Interreg

Latvia – Lithuania

Co-funded by the European Union

Deliverable No. D.1.2.1

Report about the efficiency of probiotic use in recirculation aquaculture systems

Document is produced by the Fisheries Service under the Ministry of Agriculture of the Republic of Lithuania within the INTERREG project SPPwelfare “Latvian and Lithuanian conjunction - Improvement of Baltic salmon and pikeperch rearing methods for more sustainable, resilient and healthy fish populations”

CONTENT

Introduction

In recent years, the aquaculture sector has been actively seeking sustainable and environmentally friendly solutions that can improve fish growth rates and disease resistance. The rapidly developing aquaculture industry faces challenges related to frequent outbreaks of infectious diseases caused by increased stress on fish in intensive farming conditions. Antibiotics are widely used to control these diseases, but their prophylactic use promotes the spread of antibiotic-resistant pathogens and poses a threat to public health. Therefore, strict measures to control the use of antibiotics in aquaculture are being implemented in many countries. Probiotics have become a sustainable alternative to synthetic antibiotics and other chemical products used in the aquaculture sector (Jamal et al., 2020). One of the most promising areas is the use of probiotic-enriched feed in aquaculture. Probiotics are live microorganisms that, when used properly, have a positive effect on the organism's microbiota, immunity, and metabolism. The use of probiotics in aquaculture is associated with improved fish digestion, more efficient nutrient absorption, and optimal microbiological balance in the aquatic environment (Chandra and Joshi 2024). In addition, numerous studies have shown that probiotics can positively modulate fish reproductive processes, increasing fertility and embryo viability through the regulation of the intestinal microbiota and the activation of related hormonal and enzymatic mechanisms (Akbari Nargesi et al., 2020; Giri et al., 2018). The inclusion of probiotics in fish nutrition is most commonly achieved through feed, water, or injections, and their combination with plant-based feeds is considered a significant step toward enhancing the sustainability of the aquaculture sector (Mugwanya et al., 2022).

Although interest in the use of probiotics in aquaculture is not a new phenomenon, significant progress has been made in researching the effects of probiotics, particularly in fish of the genus Salmo (Dindial 2022). Particular attention is paid to their effect on Atlantic salmon (*Salmo salar*) farmed in closed recirculating aquaculture systems (RAS), where environmental conditions and microbiological equilibrium directly influence fish health and productivity..

Studies have shown that supplementing feed with probiotics can significantly improve fish growth rates, feed utilization, and the activity of the immune system. As with all animals, the gut microbiome of fish consists of a complex, dynamic ecosystem that can host trillions of microorganisms. This microbiome plays an important role in maintaining the health of the

organism, including the regulation of digestive processes, modulation of immune system functions, and nutrient absorption. Such functions of the microbiome can directly affect fish growth, development, and metabolic processes (Talwar et al., 2018).

The beneficial bacteria in the gut can have a positive effect on fish health in several ways. They can strengthen immunity by producing substances that inhibit the growth of pathogenic microorganisms and reduce intestinal damage caused by pathogens. In addition, some bacteria stimulate the immune system of fish by increasing leukocyte activity and regulating the expression of cytokines and chemokines (Lazado and Caipang, 2014). On the other hand, it has been found that certain bacteria can improve fish digestion and nutrient absorption by increasing the length of microvilli, enhancing the fold length of the mucosa, secreting various digestive enzymes, and by fermenting non-digestible compounds (e.g., complex carbohydrates), converting them into absorbable forms (Abid et al., 2013). Given the growing potential of probiotics, it is necessary to systematically evaluate their effect on the physiological parameters of fish of the genus Salmo in order to optimize aquaculture practices and ensure the sustainable development of this sector.

Recirculating aquaculture systems (RAS) enable effective control of environmental parameters and significantly reduce water consumption, which makes them widely used for intensive salmonid production. However, due to high stocking densities and limited water exchange, these systems create favourable conditions for the accumulation of microbial biofilms, organic matter, and the proliferation of potentially pathogenic microorganisms. Such factors can negatively affect fish growth, stress response, and the functioning of the immune system.

In recent years, the use of probiotics in RAS has gained increasing importance as a biological alternative to conventional disease prevention methods. Probiotic microorganisms incorporated into feed or water help stabilize the microbial community both in the fish gut and within the system itself – particularly in biofilters, sediments, and water. This contributes to reducing the accumulation of ammonia, nitrites, and suspended solids, while inhibiting the proliferation of pathogenic bacteria such as *Aeromonas*, *Vibrio*, and *Pseudomonas* spp. (Nurhasanah et al., 2023).

Studies have shown that probiotics in recirculating aquaculture system (RAS) environments can improve fish growth performance (body weight, length, and condition factor), enhance survival rates, and strengthen intestinal barrier integrity. Moreover, certain *Bacillus* and *Lactobacillus* strains help reduce organic waste loads by promoting nitrification and

denitrification processes. In this way, probiotics not only enhance fish health but also contribute to the overall microbiological stability and sustainable functioning of RAS.

An important practical aspect involves the selection and application method of probiotics, as their effectiveness depends on the microbial strain, dosage, frequency of administration, and environmental parameters such as temperature, pH, and oxygen concentration. When appropriately selected and integrated into RAS management strategies, probiotic formulations can reduce the need for chemical treatments and help maintain ecosystem balance, thereby improving fish productivity and overall physiological condition (Nurhasanah et al., 2023; Calcagnile et al., 2024; Mohammed et al., 2025; de Oliveira et al., 2025).

2. Methods

The aim of this study was to evaluate the effect of probiotics on the growth of Atlantic salmon (*Salmo salar* L.) at different stages of their development. The studies were conducted in recirculating aquaculture systems (RAS) to ensure controlled environmental conditions and standardized feeding. During the experiment, a comparison was made between the control and test groups of fish. The fish in the control group were fed commercial feed, while the test group was fed the same feed supplemented with probiotics. *Salmo salar* individuals that were fed from the early fry and parr (0+ age) stages of development were evaluated. The study analysed fish at both stages of their development in order to assess the effect of probiotics at different stages of their ontogenesis. A description of the study object and the experimental design scheme are presented below.

2.1 Study species

The studies were conducted with Atlantic salmon (*Salmo salar* L.) to assess the growth characteristics of individuals at different stages of their development. Two separate experiments were carried out:

- **Experiment I:** involved parr (0+ age) Atlantic salmon;
- **Experiment II:** involved early fry of Atlantic salmon.

The fish were reared in recirculating aquaculture systems at the Eastern Region Pisciculture Division of the Fishery Service under the Ministry of Agriculture of the Republic of Lithuania (Meškerinė village, Pabradė Eldership, Švenčionys District).

2.2 Description of the RAS at Eastern Region Pisciculture Division

The study was conducted at the Eastern Region Pisciculture Division of the Departament of Pisciculture (Meškerinė). This facility is equipped with modern recirculating aquaculture systems (RAS), which allow for the maintenance of stable and controlled environmental conditions during fish rearing (Fig. 1).

