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SPPwelfare project Report

Efficiency of Probiotic Use in Different Rearing Systems (Recirculation and Flow-Through)

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1. Foreword

Aquaculture plays a vital role in ensuring sustainable food production in the face of growing global demand and environmental challenges. Within this context, fish welfare and the ecological efficiency of aquaculture systems are increasingly recognized as key pillars for responsible aquaculture practices. The use of probiotics represents one of the most promising tools to enhance both fish health and the stability of the environment, particularly under intensifying climate pressures and stricter environmental regulations.

This report, prepared as part of the SPPwelfare project (Deliverable No. D.1.2.2), presents comprehensive insights into the application and effects of probiotic supplementation in two distinct aquaculture systems—recirculating aquaculture systems (RAS) and traditional flow-through systems. It explores how targeted probiotic strategies influence fish growth, immune status, microbial community dynamics, and overall system performance.

Particular emphasis is placed on species of economic importance to the Baltic region, salmon and pikeperch. The research also considers the impact of rising water temperatures - an increasingly pressing concern in the context of climate change - on fish welfare and performance, and how probiotics may offer mitigation strategies.

We extend our sincere thanks to the researchers, aquaculture practitioners, and project partners whose collaborative efforts made this work possible. We hope this report will serve not only as a contribution to scientific knowledge but also as a practical reference for aquaculture professionals and policymakers working on sustainability and animal welfare in fish farming.

Contents

2. Introduction

1. Objectives and purpose of the project

In the Baltic Sea region, both in Latvia (LV) and Lithuania (LT), salmon and pikeperch are highly valued for their ecological, economic, and cultural importance. These species are among the most valuable for both recreational and commercial fisheries. In Latvia, the Institute of Food Safety, Animal Health and Environment (BIOR) is responsible for their reproduction and management, while in Lithuania this role is carried out by the Fisheries Service under the Ministry of Agriculture of the Republic of Lithuania.

Artificial reproduction of Baltic salmon resources is performed, because salmon is threatened due to historical overexploitation (overfishing), climate change, eutrophication and reduced access to spawning rivers (damming of rivers). Several salmon stocks of Baltic rivers are endangered or have even been lost therefore total salmon catches have been decreasing continuously since the 1990s.

Both Latvian and Lithuanian researchers are deeply concerned about the future of Baltic salmon resources, as the common goal is long-term maintenance of sustainable salmon populations. Addressing this pressing issue requires close cooperation between Latvia and Lithuania and the integration of our knowledge and values in fish farming management. Consequently, we have initiated this problem framing exercise for salmon rearing, aiming to produce healthier salmon smolts and thereby contribute to more sustainable salmon populations.

Pikeperch is also a highly valuable recreational and commercial fish specie whose resources are being actively replenished in inland waters. Being the top-predator, the presence of this fish in water bodies helps to control the abundance of low-value fish such as cyprinids. Pikeperch is a biologically flexible species adapted for the water bodies and climate changes of the Latvian and Lithuanian region.

The methods of artificial reproduction of pikeperch and rearing of fingerlings are complex and require adaptation to different conditions and rearing systems. Pikeperch is one of the most difficult fish to reproduce artificially, handling and compliance with welfare rules are essential aspects to ensure the survival and health of breeders and fingerlings. Due to its fast growth, high quality flesh and high economic expectation, pikeperch is one of the most promising freshwater fish species for the diversification of European inland aquaculture. However, its culture is still limited by impairment in growth rate and high mortality during rearing. At this moment, only several farms produce pikeperch in RAS systems in Europe, including Latvia and Lithuania.

Salmon and pikeperch broodstock fishery usually is carried out in natural waters, but final maturation of breeding fish is performed artificially, in the fish farms. For this reason, a persistent risk of disease is always a threat, as during juvenile rearing, many bacterial diseases can infect fish. Usually, antibiotics are used for disease treatment.

Antibiotics have traditionally played a significant role in managing bacterial infections in aquaculture, particularly in salmon hatcheries where high stocking densities and intensive production systems increase the risk of disease outbreaks. In these settings, antibiotics are often administered via medicated feed to control common pathogens such as *Aeromonas salmonicida*, and others. However, growing concerns over antimicrobial resistance and regulatory restrictions have led to the necessity to reduce their prophylactic use and putting a stronger emphasis on preventive measures, including improved husbandry, vaccination a.o.. In contrast, antibiotic use in

Pikeperch aquaculture remains relatively limited, largely due to the species' more recent domestication and smaller scale of commercial production. Nevertheless, when bacterial infections occur - typically caused by *Aeromonas spp*. or *Flavobacterium spp*. - antibiotics may be applied. This highlights the importance of alternative health management strategies, such as probiotics, especially in closed or recirculating aquaculture systems (RAS) where microbial balance is critical to maintaining fish health.

The future of salmon and pikeperch resources is a pressing concern for both Project partners, arising from the adverse effects of pollution in natural water bodies, disease outbreaks, growth of antimicrobial resistance, and unsustainable rearing practices. **Recognizing these challenges, the main aim of the project is to improve salmon and pikeperch rearing methods, resistance to diseases and biosecurity measures for more sustainable, resilient and healthy fish populations, thereby promoting ecosystems in the Program area.**

2. Relevance of the study: the role of probiotics in aquaculture

The word "probiotic" comes from the a Greek origin "pro bios" which means "for life" was first coined by Lilly and Stillwell (1965). The definition of a probiotic differs greatly depending on the source, but the first generally accepted definition was proposed by Fuller (1989) as "...a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance".

In aquaculture as probiotics are used different species of bacteria, yeasts, micro-algae and bacteriophages, gaining benefits such as improved growth, health immunomodulation and protection against diseases.

Aquaculture fish can be infected by a variety of bacterial diseases, leading to significant losses. To reduce mortality, maintain stock and fish health, antibiotics are usually applied. However, the repeated or improper use of antibiotics can lead to the development of antimicrobial resistance (AMR) in bacteria. This resistance not only reduces the effectiveness for future treatments but also poses a risk to environmental and public health. Resistant bacteria can persist in aquatic systems and potentially transfer resistance genes to other microbial communities. As a result, there is a growing need for sustainable disease management strategies in aquaculture, including improved biosecurity, vaccination, and the application of alternatives such as probiotics and phytochemicals to reduce antibiotic dependence.

In pursuit of this goal, the partners will carry out knowledge transfer in salmon and pikeperch artificial breeding and restocking, testing the effectiveness of probiotics for juvenile salmon and pikeperch. T**hrough concerted action and knowledge exchange, the project will contribute to the conservation and sustainable management of salmon and pikeperch resources in the cross-border region of Latvia and Lithuania**.

3. Overview of Recirculating Aquaculture Systems (RAS) and Flow-Through Systems (FTS)

Recirculating Aquaculture Systems (RAS) and Flow-Through Systems (FTS) are two fundamental approaches to fish farming, each with distinct operational models and environmental impacts. RAS are closed-loop systems where water is continuously filtered, purified and reused, allowing precise control of water quality, temperature, and biosecurity. This makes RAS ideal for land-based, intensive aquaculture near urban centers or areas with limited water resources. In contrast, FTS operates by continuously supplying natural water, typically from rivers or wells, through fish tanks before discharging it back into the environment. While simpler and more cost-effective, FTS is more dependent on natural water availability and poses greater risks for environmental discharge and disease spread. Both systems play essential roles in sustainable aquaculture.

