Effect of size sorting on the survival, growth and cannibalism in pikeperch (*Sander lucioperca* L.) larvae during intensive culture in RAS

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ABSTRACT: The aim of the experiment was to determine the impact of sorting on the growth, survival, and cannibalism of pikeperch larvae during intensive culture in recirculation systems. Larvae aged 48 days post-hatch (DPH) were reared in three groups – small specimens (group S – average body weight 40 mg), large specimens (group L – average body weight 76 mg), and unsorted ones (group U – average body weight 55 mg). After three weeks of rearing, there were no statistically significant differences in specific growth rates among the groups. However, increases in biomass were higher in the sorted groups. Survival exceeded 50% in the sorted groups and 39% in group U. Higher cannibalism was noted in group U than in the sorted groups. Significant differences among the sorted groups were observed in cannibalism, which was higher in group L, and in natural and manipulation losses, which were higher in group S ($P < 0.05$). During the first two weeks of rearing, the lowest cannibalism rates were observed in group S, the difference between groups S and U was statistically significant ($P < 0.05$). The results of the experiment indicate that the sorting of pikeperch larvae has a positive impact on the survival rate, however, it has no impact on growth during their rearing in the RAS.

Keywords: pikeperch; larvae; sorting; RAS; growth; survival; cannibalism

The pikeperch, *Sander lucioperca* (L.), has long been farmed in ponds, and currently research is focused on methods of intensive aquaculture, including production in recirculating aquafarming systems (RAS) (Kestemont and Mélard, 2000; Zakęś et al., 2000). So far, the rearing of pikeperch larvae has presented the greatest challenge. Among many problems encountered during this stage of production, the most important are the failure of swim bladder inflation in some individuals and increased incidences of cannibalism (Kindschi and MacConnell, 1999; Kestemont and Mélard, 2000; Szkudlarek and Zakęś, 2007).

Cannibalism occurs among pikeperch during intensive rearing when the first individuals attain 15 mm in body length (Szkudlarek and Zakęś, 2007). One of the major factors contributing to this phenomenon is the excessive variation in the size of fish in the cohort (Baras and Jobling, 2002; Kestemont et al., 2003; Puvanendran et al., 2008). The variation of the fish size may be due to behavioural factors like territoriality and dominance hierarchies (Jobling et al., 1993). A factor conducive to cannibalism is also the presence of larvae with non-inflated swim bladders (NSB), which are easy victims due to the impairment of motor skills (Summerfelt, 1996; Czesny et al., 2005).

Sorting is a common procedure used during intensive fish rearing to reduce size variations (Jensen, 1990), and as a result of this treatment the market size of farmed fish can also be obtained more quickly (Wallat et al., 2005). In addition to reducing cannibalism, sorting also optimizes feeding since granulation and ration sizes can be tailored to the fish size (Saoud et al., 2005). In the literature studies that focus on the effects of sorting on pikeperch larvae are missing. Zakęś et al. (2004) investigated the impact of the sorting of only juvenile pikeperch in relation to the effects of rearing under RAS.

The aim of the present study was to determine the effect of sorting according to size on growth, survival, and cannibalism in pikeperch larvae during intensive culture under RAS.

MATERIAL AND METHODS

Pikeperch larvae were obtained through artificial out-of-season reproduction conducted at the Department of Sturgeon Fish Breeding (DSFB) in Pieczarki of the Inland Fisheries Institute in Olsztyn. Cultured pikeperch spawners were stimulated with human chorionic gonadotropin (hCG) (Zakęś and Szczepkowski, 2004; Zakęś and Demska-Zakęś, 2009). The larvae were reared on a mixed diet (artificial feed + *Artemia* sp.) using the procedure described by Szkudlarek and Zakęś (2007). After 44 days of rearing (larvae aged 48 days post hatch – DPH) the fish were sorted to separate NSB individuals from larvae with inflated swim bladders (ISB). Sorting was performed in an aqueous solution of sodium chloride and the anaesthetic etomidate (10 g NaCl/l + 1 ml Propiscin/l) (Kazuń and Siwicki, 2001; Zakęś, 2009). Then a portion of the ISB fish was divided into two groups with a plastic 1.8 mm slot sorter. The other ISB individuals were not sorted. Three experimental groups were obtained in this way: (1) unsorted fish (group U), (2) smaller fish (group S) and (3) larger fish (group L).