In systems operating on the RAS principle, water circulates continuously through mechanical and biological filters, ensuring its effective purification and reuse. This technology significantly reduces water consumption while maintaining consistent water quality throughout the experiment.

The RAS facilities at the Eastern Region Pisciculture Division (Meškerinė) allow precise control of temperature, oxygen levels, pH, and nitrogen compound concentrations, creating optimal conditions for salmonid rearing from the embryonic stage onward. To ensure the welfare of the fish, water quality is monitored continuously and microbiological tests are performed periodically. In addition, advanced broodstock management practices are applied: broodstock are captured from Lithuanian rivers using electrofishing, held in concrete tanks, and anaesthetics are used during gamete collection to minimize stress and ensure high-quality reproduction. These technological measures and environmental control methods ensure precise experimental conditions, allowing for an objective assessment of the effect of probiotics on the growth of Atlantic salmon at different stages of their development.

Fig. 1. Recirculating aquaculture systems (RAS) at the Eastern Region Pisciculture Division.

2.3 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 *Salmo salar*. Ingredients: Lactobacterias and yeast cultures, sugar cane molasses, natural minerals, sea salt, herbs extracts, chlorine-free water.

Dosage and incorporation into feed: 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.

2.4 Experimental design and procedure

Experiment I: On 12 November 2024, following sorting by body weight, the smallest fraction of Atlantic salmon (*Salmo salar*) juveniles (average weight 2.98 g) was placed into tanks (total volume of 2 m^3 and a water filling level of 50 %) of the recirculating aquaculture system (RAS). Two experimental groups were established for the study: a control group (hereafter referred to as "Control") and a probiotic-fed group (hereafter referred to as "Probiotic"). The Control group, comprising 500 individuals, was fed a standard commercial diet (Aller Aqua Futura Ex Gr, pellet size 0.9–1.6 mm). The Experimental (Probiotic) group, consisting of 1,000 individuals, received the same diet supplemented with the Smart Fishery probiotic preparation (Table 1). The probiotic was prepared by diluting it at a 1:50 ratio with water, at a dosage of 1.5 mL per kilogram of feed. The resulting solution was uniformly applied to the feed pellets immediately prior to feeding to ensure the viability and even distribution of the microorganisms.

The fish were administered a commercial, nutritionally balanced diet specifically formulated for the growth phase of salmonid juveniles. Individuals in each tank were fed once daily, with the feeding ration corresponding to approximately 2 % of the total body mass. All specimens were maintained under standardized RAS conditions. The mean water temperature was 11 °C (±1 °C), pH 7.8, and dissolved oxygen saturation was approximately 92 %. 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.

On 11 December 2024, the initial biometric assessment of the fish was performed. The mean body weight across all experimental tanks was 3.6 g. Due to technical issues, the data from this measurement were not preserved. Observations indicated a reduction in feeding activity in all

tanks; however, no mortality occurred during this period. A second measurement of body length and body weight was conducted on 1 January 2025. Data obtained from this assessment were subsequently used for further analyses, the results of which are presented in the Results section.

Table 1. Characteristics of the experimental groups of *Salmo salar* parr (0+ age) – Experiment I.

Group	Fish age	Description	Sample size (n)	Feed
Control	0+	Fed standard commercial feed without probiotics	500	Commercial*
Probiotic	0+	Fed commercial feed supplemented with probiotics	1000	Commercial + Probiotics

* Aller Aqua Futura Ex Gr, 0.9-1.6 mm.

Experiment II: On 28 October 2024, Atlantic salmon (*Salmo salar* L.) eggs were fertilized and transferred to an incubator. Between 13 and 16 March 2025, fry at the early developmental stage were transferred to RAS tanks, and feeding was initiated. In Tank 1, containing 5,500 individuals, the fish comprised the experimental group and were fed feed treated with the Smart Fishery probiotic. In Tank 2, containing 6,000 individuals, the fish comprised the control group and were fed standard commercial feed without probiotics (Table 2).

The probiotic preparation was formulated by diluting it at a 1:50 ratio with water, at a dose of 1.5 mL of product per kilogram of feed. The resultant solution was uniformly applied to the feed pellets immediately prior to feeding to preserve the viability of the microbial population. Fish were administered a commercial, nutritionally balanced diet specifically formulated for the growth phase of salmonid juveniles. Feeding began in March with Aller Aqua Infa Ex (0.2 mm) and continued until September–October using Aller Aqua Futura Ex Gr (0.9–1.6 mm). The fish in each tank were fed seven times daily, with the feed ration corresponding to approximately 2 % of the total biomass.

All fish were maintained under standardized recirculating aquaculture system (RAS) conditions: mean water temperature was 11 °C (±1 °C), pH 7.8, and dissolved oxygen saturation approximately 92 %. Water quality parameters were monitored daily to ensure environmental stability. The photoperiod was maintained on a 12 h light:12 h dark cycle to simulate natural diurnal rhythms and provide physiologically optimal conditions for growth.

Morphometric assessments, including body weight, total length, and dorsal fin condition, were conducted twice: on 23 September 2025 (first measurement – Measurement I) and on 15 October 2025 (second measurement – Measurement II), when the salmon were at the 0+ age stage.

Table 2. Characteristics of the experimental groups of *Salmo salar* at the early fry stage — Experiment II.

Group	Fish stage	Description	Sample size (n)	Feed
Control	Early fry	Fed standard commercial feed without probiotics	6000	Commercial
Probiotic	Early fry	Fed commercial feed supplemented with probiotics	5500	Commercial + Probiotics

2.5 Morphometric data collection

For each experimental individual, total body length (TL) and body weight (W) were recorded, and the condition factor (CF) was calculated. This index 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:

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

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 Assessment of dorsal fin damage

The degree of dorsal fin damage (necrosis) was assessed visually using a four-point scale, adapted from methods commonly applied to evaluate the morphological condition and welfare of salmonids (according to Person-Le Ruyet et al., 2007). During evaluation, each fish was positioned on a moist measuring board to minimize stress and ensure compliance with animal welfare standards.

The scoring system was defined as follows:

0 – Intact fin: fin is unaltered, without deformities or evidence of tissue necrosis.

1 – Mild damage: minor tissue necrosis or slight irregularities along the fin margins.

2 – Moderate damage: noticeable tissue loss and reduction in fin area.

3 – Severe damage: extensive tissue necrosis, marked deformity, or substantial loss of fin tissue.

2.7 Statistical analyses

Morphometric parameters, including total body length, body weight, and condition factor, were analyzed to assess differences between experimental 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 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 ± standard deviation (SD). Statistical significance was defined as $p < 0.05$. Analyses were performed using GraphPad Software, LLC (version 10.6.0, USA).

3. Results

The present section reports the findings of experimental investigations conducted under controlled recirculating aquaculture system conditions, aimed at assessing the impact of probiotic supplementation on the growth performance of *Salmo salar*. Growth parameters of fish from different experimental groups are summarized using descriptive statistics, including sample size (n), minimum and maximum values, mean values, and standard deviations (SD). These data facilitate a comparative evaluation of growth performance between control and probiotic-supplemented groups across both 0+ age juveniles and early fry stage individuals.