In recent years, the need to improve salmon rearing, particularly by estimation of fish health indices and feed supplements has become increasingly urgent in Latvia. This urgency was driven by the observable effects of climate change, the rising of temperatures in FTS. Warmer water exacerbates stress and disease susceptibility in salmon, complicating rearing and reducing the outcome.

2. Literature Review

4. Definition of probiotics and their mechanisms of action

Probiotics are viable microorganisms that enhance the health of aquatic organisms after consumption, promoting immune function and disease resistance. They are non-pathogenic microbes that exert health benefits to the host when administered in adequate quantity (Ringø et al., 2018; Newaj-Fyzul & Austin, 2015). The mechanisms by which probiotics exert their action are competitive exclusion of pathogens from adhesion sites, improvement of the intestinal mucosal barrier, gut immunomodulation, and neurotransmitter synthesis. There are several mechanisms of probiotics action: (1) probiotics perform their function by competing with pathogens for nutrients and receptors for binding thereby making their survival and adherence to gut mucosa difficult; (2) probiotics produce anti-microbial substances which inhibit pathogens growth; (3) probiotics promote epithelial barrier function by enhancing mucus production and increasing the expression of tight junction proteins which prevents the translocation of pathogens from intestine into the blood; (4) probiotics regulate immunity of the host by modulating maturation and function of dendritic cells subsequently increasing the activity of T cells which play important role in immune homeostasis; (5) Probiotics also regulate the production of neurotransmitters including serotonin, dopamine and gamma aminobutyric acid (GABA) (Latif et al., 2023). In aquaculture probiotics have been shown to inhibit the growth of harmful pathogens, improve disease resistance, improve nutrient digestibility, and increase stress tolerance (Calcagnile et al, 2024).

Probiotics have an impact on microbial biofilm formation in RAS, as closed-loop systems rely on microbial biofilms providing nutrient cycling, water purification, and system stability. The biofilm microbiota in RAS is highly dynamic and influenced by numerous factors, including water chemistry, feed composition, system design, and the microbial load introduced by fish (Martins et al., 2010). Beneficial microbes in the biofilm, such as nitrifying bacteria (e.g., Nitrosomonas, Nitrobacter), are essential for ammonia and nitrite conversion (van Rijn, 2013). However, opportunistic and pathogenic bacteria can also establish themselves in biofilms, potentially compromising fish health (Vadstein et al., 2018). Probiotic administration in RAS, typically via feed or water supplementation, can significantly influence the microbial composition and functionality of biofilms. Must tested probiotic strains are *Bacillus spp*., *Lactobacillus spp*., and *Pseudomonas spp*. (Ganguly et al., 2013; Vadstein et al., 2018; Verschuere et al., 2000; Moriarty, 1999).

By influencing biofilm composition, probiotics help stabilize water parameters such as ammonia, nitrite, and nitrate levels (Liu et al., 2018). Improved microbial balance in biofilms also correlates with lower disease incidence, reduced reliance on antibiotics, and enhanced immune responses in fish (Kesarcodi-Watson et al., 2008; Dimitroglou et al., 2011). The overall result is better growth performance, feed conversion ratios, and welfare indicators in RAS-reared species (Zhou et al., 2009).

While the benefits are promising, probiotic efficiency in modifying biofilms depends on strain specificity, dosage, application frequency, and compatibility with existing microbial populations (Hai, 2015). Additionally, excessive or unregulated use may disrupt microbial equilibrium or lead to unwanted shifts in community structure (Balcázar et al., 2006).

5. Previous research on probiotic use in fish farming

Research on probiotic use in fish farming has demonstrated promising effects on both fish health and aquaculture system performance. A notable example is the study conducted under the Baltic Blue Biotechnology Alliance project by Klaipeda University, where two new probiotic-based products—one as a water treatment and the other as a feed additive—were tested on predatory pikeperch (*Sander lucioperca*) and omnivorous carp (*Cyprinus carpio*) in both recirculating aquaculture systems (RAS) and pond systems. The experiments revealed that the application of probiotics, particularly through water treatment, significantly improved fish growth rates and intestinal morphology. In carp, for example, the length of intestinal villi increased, indicating enhanced nutrient absorption efficiency. Although the water purification capacity of probiotics in RAS did not show conclusive improvements, probiotic bacteria were successfully identified in the intestines of treated fish, confirming colonization. In pond-based trials, probiotic application led to better water quality parameters (e.g., lower levels of NH_4^+, NO_2^-, and PO_4^{3-}) and up to a 115% increase in fish biomass production compared to the control group. These findings support the growing body of evidence that probiotics can play a key role in enhancing aquaculture productivity and sustainability by improving fish health, digestion, and overall system stability (Nika et al., 2020).

Recent research in salmon aquaculture further reinforces these findings, highlighting the relevance of probiotics in intensive farming environments, especially under the growing pressure of climate change. Studies on Atlantic salmon (*Salmo salar*) have shown that dietary supplementation with specific probiotic strains, such as *Lactobacillus plantarum*, *Bacillus subtilis*, and *Pediococcus acidilactici*, can significantly enhance gut microbiota balance, improve feed conversion ratios (FCR), and stimulate non-specific immune responses (Ringø et al., 2018; Merrifield et al., 2010). In smolt production, probiotics have been linked to reduced mortality during seawater transfer and increased resistance to bacterial pathogens such as *Aeromonas salmonicida* and *Vibrio anguillarum*. Moreover, in RAS systems probiotics have been investigated not only for growth and immune benefits but also for their potential interactions with biofilter microbial communities and system-level nitrogen cycling (Menanteau-Ledouble et al., 2020; Bugten et al., 2022).

In flow-through (FT) hatcheries of the Baltic region, including Latvia, rising water temperatures linked to climate change are creating increasing challenges for salmon rearing. While probiotics have been studied more extensively in recirculating aquaculture systems, their application in FT hatcheries remains only sparsely investigated. Nevertheless, existing evidence suggests that probiotics may help mitigate thermal stress by supporting intestinal barrier integrity, enhancing antioxidant responses, and reducing inflammation.

Taken together, current research across species and systems underlines the importance of continued probiotic development and adaptation to specific aquaculture environments. This is particularly important for countries like Latvia and Lithuania, where both flow-through and RAS systems are used, and where rising temperatures demand innovative solutions for FTS to maintain fish health and system efficiency.

6. Effects on fish health, growth, and immunity

The application of probiotics in aquaculture has demonstrated a wide range of beneficial effects on fish health, growth performance, and immune system function. These effects are especially significant in intensive rearing systems such as Recirculating Aquaculture Systems (RAS) as well as for flow-through hatcheries, where fish are often subjected to various environmental and physiological stressors (Fuller, 1989; Hai, 2015).

Improved Growth and Feed Utilization

Probiotic supplementation, whether administered via feed or directly into the water, has been associated with enhanced growth rates and improved feed conversion ratios (FCR) in multiple fish species. These effects are primarily due to improved digestion and nutrient absorption, enhanced enzymatic activity, and stabilization of gut microflora (Merrifield et al., 2010). In carp juveniles, for instance, water-based probiotics resulted in significantly longer intestinal villi, increasing the surface area for nutrient uptake (Nika et al., 2020).

Nika et al. (2020) conducted trials using probiotics produced by Baltic Probiotics, which showed that both pikeperch and carp juveniles exhibited significantly improved growth in water-treated groups compared to controls. This suggests that environmental microbial modulation can be more effective than feed-only applications during early life stages.

