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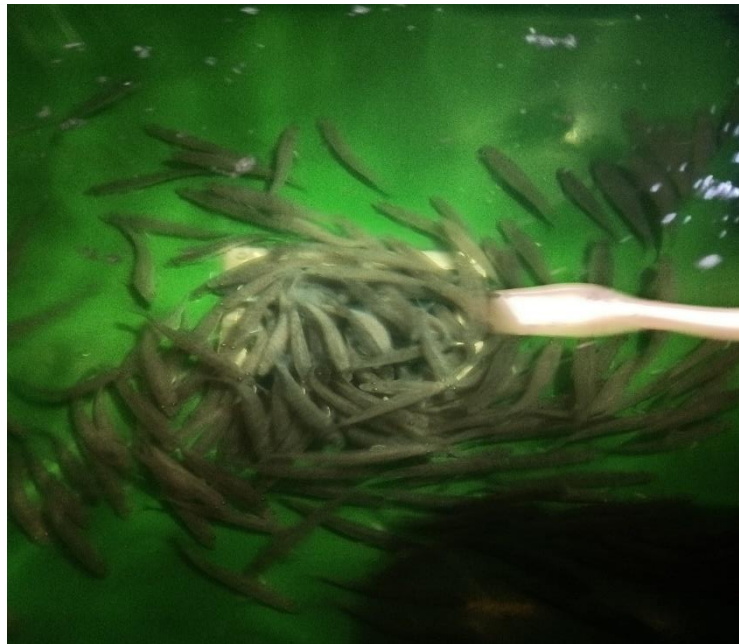
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Report about the effect of applied probiotics on pikeperch juvenile growth and survival rate.



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Introduction

Pikeperch (*Sander lucioperca*), a freshwater teleost of the Percidae family, holds significant commercial value due to its nutritional and economic importance (Kestemont et al., 2015). However, larviculture of this species in Recirculating Aquaculture Systems (RAS) remains challenging, primarily because of low stress tolerance, cannibalistic behavior, and consequently, low survival rates during early developmental stages (Steenfeldt, 2015; Policar et al., 2019).

Recirculating aquaculture systems are a sustainable and efficient approach to modern fish farming, but they are often challenged by the proliferation of opportunistic pathogens resulting from high stocking densities and stress (Derome et al., 2016). Using probiotics has emerged as a promising strategy to improve fish health and growth while reducing reliance on antibiotics. According to the 2002 FAO/WHO definition (FAO, 2002; Gatesoupe, 1999; Balcázar et al., 2006), probiotics are live microorganisms that, when administered in adequate amounts, help maintain intestinal microbial balance and promote overall host well-being. The concept of probiotics has evolved over time. The definition by Merrifield et al. (2010) is widely regarded as the most comprehensive. According to this definition, a probiotic is a microbial component, either living or non-viable, that, when administered through feed or water, confers benefits to the host by enhancing disease resistance, health status, growth performance, feed efficiency, stress tolerance, or overall vitality. Lactic acid bacteria (LAB) are the most extensively studied probiotic microorganisms (Zorriehzahra et al., 2016).

Common probiotic groups in aquaculture include *Pseudomonas*, *Bacillus* species, and lactic acid bacteria, with lactobacilli being the most widely utilized (Martinez-Cruz et al., 2012). Probiotic LAB improves growth performance by increasing digestive enzyme activity (Vand et al., 2014), elevating insulin-like growth factor 1 (IGF1) expression, and reducing cortisol levels in juvenile fish (Carnevali et al., 2006). They also promote bone mineralization (Avella et al., 2012), enhance immune responses, and protect against pathogenic infections (Pirarat et al., 2011; Abumourad et al., 2013). Probiotic bacteria may also hinder the growth of harmful microorganisms in the intestinal tract by competing for nutrients and adhesion sites. At the same time, these bacteria can stimulate the immune system and improve the host's ability to handle stress and disease (Hai, 2015).

In summary, several advantages for fish are provided by dietary supplementation with probiotics, including enhanced nutrient utilization, improved growth, and increased survival in recirculating aquaculture systems (Nayak, 2010). Studies in pikeperch have highlighted the beneficial effects of incorporating lactic acid bacteria probiotics into their diets (Ljubobratović et al., 2017, 2019; Yanes-Roca et al., 2020). As antibiotic resistance continues to limit traditional disease management strategies, probiotic supplementation in RAS offers a viable, environmentally friendly alternative to improve fish welfare, stabilize microbiota composition, and support sustainable aquaculture production.

The aim of this study was to evaluate the effects of probiotic supplementation on juvenile pikeperch (*Sander lucioperca*) reared in recirculating aquaculture systems (RAS) by monitoring growth performance over an extended period. Specifically, the study focused on measuring body length, weight, feed conversion ratio (FCR) and specific growth rate (SGR) to assess the efficiency of probiotic use in promoting juvenile growth and overall development under controlled RAS conditions.

2. Methods

The study evaluated the effect of probiotics on the growth of pikeperch (*Sander lucioperca*) reared in recirculating aquaculture systems (RAS). Comparative analyses were conducted between control and trial fish groups. The control group was fed a commercial diet, while the trial group received the same diet supplemented with probiotics. The trials were performed on *Sander lucioperca* individuals that were initially fed from 0+ age. The following section presents a description of the study subject and the trial design.

2.1 Study species

Three separate trials were conducted on juvenile pikeperch (*Sander lucioperca*). **Trial I** was initiated in November 2024 and continued until April 18, 2025, while **Trial II** began in June 2025 and lasted until October 21, 2025. The fish were reared in recirculating aquaculture systems (RAS) at the Fisheries Service under the Ministry of Agriculture of the Republic of Lithuania, Western region Pisciculture division of the Department of Pisciculture, Rusnė (Šyškrantės St. 50, LT-99352, Šyškrantės village, Rusnė eldership, Šilutė district). **Trial III** main data collection was performed from August

2025 till October 1 st, 2025. Pikeperch juveniles 0+ were reared with improved methods and kept in the RAS at the Institute of Food Safety, Animal Health and Environment "BIOR", Aquaculture Innovation Infrastructure Centre (Tome Parish, Ogre Municipality, Latvia, LV-5020).