3.1 Experiment I: sample size

This subsection presents data characterizing the sample size of 0+ age *Salmo salar* juveniles (parr), 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 experimental group, allowing comparison of growth performance and body condition between control and probiotic-supplemented groups.

Table 4 provides the corresponding data after the removal of outliers identified using the ROUT method, enabling evaluation of their influence on morphometric parameters and statistical comparisons between experimental groups.

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

Group	Sample size (n)	L (cm) min–max	L (cm) mean ± SD	W (g) min–max	W (g) mean ± SD	CF mean ± SD
Control	493	5 – 12.1	8.67 ± 0.91	2.6 – 14.2	6.20 ± 1.85	0.94 ± 0.17
Probiotic	502	4.2 – 12.0	8.53 ± 1.03	2.9 – 12.1	6.42 ± 1.69	1.05 ± 0.33

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

Group/Parameter	Control			Probiotic		
	Sample size (n)	min–max	mean ± SD	Sample size (n)	min–max	mean ± SD
L (cm)	492	6.20 – 12.1	8.68 ± 0.90	499	5.5 – 12.0	8.56 ± 0.98
W (g)	480	2.6 – 11.40	6.03 ± 1.53	502	2.9 – 12.1	6.42 ± 1.69
CF	485	0.53 – 1.29	0.93 ± 0.12	482	0.55 – 1.48	1.00 ± 0.15

3.2 Experiment I: body length and weight

Initial analysis of total body length showed that *Salmo salar* (0+ age) in the control group were on average 1.6 % longer than those receiving the probiotic-supplemented diet (Figure 2A). The difference between groups was statistically significant according to the Mann–Whitney U test ($p = 0.044$).

After excluding outliers identified using the ROUT method (one fish from the control group and three fish from the probiotic group; Table 4), fish in the control group remained on average 1.4 % longer than those in the probiotic-fed group; however, this difference was no longer statistically significant (Figure 2B, $p = 0.06$).

These results suggest that the initially observed significant difference in body length was primarily influenced by atypical values, and their removal eliminated the statistical difference between the experimental groups.

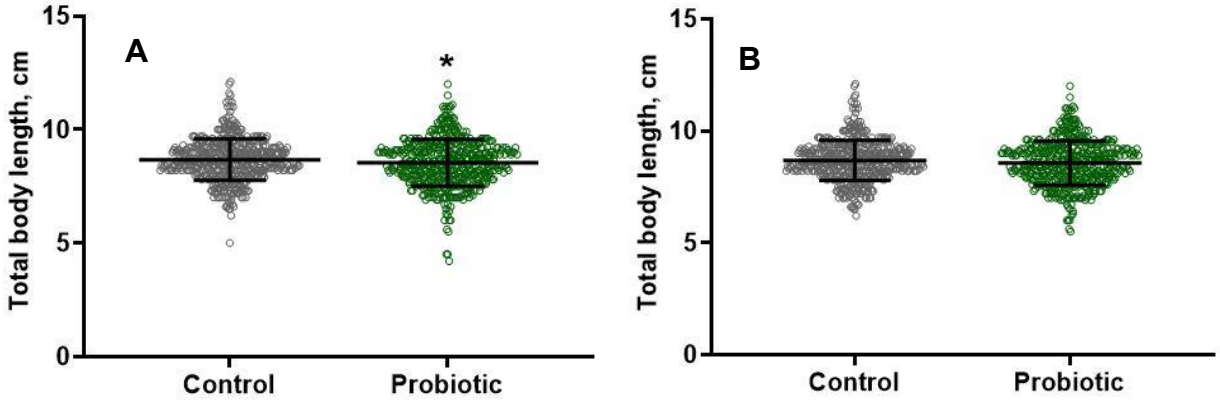

Fig. 2. Mean total body length (cm ± SD) of *Salmo salar* (0+ age) in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$). (A) Including all data; (B) after removal of outliers identified using the ROUT method.

Analysis of body weight in 0+ age *Salmo salar* demonstrated that individuals receiving the probiotic-supplemented diet exhibited a significantly greater mean body weight compared with control fish fed standard commercial feed. In the initial dataset, fish in the probiotic group were on average 3.5 % heavier than control fish (6.42 g vs. 6.20 g, respectively), with the difference reaching statistical significance (Mann–Whitney U test, $p = 0.005$; Figure 3A).

Following exclusion of outliers identified via the ROUT method (13 individuals removed from the control group; none removed from the probiotic group; Table 4), mean body weight values were slightly adjusted. After this correction, probiotic-fed fish remained on average 6.5 % heavier than controls, and the difference persisted as statistically significant ($p = 0.0004$; Figure 3B).

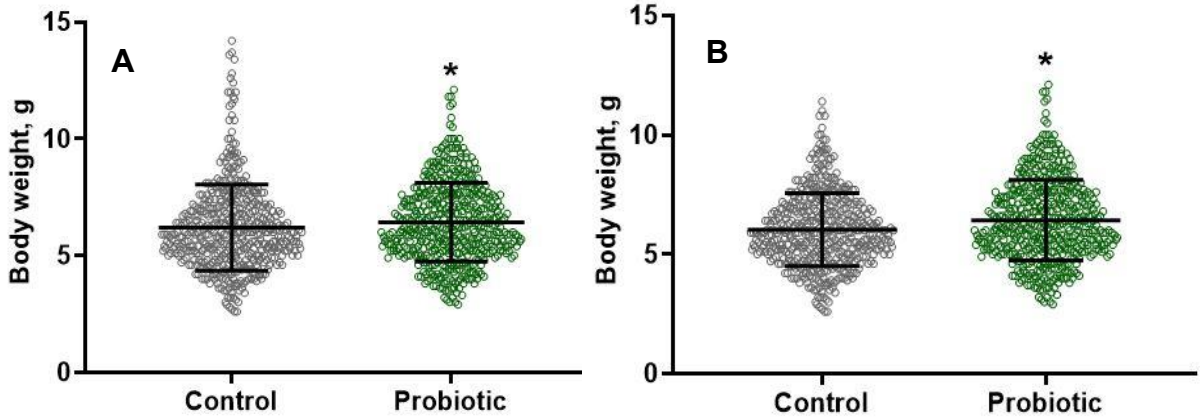

Fig. 3. Mean body weight (g ± SD) of *Salmo salar* (0+ age) in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$). (A) Including all data; (B) after removal of outliers identified using the ROUT method.

3.3 Experiment I: condition factor

Analysis of the condition factor (CF) in 0+ age *Salmo salar* revealed that fish fed the probiotic-supplemented diet exhibited a higher body condition compared with those receiving standard commercial feed. In the initial dataset, the mean CF in the probiotic group was 11.7 % greater than in the control group (1.05 vs. 0.94, respectively), and the difference was highly statistically significant ($p < 0.0001$; Figure 4A).

Following the exclusion of outliers identified via the ROUT method, the intergroup difference decreased to 7.5 %, but remained statistically significant (1.00 in the probiotic group vs. 0.93 in the control group; $p < 0.0001$; Figure 4B).