Enhanced Fish Health and Disease Resistance

Probiotics support fish health by maintaining gut microbial balance and competing with pathogenic bacteria through mechanisms such as competitive exclusion, production of antimicrobial compounds (e.g., organic acids and bacteriocins), and improved mucosal barrier integrity (Ringø et al., 2018). Studies have shown reduced disease outbreaks in probiotic-treated fish exposed to common aquaculture pathogens such as *Aeromonas hydrophila* and *Vibrio anguillarum* (Balcázar et al., 2006).

In experimental trials, fish from probiotic groups also exhibited fewer signs of inflammation and gut disruption, which translates into reduced energy loss from immune overactivation and better resilience under intensive culture conditions (Zivkovic, 1999).

Immune System Modulation

In addition to local gastrointestinal effects, probiotics have been shown to modulate systemic immunity. They enhance the activity of innate immune responses by increasing macrophage activity, phagocytic efficiency, and the production of immune-related enzymes like lysozyme (Wang et al., 2008). Some probiotics also influence cytokine profiles, balancing pro- and anti-inflammatory responses (Lazado & Caipang, 2014).

In salmonid farming, species such as *Pediococcus acidilactici* and *Lactobacillus plantarum* have demonstrated significant effects on mucosal immunity and disease resistance, especially during stress events like smoltification or seawater transfer (Merrifield et al., 2010; Ringø et al., 2018).

Stress Tolerance and Climate Adaptation

With the ongoing rise in water temperatures due to climate change, especially in flow-through systems (FTS), probiotic use is gaining importance as a strategy to support thermal stress adaptation. Studies have shown that probiotic-treated fish often exhibit lower cortisol levels, improved antioxidant capacity (e.g., SOD, CAT enzymes), and stronger osmoregulatory performance (Hai, 2015; Hoseinifar et al., 2018).

This is particularly relevant in Latvian hatcheries, where rearing salmon under elevated flow-through water temperatures has become increasingly challenging. In such settings, probiotics can help maintain fish robustness and survival during critical early life stages.

7. Limitations for Probiotic Use in Aquaculture

While probiotics are widely recognized for their benefits promoting fish health and improving water quality, literature data also highlights considerations regarding their misuse or overapplication, as the effectiveness and safety of probiotics in aquaculture are not universally guaranteed and depend on several key variables including strain specificity, appropriate dosage, application frequency, and the compatibility of probiotic strains with already existing microbial communities (Hai, 2015).

One critical concern is the potential for ecological disruption within microbial biofilms and the surrounding aquatic environment. Probiotics can influence the composition and balance of microbial communities not only by enhancing beneficial bacteria, but also by outcompeting or suppressing native microbes that play important roles in nutrient cycling or pathogen regulation. In some cases, an unintended dominance of introduced strains can result in microbial imbalance, reducing the overall diversity and resilience of the system (Balcázar et al., 2006; Merrifield et al., 2010).

Moreover, certain probiotic applications have been associated with transient increases in opportunistic pathogens or shifts toward undesired metabolic profiles, especially when probiotic strains are poorly adapted to the host species or environmental conditions. For instance, probiotics introduced in incompatible salinity or temperature regimes may fail to colonize effectively, leading to unpredictable microbial succession or even pathogen overgrowth due to destabilization of the microbial community (Tinh et al., 2008).

Another important aspect is strain-specific effects. Not all probiotic species or even strains within a species exhibit the same mode of action or safety profile. Some *Bacillus* strains, for example, have been shown to produce antimicrobial compounds that can suppress pathogens but also impact beneficial nitrifying bacteria if not properly selected or dosed (Gatesoupe, 2007). This reinforces the importance of targeted strain validation, especially in recirculating aquaculture systems (RAS) where microbial communities are critical for water purification and nutrient cycling.

3. Materials and Methods

1) Experiment design

Assessment of probiotic efficiency on health and growth performance of Baltic salmon **fry,** FTS

For experiment were randomly chosen Baltic salmon (*Salmo salar*) fry with an average weight 0,15 g. Experiment started in 1.05.2025. Fish were distributed evenly across 10 tanks (1.8 m^3 each), resulting in a stocking density of 10,000 individuals per each tank. The fish were reared in a flow-through system. The experiment lasted 31 days. Start date: 01.05. end date - 31.05.2025. The tanks were assigned into 10 treatment groups, each in several replicates:

Control group (Control) – fish fed with an age-appropriate basic commercial feed Aller aqua.

Probiotic feeding group (ProbFeed) – fish fed daily with basic feed, supplemented with Baltic Probiotics additive, counting 1 ml kg^{-1} of Smart Fishery probiotics (Baltic Probiotics Ltd., Latvia).

Probiotic bathing group (ProbBath) – fish fed with basic feed and additionally subjected to probiotic bathing (15 ml m^{-3}) for 15 minutes once per week (two treatments in total). Bathing of fish was performed on Day 5, 12, 19 and 26.

The experiment concluded on May 31. Monitoring of fry development continued throughout the entire project implementation time.

Assessment of probiotic efficiency on health and growth performance of Baltic salmon **presmolts**, FTS (September 2024)

For the experiment were randomly chosen 9,000 Baltic salmon (*Salmo salar*) presmolts with an average weight 34.39 ± 6.07 g. Fish were distributed evenly across six tanks (1.8 m^3 each), resulting in a stocking density of 1,500 individuals per tank. The fish were reared in a flow-through aquaculture system. The experiment lasted 14 days. The tanks were randomly assigned into three treatment groups, each in two replicates:

Control group (Control) – fish fed with an age-appropriate basic commercial feed Aller aqua.

Probiotic feeding group (ProbFeed) – fish fed daily with basic feed, supplemented with Baltic Probiotics additive, counting 1 ml kg^{-1} of Smart Fishery probiotics (Baltic Probiotics Ltd., Latvia).

Probiotic bathing group (ProbBath) – fish fed with basic feed and additionally subjected to probiotic bathing (15 ml m^{-3}) for 15 minutes once per week (two treatments in total).

Fish were fed daily according to standard salmonid feeding protocols. For the ProbFeed group, probiotics were mixed with feed prior to feeding, whereas for the ProbBath group, probiotic bathing was conducted in restricted tanks under controlled conditions.

All basins were controlled daily checking for fish with inadequate behavior. **Mortality was not detected in no one of experiment group during all 14 day trial.**

At the start and conclusion of the experiment, 50 salmon presmolts with age 1+ were randomly sampled from each tank to measure body weight and total length. The following growth parameters were calculated:

Weight Gain (WG): final weight − initial weight

Fulton's Condition Factor (K): $(W / L^3) \times 100$, where W is body weight (g) and L is body length (cm)

Assessment of probiotic efficiency on the skin-mucus microbiota, health and growth performance of Baltic salmon **parr** reared in FTS (August 2025)

A two-week trial was conducted with 30,000 Baltic salmon presmolts (2,37 ± 0,33 g mean weight; 2,53± 0,42 cm length) reared in FTS, in six 1.8 m^3 tanks (n = 5000 per tank). Fish were allocated to three experimental groups in duplicate: (Control) control, fed with basic commercial feed Aller aqua; (ProbFeed) probiotic feeding, basic diet supplemented with 5 ml kg^{-1} Smart Fishery probiotics (Baltic Probiotics Ltd., Latvia); and (ProbBath) probiotic bathing, basic diet with daily baths in 15 ml m^{3-1} probiotics for 15 min. Fish were fed daily according to standard salmonid feeding norms.

Growth performance was evaluated by recording the length and weight of 50 randomly selected fish per tank at the start and end of the trial.