2.2 Probiotics and their application

In this study, the biological preparation Smart Fishery (series no. F920R030565, code no. F9), produced by Ltd. Baltic Probiotics (Ceptuve, Rucava pag., Rucava Municipality, LV-3477, Latvia), was used. According to the certificate of analysis (28 April 2020), the product is a biological formulation effective microorganisms, fermented herbs and phyto-ferments for improving the microbiological quality of water. The preparation is designed to improve water microbiological quality in recirculating aquaculture systems, is based on natural fermentation, and it is biodegradable and safe for humans, animals, and plants.

General properties: pH 3.5 ± 0.2 ; Microbes total count 2.2×10^6 CFU/ml; *Salmonella* - not detected/ 25 ml. Analytical composition: water 96.5 %, crude protein 0.56 %, crude fat < 0.1 %, crude fiber < 0.5 %, crude ash 0.57 %, carbohydrates < 1. These parameters ensure the safety of the biological preparation, the activity of the effective microorganisms, and its suitability for incorporation into the diet of *Sander lucioperca*. Ingredients: Lactobacteria and yeast cultures, sugar cane molasses, natural minerals, sea salt, herbs extracts, chlorine-free water.

Dosage and incorporation into feed: for the trial I and II the probiotic was prepared by diluting it at a ratio of 1:50 with water, with a usage rate of 1.5 mL of product per 1 kg of feed. The resulting solution was thoroughly mixed with commercial feed to ensure uniform distribution and stability of the microorganisms.

2.3 Study design and procedure

Trial I: In November 2024, juvenile pikeperch were transferred from ponds to recirculating aquaculture system (RAS) tanks (total tank volume: 1 m³; water volume: 50% of capacity). Two trial groups were established: a control group (hereafter referred to as Control) and a probiotic-fed group (hereafter referred to as Probiotics). Each group was assigned two replicate tanks containing 0+ juvenile pikeperch. Fish in the *Control* group were fed a standard commercial diet (Aller Aqua Futura Ex Gr, 1.6–2.0 mm pellet

size). The diet for the *Probiotics* group was supplemented with the probiotic preparation Smart Fishery (Table 1). The probiotic solution was prepared by diluting the product 1:50 with water and applied at a dosage of 1.5 mL per kilogram of feed. The diluted solution was evenly sprayed onto the feed pellets immediately prior to feeding to ensure uniform coating and to maintain the viability of the microorganisms.

Fish in each tank were fed using automatic feeders, with the daily feed ration corresponding to 5% of the total fish biomass. All fish were maintained under identical RAS conditions. The mean water temperature was 15 °C (± 1 °C), pH was 8.0, and dissolved oxygen levels were approximately 95% saturation. Water quality parameters were monitored on a daily basis to ensure environmental stability. The photoperiod was maintained at a 12 h light:12 h dark cycle to simulate natural diurnal fluctuations and provide physiologically optimal conditions for growth.

Morphometric measurements were conducted over a six-month period on November 7 and December 20, 2024, and on January 20, February 25, March 25, and April 18, 2025. The data obtained from these measurements were used for further analysis, and the results are presented in the Results section.

Table 1. Characteristics of the trial groups of *Sander lucioperca* (0+ age) – Trial I.

Group	Fish age	Description	Sample size (n)	Feed
Control	0+	Fed standard commercial feed without probiotics	252	Commercial*
Probiotic	0+	Fed commercial feed supplemented with probiotics	252	Commercial + Probiotics

* Aller Aqua Futura Ex Gr, 1,6-2,0 mm.

Trial II:

In June 2025, juvenile pikeperch (*Sander lucioperca*) were distributed into four tanks. Tanks No. 4 and No. 5, containing 402 individuals in total, comprised the trial group and were fed a diet supplemented with the Smart Fishery probiotic. Tanks No. 2 and No. 3, containing 402 individuals in total, comprised the control group and were fed a standard commercial diet without probiotic supplementation (Table 2).

The probiotic solution was prepared by diluting the product 1:50 with water and applied at a dosage of 1.5 mL per kilogram of feed. The diluted solution was evenly sprayed onto the feed pellets immediately prior to feeding to ensure uniform coating and to maintain the viability of the microorganisms. Fish in each tank were fed using

automatic feeders, with the daily feed (Aller Aqua Futura Ex Gr, 1.6–2.0 mm pellet size) ration corresponding to 5% of the total fish biomass.

All fish were maintained under identical RAS conditions: mean water temperature 15 °C (± 1 °C), pH 8, and dissolved oxygen approximately 95% saturation. Water quality parameters were monitored daily to ensure stability. The photoperiod was set to a 12-hour light / 12-hour dark cycle to simulate natural day–night conditions and provide physiologically optimal growth conditions. Morphometric measurements were conducted four times: on July 16, August 22, September 18, and October 21, 2025.

Table 2. Characteristics of the trial groups of *Sander lucioperca* – Trial II.

Group	Fish age	Description	Sample size (n)	Feed
Control	fry	Fed standard commercial feed without probiotics	402	Commercial*
Probiotic	fry	Fed commercial feed supplemented with probiotics	402	Commercial + Probiotics

* Aller Aqua Futura Ex Gr, 1,6-2,0 mm.

Trial III:

Pikeperch juvenile fish (0+) obtained and pre-reared in a recirculating aquaculture system (RAS) were transferred to a separate isolated RAS with longitudinal flow-through tanks. The fish were distributed at a density of **50** individuals per tank (two control tanks and two test tanks).