The experiments were conducted in a RAS on a laboratory scale. The larvae were held in round tanks of 40 dm^3 in volume. The stock numbered 100 individuals per tank. Each group of fish was reared in three replications, and the rearing time was 21 days.

Water quality parameters were monitored during the experiment, and water samples were taken from the outflows of the rearing tanks. Water temperature was monitored daily (± 0.1°C). The oxygen content in water (± 0.01 mg/dm^3) and pH (to ± 0.01) were determined with a CyberScan PCD 5500 meter (Eutech Instruments, Vernon Hills, USA). Total ammonia nitrogen (CAA = NH_4^+ + NH_3) was determined by the Nesslerization method, while nitrites were determined by the sulphanilic method (Hermanowicz et al., 1999) using a Carl Zeiss 11 spectrophotometer (Carl Zeiss Jena, Germany). Water temperature during the rearing was 20.0 ± 0.1°C. Oxygen content was ≥ 90% saturation, and pH ranged from 7.97 to 8.10. The maximum concentrations of ammonia and nitrite did not exceed 0.16 mg/dm^3 CAA and 0.08 mg/dm^3 NO_2, respectively. The rearing tanks were illuminated 24 h/day, and the average light intensity measured at the water surface in the central part of the rearing tank was 170 lux (meter L-100, Sonopan, Bialystok, Poland).

The fish were fed Nutra Amino Balance 3.0 (NAB 3.0) (Skretting, France) commercial feed during the first six days of the experiment (48–54 DPH), followed by a mixture of smaller granules of NAB 3.0 and larger NAB 2.0 feeds at a ratio of 1:1 for the next two days. After this period, only NAB 2.0 was supplied. NAB 3.0 feed contained 55% protein and 16% fat (19.0 MJ digestible energy/kg), and NAB 2.0 contained 54% protein and 18% fat (19.5 MJ/kg). The feed was supplied in excess at a daily feed ration of 55% of fish biomass per day at the beginning of the experiment and 30% of fish biomass per day at the end of the experiment. Feed was supplied 24 h per day with a 4305 FIAP automatic band feeder (Fischtechnik GmbH, Nienburg, Germany). Once a day the bottoms of the tanks were cleared of fish excrements, and unconsumed feed and dead fish were noted. Losses after the first day of the experiment were defined as manipulation losses associated with the separation of ISB fish and size sorting.

Total body length TL (± 0.1 mm) and body weight BW (± 0.001 g) measurements were done at the beginning and at the end of the experiment. Random samples of 30 individuals were taken from each variant studied. Total biomass was determined by weighing all the fish from a given tank. On days 7 and 14 of the experiment, film documentation was done to determine the number of fish in the tanks. A Handycam HDR-SR1E (Sony, Japan) was used to record the visual documentation, these data were used for the calculation of cannibalism during the rearing.

After the completion of the experiment, the following rearing indexes were calculated:

$$SGR = 100 \times (\ln BW_2 - \ln BW_1)/D \quad (1)$$

where:

SGR = specific growth rate (%/day)

BW_1, BW_2 = mean body weight at the beginning and at the end of the experiment (g)

D = rearing time (days)

$$V = (SD/BW) \times 100 \quad (2)$$

where:
V = coefficient of variation of body weight (%)
SD = standard deviation of fish body weight (g)
BW = mean body weight (g)

$$CF = 100 \times (BW \times Sl^{-3}) \quad (3)$$

where:
CF = Fulton's condition coefficient
BW = body weight (g)
Sl = body length (mm)

$$S = 100 \times (LC/LS) \quad (4)$$

where:
S = survival (%)
LC = number of fish caught at the end of the experiment (individuals)
LS = number of fish stocked at the beginning of the experiment (individuals)

The rate of cannibalism (C) was calculated from the difference between the number of fish stocked at the beginning of the experiment minus natural mortality (Szkudlarek and Zakęś, 2007) and the number of fish at the end of the experiment.

$$C = 100 \times [(LS - M - LC)/LS] \quad (5)$$

where:
C = cannibalism (%)
M = natural mortality (individuals)

Statistical calculations were performed by the Statistica 8.0PL programme (StatSoft Inc., Kraków, Poland). Single-factor analysis of variance (ANOVA) was applied to confirm the significance among the mean values of the rearing indexes studied. All three groups (sorted fish – group S and group L, unsorted fish – group U) were analysed together. Tukey's test was used to evaluate the significance of differences, which were considered statistically significant at $P \leq 0.05$.