Assessment of probiotic efficiency on health and growth performance of Baltic salmon **parr and presmolts,** RAS

For experiment were randomly chosen Baltic salmon 7000 (*Salmo salar*) parr with an average weight 3,6 g. The probiotic test experiment started on 06.12.2024. Fish were reared in RAS system until their release in natural waters in May 2025. For ProbFeed were selected 3060 parr, but for Control 3040 parr and placed in separate tanks. Fish distributed evenly across tanks (15 m^3 and 7 m^3 tanks, subsequently). The average rearing temperature during the trial was 11 °C, with water pH at 7.8 and dissolved oxygen levels averaging 92%. Daily measurements of water quality parameters and fish performance were conducted throughout the experimental period. The experiment lasted six months, until smolt release in natural waters. Subsequent treatments were used:

Control – fish fed with an age-appropriate basic commercial feed.

ProbFeed group – fish fed daily with basic feed, supplemented with Baltic Probiotics additive, counting 1 ml kg^{-1} of Smart Fishery probiotics (Baltic Probiotics Ltd., Latvia).

For probiotic treatment Smart Fishery probiotics (Baltic Probiotics Ltd., Latvia) was diluted at a ratio of 1:50 with water. The prepared solution was applied to fish feed at a dosage of 1.5 ml per 1 kg of feed.

8. Bacterial Isolation and Identification

Skin and gill mucus samples were collected from ten to twenty fish per tank at day 0 and day 14 using sterile cotton swabs. Samples were immediately placed in semisolid Amies transport medium and transported to the Institute of Food Safety, Animal Health and Environment "BIOR" (Riga, Latvia) for analysis.

9. Pathogen isolation

Samples were cultivated on tryptone soya agar, blood agar, and MacConkey agar, and incubated at 22 °C and 37 °C for 24–48 hours. Colonies were sub-cultured to obtain pure strains, which were identified based on colony morphology, growth temperature, and phenotypic traits including catalase, oxidase, and indole activity. For precise species identification, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was used. All procedures for microbial cultivation, identification, and phenotypic characterization followed standardized methods, including those recommended by the Clinical and Laboratory Standards Institute (CLSI) and other recognized microbiological protocols.

10. Antimicrobial Susceptibility Testing

One representative bacterial isolate from each culture was selected for susceptibility testing against four commonly used antibiotics: doxycycline, enrofloxacin, florfenicol, and oxytetracycline. The disk diffusion method was conducted in accordance with CLSI guidelines (CLSI, 2014). Bacterial suspensions were spread on Mueller-Hinton agar plates, and antibiotic-impregnated disks were applied. Following incubation (at 22°C for *Aeromonas salmonicida* and 37°C for other bacteria), and inhibition zones were measured. Isolates were classified as susceptible or resistant according to CLSI criteria.

11. Probiotics additives

The probiotic used in this study was SMART FISHERY provided by Ltd. Baltic Probiotics (Lithuania). SMART FISHERY probiotics are produced through a controlled natural fermentation process and do not contain chemically synthesized components or genetically modified organisms (Non-GMO). Probiotics consists of a synergistic blend of active ingredients: Lactic acid bacteria (*Lactobacillus* spp.), yeast cultures, fermented plant and herb extracts, phyto-ferments, sugar cane molasses, natural minerals and sea salt, chlorine-free water.

12. The Multiple Antibiotic Resistance (MAR) Index

MAR = a / b, where a is the number of antibiotics to which the isolate was resistant, and b is the total number of antibiotics tested. A MAR index above 0.2 was considered indicative of a high-risk contamination source.

4. Results

13. Assessment of probiotic efficiency on health and growth performance of Baltic salmon **fry,** FTS

The evaluation of probiotic effects on Baltic salmon fry performance was conducted from 1 to 31 May 2025, after which the experimental groups continued to be monitored within the framework of the project. Water temperature fluctuated from 12.23±2.65°C up to 16.33±1.60°C (Fig.1). Saturation of oxygen in FTS was optimal for salmon all the experiment time.

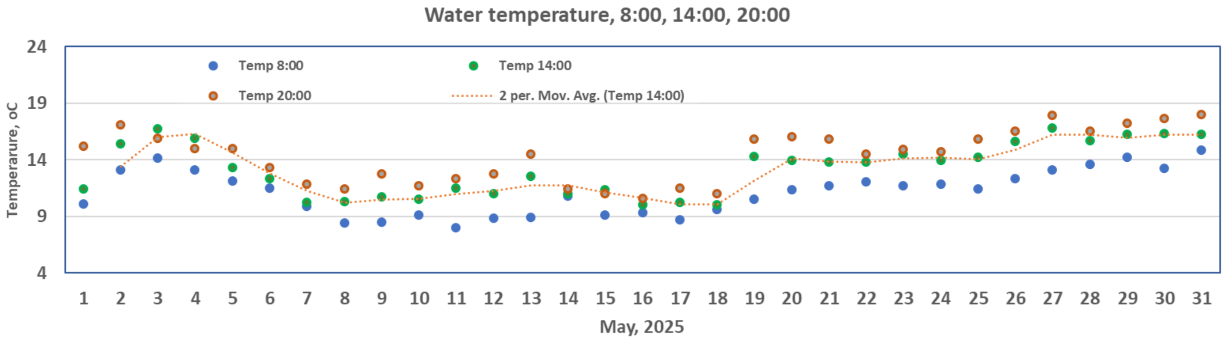

Fig. 1. Temperature increment during 31 experiment days, from 1st till 31st of May, measured daily at 8:00, 14:00 and 20:00.

Control Group: Fish survival remained consistently moderate throughout the month. Peak mortality values occurred on May 5 (21 fry) and May 9 (18 fry), with smaller but steady daily losses ranging from 2 to 18 fry/day. **No strong decreasing trend was evident, suggesting persistent baseline mortality under standard conditions**.

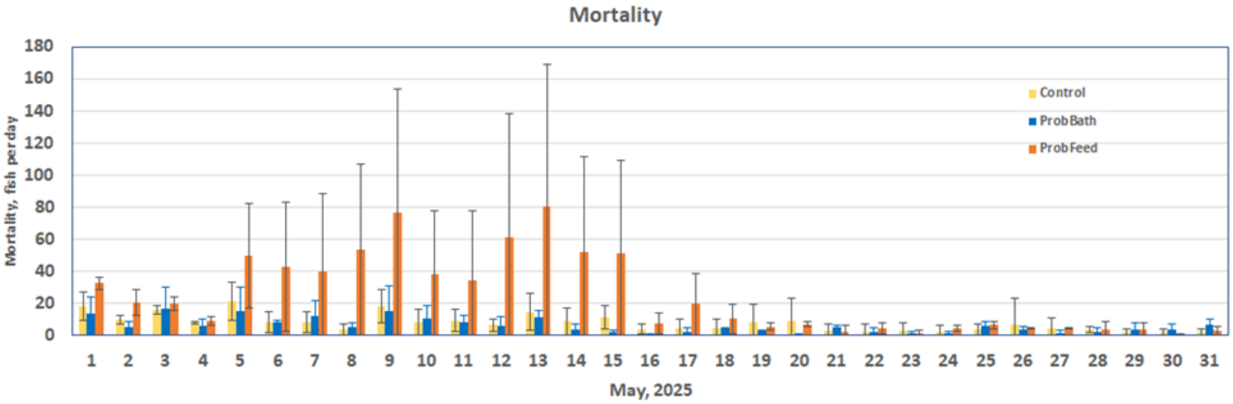

Fig.2. Baltic salmon fry loss during initial rearing period from 1st till 31st of May.