The photoperiod was set to 12 hours of light and 12 hours of darkness. Light intensity differed between paired tanks, with one control tank and one trial tank maintained at 5% light intensity, and the other control tank and test tank maintained at 30% light intensity.

The trial was conducted with pikeperch juveniles that have started feeding on artificial (extruded) feed, for the basis the *Aller Futura* EX GR 0.5–1.0. feed was used. Fish were fed manually each day from 8:00 till 16:00 (once per hour), each portion prepared freshly before the feeding time, ration was calculated as 5% of fish total biomass. Fish tanks were cleaned before the first feeding and within an hour after the last one.

Water parameters were controlled and maintained daily, temperature was maintained at around $21 \pm 1^\circ\text{C}$; pH 7,75-7,96; ammonia concentration monitored every second day to

calculate non-ionized ammonia levels and to ensure it does not reach the critical level of 0,01 mg/l. that would negatively affect fish. Mortality and occurrence of morphological abnormalities were registered every day. Individual body mass measurements were carried out at the beginning and at the end of the trial.

2.5 Morphometric data collection

For each trial individual in trials I and II, total body length (TL) and body weight (W) were recorded, and the condition factor (CF) was calculated, for the trial III the body weight (W) and feed conversion ratio (FCR) were calculated. Condition factor was used as an integrative morphometric parameter to assess individual growth performance, physiological condition, and overall fitness, and to indirectly evaluate feeding efficiency and the influence of environmental conditions on organismal homeostasis.

The following measurements were performed for each fish in trials I and II:

- **Total body length (TL):** measured from the tip of the snout to the end of the caudal fin using a millimeter ruler (accuracy ± 0.1 cm).
- **Body weight (W):** measured using electronic scales (precision 0.1 g) (the same procedure for trial III).

All measurements were conducted after a 24-hour fasting period to avoid weight fluctuations due to gut content.

- **Condition factor (CF):** calculated according to Fulton, representing the relationship between fish mass and body length, using the formula:

$$CF=100 (W/TL^3)$$

where W is body weight and TL is total body length.

- **Specific growth rate (SGR):** calculated using the formula (Márquez et al., 2024):

$$SGR=(\ln(W_2)-\ln(W_1))/t \times 100$$

where W_1 and W_2 are initial and final body weights, respectively, and t is the time interval in days.

2.6 Statistical analyses

Morphometric parameters, including total body length, body weight, and condition factor, were analysed to assess differences between trial groups. Data distribution was examined and found to deviate from normality; therefore, intergroup comparisons were conducted using the non-parametric Mann–Whitney U test.

Potential outliers in trials I and II were identified using the ROUT (Robust regression and Outlier removal) method with a false discovery rate of $Q = 1\%$. Results are reported both including outliers and following outlier removal to evaluate their impact on statistical outcomes.

All data are presented as mean \pm standard deviation (SD). Statistical significance was defined as $p < 0.05$. Analyses were performed using R (ver 4.5.1.) and GraphPad Software, LLC (version 10.6.0, USA).

3. Results

The Results section presents data from studies conducted under RAS conditions to evaluate the effect of probiotics supplemented diet on pikeperch growth. Growth parameters of fish from different trial groups are summarized using descriptive statistics, including sample size (n), minimum and maximum values, mean values, and standard deviations (SD). These data allow for a comparison of growth differences between control and probiotic-fed fish.

3.1 Trial I: sample size

This subsection presents data characterizing the sample size of 0+ age pikeperch, including total body length (TL), body weight (W), and condition factor (CF). Table 3 summarizes the key descriptive statistics – minimum and maximum values, means, and standard deviations (SD) – for each test group, allowing comparison of growth performance and body condition between control and probiotic-supplemented groups.

Table 4 presents the data obtained after the removal of outliers identified using the ROUT method, allowing assessment of their impact on morphometric parameters and statistical comparisons between trial groups.

Table 3. Sample size, total body length, body weight, and condition factor in the trial groups.

Group	Sample size (n)	L (cm) min-max	L (cm) mean \pm SD	W (g) min-max	W (g) mean \pm SD	CF mean \pm SD
November						
Control	252	9 – 12.5	10.36 \pm 0.64	5.0 – 15.0	8.75 \pm 1.65	0.78 \pm 0.08
Probiotic	252	9 – 13.0	10.25 \pm 0.60	5.0 – 15.0	8.34 \pm 1.40	0.77 \pm 0.08
December						
Control	248	9 – 14.5	11.19 \pm 1.19	4.0 – 29.0	12.26 \pm 6.51	0.80 \pm 0.22
Probiotic	249	8.5 – 15.0	11.00 \pm 1.09	3.0 – 32.0	11.39 \pm 5.73	0.79 \pm 0.22
January						
Control	149	10.5 – 16.0	13.55 \pm 1.18	7.0 – 40.0	23.13 \pm 6.83	0.91 \pm 0.16
Probiotic	145	10,0 – 17,0	13.37 \pm 1.10	8.0 – 41.0	22.50 \pm 5.79	0.92 \pm 0.13
February						
Control	148	11.5 – 18.5	15.15 \pm 1.34	9.0 – 54.0	32.10 \pm 8.75	0.90 \pm 0.10
Probiotic	145	10,5 – 17,5	15.13 \pm 1.27	9.0 – 55.0	32.27 \pm 7.83	0.92 \pm 0.09
March						
Control	146	11.5 – 19.5	16.01 \pm 1.52	10.0 – 59.0	37.04 \pm 10.19	0.89 \pm 0.12
Probiotic	142	11.0 – 20.0	16.16 \pm 1.40	9.0 – 64.0	37.46 \pm 9.62	0.87 \pm 0.10
April						
Control	146	11.5 – 20.5	17.01 \pm 1.63	10.0 – 76.0	43.32 \pm 11.90	0.86 \pm 0.10
Probiotic	142	11.0 – 20.5	17.01 \pm 1.58	10.0 – 73.0	42.85 \pm 11.22	0.85 \pm 0.09

Table 4. Sample size, total body length, body weight, and condition factor in the trial groups after removal of outliers identified using the ROUT method.