Table 1. Initial and final indicators of pikeperch rearing after size grading (mean ± SD, $N = 3$)

Specification	Experimental groups		
	S	L	U
Initial body weight (g)	0.04 ± 0.011	0.08 ± 0.023	0.06 ± 0.019
Final body weight (g)	0.33 ± 0.028^{b}	0.53 ± 0.031^{a}	0.45 ± 0.039^{ab}
Specific growth rate, SGR (%/day)	10.1 ± 0.4^{a}	9.2 ± 0.3^{a}	10.0 ± 0.4^{a}
Fish biomass gain (% of initial biomass)	334.7 ± 20.1^{a}	295.8 ± 27.9^{ab}	223.6 ± 13.0^{b}
Initial total body length, TI (mm)	14 ± 1.1	17 ± 1.6	15 ± 1.6
Final total body length, TI (mm)	29 ± 0.7^{b}	34 ± 0.8^{a}	32 ± 1.1^{ab}
Initial body weight variation coefficient, *V* (%)	27.9 ± 2.5	33.8 ± 4.6	34.2 ± 4.8
Final body weight variation coefficient, *V* (%)	37.6 ± 2.7^{a}	44.7 ± 10.7^{a}	44.8 ± 11.0^{a}
Fulton condition coefficient, *CF*	1.3 ± 0.01^{a}	1.3 ± 0.01^{a}	1.3 ± 0.02^{a}
Survival (%)	52.7 ± 4.7^{ab}	59.7 ± 4.4^{b}	39.0 ± 3.5^{a}
Cannibalism (%)	11.3 ± 0.7^{a}	21.3 ± 3.0^{b}	29.0 ± 2.0^{b}
Natural losses (%), where:	36.0 ± 4.0^{b}	19.0 ± 2.1^{a}	32.0 ± 2.9^{ab}
Manipulation losses	14.7 ± 3.4^{b}	2.7 ± 1.5^{a}	8.0 ± 2.5^{ab}
Other losses	21.3 ± 0.9^{ab}	16.3 ± 0.7^{a}	24.0 ± 1.7^{b}

Group L = larger sorted fish, group S = smaller sorted fish, group U = unsorted fish
Data in rows with different letter indexes differ significantly statistically ($P \leq 0.05$)

RESULTS

The body weight growth of pikeperch larvae in all the analysed groups expressed as relative values (SGR) was similar. The SGR ranged from 9.2%/day in group L to 10.1%/day in group S (Table 1). The final body weights of larvae were 0.33 g in group S, 0.45 g in group U and 0.55 g in group L (Table 1). The final body weight of the sorted larvae analysed together was 0.43 g. Increases in fish biomass were higher in each of the sorted groups (group S and group L) than in group U. The difference between group S and group U was statistically significant ($P \leq 0.05$). As a result, the value of this ratio calculated for the sorted groups treated together (groups S + L) was significantly higher than in group U. The values of Fulton's condition coefficient (*CF*) in all analysed groups were similar (Table 1). The variation of the fish size in groups expressed as the value of the coefficient of variation of body weight at the end of the experiment did not differ significantly either.

The survival of fish in groups S and L was similar (52.7–59.7%), and it was significantly higher in group L than that observed in the unsorted group ($P < 0.05$, Table 1). When the sorted fish were analysed together, the survival rate was higher by more than 17% in comparison with the unsorted group U (Table 1). The highest natural losses (including manipulation losses) were noted in group S in comparison with the significantly lower value recorded in group L. It was linked particularly to manipulation losses, which were more than five times lower than in group S ($P < 0.05$). Natural losses (excluding manipulation losses) ranged from 16.3% (group L) to 24.0% (group S) ($P < 0.05$).

