



Effect of stocking density on growth, survival and cannibalism of juvenile pikeperch, *Sander lucioperca* (L.), in a recirculating aquaculture system

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Abstract

The effect of stocking density on the rearing performance of pikeperch juveniles was studied. Three separate experiments (I, II and III) were conducted with fish of an initial average body weight of 1.3, 6.7 and 19.2 g, respectively. Each experimental design consisted of three treatments (three replicates) with different initial stocking densities (low, medium and high). Experiments lasted 28 days in a recirculating aquaculture system (21°C, 24L:0D) with an initial stocking density of 0.78, 1.04 and 1.30 kg m⁻³ in experiment I, 2.68, 4.02 and 5.36 kg m⁻³ in experiment II, and 3.84, 7.68 and 11.52 kg m⁻³ in experiment III. The results of our study showed that in experiment I, the use of a stocking density of 1.04 kg m⁻³ resulted in the highest body weight and survival, as well as the lowest feed conversion ratio and cannibalism. In experiments II and III, the pikeperch growth rate decreased, and their feed conversion ratio increased gradually with increasing stocking density. Our study demonstrated that based on growth parameters, densities of 1.04, 2.68 and 3.84 kg m⁻³ can be used for pikeperch with an initial body weight of 1.3, 6.7 and 19.2 g, respectively.

Keywords *Sander lucioperca* · Stocking density · Growth parameters · RAS

Introduction

Intensive aquaculture in recirculating aquaculture systems (RAS) has advantages (low water usage, constant water quality and low environmental impact) and disadvantages (high initial costs and high operating costs) in comparison to traditional pond systems (Martins et al. 2010; Nagy et al. 2022). Pikeperch *Sander lucioperca* (L.) is one of the most promising species cultured in RAS (Policar et al. 2019). In terms of diversification, pikeperch

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is an attractive alternative to other inland species reared in aquaculture (Dalsgaard et al. 2013; Baekelandt et al. 2018; Nagy et al. 2022). Due to its high-quality meat, high market and fast growth, the species has been considered for aquaculture intensification primarily in Western and Central Europe (Polcar et al. 2019). Nevertheless, pikeperch culture remains limited mainly because of low survival rates, cannibalism and deformities in larval stages (Szkudlarek and Zakeš 2007; Dalsgaard et al. 2013; Colchen et al. 2020). Previous studies have indicated that juvenile pikeperch can be cultured successfully in RAS. Many studies have evaluated the influences of temperature (Rónyai and Csengeri 2008; Wang et al. 2009), light intensity (Luchiaro et al. 2006; Kozłowski et al. 2010), water quality (Steenfeldt et al. 2010; Dalsgaard et al. 2013), feeding frequency (Zakeš et al. 2006; Wang et al. 2009; Pěnka et al. 2023), feed pellet size (Mattila and Koskela 2017; Kozłowski et al. 2021) and stocking density (Szkudlarek 2002; Molnár et al. 2004; Steenfeldt et al. 2010) on the growth of pikeperch.

Stocking density is an important factor determining production efficiency in intensive aquaculture systems (Riche et al. 2013), influencing growth, water quality and fish welfare (Salas-Leiton et al. 2010; Oppedal et al. 2011; Manley et al. 2014; Yang et al. 2020). Depending on fish species, extreme low or high densities can have either positive (Liu et al. 2019; Ni et al. 2016; Yang et al. 2020) or negative effects on fish welfare and growth (Adams et al. 2007; Jørgensen et al. 1993; Millán-Cubillo et al. 2016). The complex relationship between fish welfare and stocking density means that the determination of optimal stocking densities is difficult. One species' reactions to stocking density can differ significantly depending on fish size and the system in which they are farmed (Ellis et al. 2002). Thus, stocking density is a key factor influencing the health of fish in commercial production, and it is of particular concern regarding fish welfare (Li et al. 2021). In the case of pikeperch, information on the stocking density is limited to small (< 1 g) fish (Szkudlarek 2002; Molnár et al. 2004). For fish bigger than 1 g, most of the literature data is based only on the report of Steenfeldt et al. (2010), who demonstrated that pikeperch weighing from 10 to 50 g should be kept at densities lower than 15–30 kg m⁻³, while fish weighing up to 2 kg can be kept at densities of approximately 30–60 kg m⁻³. Such densities do not increase stress levels, growth rates or the feed conversion ratio. Currently, there is no information on the effects of stocking density on production performance of pikeperch sizing 1 to 10 g.