Group/Parameter	Control			Probiotic		
	Sample size (n)	min-max	mean \pm SD	Sample size (n)	min-max	mean \pm SD
March						
L (cm)	141	11.5 – 20.0	16.20 \pm 1.33	–		
November						
W (g)	247	5.0 – 13.0	8.64 \pm 1.46	250	5.0 \pm 12.0	8.29 \pm 1.31
January						
CF	144	0.53 – 1.31	0.92 \pm 0.14	144	0.59 – 1.23	0.93 \pm 0.12
February						
CF	142	0.64 – 1.15	0.90 \pm 0.09	143	0.73 – 1.18	0.90 \pm 0.08
April						
CF	142	0.66 – 1.10	0.85 \pm 0.09	–		

3.2 Trial I: body length and weight

Mean body length increased in both groups throughout the trial period. In the control group, mean body length increased from 10.36 cm to 17.01 cm, while in the probiotic-fed group it increased from 10.25 cm to 17.01 cm (Figure 1). Statistical analysis revealed no significant differences between the groups ($p > 0.05$).

Outlier analysis using the ROUT method identified and removed one individual from the control group, while no outliers were detected in the probiotic group. The graph is not presented, as this correction did not significantly alter the results (Table 4).

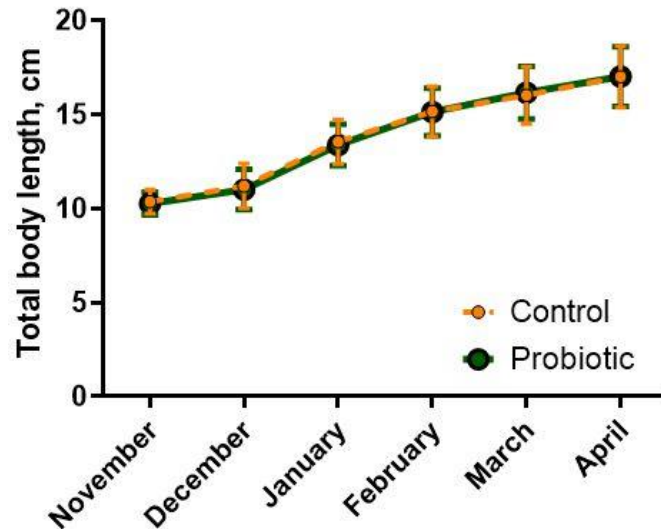


Fig. 1. Mean total body length (cm \pm SD) of *Sander lucioperca* (0+ age) in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics.

Throughout the trial period, body weight (g) of pikeperch increased steadily in both the control and probiotic-supplemented groups. The initial mean body weight was 8.75 ± 1.65 g in the control group and 8.34 ± 1.40 g in the probiotic group, increasing to 43.32 ± 11.90 g and 42.85 ± 11.22 g, respectively, by the end of the trial. Although mean body weights in the probiotic group were slightly lower than those of the control group at certain time points, these differences were not statistically significant. A statistically significant difference was observed only during the November measurement, when individuals in the control group exhibited higher body weight than those in the probiotic group (Figure 2; $p = 0.01$, Mann–Whitney U test).

Outlier detection using the ROUT method resulted in the removal of five individuals from the control group and two individuals from the probiotic group (November measurement). This correction did not significantly alter the results; therefore, a separate graph is not presented (Table 4).

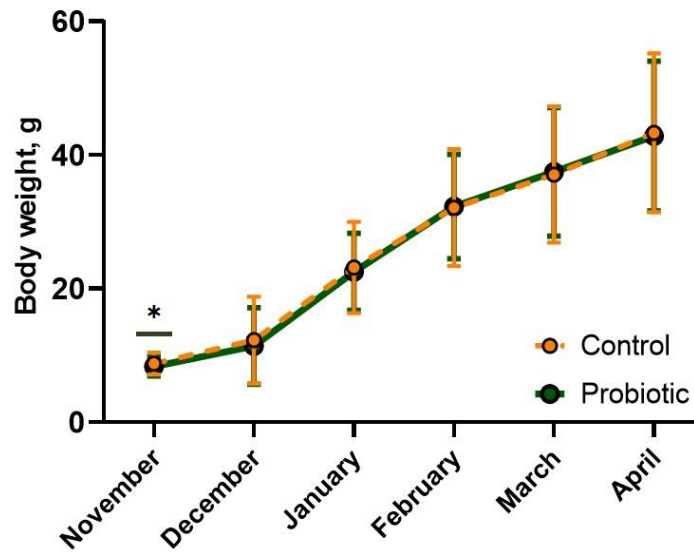


Fig. 2. Mean body weight (g ± SD) of *Sander lucioperca* (0+ age) in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

3.3 Trial I: condition factor

During the trial period, the condition factor (CF) of pikeperch gradually increased in both the control and probiotic-fed groups. At the first measurement, mean CF values were 0.78 ± 0.08 in the control group and 0.77 ± 0.08 in the probiotic group. By the final measurement, CF had increased to 0.86 ± 0.10 and 0.85 ± 0.09 , respectively. Although mean CF values in the probiotic group were slightly lower than in the control group at some sampling points, these differences were not statistically significant (Figure 3; $p > 0.05$), indicating a similar condition factor pattern in both groups.

Outlier detection using the ROUT method resulted in the removal of five individuals from the control group and one from the probiotic group in January, six and two individuals, respectively, in February, and four control group individuals in April. These adjustments did not substantially alter the overall trends (Table 4).