Significant differences in losses due to cannibalism were observed among the groups analysed. Cannibalism in the groups of sorted fish was nearly twice as high in group L as it was in group S ($P < 0.05$). No significant differences were noted between groups L and U (Table 1). Cannibalism was 29.0% in group U, which was approximately by 13% higher than that recorded in the groups of sorted fish analysed together ($P < 0.05$).

The rate of cannibalism during rearing varied in each of the groups. During the first two weeks of rearing, the lowest cannibalism rates were observed in group S, while they were higher in groups L and U. The difference between groups S and U was statistically significant ($P < 0.05$, Figure 1). It was not until the third week of rearing that the level of cannibalism in all groups was similar (Figure 1). At this point, increased cannibalism was noted in the sorted groups (groups S and L). As a result, the volume of losses due to cannibalism was similar in all groups and ranged from 12.0% (group S) to 15.6% (group U) ($P > 0.05$, Figure 1).

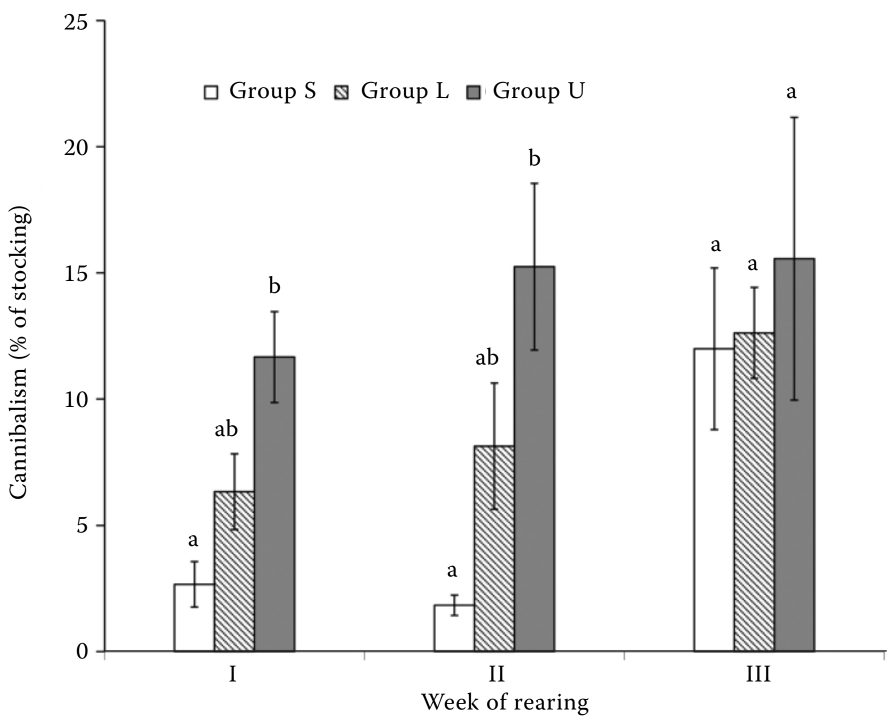

Figure 1. Losses from cannibalism in subsequent weeks of pikeperch larvae rearing

Group L = larger sorted fish
Group S = smaller sorted fish
Group U = unsorted fish
Groups marked by the same letter index in the same week of rearing do not differ significantly ($P > 0.05$) (mean ± SD, $N = 3$)