The aim of the present study was to assess the influence of stocking density on the growth, survival and cannibalism of juvenile pikeperch in three-size groups fed commercial food and reared under controlled conditions. Three separate experiments were conducted in controlled conditions with fish of different initial mean body weights (1.3, 6.7 and 19.2 g).

Materials and methods

Rearing conditions and experiment design

The study was conducted at the Department of Sturgeon Breeding in Pieczarki of the National Inland Fisheries Research Institute in Olsztyn, Poland. Three separate experiments (I, II and III) were performed using different fish sizes. In experiment I (fish age 67 days post-hatch [DPH]), the mean pikeperch body weight was 1.3 ± 0.1 g (mean values \pm standard deviation) with a mean body length of 5.4 ± 0.1 cm. In experiment II, pikeperch (100 DPH) weighed 6.7 ± 0.2 g with a mean body length of 8.1 ± 0.2 cm, while in

experiment III (130 DPH), they weighed 19.2 ± 0.2 g and had a body length of 11.7 ± 0.2 cm. Each experiment (I, II and III) was performed in three experimental groups and three replicates and each lasted 28 days. The fish were reared in 9 tanks with working volumes of 1.0 m^3 each ($1.2 \text{ m} \times 1.2 \text{ m} \times 0.7 \text{ m}$). All experiments were performed in the same recirculating system one after the other. The tanks were part of a RAS equipped with an oxygen generator (OGP 4, Atlas Copco, Netherlands), micro sieve (Hydrotech filter, Veolia, Sweden) and a biofilter with a volume of 3.2 m^3 (SDK CN 3.2, SDK, Poland) with plastic filling (Light Bioelementer RK Plast A/S, Denmark) of a total volume of 1.5 m^3 . The filter thickness was 0.93 g cm^{-3} , and its surface area proper was $750 \text{ m}^2 \text{ m}^{-3}$. Throughout the experiments, the photoperiod was 24-h light at an intensity of 100 lux measured above water surfaces.

Each experiment consisted of three treatments with different (low, L; medium, M; and high, H) stocking densities:

Experiment I: LI, stocking density 600 inds. m^{-3} (0.78 kg m^{-3}); MI, stocking density 800 inds. m^{-3} (1.04 kg m^{-3}); HI, stocking density $1000 \text{ inds. m}^{-3}$ (1.30 kg m^{-3})
Experiment II: LII, stocking density 400 inds. m^{-3} (2.68 kg m^{-3}); MII, stocking density 600 inds. m^{-3} (4.02 kg m^{-3}); HII, stocking density 800 inds. m^{-3} (5.36 kg m^{-3})
Experiment III: LIII, stocking density 200 inds. m^{-3} (3.84 kg m^{-3}); MIII, stocking density 400 inds. m^{-3} (7.68 kg m^{-3}); HIII, stocking density 600 inds. m^{-3} (11.52 kg m^{-3})

The daily feed rations and feed pellet sizes for the different experiments were determined based on previous studies (Kozłowski et al. 2021). The fish were fed commercial sinking feed manufactured by Aller Aqua (Denmark). *Thalassa Ex GR* 0.9–1.6 mm was used in experiment I, *Thalassa Ex GR* 1.3–2.0 mm in experiment II and *Thalassa Ex GR* 1.6–2.4 mm in experiment III. All of the feeds contained 54% protein, 15% fat and 8.5% carbohydrates. Feed was delivered by automatic band feeders (Fischtechnik GmbH, Germany) for 18 h day⁻¹.