ProbBath group: Fry mortality was consistently lower than the Control group. In early May some higher mortality values occurred (e.g., 15 on May 5, 15 on May 9), but daily losses in all groups significantly decreased after mid-May, with some days reporting near-zero mortality (e.g., 0.5 on May 16 and 20). **Overall, ProbBath treatment demonstrated moderate effectiveness in reducing mortality, especially in the second half of May.**

ProbFeed group: Highest mortality observed among all groups in the first half of May, with peaks on: May 5 (50 fry), May 12 (61 fry), May 13 (80 fry). **Mortality dropped sharply after mid-May, stabilizing at low levels, between 0.5 and 7.0 fry per day**.

A Kruskal–Wallis test confirmed that the differences between groups were statistically significant ($p < 0.001$). Post-hoc pairwise comparisons (Dunn's test with Bonferroni correction) revealed that mortality in the ProbFeed group was significantly higher than in both the Control ($p < 0.001$) and ProbBath ($p < 0.001$) groups. Additionally, the ProbBath group had a modest but statistically significant reduction in mortality compared to the Control group ($p = 0.02$).

All groups started with the same average weight (0.15 g), and similar initial growth conditions. After 31 days: **Control group** had the highest increment in weight (0.86±0,08 g), reflecting most efficient feed conversion in the presence of natural microflora, and in absence of probiotic additives. **ProbBath** group showed lower growth increment (0.76 ± 0,04 g) in comparison with Control, whereas the **ProbFeed** group achieved the lowest weight increment (0.64±0,04 g), indicating the most inefficient feed utilization.

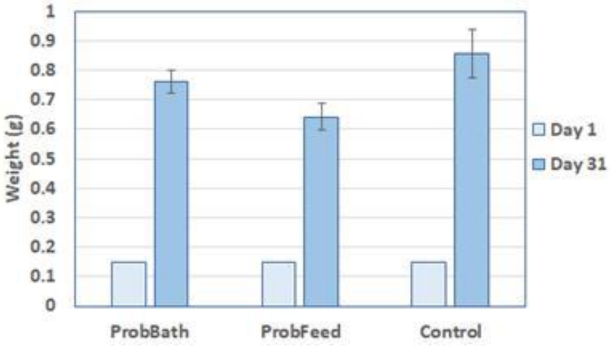

Fig. 3. Salmon fry average weight increment during 31 day trial, May

A statistical comparison of final weights between groups revealed significant differences (Kruskal–Wallis, $p \approx 0.03$). The Control group had the highest average increment in weight (0.86 g), followed by the ProbBath group (0.765 g), and the lowest in the ProbFeed group (0.64 g). Pairwise comparisons indicated that the Control group had significantly higher weight gain compared to the ProbFeed group, while differences between Control and ProbBath were not statistically significant. These results suggest better growth performance in the Control and ProbBath groups during May.

After the experiment concluded, monitoring of salmon fry continued for all experimental groups.

After the experimental phase, the rearing of salmon fry in the ProbFeed, ProbBath, and Control groups continued under rising temperature conditions. In June, water temperatures ranged from 13.6°C to 19.3°C (mean 16.3°C), while in July, temperatures increased further from 14°C to 22°C (mean 18°C), approaching the upper thermal tolerance threshold for the fry.

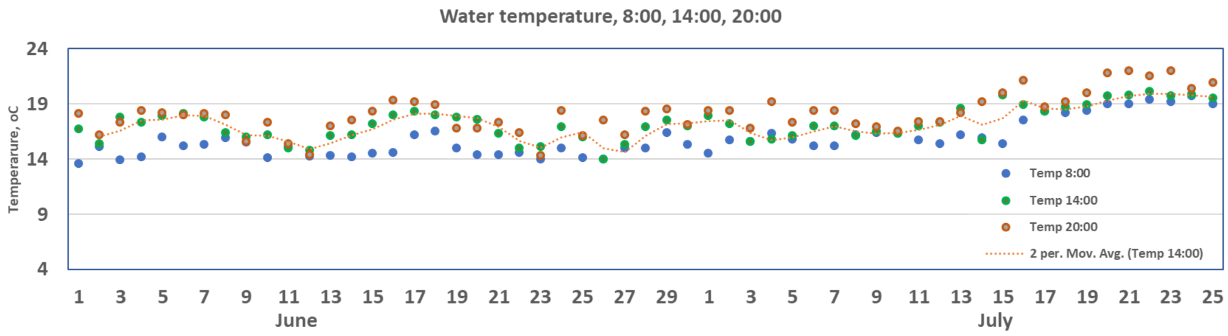

Fig.4. Temperature fluctuation in FTC rearing system, July 2025.

In June, fry in all groups (**ProbFeed**, **ProbBath**, and **Control**) showed stable performance, with low mortality and normal feeding behavior. Average growth rates were similar across the groups, and water temperature remained moderate. In July, as water temperatures increased, signs of thermal stress began to emerge, particularly in the Control groups. Salmon fry in the ProbFeed and ProbBath groups demonstrated greater resilience, with lower incidence of stress-related symptoms and more stable feeding activity. Mortality remained lowest in the ProbFeed group, suggesting a potential protective effect of the probiotic treatment under elevated temperature conditions.

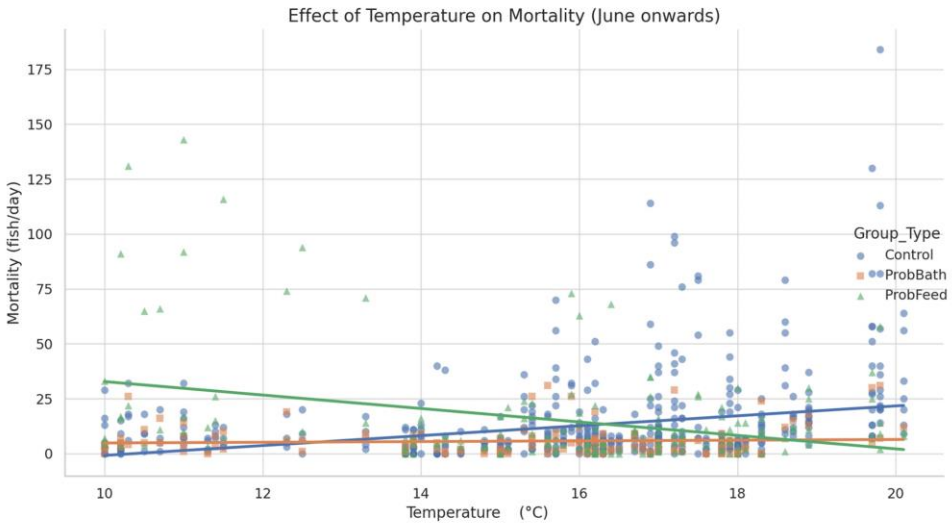

Fig.5. Effect of temperature on salmon fry mortality in FTC rearing system.

A statistical analysis was conducted to evaluate the relationship between temperature and mortality rates in each group. In the **Control group,** a moderate positive correlation was observed (r = +0.29, $p < 0.00000000004$), indicating that higher temperatures were significantly associated with increased mortality. In contrast, the **ProbFeed** group showed a moderate negative correlation (r = –0.33, $p < 0.000011$), suggesting that mortality decreased as temperature rose—potentially indicating a protective effect of the probiotic feed. The **ProbBath** group exhibited a very weak and nonsignificant correlation (r = +0.06, p = 0.48), suggesting no clear relationship between temperature and mortality, possibly reflecting stable thermal tolerance regardless of environmental variation.