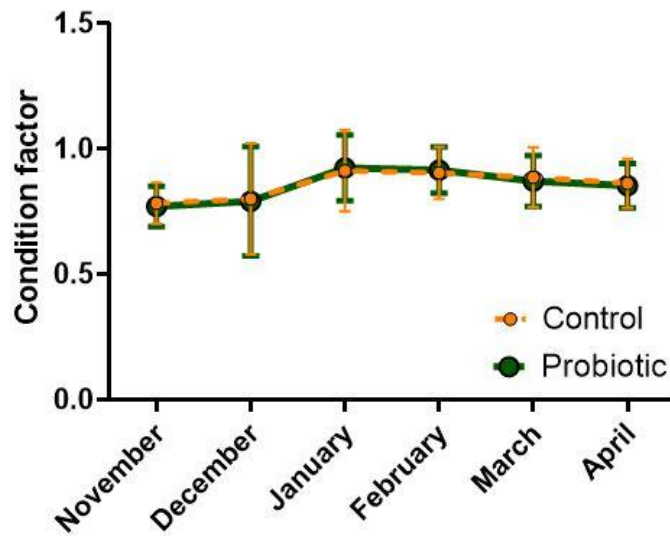


Fig. 3. Mean condition factor (CF ± SD) of 0+ age *Sander lucioperca* in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics.

3.4 Trial I: mean specific growth rate

Table 5. Specific growth rate (SGR, %/d) of 0+ age pikeperch (*Sander lucioperca*) in the control and probiotic groups during the trial period (November 2024 – April 2025).

Interval	Period	Days	Control SGR (%/d)	Probiotics SGR (%/d)
November – December	2024-11-07 → 2024-12-20	43	0.784	0.725
December – January	2024-12-20 → 2025-01-20	31	2.048	2.196
January – February	2025-01-20 → 2025-02-25	36	0.91	1.002
February – March	2025-02-25 → 2025-03-25	28	0.511	0.533
March – April	2025-03-25 → 2025-04-18	24	0.653	0.560
Total period	2024-11-07 → 2025-04-18	162	0.987	1.010

During the trial, the specific growth rate (SGR, %/d) of pikeperch varied depending on the period, but no significant differences were observed between the groups. In the first period (November–December), SGR was 0.784 %/d in the control group and 0.725 %/d in the probiotic group, while the highest growth rate was recorded during the December–January period, at 2.048 %/d and 2.196 %/d, respectively. Subsequently, growth rates gradually declined, with an overall mean of 0.987 %/d in the control group

and 1.010 %/d in the probiotic group (Table 5). These results indicate that probiotic supplementation slightly increased the growth rate of pikeperch, although the overall growth patterns remained similar.

3.5 Trial II: sample size

This subsection presents data characterizing the trial sample of pikeperch fed a probiotic-supplemented diet from the juvenile stage, including total body length (L), body weight (W), condition factor (CF). summarizes the key descriptive statistics for each trial group, including minimum and maximum values, means, and standard deviations (SD), enabling comparison of growth performance and physiological condition between fish fed standard commercial feed (control) and those receiving probiotic-supplemented feed.

Table 7 presents the dataset obtained after the exclusion of outliers identified using the ROUT method, allowing assessment of their impact on morphometric parameters and statistical comparisons between groups.

Table 6. Sample size, total body length (L), body weight (W), and condition factor (CF) of pikeperch in the trial groups.

Group	Sample size (n)	L (cm) min-max	L (cm) mean ± SD	W (g) min-max	W (g) mean ± SD	CF mean ± SD
July						
Control	402	7.0 – 10.5	8.49 ± 0.83	3.0 – 12.0	6.17 ± 1.97	0.984 ± 0.15
Probiotic	402	5.5 – 11.0	8.80 ± 1.06	3.0 – 14.0	7.03 ± 2.53	1.00 ± 0.20
August						
Control	393	11.0 – 16.5	13.48 ± 1.10	14.0 – 57.0	26.41 ± 7.56	1.06 ± 0.14
Probiotic	395	10.5 – 19.0	13.45 ± 1.32	11.0 – 51.0	27.38 ± 7.81	1.11 ± 0.15
September						
Control	393	12.0 – 19.5	15.32 ± 1.18	20.0 ± 74.0	37.12 ± 9.21	1.02 ± 0.10
Probiotic	395	12.0 – 19.0	15.61 ± 1.27	19.0 ± 78.0	40.95 ± 10.1	1.06 ± 0.12
October						
Control	385	12.5 – 21.5	17.27 ± 1.34	19.0 ± 91.0	47.11 ± 11.47	0.90 ± 0.09
Probiotic	386	14.0 – 21.0	17.45 ± 1.37	24.0 ± 90.0	50.96 ± 11.78	0.95 ± 0.10

Table 7. Sample size, total body length (L), body weight (W), and condition factor (K) of pikeperch in the trial groups after removal of outliers identified using the ROUT method.

Group/Parameter	Control			Probiotic		
	Sample size (n)	min–max	mean \pm SD	Sample size (n)	min–max	mean \pm SD
August						
W	386	14.0 – 42.0	25.90 \pm 6.57	–	–	–
September						
W	392	20.0 – 68.0	37.02 \pm 9.03	–	–	–
October						
W	384	19.0 – 84.0	46.99 \pm 11.26	–	–	–
July						
CF	401	0.70 – 1.37	0.98 \pm 0.13	397	0.71 – 1.30	0.98 \pm 0.12
August						
CF	–	–	–	388	0.87 – 1.48	1.12 \pm 0.12
September						
CF	392	0.69 – 1.31	1.02 \pm 0.10	–	–	–
October						
CF	380	0.69 – 1.18	0.90 \pm 0.08	384	0.71 – 1.27	0.94 \pm 0.09

3.6 Trial II: body length and weight

Pikeperch body length (cm) differed between groups and across measurement periods. Fish fed the probiotic-supplemented diet exhibited greater body lengths compared to the control group during most measurement periods. Mean values differed significantly between the control and Probiotics groups at all measurement periods ($p < 0.04$), except in August. ROUT analysis detected no outliers in the body length dataset.