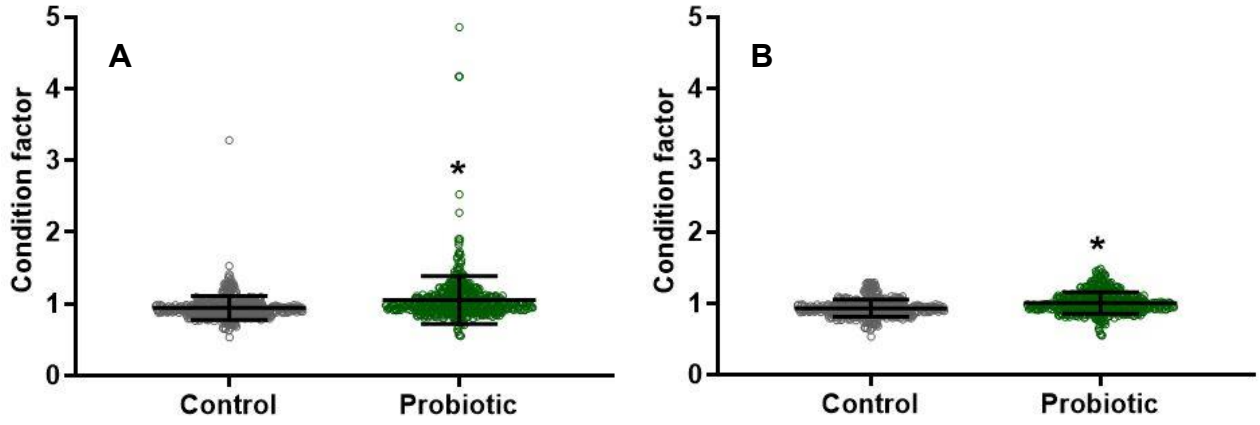

Fig. 4. Mean condition factor (CF ± SD) of 0+ age *Salmo salar* in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$). (A) Including all data; (B) after removal of outliers identified using the ROUT method.

3.4 Experiment II: sample size

This subsection presents data characterizing the experimental sample of *Salmo salar* fed a probiotic-supplemented diet from the early fry stage, including total body length (L), body weight (W), condition factor (CF), and dorsal fin damage. Table 5 summarizes the key descriptive statistics for each experimental 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 6 presents the dataset following the exclusion of outliers identified using the ROUT method, allowing assessment of their impact on morphometric parameters and statistical comparisons between groups. Outlier removal was applied exclusively to condition factor data at both the first and second measurement and to body length data at the second measurement in the probiotic group. No outliers were detected for the remaining parameters (Table 6).

Table 5. Sample size, total body length (L), body weight (W), and condition factor (CF) of *Salmo salar* in the experimental groups.

Group	Sample size (n)	L (cm) min–max	L (cm) mean ± SD	W (g) min–max	W (g) mean ± SD	CF mean ± SD
Measurement I						
Control	200	2.6 – 9.0	6.56 ± 1.0	1.0 – 6.7	2.99 ± 1.08	1.13 ± 0.94
Probiotic	200	4.2 – 8.5	6.57 ± 0.91	0.70 – 6.7	3.02 ± 1.16	1.06 ± 0.46
Measurement II						
Control	200	5 – 8.5	7.04 ± 0.67	1.6 – 7.0	3.65 ± 1.06	1.03 ± 0.21
Probiotic	200	3 – 8.5	7.01 ± 0.74	1.3 – 7.2	3.62 ± 1.03	1.06 ± 0.55

Table 6. Sample size, total body length (L), body weight (W), and condition factor (K) of *Salmo salar* in the experimental groups after removal of outliers identified using the ROUT method.

Group/Parameter	Control			Probiotic		
	Sample size (n)	min–max	mean ± SD	Sample size (n)	min–max	mean ± SD
Measurement I						
K	184	0.57 – 1.43	0.96 ± 0.14	190	0.56 – 1.64	0.98 ± 0.22
Measurement II						
L (cm)	-			199	5 – 8.5	7.03 ± 0.69
K	194	0.66 – 1.42	1.00 ± 0.13	192	0.73 – 1.34	1.01 ± 0.12

3.5 Experiment II: body length and weight

Analysis of total body length (L) in 0+ age *Salmo salar* fed a probiotic-supplemented diet from the early fry stage showed that the mean body length of the control group was 6.56 cm, while the probiotic group averaged 6.57 cm, corresponding to a 0.15 % increase in the probiotic group. Statistical analysis using the Mann–Whitney U test revealed no significant difference between the experimental groups ($p = 0.935$; Figure 5A). No outliers were detected using the ROUT method, so no data adjustments were required.

In the measurement II, the mean total body length of *Salmo salar* in the control group was 7.04 cm, while individuals in the probiotic-supplemented group averaged 7.01 cm, representing a 0.43 % higher value in the control group. Statistical comparison using the Mann–Whitney U test indicated no significant difference in body length between the groups ($p = 0.713$; Figure 5B). Outlier analysis performed using the ROUT method identified a single extreme value within the probiotic group; exclusion of this data point had a negligible effect on the statistical outcome ($p = 0.779$; Table 6).

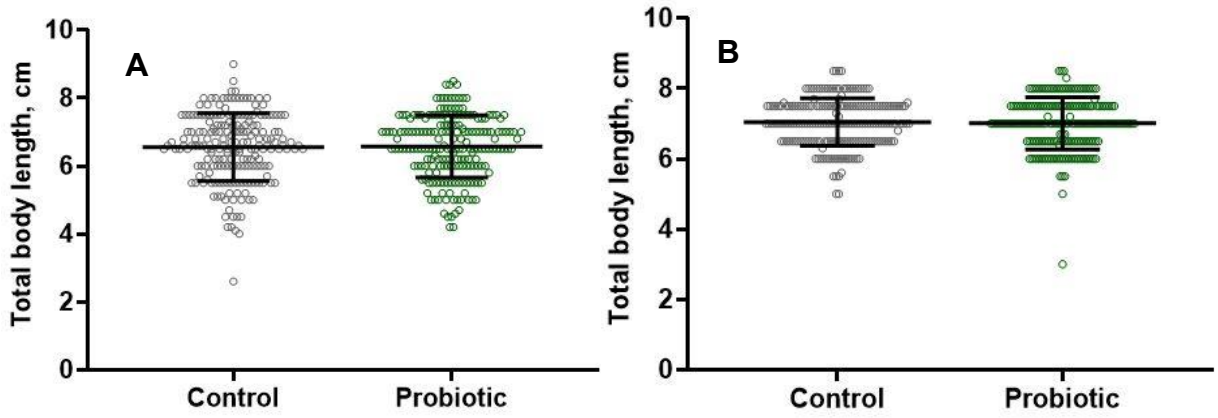

Fig. 5. Mean total body length (cm ± SD) of *Salmo salar* (0+ age) in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. A – Measurement I; B – Measurement II.