In July, critically low dissolved oxygen levels were observed (3,4 mg/l), particularly in the early morning hours. Under these conditions, fry in the ProbFeed and ProbBath groups demonstrated markedly better resilience compared to the Control group, further supporting the positive impact of probiotics and their role in enhancing fry robustness and overall health.

14. Assessment of probiotic efficiency on the skin-mucus microbiota, health and growth performance of Baltic salmon **presmolts**, FTS (september 2024)

The initial average weight for the **Control** group was 34,5±6,45 g and length 14,81±1,29 cm. During 14 incubation days, salmon weight using standard feeding protocol (**Control group**) increased for 4,50 g and length for 0,55 cm. Feed applied together with probiotics caused weight increment for 11,26 g, and length increment for 0,9 cm (**ProbFeed group**). Salmon weight and length increment in this group was approximately twice times larger than in **Control**. Standard feeding protocol applied together with probiotic baths (**ProbBath group**) provided salmon weight increase for 9,26 g, and length increase for 0,98 cm. Salmon weight and length increment in **ProbBath group** was approximately twice times larger than increment in **Control**.

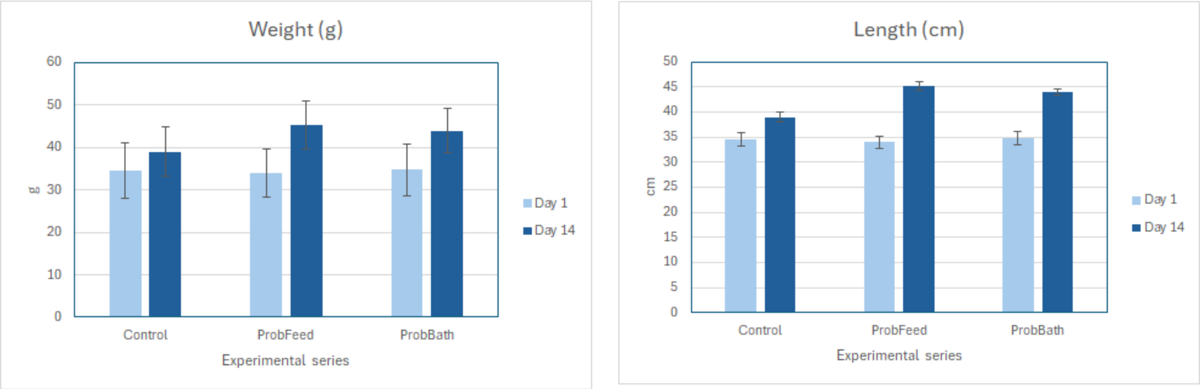

Fig. 6. The weight and length increment during 14 experiment days, Baltic salmon presmolts, Control, Probiotics in feed (ProbFeed), Probiotic baths (ProbBath)

After 14 incubation days Condition factor (K) values were slightly higher in both probiotic treatments (ProbFeed: 1.09 ± 0.08; ProbBath: 1.11 ± 0.09) compared to the Control (1.03 ± 0.16), suggesting better overall body condition in probiotic-treated fish.

Table 1

Growth performance of Baltic salmon presmolts

	Wi (g)	Wf (g)	Li (cm)	Lf (cm)	Wg (g)	K

Control	34,50 ± 6,46a	39,00 ± 5,82a	14,81 ± 1,29a	15,36 ± 1,02a	4,50	1.03 ± 0.16a
ProbFeed	33.99 ± 5,74a	45,27 ± 5,54b	15,01 ± 1.24a	15,91 ± 0,84a	11,26	1.09 ± 0.08a
ProbBath	34.67 ± 5,98a	43,93 ± 5,14b	14,99 ± 1.32a	15,97 ± 0,64a	9,26	1.11 ± 0.09b

Abbreviations: W_I initial mean weight, W_F final mean weight], L_I initial mean length, L_F final mean length, Wg weight gain, *K* condition factor.
Values are mean ± SE. Mean values with different superscript letters in a row are significantly different ($P < 0.05$).

Hatchery Pelči FTS system has natural water source, therefore fish in aquaculture systems are subjected to the potential impact of pathogens from incoming water source.

On Day 1, the following pathogens were isolated from the Control and ProbFeed fish groups — *Aeromonas salmonicida*, *A. veronii*, and *Pseudomonas* spp.; from the ProbBath group — *A. salmonicida*, *A. bestiarum*, and *A. veronii*.

On Day 1 microbial resistance to florfenicol was detected in *A. veronii* isolated from the ProbFeed group. Additionally, resistance to both florfenicol and oxytetracycline was observed in *A. salmonicida*, *A. veronii*, and *Pseudomonas* spp. isolated from ProbFeed fish. In contrast, no antibiotic-resistant pathogens were detected in the ProbBath group.

After 14 days, a single pathogenic bacterial species was identified in each experimental group: *A. bestiarum* in both the Control and ProbFeed groups, and *A. salmonicida* in the ProbBath group. None of these isolates showed antimicrobial resistance (Table 2).

Table 2

Pathogenic bacteria isolated and their susceptibility to antibiotics

	Experiment group	**Pathogen isolate**	**Antibiotics tested for AMR**				**MAR**
			Doxycycline	Enrofloxacin	Florfenicol	Oxytetracycline	
Day 1	**Control**	*A.salmonicida*	Susceptible	Susceptible	Susceptible	Susceptible	0
		A.veronii	Susceptible	Susceptible	**Resistant**	Susceptible	0,25
		Pseudomonas spp.	Susceptible	Susceptible	Susceptible	Susceptible	0

	ProbFeed	*A.salmonicida*	Susceptible	Susceptible	**Resistant**	**Resistant**	0,5
		A.veronii	Susceptible	Susceptible	**Resistant**	**Resistant**	0,5
		Pseudomonas spp.	Susceptible	Susceptible	**Resistant**	**Resistant**	0,5
	ProbBath	*A.salmonicida*	Susceptible	Susceptible	Susceptible	Susceptible	0
		A.bestiarium	Susceptible	Susceptible	Susceptible	Susceptible	0
		A.veronii	Susceptible	Susceptible	Susceptible	Susceptible	0
Day 14	**Control**	*A.bestiarium*	Susceptible	Susceptible	Susceptible	Susceptible	0
	ProbFeed	*A.bestiarium*	Susceptible	Susceptible	Susceptible	Susceptible	0
	ProbBath	*A.salmonicida*	Susceptible	Susceptible	Susceptible	Susceptible	0

MAR - The Multiple Antibiotic Resistance

Results show that:

Initial AMR was more frequent in the ProbFeed group on Day 1, particularly against florfenicol and oxytetracycline.

The absence of AMR on Day 14 across all groups may indicate either to the loss of resistant strains over time or replacement by susceptible bacteria.

The MAR values confirm that only a small proportion of isolates initially exhibited resistance, and none persisted by the end of the observation period.

15. Assessment of probiotic efficiency on the, health and growth performance and skin-mucus microbiota of Baltic salmon **parr,** FTS (August 2025)

Growth performance. Analysis of variance (ANOVA) revealed significant differences among treatment groups for length ($p = 0.0078$), weight ($p < 0.001$), and condition factor ($p = 0.0030$). Tukey's post-hoc comparisons indicated that parr exposed to the ProbBath treatment grew significantly longer than the Control group (p = 0.007). In contrast, no significant differences in length were observed between Control and ProbFeed, or between ProbFeed and ProbBath.

