crucian carp, *Carassius carassius* (ŻARSKI et al. 2011b), where a large stocking density did not affect the growth rate of larvae. Therefore, based on the data published to date, it can be claimed that a negative effect of the stocking density on the final rearing result is most probably related to the size of the fish and its developmental stage (IRWIN et al. 1999, AMBROSIO et al. 2008), where fish in a higher developmental stage are able to more intensely react to stress-inducing stimuli, such as stocking density. This dependency, as a result of stress related to application of large stocks, can have an immediate effect on physiological conditions, and consequently, contribute to a reduction in the growth rate (COSTAS et al. 2008, ŻARSKI et al. 2011a, b). However, this aspect should be more closely studied and in future research the analysis of the cortisol level in the blood plasma should be included because data on the physiological responses (such as excretion of the cortisol) of fish larvae due to the stressful culture conditions is very limited (BOLASINA et al. 2006).

As results from the data presented showed there are no contraindications as to applying large stocking densities in larval rearing and early juvenile stages of the common barbel. No negative effect in applying large stocks on the growth rate and individual development of fish was found, regardless of the method of density examination.

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