Analysis of body weight in *Salmo salar* (0+ age), reared from the early fry stage under probiotic feeding conditions, revealed that the mean body weight of fish in the control group was 2.99 g, while those in the probiotic-supplemented group averaged 3.02 g, representing an approximately 1.0 % higher weight in the probiotic group. Statistical analysis using the non-parametric Mann–Whitney U test showed no significant difference between groups ($p = 0.641$; Figure 6A). Outlier detection using the ROUT method identified a single extreme value in the probiotic group.

In the measurement II, the mean body weight of control group fish was 3.65 g, compared with 3.62 g in the probiotic-supplemented group, corresponding to a 0.82 % higher average weight in the control group. The Mann–Whitney U test indicated no statistically significant difference between groups ($p = 0.821$; Figure 6B). No outliers were detected by the ROUT method.

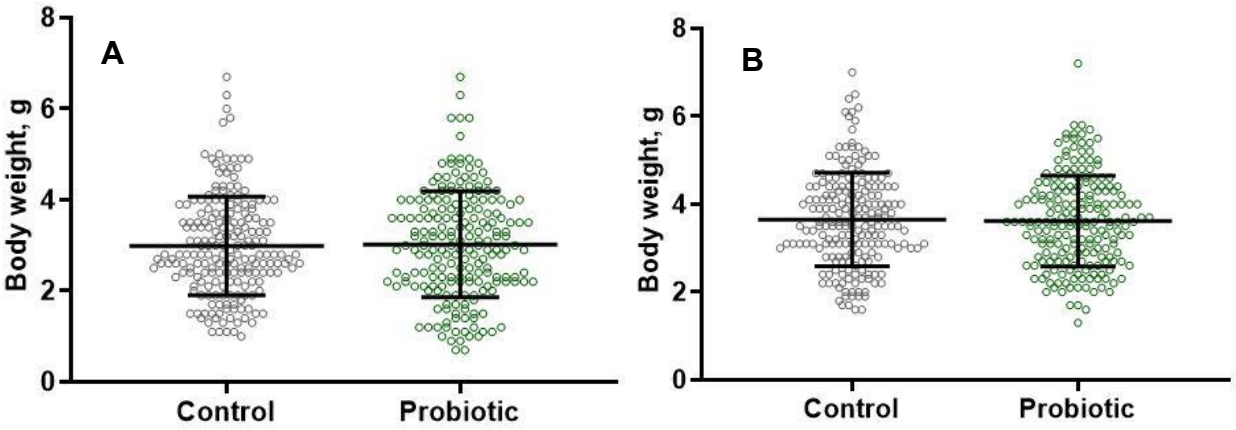

Fig. 6. Mean body weight (g) of *Salmo salar* (±SD) in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. A – Measurement I; B – Measurement II.

3.6 Experiment II: condition factor

Analysis of the condition factor (CF) in 0+ age *Salmo salar* fed the probiotic-supplemented diet from the early fry stage indicated that the mean CF was 1.13 in the control group and 1.06 in the probiotic group, corresponding to an approximate 6.2 % higher condition in the probiotic-fed fish. However, non-parametric Mann–Whitney U testing revealed no statistically significant difference between the groups ($p = 0.1365$; Figure 7A).

Following outlier removal using the ROUT method, 16 individuals were excluded from the control group and 10 from the probiotic group (Table 6). After this adjustment, the mean condition factor was 0.96 in the control group and 0.98 in the probiotic group, corresponding to approximately 2.1 % higher condition in the probiotic-fed fish. The intergroup difference remained statistically non-significant ($p = 0.266$; Figure 7B).

Analysis of the condition factor (K) data from the measurement II revealed that the mean condition factor of the control group fish was 1.03, while that of the group fed a probiotic-supplemented diet was 1.06. This indicates that fish in the probiotic group were approximately 2.9% in better condition than those in the control group. The nonparametric Mann–Whitney U test showed no statistically significant differences between the experimental groups ($p = 0.725$, Fig. 7C). Using the ROUT method, 6 individuals were excluded from the control group and 8 from the probiotic group (Table 6). After data correction, the mean condition factors were 1.00 for the control group and 1.01 for the probiotic group. The difference between groups remained statistically non-significant (Fig. 7D, $p = 0.843$).

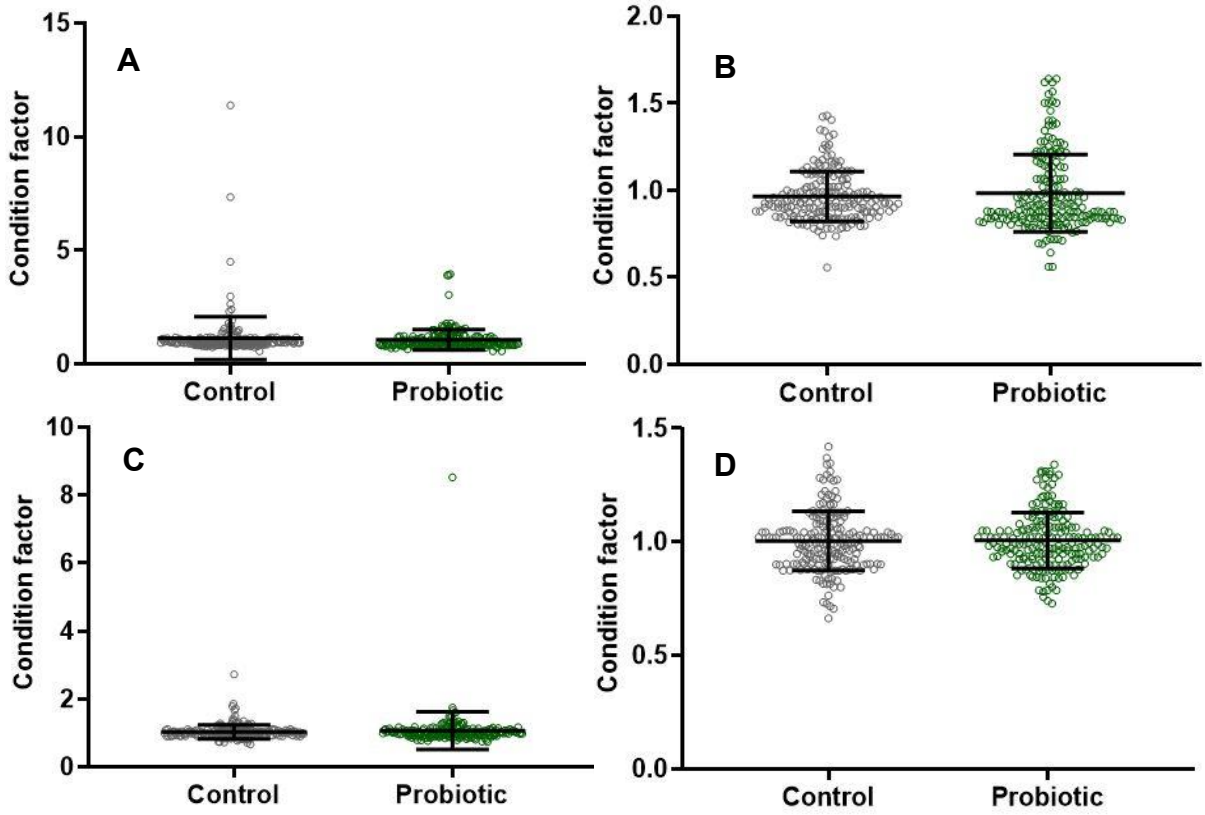

Fig. 7. Condition factor (±SD) of *Salmo salar* in the experimental groups: Control – fed standard commercial feed; Probiotic – fed commercial feed supplemented with Smart Fishery probiotics. Measurement I: A – including all data; B – after removal of outliers identified by the ROUT method. Measurement II: C – including all data; D – after removal of outliers identified by the ROUT method.