Physical and chemical analyses of water

Oxygen content and pH were measured with a CyberScan PCD 5500 (Eutech Instruments, USA). Total ammonia nitrogen ($\text{TAN} = \text{NH}_4^+\text{-N} + \text{NH}_3\text{-N}$) and nitrite ($\text{NO}_2\text{-N}$) concentration were determined with an Aquamate Plus UV-Vis spectrophotometer (Thermo Scientific, England). Water quality parameters were measured at least three times weekly, and water temperature was measured daily. The average water temperature during the experiments was 21.0 ± 0.8 °C. Water oxygen concentration at the tank outflows did not decrease below $7.0 \text{ mg O}_2 \text{ l}^{-1}$, and pH ranged from 7.6 to 7.8. The maximum concentrations of ammonia nitrogen and nitrite did not exceed 0.27 mg l^{-1} or 0.36 mg l^{-1} , respectively. All water quality parameters did not differ among the experiments and in between treatments ($P > 0.05$). The water flow in the experimental tanks was 12 l min^{-1} .

Data collection and statistical analyses

The biomass and number of fish in each tank were determined at the beginning and the end of each of the three experiments. The biomass was estimated by bulk-weighing the whole fish population from each tank. Additionally, on days 1, 7, 14, 21 and 28 of the experiments, individual measurements of body weight (BW) and total length (TL) were performed for 50

specimens from each tank to determine growth parameters and weekly feed rations. Before the measurements, fish were anaesthetized in a solution of Propiscin (active ingredient—etomidate) at a concentration of 0.8 ml l^{-1} . Fish mortality was monitored and recorded daily. These data were used to calculate the following parameters:

$$\text{Specific growth rate: SGR (\% d}^{-1}\text{)} = 100 \times (\ln \text{BW}_2 - \ln \text{BW}_1) \times \text{T}^{-1}$$

$$\text{Condition factor: K} = 100 \times \text{BW} \times \text{BL}^{-3}$$

$$\text{Feed conversion ratio: FCR} = \text{TFC} \times (\text{FB} - \text{IB})^{-1}$$

$$\text{Daily feed intake: DFI (\%BW d}^{-1}\text{)} = \text{FC} \times 100 [(\text{IB} + \text{FB}) \times 2^{-1}]^{-1} \times \text{T}^{-1}$$

$$\text{Coefficient of variation for body weight: CV (\%)} = 100 \times \text{SD} \times \text{BW}^{-1}$$

$$\text{Survival: S (\%)} = 100 (\text{FN IN}^{-1})$$

$$\text{Cannibalism: C (\%)} = 100 [\text{IN} - (\text{FN} + \text{DI})] \text{IN}^{-1}$$

$$\text{Biomass gain: BG (\%)} = 100 \times (\text{FB} - \text{IB}) \times \text{IB}^{-1}$$

where BW_1 is the initial body weight (g), BW_2 is the final body weight (g), BW is the body weight (g), T is the rearing period (days), BL is the body length (cm), SD is the body weight standard deviation, IB is the initial fish biomass (g), FB is the final fish biomass (g), IN is the initial number of fish (individuals), FN is the final number of fish (individuals), TFC is the total feed consumption (g) and DI is the number of dead specimens during rearing (individuals).

Mean values and standard deviations (SD) are presented. The normality of parameter distribution was tested by the Shapiro-Wilk test and, to confirm the homogeneity of variance, Levene's test. The data expressed in percentages were *arcsin* transformed before the statistical analysis. Data were compared using one-way ANOVA. The significance of differences was estimated using a post hoc HSD Tukey test ($P < 0.05$). Analyses were performed using STATISTICA 12 PL software (StatSoft, Poland).

Results

In experiment I, fish in the medium stocking density presented significantly higher final body weight than in the low and high stocking densities ($P < 0.05$). A similar trend was observed for FCR, with fish at medium density presenting significantly lower FCR than in low stocking density (Table 1; $P < 0.05$). Survival and cannibalism were also significantly better at 1.04 kg m^{-3} densities ($P < 0.05$) when comparing with the other stocking densities tested, demonstrating that fish performed better when reared at medium stocking density. In experiment II, pikeperch of initial body weight of 6.7 g performed (final body weight, SGR and FCR) significantly better and presented lower DFI at low density when compared with higher density (Table 2; $P < 0.05$). In experiment III, fish final body weight and SGR were significantly higher at low stocking density when compared with the medium and high stocking density (Table 3; $P < 0.05$). A similar trend was observed for FCR, with fish at low densities presenting significantly lower FCR than the other treatments ($P < 0.05$).