Weight. Fish from the ProbBath group reveal a marked increase in body weight in relation to the Control (p < 0.001) and ProbFeed groups (p = 0.001). No significant difference was detected between ProbFeed and Control fish.

Condition factor. Condition factor was also significantly improved in the ProbBath group in comparison to the Control (p = 0.002) and ProbFeed treatments (p = 0.004). ProbFeed did not differ significantly from Control fish in this parameter (p ≈ 1.0). **Gill cover necrosis**

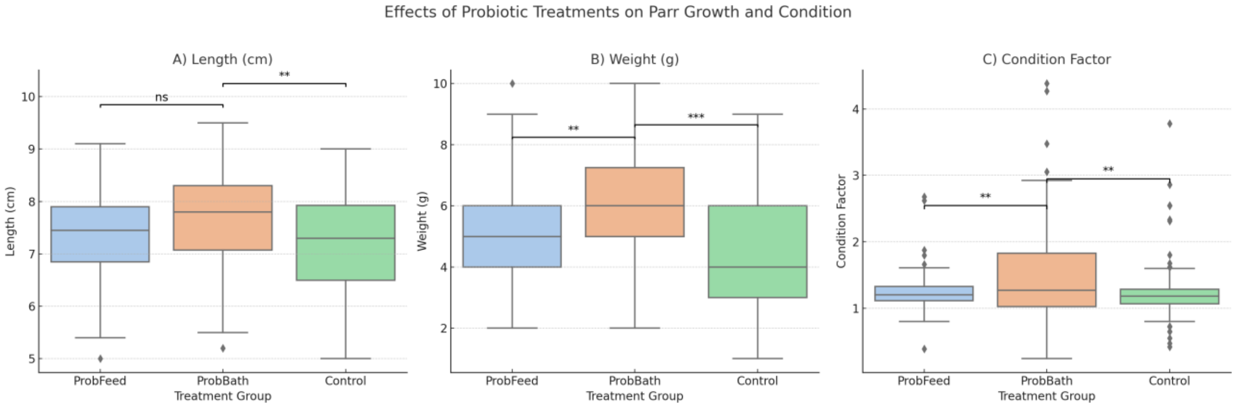

Fig. 7. Comparison of ProbFeed, ProbBath, and Control treatments for A) Length (cm); B) Weight (g), C) Condition Factor

Statistical significance markers:
ns = not significant (no reliable difference between groups)
* = p < 0.05 (significant difference)
** = p < 0.01 (highly significant difference)
*** = p < 0.001 (very highly significant difference)

Gill cover necrosis was recorded in 59% of Control fish, compared with 38% in ProbBath and 34% in ProbFeed. Tukey's post-hoc tests revealed that both ProbBath and ProbFeed significantly reduced necrosis compared with Control. The prevalence decreased from 59% in Control to 38% in ProbBath (p = 0.0069) and 34% in ProbFeed (p = 0.0009), corresponding to ~21–25% fewer affected fish. No significant difference was detected between ProbBath and ProbFeed (p = 0.83).

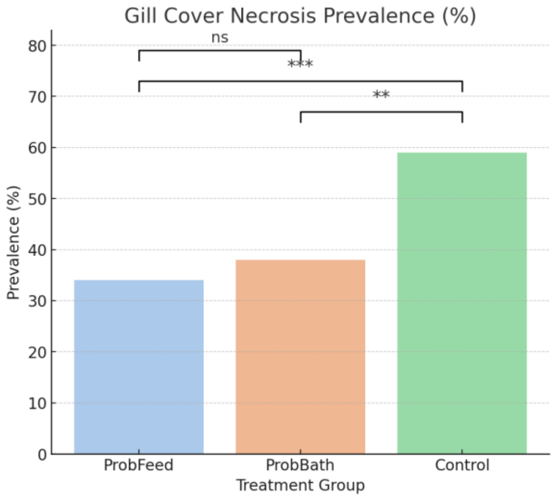

Fig.8. Occurrence of gill cover necrosis in treatments ProbFeed, ProbBath and Control. Prevalence of gill cover necrosis (%) is shown as bar plots for the same groups. Boxes represent median and interquartile range, whiskers indicate the 1.5× IQR range, and dots represent individual data points.

Star symbols note significance levels between groups:
ns = not significant
* = p < 0.05
** = p < 0.01
*** = p < 0.001

Results demonstrate that ProbBath administration enhanced growth performance and body condition compared with Control. In contrast, ProbFeed supplementation was less efficient and did not reveal measurable improvements relative to Control across all evaluated growth parameters. Results reveal that in both probiotic treatments occurred reduction of the prevalence of necrosis in comparison to the Control group, with broadly similar levels of protection between feed and bath application.

16. Assessment of probiotic efficiency on health and growth performance of Baltic salmon **parr and presmolts,** RAS

At the beginning of the experiment, the **average body length** of fish in both groups was 7.01±0,78 cm. Over six months, fish reared without probiotics (Control) exhibited a slightly larger increase in length compared to the ProbFeed. At the end of the study, the Control group reached an average length of 14.33±1.24 cm, while the ProbFeed group attained 13.03±2.07 cm, $p = 5.3\times10^{-5}$ showing statistically significant slower length growth in ProFeed tank.

Initial **average weight** across the groups was 3.6±1,19 g. At the end of the experiment the Control showed reached average weight 34.42±9,35 g, while the group receiving probiotics, 27.98±12.39 g (ProbFeed). The maximal recorded individual weight was recorded for the ProbFeed group (56.1 g). Standard deviation in weight increased in both groups along with increment in weight, the ProbFeed group showed slightly higher weight variability at the final time point as Control (StDev 12.39 vs. 9.35, subsequently). p = 0.022 showing significantly slower weight gain in the ProFeed tank.

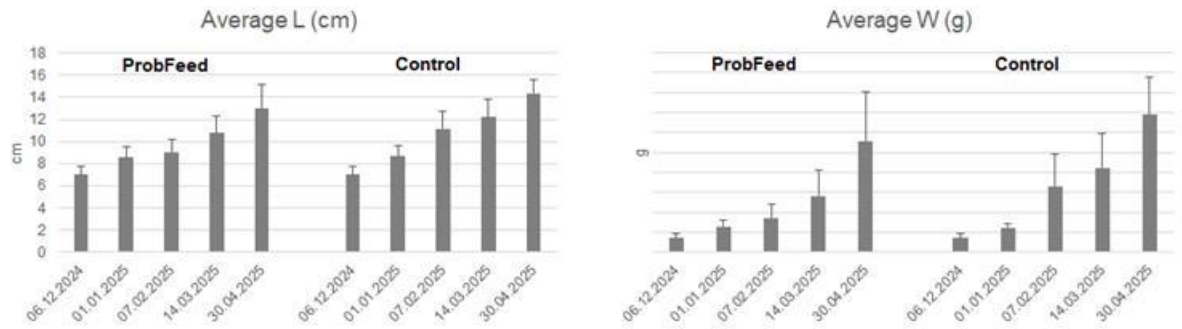

Fig. 9. Increment in length (L, cm) and weight (W, g) in ProbFeed and Control series.

Fulton's condition factor (K) was calculated to evaluate the effect of dietary probiotics on the body condition of Baltic salmon (Salmo salar) presmolts. A linear mixed-effects model was applied to assess the main effect of treatment (Control vs. ProbFeed) on K, with sampling data included as a random effect to account for repeated measures. In this preliminary analysis (based on the available subset of the dataset), the estimated difference between the ProbFeed and Control groups was −0.027 K units (95% CI: −0.101 to 0.046), indicating slightly lower values in the ProbFeed group. This difference was not statistically significant ($p = 0.468$).