3.7 Experiment II: dorsal fin damage

Table 7. Distribution of dorsal fin damage (necrosis degree) in Atlantic salmon (*Salmo salar*) between experimental groups.

Measurement I			
Damage degree	**Description**	**Control (n, %)**	**Probiotic (n, %)**
0	Intact fin	42 (40.78 %)	21 (17.5 %)
1	Mild damage	36 (34.95 %)	44 (36.67 %)
2	Moderate damage	24 (23.30 %)	52 (43.33 %)
3	Severe damage	1 (0.97 %)	3 (2.5 %)
Total		**103 (100 %)**	**120 (100 %)**
Measurement II			
Damage degree	**Description**	**Control (n, %)**	**Probiotic (n, %)**
0	Intact fin	83 (41.5 %)	43 (21.5 %)
1	Mild damage	101 (50.5 %)	124 (62 %)
2	Moderate damage	16 (8 %)	33 (16.5 %)
3	Severe damage	0 (0 %)	0 (0 %)
Total		**200 (100 %)**	**200 (100 %)**

Table 7 presents the degrees of dorsal fin necrosis (ranging from 0 to 3) and their distribution between the control and probiotic treatment groups. In the control group, most fish had intact dorsal fins (degree 0), accounting for over 40% of individuals in both measurements. In the probiotic group, only 17.5% of fish had undamaged fins during measurement I and 21.5% during measurement II (Fig. 8). The probiotic group showed a higher proportion of mild damage (degree 1) and nearly twice the proportion of moderate damage (degree 2; 43.33% and 16.5%) compared to the control group (23.3% and 8%). Severe dorsal fin damage (degree 3) was rare in both groups, occurring in 0.97% of control fish and 2.5% of probiotic fish during measurement I. No severe damage was observed in either group during measurement II.

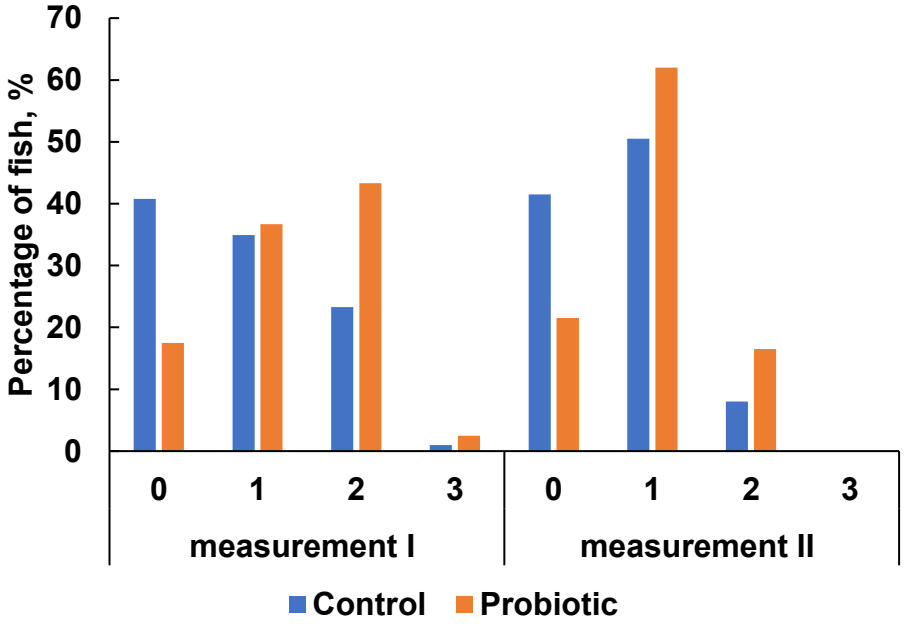

Fig. 8. Percentage distribution of dorsal fin damage in *Salmo salar* experimental groups.

3.8 Experiment II: mean specific growth rate

Table 8. Mean body weight and specific growth rate (SGR) of *Salmo salar* in the control and probiotic groups.

Group	Initial weight (g)*	Final weight (g)#	Time (days)	SGR (%/day)
Control	2.99	3.65	22	0.91
Probiotic	3.02	3.62	22	0.81

* - measurement I, # - measurement II.

Table 8 summarizes the mean body weights of *Salmo salar* from the control and probiotic groups at Measurement I and Measurement II, along with the calculated specific growth rate (SGR, %/day). The control group exhibited an average SGR of 0.91% per day, whereas the probiotic group showed an average SGR of 0.81% per day, indicating that the growth rate of the control fish was approximately 11% higher. These findings suggest that the probiotic supplementation did not significantly improve weight gain over the course of the experimental period.

4. Discussion

The application of probiotics in aquaculture of the genus Salmo can be highly beneficial, as it helps strengthen fish immune systems, improve nutrient utilization and growth, and reduce mortality risk. These effects are particularly critical given the increasing implementation of intensive aquaculture systems, including RAS. Fish reared at high stocking densities are continuously exposed to elevated pathogen pressure; therefore, enhanced immunity becomes a key factor in maintaining good health status (Dindial, 2021).

This study assessed the impact of Smart Fishery probiotics incorporated into *Salmo salar* feed at different growth stages on condition and morphological parameters. Data analysis indicated that the effects of probiotics were not consistent across experimental groups: in some instances, a positive influence on body weight and condition was observed, whereas other parameters, such as growth rate and incidence of fin lesions, did not show significant improvement compared to control fish groups. The following discussion presents the results of the two experiments, analysed separately.

Salmo salar parr (0+ age) fed commercial feed (Aller Aqua Futura Ex Gr, 0,9-1,6 mm) supplemented with Smart Fishery probiotics showed positive changes in growth-related parameters. Although the control group exhibited slightly greater mean length, this difference was not statistically significant. In contrast, body weight in the probiotic group was higher, and the condition factor was also significantly greater. These results suggest that probiotic supplementation may have enhanced feed utilization efficiency or metabolic performance.