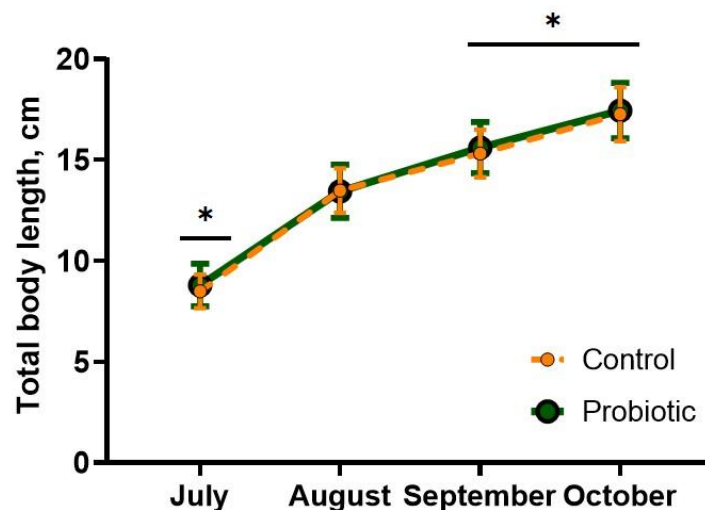


Fig. 4. Mean total body length (cm \pm SD) of *Sander lucioperca* in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with

Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

Across all four measurement periods, body weight (g) of pikeperch differed significantly between the control and probiotics groups ($p < 0.01$), with fish fed the probiotic-supplemented diet exhibiting higher mean body weights than those in the control group. Outlier analysis using the ROUT method led to the removal of values only from the control group; this adjustment did not significantly affect the overall results (Table 7).

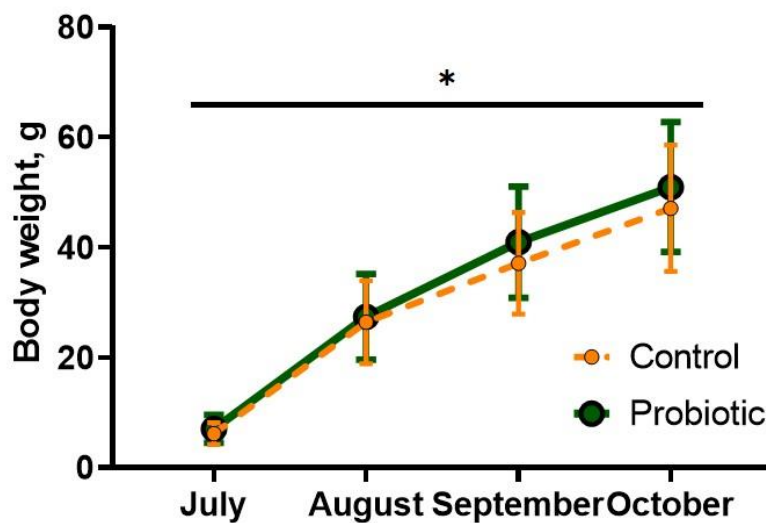


Fig. 5. Mean body weight (g) of *Sander lucioperca* (\pm SD) in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

3.7 Trial II: condition factor

The condition factor (CF) differed significantly between groups ($p < 0.05$), with fish receiving the probiotic-supplemented diet exhibiting higher mean CF values than the control group (Fig. 6). These results indicate that dietary probiotic supplementation positively influenced the average condition factor.

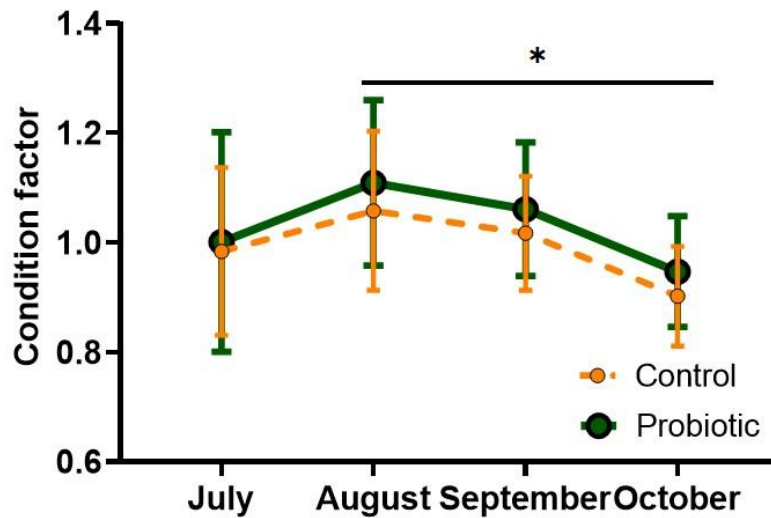


Fig. 6. Condition factor (\pm SD) of *Sander lucioperca* in the trial groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

3.9 Trial II: mean specific growth rate

Table 8. Mean specific growth rate (SGR) of *Sander lucioperca* in the control and probiotic groups during the period July 16 – October 21, 2025.

Interval	Period	Days	Control SGR (%/d)	Probiotics SGR (%/d)
July – August	2025-07-16 → 2025-08-22	38	3.83	3.58
August – September	2025-08-22 → 2025-09-18	27	1.26	1.49
September – October	2025-09-18 → 2025-10-21	33	0.72	0.66
Total period	2025-07-16 → 2025-10-21	98	2.08	2.02

The specific growth rate (SGR) of pikeperch varied across measurement periods, with the highest values recorded from July to August (3.83 %/d in the control group and 3.58 %/d in the probiotic group), followed by a gradual decline. Over the entire 98-day trial period, mean SGR was 2.08 %/d in the control group and 2.02 %/d in the probiotic group. No statistically significant differences were observed between groups.