DISCUSSION

Sorting the pikeperch larvae by size did not result in increased growth rates, and the growth of fish from the unsorted group did not differ significantly from that of the sorted fish. Similar results were obtained during the rearing of juvenile perch (initial BW from 7.1 to 88.7 g) (Mélard et al., 1995), pikeperch (initial BW ca. 40 g) (Zakęś et al., 2004), yellow perch *Perca flavescens* Mitchill (Wallat et al., 2005) and turbot, *Scophthalmus maximus* L. (Sunde et al., 1998). Moreover, sorting was found to have a negative impact on European perch, *Perca fluviatilis* L., and greater increases in body weight were reached in fish that were not sorted (Mélard et al., 1996). However, unlike the juvenile pikeperch, significantly higher increases in fish biomass were recorded in the group of sorted fish in the present study. This was because of the higher survival rates of fish in the sorted groups that were primarily the result of decreased losses due to cannibalism. Cannibalism was almost twice as low in the combined sorted fish groups as in the unsorted group. Similar results were obtained when the size sorting was applied to pike, *Esox lucius* L., during intensive rearing in RAS, and significant increases in survival and the reduction of losses associated with cannibalism were noted (Szczepkowski, 2009). Cannibalism is particularly significant in determining the effects of rearing fish larvae, including those of pikeperch (Kestemont et al., 2007; Szkudlarek and Zakęś, 2007). Variation in the fish size during rearing leads to higher rates of cannibalism (Baras and Jobling, 2002). The present study indicated that the sorting of pikeperch larvae according to size during rearing and the consequent decrease in the fish size variation significantly reduced losses and improved rearing efficiency. Cannibalism is not a significant phenomenon among juvenile pikeperch (at body weight above 5 g), and therefore the sorting did not have a significant impact on the results of their rearing (Zakęś et al., 2004; Zakęś, 2009). It was also confirmed among other species, such as cod, *Gadus morhua* L., that the level of cannibalism is highly dependent on the stage of ontogenetic development of the fish (Folkvord and Otterå, 1993).

In the present experiment, the positive impact of sorting was visible immediately following the procedure. Cannibalism among the unsorted fish (group U) occurred from the very beginning of rearing and it was significantly higher in the first two weeks of rearing than that observed in sorted group S. Notably, losses due to cannibalism were higher in the third week of rearing in the groups of sorted fish, which was probably connected with the increasing variation of the fish size during rearing. Thus, the fish should be sorted after two weeks of rearing again. It is also noteworthy that although the feed was supplied in excess, cannibalism occurred in all groups of pikeperch. Thus, the large quantity of available food (artificial feed) was not a significant limiting factor in cannibalism. Although insufficient food resources increase cannibalism and losses associated with it (Smith and Reay, 1991), high levels of this phenomenon were also noted when larger rations of feed were supplied, as was observed for example in perch (Kestemont et al., 2003).

The high manipulation losses recorded following the separation of ISB fish from NSB fish and the sorting of ISB fish by size were noteworthy. It should be noted that manipulation losses in the combined groups of sorted fish (groups S + L) did not differ from those observed in the unsorted fish (group U). Thus, the sorting of ISB fish from NSB fish using an aqueous solution of sodium chloride and anaesthetic resulted in nearly all the manipulation losses.

This was particularly apparent in the group of small sorted fish (group S), which proved to be very sensitive to this treatment. Unquestionably, non-inflated swim bladders are a major cause of high larval mortality among walleye and pikeperch in the initial period of intensive rearing in RAS (Chatain and Ounais-Guschemann, 1990; Lim, 1993; Barrows et al., 1993; Szkudlarek and Zakęś, 2007). Therefore the NSB fish should be separated from the other larvae as soon as possible during the rearing of Percidae larvae. However, this procedure might have been performed too early in the present study, as it was indicated by the high losses of smaller fish (average weight 0.040 g) after manipulation. To guarantee lower manipulation losses, the pikeperch larvae should be of an adequate size as were the fish from group L, which weighted about 0.080 g.

The same size sorting procedure with a slot sorter did not result in a significantly higher volume of manipulation losses. It is supported by the fact that in the groups subjected to this procedure (groups S + L) manipulation losses were not higher than in unsorted fish (group U). It is difficult to assess the cause of the other losses. It seems that in sorted group L and in unsorted fish (group U) they were partly a result of mutual ineffective attacks of fish and in the group of smaller fish they were a result of stress after separating the NSB fish.

The results of the present experiment indicate that the sorting of pikeperch larvae according to size has a positive impact on the effectiveness of their rearing in RAS. Although this treatment does not result in improved growth rates, significant improvements were noted in rearing results (higher biomass gain) thanks to higher survival that was mainly due to lower losses caused by cannibalism. Future research should focus on determining the optimal procedure of sorting and its frequency, because sorting helps to reach high survival rates and minimizes stress that accompanies this treatment.

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