Assessment of the effects of probiotics on *Salmo salar* fed from the early fry stage revealed no statistically significant differences between the experimental and control groups in body length, body weight, or condition factor ($p > 0.05$). Fish receiving probiotic-supplemented feed exhibited a slightly higher condition factor (up to 6.2%), although this increase was not statistically significant. Notably, the distribution of dorsal fin lesions differed between groups: the proportion of intact fins in the probiotic group was only 17.5–21.5%, compared to over 40% in the control group. While severe lesions were infrequent in both groups, the higher incidence of mild and moderate dorsal fin damage in the probiotic group may reflect environmental or behavioural stressors that were not mitigated by probiotic supplementation. In summary, long-term probiotic supplementation from the early developmental stages of *Salmo salar* did not result in statistically significant changes in

growth or condition indices. Nevertheless, certain trends were observed that may warrant further investigation, particularly regarding the effects of probiotics on tissue regeneration and morphological condition. It should be noted that Smart Fishery probiotics were administered in the RAS by spraying onto the feed. However, as the system operated as a shared recirculating setup, partial dispersion of probiotics into the water may have occurred, potentially resulting in low-level incidental exposure of the control group fish.

The application of *Lactobacillus* genus probiotics, particularly *L. delbrueckii* and *L. plantarum*, demonstrates significant potential in the aquaculture of *Salmo* species. In *S. salar*, *L. delbrueckii* lactis has been shown to adhere to the intestinal epithelium and protect tissues against the pathogenic effects of *Aeromonas salmonicida salmonicida*, thereby enhancing innate immune responses. In *S. trutta caspius*, *L. plantarum*-based synbiotics, when combined with prebiotics, have been reported to improve growth performance, protein utilization efficiency, and immune system parameters, while concurrently reducing stress-related biomarkers such as cortisol and glucose.

Although the application of *Lactobacillus* spp. in *Salmo* species remains relatively limited, current evidence suggests considerable potential for enhancing growth, health, and feed utilization efficiency, justifying further investigation. Moreover, the use of lactic acid bacteria in salmonid aquaculture has been reported to increase intestinal microbiota diversity and support microbiome ecosystem stability (Gupta et al., 2019).

Probiotic baths with *Aliivibrio fischeri* significantly reduced ulcer incidence and improved growth performance in *S. salar* (Klakegg et al., 2020). In addition, feeding rainbow trout (*Oncorhynchus mykiss*) with feed supplemented with *Lactobacillus plantarum* R2 strain enhanced growth parameters and stimulated immune responses in the gills and kidneys (Chomová et al., 2025). Atlantic salmon reared under freshwater conditions and fed *Lactobacillus curvatus*-supplemented diets for seven weeks exhibited a 4.2 % increase in final body weight, while fish fed *L. curvatus*-supplemented diets under saltwater conditions for 11 weeks showed a 4.7 % increase in body weight (Cathers et al., 2022). These findings are consistent with the present study, in which 0+ age *Salmo salar* fed probiotic-supplemented commercial feed were, on average, approximately 3.5 % heavier than control fish; after data correction, the mean body weight difference increased to 6.5 % and remained statistically significant. Furthermore, previous research demonstrated that Smart Fishery probiotics significantly enhanced the growth of *Silurus glanis* in RAS (Zibiene, 2023).

Previous studies have demonstrated that *Pediococcus acidilactici* probiotics exert multifaceted effects in fish, including the promotion of growth, enhancement of intestinal morphological and microbiological parameters, and stimulation of immune function (Zhao et al., 2024; Brown et al., 2024). However, Díaz et al. (2025) reported that supplementation with *P. acidilactici* did not significantly affect the body weight of *Salmo salar*, an outcome potentially attributable to the advanced reproductive stage of the fish, during which feed intake is generally reduced. Comparable results were observed in Nile tilapia (*Oreochromis niloticus*), where *P. acidilactici* supplementation had no measurable impact on growth performance but induced alterations in intestinal microbiota composition and enhanced nonspecific immune responses (Ferguson et al., 2010).

Importantly, *P. acidilactici* administration was shown to markedly improve reproductive performance and offspring viability in *S. salar* males. After 120 days of probiotic supplementation, treated fish exhibited increased gonad mass, gonadosomatic index (GSI), and sperm concentration, along with enhanced sperm fertilization capacity and higher embryo survival across all developmental stages assessed. The incidence of embryonic deformities and mortality was reduced in the probiotic-treated groups. Furthermore, a significant interaction between the duration of probiotic administration and embryo survival was observed, suggesting that prolonged supplementation may further enhance reproductive outcomes. These findings indicate that *P. acidilactici* probiotics can positively influence male reproductive parameters, offspring quality, and survival, highlighting their potential application in salmon breeding programs within aquaculture. Overall, these findings highlight that the effects of probiotic supplementation on fish growth, development, and reproductive performance are highly context-dependent, influenced by species, probiotic strain, and experimental conditions, and should be carefully considered in the design of aquaculture management strategies.

Studies have demonstrated the beneficial effects of probiotics on growth, immune function, and overall physiological performance in Salmo species. However, significant gaps remain in our understanding of their mechanisms of action. Further research is needed to identify the most effective probiotic species, strains, and combinations in Salmo species and to clarify the biochemical pathways through which they modulate host immune responses. Additionally, limited knowledge exists regarding why different probiotics produce contrasting physiological effects, such as variations in cortisol or immunoglobulin levels. Understanding these processes would improve our knowledge of the relationship between intestinal

microbiota and fish health and establish a basis for optimizing microbiota using biotechnological methods (Dindial, 2021).

In conclusion, the use of probiotics in Salmo aquaculture warrants further investigation. Current evidence suggests that these microorganisms have the potential to improve growth performance, strengthen immune function, and reduce mortality. This supports their application as a strategy for sustainable aquaculture development.

5. Conclusions

1. *Salmo salar* parr (0+ age) fed commercial feed supplemented with Smart Fishery probiotics did not differ significantly in body length from control fish. Although control fish were initially about 1.6 % longer, this difference was not statistically significant after data correction using the ROUT method.
2. *Salmo salar* parr (0+ age) receiving probiotic-supplemented feed exhibited a significant increase in body weight compared to control fish. After data correction, the mean weight difference reached 6.5 %, indicating that probiotic supplementation positively influenced growth.
3. *Salmo salar* parr (0+ age) that were fed probiotic-supplemented feed had a higher condition factor, which was 7.5% greater than that of the control group, reflecting improved overall condition in the treatment group.
4. *Salmo salar* (0+ age) fed probiotic-supplemented feed from the early fry stage did not differ significantly from control fish in total body length or body weight.
5. The condition factor of *S. salar* fed probiotic-supplemented feed from the early fry stage was higher (up to 6.2 %) than in control fish, but this difference was not statistically significant.
6. In *S. salar* fed probiotic-supplemented feed from the early fry stage, the proportion of intact dorsal fins ranged from 17.5 % to 21.5 %, compared to over 40 % in the control group. Mild (37–62 %) and moderate (16.5–43.3 %) fin lesions were more frequent in the probiotic-supplemented group, whereas severe lesions were rare in both groups and were not observed at the second measurement.
7. Analysis of specific growth rate (SGR) showed that control fish exhibited higher growth performance (0.91 %/day) than fish receiving probiotic-supplemented feed (0.81 %/day), corresponding to approximately 11 % greater growth in the control group under the experimental conditions.