3.10 Trial III: sample size

Pikeperch juveniles were selected by individual body mass and length, individual body mass did not differ significantly (p -value $>0,05$) between fish before beginning of trial. Fish were sorted twice in July, 2025, allowing to exclude the cannibals from the trial groups. K1 and T1 groups (Table 9.) correspond to 30% light intensity factor.

Table 9. Sample size, body weight (W), mortality, and feed conversion ratio (FCR) of pikeperch in the trial groups.

Group	Sample size (n)	W (g) mean \pm SD	Mortality, %	FCR
August				
Control (K1)	50	1.20 \pm 0.06	-	-
Control (K2)	50	1.28 \pm 0.08	-	-
Probiotic (T1)	50	1.22 \pm 0.06	-	-
Probiotic (T1)	50	1.22 \pm 0.06	-	-
September				
Control (K1)	50	4.52 \pm 0.23	8	0,510
Control (K2)	50	5.18 \pm 0.31	12	0,451
Probiotic (T1)	50	4.37 \pm 0.18	14	0,572
Probiotic (T2)	50	5.20 \pm 0.31	5	0,424

3.11 Trial III: body weight

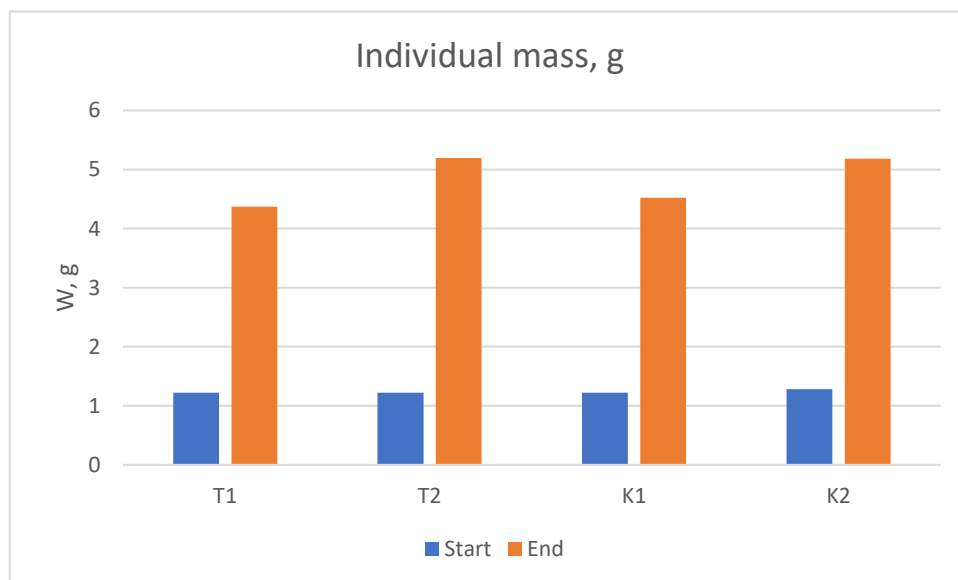


Fig. 7. Individual mass of *Sander lucioperca* in the trial groups: T1 – fed standard commercial feed supplemented with Smart Fishery probiotics under light intensity regime of 30%; T2 – fed standard commercial feed supplemented with Smart Fishery probiotics under light intensity regime of 5%; K1 – fed standard commercial feed

under light intensity regime of 30%; K2 – fed standard commercial feed under light intensity regime of 5%

Pikeperch body weight (g) differed between groups. Fish fed the probiotic-supplemented diet did not exhibit significantly greater body weight compared to the control group of the same light intensity. Groups T2 and K2 demonstrated significantly higher body weight at the end of the trial, indicating that the light intensity could be the major factor affecting pikeperch juvenile growth at current trial conditions (Fig. 7). Darker conditions improved fish growth, resulting in 18% higher individual mass after a month of trial.

3.12 Trial II: mean specific growth rate

Table 10. Mean specific growth rate (SGR) of *Sander lucioperca* in the control and probiotic groups.

Group	Days	Control SGR (%/d)
Control (K1)	31	4.25
Control (K2)	31	4.66
Probiotic (T1)	31	4.17
Probiotic (T2)	31	4.83

The specific growth rate (SGR) of pikeperch varied across measurement periods. Although, the highest SGR value corresponded to probiotic group (T2, Table 10), according to statistical analysis revealed that significant difference between groups related to different light regimes, which corresponds to weight parameters to the same groups: 4.66 and 4.83 in K2 and T2 (light intensity 5%) and 4.25 and 4.17 in K1 and T1 (light intensity 30%) accordingly. Compared to other trials (I and II) the SGR is higher in the case of trial III, which is related to the age of the juveniles – growth rates are higher in smaller fish.

4. Discussion

This study examined how probiotic supplementation affects the growth of juvenile pikeperch (*Sander lucioperca*) in Recirculating Aquaculture Systems (RAS). In the 0+ age group, probiotics had no significant impact on fish body length, weight,

condition factor, or specific growth rate. This suggests that overall growth was unaffected by probiotics at this developmental stage. However, juveniles at earlier developmental stages that received probiotic-supplemented feed exhibited significantly greater body length, weight, and condition factor than the control group, suggesting that probiotics are more beneficial during the early stages of development when applied for a longer period of time. While the specific growth rate varied across feeding periods, no significant differences were observed between groups, as both exhibited similar growth patterns over time under same conditions. This pattern aligns with previous findings in other freshwater fish species, in which early exposure to lactic acid bacteria was shown to positively influence the growth and immune function of juveniles (Martinez Cruz et al., 2012; Avella et al., 2012; Abumourad et al., 2013). Together, these studies underscore the significance of timing in probiotic supplementation, indicating that beginning administration at the earliest juvenile stage can maximize its favourable impact on growth performance and physiological development in pikeperch reared in RAS. It is extremely important to consider other physical factors, such as lighting, as its significant effect may completely mask the positive impact of probiotics on growth due to strong stimulation and a higher risk of handling stress. Therefore, the use of automatic feeders in combination with probiotic-supplemented feed is possible and preferable.