Discussion

Stocking density is an environmental stress factor that influences fish growth (Liu et al. 2017). In the present study, low initial stocking densities were applied to the three size groups ($0.78\text{--}11.52 \text{ kg}^{-3}$) because of the better understanding of how optimal growth

Table 1 Growth parameters ($n = 3$ replicates) of pikeperch (initial body weight of 1.3 g) reared under different stocking densities in experiment I (LI, 0.78 kg m⁻³; MI, 1.04 kg m⁻³; HI, 1.30 kg m⁻³)

Parameter	Treatments			F	P
	LI	MI	HI		
Final weight (g)	4.9 ± 0.1 ^b	5.3 ± 0.1 ^a	4.8 ± 0.2 ^b	9.69	0.013
Final total length (TL, cm)	8.6 ± 0.2 ^a	8.5 ± 0.1 ^a	8.4 ± 0.3 ^a	0.87	0.463
Specific growth rate (SGR, % d ⁻¹)	4.88 ± 0.07 ^a	4.99 ± 0.21 ^a	4.80 ± 0.03 ^a	1.68	0.262
Condition factor (CF)	1.22 ± 0.00 ^a	1.25 ± 0.01 ^a	1.23 ± 0.03 ^a	2.52	0.160
Feed conversion ratio (FCR)	0.72 ± 0.02 ^b	0.64 ± 0.04 ^a	0.70 ± 0.01 ^{ab}	7.66	0.022
Daily feed intake (DFI, %BW d ⁻¹)	2.94 ± 0.09 ^a	2.34 ± 0.79 ^a	2.00 ± 0.70 ^a	1.81	0.242
Body weight variation coefficient (CV, %)	32.2 ± 4.9 ^a	29.8 ± 7.3 ^a	35.8 ± 14.6 ^a	0.27	0.768
Survival (%)	93.0 ± 0.3 ^c	97.2 ± 0.8 ^a	94.6 ± 0.2 ^b	66.67	< 0.001
Cannibalism (%)	4.7 ± 0.7 ^b	1.0 ± 0.1 ^a	3.7 ± 0.3 ^b	54.14	< 0.001
Final biomass (kg m ⁻³)	2.72 ± 0.06 ^c	4.27 ± 0.03 ^b	4.51 ± 0.21 ^a	164.55	< 0.001
Biomass gain (BG, %)	291.9 ± 8.1 ^a	305.2 ± 23.1 ^a	283.8 ± 3.3 ^a	1.73	0.255

All data are expressed as mean ± SD. Values marked with different letters show significant differences ($P < 0.05$)

Table 2 Growth parameters ($n = 3$ replicates) of pikeperch (initial body weight of 6.7 g) reared under different stocking densities in experiment II (LII, 2.68 kg m⁻³; MII, 4.02 kg m⁻³; HII, 5.36 kg m⁻³)

Parameter	Treatments			F	P
	LII	MI	HII		
Final weight (g)	19.3 ± 0.3 ^a	18.2 ± 0.7 ^{ab}	17.2 ± 0.3 ^b	12.71	0.007
Final total length (TL, cm)	13.9 ± 0.2 ^a	13.5 ± 0.3 ^a	13.4 ± 0.3 ^a	3.29	0.108
Specific growth rate (SGR, % d ⁻¹)	3.79 ± 0.12 ^a	3.59 ± 0.05 ^{ab}	3.39 ± 0.18 ^b	7.19	0.025
Condition factor (CF)	1.28 ± 0.03 ^a	1.27 ± 0.03 ^a	1.24 ± 0.01 ^a	2.34	0.177
Feed conversion ratio (FCR)	0.74 ± 0.02 ^a	0.80 ± 0.03 ^{ab}	0.87 ± 0.06 ^b	7.28	0.025
Daily feed intake (DFI, %BW d ⁻¹)	2.54 ± 0.01 ^b	2.62 ± 0.06 ^{ab}	2.69 ± 0.05 ^a	8.33	0.019
Body weight variation coefficient (CV, %)	39.5 ± 1.5 ^a	35.9 ± 7.5 ^a	36.8 ± 0.1 ^a	0.54	0.607
Survival (%)	97.9 ± 0.9 ^a	98.6 ± 0.9 ^a	97.9 ± 1.3 ^a	0.43	0.667
Cannibalism (%)	1.9 ± 0.6 ^a	1.0 ± 0.9 ^a	1.8 ± 1.2 ^a	0.89	0.457
Final biomass (kg m ⁻³)	7.54 ± 0.09 ^c	10.78 ± 0.52 ^b	13.51 ± 0.41 ^a	178.71	< 0.001
Biomass gain (BG, %)	188.9 ± 9.8 ^a	172.9 ± 4.0 ^{ab}	158.8 ± 12.9 ^b	7.33	0.024