6. References

1. Abid, A., Davies, S. J., Waines, P., Emery, M., Castex, M., Gioacchini, G., & Merrifield, D. L. 2013. Dietary synbiotic application modulates Atlantic salmon (Salmo salar) intestinal microbial communities and intestinal immunity. Fish & shellfish immunology, 35(6), 1948–1956.
2. Akbari Nargesi, E., Falahatkar, B., Sajjadi, M.M. 2020 Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (Oncorhynchus mykiss) broodstock. Aquacult. Nutr., 26, 98–108.
3. Brown, R., Zheng, R., Jacobi, S.K. 2024. PSV-21 Pediococcus acidilactici survives an in vitro simulated digestion. J. Anim. Sci., 102 (Suppl. 2), 316–317
4. Calcagnile, M., Tredici, S.M., Alifano P., 2024. A comprehensive review on probiotics and their use in aquaculture: Biological control, efficacy, and safety through the genomics and wet methods. Heliyon, 10 (24), e40892.
5. Cathers, H.S., Mane, S.P., Tawari, N.R. *et al.* 2022. In silico, in vitro and in vivo characterization of host-associated *Latilactobacillus curvatus* strains for potential probiotic applications in farmed Atlantic salmon (*Salmo salar*). *Sci Rep* **12**, 18417, https://doi.org/10.1038/s41598-022-23009-y.
6. Chandra, S., Joshi, N. 2024. Probiotics in aquaculture. In Handbook of Aquatic Microbiology, 1st ed.; CRC Press: Boca Raton, FL, USA, pp. 90–99.
7. Chomová, N., Ratvaj, M., Mudroňová, D. *et al.* 2025. Feed enriched by autochthonous probiotic bacteria and its effect on growth and immune response under different feeding regimes and immune response after *Aeromonas salmonicida* infection in rainbow trout (*Oncorhynchus mykiss*). *Aquacult Int* **33**, 267, https://doi.org/10.1007/s10499-025-01950-9.
8. Díaz, R., Carrasco, D., Quiñones, J., Martínez, A., Sepúlveda, G., Pérez-Núñez, I., Huaiquipán, R., Cancino-Baier, D., Beltrán, J. F., Farías, J. G., Paz, E. A., & Sepúlveda, N. 2025. The Probiotic *Pediococcus acidilactici* in the Feed of Salmonids: A Strategy to Improve Reproductive Parameters. *Animals*, *15*(11), 1659, https://doi.org/10.3390/ani15111659.
9. Dindial, A. (2022). Developments in Probiotic Use in the Aquaculture of Salmo Spp. IntechOpen, https://doi.org/10.5772/intechopen.99467.
10. Ferguson, R.M., Merrifield, D.L., Harper, G.M., Rawling, M.D., Mustafa, S., Picchietti, S., Balcàzar, J.L., Davies, S.J. 2010. The effect of Pediococcus acidilactici on the gut microbiota and immune status of on-growing red tilapia (Oreochromis niloticus). J. Appl. Microbiol., 109, 851–862
11. Giri, S.S., Yun, S., Jun, J.W., Kim, H.J., Kim, S.G., Kang, J.W., Kim, S.W., Han, S.J., Sukumaran, V., Park, S.C. 2018. Therapeutic effect of intestinal autochthonous Lactobacillus reuteri P16 against waterborne lead toxicity in Cyprinus carpio. Front. Immunol., 9, 1824.
12. Gupta, S., Fečkaninová, A., Lokesh, J., Koščová, J., Sørensen, M., Fernandes, J., Kiron, V. 2019. Lactobacillus dominate in the intestine of Atlantic salmon fed dietary probiotics. Front. Microbiol., 9, 3247
13. Jamal, M.T., Sumon, A.A., Pugazhendi, A., Al Harbi, M., Hussain, A., Haque, F. 2020. Use of probiotics in the aquaculture of commercially important fish species. Int. J. Probiotics Prebiotics, 15, 7–21.

14. Klakegg, Ø., Salonius, K., Nilsen, A., Fülberth, M., Sørum, H. 2020. Enhanced growth and decreased mortality in Atlantic salmon (Salmo salar) after probiotic bath. J. Appl. Microbiol., 129, 146–160.
15. Lazado, C. C., & Caipang, C. M. A. 2014. Mucosal immunity and probiotics in fish. Fish & shellfish immunology, 39(1), 78–89.
16. Márquez, L., Almansa, E., Ruiz Díaz, N. E., Díaz, M., Moyano, F.J. 2024. Relationships between the specific growth rate and the thermal-unit growth coefficient applied to cultured juvenile fish. Front. Mar. Sci., Sec. Marine Fisheries, Aquaculture and Living ResourceS, 1. https://doi.org/10.3389/fmars.2024.1332912
17. Mohammed, E. A. H., Ahmed, A. E. M., Kovács, B., & Pál, K. 2025. The Significance of Probiotics in Aquaculture: A Review of Research Trend and Latest Scientific Findings. *Antibiotics*, *14*(3), 242. https://doi.org/10.3390/antibiotics14030242
18. Mugwanya, M., Dawood, M.A.O., Kimera, F., Sewilam, H. 2022. Updating the role of probiotics, prebiotics, and synbiotics for tilapia aquaculture as leading candidates for food sustainability: A review. Probiotics Antimicro. Prot.,14, 130–157.
19. Nurhasanah A. *et al.,* 2023. Effect of Probiotics in Recirculating Aquaculture System (RAS) on the Concentration of Ammonia, Nitrite, and Nitrate in the Aquaculture of Catfish (*Clarias* sp.). *IOP Conf. Ser.: Earth Environ. Sci.* 1273. DOI 10.1088/1755-1315/1273/1/012018
20. Person-Le Ruyet, J., Le Bayon, N., Gros, S. 2007. How to assess fin damage in rainbow trout, Oncorhynchus mykiss? Aquatic Living Resources, 20, 191–195.
21. Talwar, C., Nagar, S., Lal, R., & Negi, R. K. 2018. Fish Gut Microbiome: Current Approaches and Future Perspectives. Indian Journal of Microbiology, 58(4), 397–414. https://doi.org/10.1007/s12088-018-0760-y
22. Zhao, M., Zhang, Y., Li, Y., Liu, K., Bao, K., Li, G. 2024. Impact of Pediococcus acidilactici GLP06 supplementation on gut microbes and metabolites in adult beagles: A comparative analysis. Front. Microbiol., 15, 1369402.
23. Zibiene G. 2023. Do Commercial Probiotics Influence The Fatty Acid Composition And Lipid Indices Of European Catfish (*Silurus Glanis* L.) Grown In a Recirculating Aquaculture System? HSOA Journal of Aquaculture and Fisheries. ISSN: 2576-5523.
24. de Oliveira, M.L., Costa, I.M., de Souza, M.R., Salotti Souza, B.M., de Melo Hoyos, D.C. 2025. Lactic acid bacteria viability and interactions in recirculating aquaculture systems for fish farming. Braz J Microbiol., 56(3):2225–2237, https://doi.org/10.1007/s42770-025-01748-0.