Previous research has explored the use of lactobacilli in pikeperch culture, demonstrating multiple benefits for larval development. Ljubobratovic et al. (2017) showed that enriching *Artemia* with lactobacilli enhanced larval growth, protein digestibility, and skeletal development. Similarly, research on weaning strategies in intensively reared pikeperch larvae has shown that supplementing live and inert feeds with *Lactobacillus* improves survival and growth. It also enhances digestive enzyme activities, such as trypsin and chymotrypsin; promotes ossification and skeletal differentiation; and reduces the proliferation of opportunistic pathogens, including *Vibrio* spp. (Ljubobratovic et al., 2020). Lactobacilli treatment has also been shown to modulate feed composition by slightly increasing the neutral lipid content of *Artemia* and reducing the lipid levels of dry feed. This may contribute to more efficient nutrient utilization. Taking together, these findings suggest that probiotics can enhance early development, optimize nutrient absorption, strengthen disease resistance, and

positively impact skeletal and metabolic processes during the critical weaning period of pikeperch larvae.

A previous study investigating the effects of lactic acid bacteria (LAB) probiotics on the early development of pikeperch during the first 21 days of feeding (Yanes-Roca et al., 2024). Larvae were exposed to probiotics either through live feed alone or through a combination of live feed and culture water and compared to a control group without probiotic supplementation. The larvae receiving LAB showed greater growth, including increased body length and myomere height, as well as higher survival rates. Probiotic treatment also altered the composition of the gut microbiota, although these changes became less distinct by the end of the 21-day period. In addition, exposure to LAB stimulated the activity of digestive enzymes such as proteases and lipases, particularly during the first week of feeding. Overall, the study indicated that administering LAB probiotics at the onset of feeding can support growth, digestive capacity, and microbial balance in intensively reared pikeperch larvae (Yanes-Roca et al., 2024).

Aquaculture is often challenged by fish health issues, and the incorporation of probiotic lactic acid bacteria (LAB) has been shown to help mitigate losses primarily caused by bacterial pathogens (Małaczewska et al., 2024). Recent studies indicate that *Levilactobacillus brevis* strains isolated from carp exhibit promising probiotic traits for pikeperch. They are easy to culture, lack harmful characteristics, and their cell-free supernatants have been shown to modulate immune responses in pikeperch head kidney cells (Małaczewska et al., 2024). These *in vitro* findings support further research into their potential benefits under *in vivo* conditions. Other studies demonstrated that treating fishmeal-based feed with the probiotic strains *Lactobacillus paracasei* BGHN14 and *Lactobacillus rhamnosus* BGT10 can enhance nutrient availability in pellets (Lukic et al., 2019). These findings suggest that feed enriched with these LAB strains can enhance digestibility and provide probiotic benefits, supporting growth and health in larval and juvenile fish. Studies on other Percidae species, such as perch (*Perca fluviatilis*), demonstrated that dietary supplementation with a mixture of *Bacillus licheniformis*, *B. subtilis*, and *B. pumilus* can positively influence larval performance (Mandiki et al., 2011). When administered through enriched Artemia at high doses, the probiotics increased growth rate,

lysozyme activity, and total immunoglobulin levels, and reduced type-2 cannibalism, particularly at higher stocking densities. In contrast, adding bacteria to the tank water alone did not affect growth or survival. These results indicate that high dietary concentrations of *Bacillus* spp. can enhance both growth and immune responses in Percidae larvae, though optimal dosing still requires further investigation (Mandiki et al., 2011).

Similar patterns have been observed in other freshwater species such as the climbing perch (*Anabas testudineus*), where dietary supplementation with the commercial multi-strain probiotics significantly enhanced growth performance, feed utilization efficiency, hematological, immune parameters and serum biochemistry, particularly at higher inclusion levels (Azaz Ibnasina et al., 2025). The study demonstrated that probiotic dosage plays a crucial role in achieving optimal physiological responses, with the highest dose (3 g/kg) producing the most pronounced improvements in growth rate, metabolic health, and innate immune activity. These results support the idea that probiotic effects are dose-dependent and influenced by feeding duration and environmental conditions (Azaz Ibnasina et al., 2025).

Our results indicate that probiotic supplementation enhances growth performance and condition factor in juvenile pikeperch. This observation aligns with previous studies in larval pikeperch, where lactic acid bacteria were shown to improve survival, growth, digestive enzyme activity, and skeletal development (Ljubobratović et al., 2020). Collectively, these findings suggest that the beneficial effects of probiotics are evident not only during the larval stage but also persist into the early juvenile stage, contributing to more efficient nutrient assimilation and overall physiological development under recirculating aquaculture system (RAS) conditions.

5. Conclusions

1. In older 0+ pikeperch reared in recirculating aquaculture systems (RAS), probiotic supplementation had no significant effect on body length, body weight, condition factor, or specific growth rate compared to the control group, indicating that probiotic supplementation did not significantly affect overall growth performance.
2. In juvenile pikeperch, body length, weight, and condition factor differed significantly between trial groups, with individuals receiving the probiotic-supplemented feed exhibiting higher values.
3. During pikeperch rearing, light intensity may significantly affect growth and potentially negate the positive effects of probiotics. Therefore, strict adherence to appropriate rearing protocols is essential.
4. Specific growth rate varied over the feeding periods; however, no significant differences were observed between groups, and both groups exhibited similar growth patterns throughout the trials.

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