All data are expressed as mean ± SD. Values marked with different letters show significant differences ($P < 0.05$)

parameters can be achieved. In experiment I, the final body weight and SGR value in LI treatment at a final stocking density of 2.72 kg m⁻³ (initial stocking density 0.78 kg⁻³) were lower than those in MI treatment (final stocking density 4.27 kg⁻³), which indicated that lower stocking density had an adverse effect on the growth of pikeperch of an initial body weight (BW_i) of 1.3 g. Other studies have also shown that low stocking density can be stressful because of social interactions among fish (Manley et al. 2014; Millán-Cubillo et al. 2016). In this study, lower body weight at low stocking densities was associated with

Table 3 Growth parameters ($n = 3$ replicates) of pikeperch (initial body weight of 19.2 g) reared under different stocking densities in experiment III (LIII, 3.84 kg m⁻³; MIII, 7.68 kg m⁻³; HIII, 11.52 kg m⁻³)

Parameter	Treatments			<i>F</i>	<i>P</i>
	LIII	MIII	HIII		
Final weight (g)	38.3 ± 0.9 ^a	36.3 ± 1.0 ^b	34.9 ± 0.7 ^b	11.48	0.009
Final total length (TL, cm)	17.0 ± 0.1 ^a	16.6 ± 0.3 ^a	16.7 ± 0.3 ^a	1.85	0.236
Specific growth rate (SGR, % d ⁻¹)	2.47 ± 0.05 ^a	2.29 ± 0.07 ^b	2.11 ± 0.04 ^c	28.17	< 0.001
Condition factor (CF)	1.25 ± 0.02 ^a	1.24 ± 0.02 ^a	1.23 ± 0.02 ^a	0.57	0.590
Feed conversion ratio (FCR)	0.82 ± 0.05 ^a	0.86 ± 0.05 ^b	0.94 ± 0.02 ^b	7.01	0.027
Daily feed intake (DFI, %BW d ⁻¹)	1.91 ± 0.05 ^a	1.90 ± 0.05 ^a	1.95 ± 0.05 ^a	0.71	0.191
Body weight variation coefficient (CV, %)	21.9 ± 1.0 ^a	25.4 ± 5.8 ^a	24.2 ± 1.9 ^a	0.76	0.506
Survival (%)	99.2 ± 1.0 ^a	99.8 ± 0.3 ^a	99.9 ± 0.2 ^a	1.21	0.362
Final biomass (kg m ⁻³)	7.60 ± 0.25 ^c	14.50 ± 0.37 ^b	20.90 ± 0.44 ^a	998.62	< 0.001
Biomass gain (BG, %)	99.8 ± 3.0 ^a	84.5 ± 5.5 ^{ab}	80.6 ± 2.3 ^b	20.54	0.02

All data are expressed as mean ± SD. Values marked with different letters show significant differences ($P < 0.05$)

higher FCR and cannibalism. Pikeperch sizing 1.3 to 5.0 g may require a minimum stocking density to achieve better performance. Low densities could be detrimental for social and/or territorial fish species as it could trigger competition for food, increased appetite (Potthoff and Christman 2006) and stressful conditions (Strand et al. 2007).

In experiments II and III, the fish performance was negatively impacted with increasing density. Similar results were also noted in studies of Amur sturgeon *Acipenser schrenckii* (von Brandt) (Yang et al. 2011), turbot *Scophthalmus maximus* (L.) (Liu et al. 2017), lenok *Brachymystax lenok* (Pallas) (Liu et al. 2019), Atlantic salmon *Salmo salar* (L.) (Oppedal et al. 2011) and pompano *Trachinotus ovatus* (L.) (Yang et al. 2020). The results of the present study showed an increase in daily feed intake with increasing stocking density. This was not reflected in the feed conversion ratio which increased with increasing stocking density. The limited growth of pikeperch at high stocking density was the result of crowding stress which triggered a rising demand for energy to activate the physiological responses to cope with stress, and led to a reduction in the available energy for growth (Yang et al. 2020). Moreover, at higher stocking densities, fish compete for food and expend more energy on movement, resulting in lower body weight gain (Yang et al. 2011).

It is well known that fish survival is negatively impacted with increase in stocking density (Hitzfelder et al. 2006). Many studies have shown that survival often decreases with increasing stocking density (Rahman et al. 2005; Hitzfelder et al. 2006; Rowland et al. 2006). In our study, differences in survival of the small pikeperch with body weight of 1.3 g resulted from higher cannibalism at the high (HI) and low (LI) stocking densities. Cannibalism also occurred in medium-sized fish of 6.7 g (BW_i) but at a low level (1–2%) compared with the small fish size. No cannibalism was observed within the larger fish size used in this experiment, with survival > 99% for the three stocking densities tested. Kozłowski et al. (2021) reported similar results when rearing pikeperch of a similar size. The results of this and previous studies (Kozłowski et al. 2021) suggest that cannibalism of pikeperch with a body length over 11 cm is stopped.

The stocking densities used in this study affected the growth of juvenile pikeperch. Another study showed no difference in growth parameters for pikeperch of 0.65 g (BW_i)

and stocking density 0.99–2.31 kg m⁻³, possibly due to high cannibalism (37–40%) (Szkudlarek 2002). Similar results were obtained by Molnár et al. (2004) for pikeperch of 0.91 g (BW_i) and stocking density of 1.25–2.08 kg m⁻³. For larger pikeperch sizing 91 g, high stocking density (15–30 kg m⁻³) did not negatively impacted growth and feed utilization (Baekelandt et al. 2018).

Stocking density is regarded as a priority topic in aquaculture research because of its influences on the welfare of cultured fish and the need for formulating further recommendations on this issue. The growth of small pikeperch (1.3 g) was slightly but significantly higher at medium stocking densities (1.04 kg m⁻³) than at low and high stocking densities (0.78 and 1.04 kg m⁻³, respectively). For the medium-sized (6.7 g) and large-sized (19.2 g) fish, growth was slightly but significantly higher at lower densities (2.68 and 3.84 kg m⁻³, respectively) than at densities ranging from 4.02 to 11.52 kg m⁻³. The results of the present study indicated that, under the same experimental conditions, pikeperch of 1.3, 6.7 and 19.2 g (BW_i) achieved faster growth at medium to low stocking density of 1.04, 2.68 and 3.84 kg m⁻³, respectively. However, low stocking densities might not be economically viable. Therefore, optimal stocking density should be assessed based on both fish performance and economic feasibility. In our study, the condition factor, which is a good indicator of health status, was similar in all the experiments and at all the stocking densities. This suggests that juvenile pikeperch were properly fed, although FCR < 1 may indicate an underestimation of the feed dose. Further studies should focus on the influence of stocking density on physiological, immune, body composition and water quality parameters.

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Author contributions Conceptualization: MK; methodology: MK, IP; formal analysis and investigation: MK, IP; writing—original draft preparation: MK; writing—review and editing: MK, IP; resources: MK, IP; supervision: MK. All the authors approved the final draft.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical approval The study was in compliance with Polish animal welfare regulations and approved by the Local Ethics Committee for Animal Experimentation of the National Inland Fisheries Research Institute in Olsztyn, Poland.

Conflict of interest The authors declare no competing interests.

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