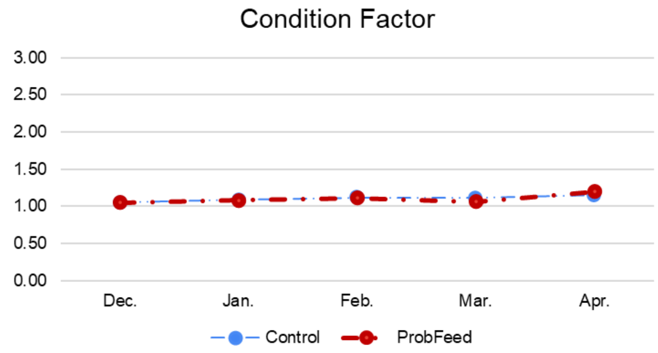

Fig 10. Fulton's condition factor (K) changes over the rearing period.

Fulton's condition factor (K) results suggest that, within the limits of the current sample, probiotic supplementation via feed did not have a detectable impact on the average condition factor of presmolts over the study period. Seasonal changes in K were evident across months in both groups, but treatment-related differences were small compared to inter-individual variability. This suggests that, based on the data provided, there is no strong evidence that the probiotics as feed additives changed the average condition factor compared to the Control group.

Smoltification process. At the beginning of the trial, all fish were classified as a parr, without any smoltification sign. In January the smoltification process started, with a shift to stage 2. In February in ProbFeed group 94.44% of parr had progressed until the stage 2, and a small fraction (5.56%) had reached highest smoltification stage 3. A marked shift occurred in March, when approximately 50 % of fish reached highest smoltification stage 3.

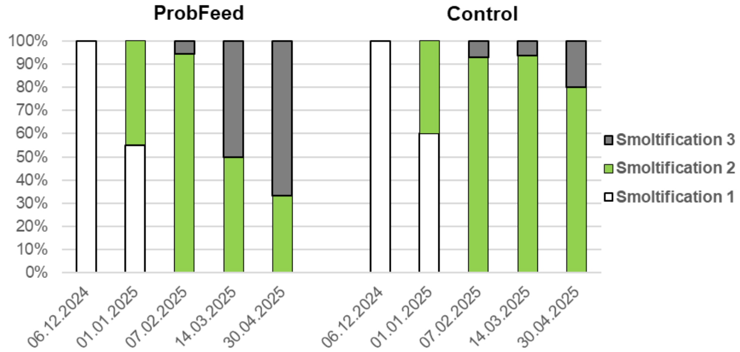

Fig. 11. Progress of smoltification in salmon stock, in percentage (%) from December 2024 till April 2025.

A similar smoltification progress was observed in the Control group. In January 40% had transitioned to stage 2, while in February and March, the vast majority of fish (93–93.75%) were already in stage 2, while small percentages (7% and 6.25%, respectively) reached stage 3. By the end of the trial, 80% of fish remained in stage 2, while 20% had reached advanced stage 3.

However, there is a visible difference in the distribution of smoltified fish in April, the Fisher's exact test gave an odds ratio of 4.89, but the difference was not statistically significant (p = 0.119).

5. Discussion

The present study evaluated the effects of probiotic supplementation, applied via feed (ProbFeed) or bath treatments (ProbBath), on the health, survival, growth performance, and smoltification dynamics of Baltic salmon (*Salmo salar*) fry, parr, and presmolts reared under both flow-through (FTS) and recirculating (RAS) aquaculture systems. The results reveal a complex interaction between probiotic application, fish developmental stage, environmental conditions, and health outcomes.

17. Effects on fry survival and growth in FTS

Fry stage is the most vulnerable stage, when salmon perform transition from yolk sac to exogenous (independent) feeding. During this initial rearing period, salmon fry are highly sensitive and vulnerable. Fry requires precise environmental conditions, optimal water quality, temperature, and nutrition. Inadequate feed intake, delayed feeding, or poor water parameters can lead to increased stress, suppressed immune function, and high mortality rates. The fry's immature digestive and immune systems make them especially susceptible to pathogens and environmental fluctuations.

During the May 2025 fry rearing trial, water temperatures gradually increased from 12.2°C to over 16°C, remaining within the optimal thermal range for salmon fry. Mortality patterns, however, differed significantly among treatments. The ProbBath group exhibited consistently lower mortality than the Control group, particularly in the second half of the month, suggesting that periodic probiotic baths may confer some protection against opportunistic pathogens or improve general resilience during early rearing. In contrast, the ProbFeed group showed markedly higher mortality rates in early May, followed by sharp improvements after mid-May.

Kruskal–Wallis and post-hoc comparisons confirmed that mortality in the ProbFeed group was significantly higher than both Control and ProbBath ($p < 0.001$), while ProbBath mortality was significantly lower than Control ($p = 0.02$). This indicates that continuous dietary supplementation at this fry stage may initially induce physiological stress or microbiota shifts detrimental to survival, while short-term bath exposure may be a more appropriate method for early fry.

Growth performance trends in May contrasted with survival results. The Control group achieved the highest weight gain (0.86 g), with ProbBath slightly lower (0.76 g) and ProbFeed the lowest (0.64 g). Differences between Control and ProbFeed were statistically significant, but not between Control and ProbBath. This pattern suggests that probiotic baths do not impair fry growth and may reduce mortality, while high-dose or continuous probiotic feed at early stages may impair feed conversion efficiency.

18. Thermal resilience and post-trial performance

Following the trial, water temperatures increased approaching the upper tolerance range for salmon fry in July (peaking at 22°C). While Control groups exhibited clear signs of thermal stress and mortality correlated positively with temperature ($r = 0.29$, $p < 0.00000000004$), the ProbFeed group displayed a moderate negative correlation ($r = -0.33$, $p < 0.000011$), indicating improved survival under elevated temperatures. This finding is important: despite early mortality in May, dietary probiotics may confer protective benefits during thermal stress, potentially via immune modulation, improved osmoregulation, or altered gut microbiota composition. The ProbBath group showed no significant correlation between temperature and mortality, suggesting consistent thermal tolerance.

19. Effects on parr in FTS

The results demonstrate that bath-administered probiotics (ProbBath) significantly enhanced growth performance and condition factor for salmon in parr stage, while feed-administered probiotics (ProbFeed) did not differ from the Control group in these metrics. These findings highlight the importance of administration route in determining probiotic efficacy. Bath exposure may promote direct colonization of external mucosal surfaces (skin, gills), leading to improved nutrient assimilation, metabolic efficiency, and reduced physiological stress. In contrast, probiotics delivered via feed may be degraded in the gastrointestinal tract, limiting their effectiveness.

In addition to growth responses, the occurrence of gill cover necrosis was substantially reduced in both probiotic groups compared with the Control. Both treatments reduced the prevalence by approximately 21–25%, and this reduction was statistically significant relative to Control. Interestingly, no difference was observed between ProbBath and ProbFeed, suggesting that probiotic exposure via either route is sufficient to confer protective effects on gill integrity. This may reflect immune or microbiome-mediated modulation of mucosal health, even when growth benefits are not observed (as in ProbFeed).

20. Effects on presmolts in FTS

For presmolts reared for 14 days in September at average temperature 18°C, both probiotic treatments significantly improved growth relative to Control. ProbFeed achieved the largest weight gain (11.26 g) and ProbBath slightly less (9.26 g), both probiotic treatments achieved approximately double the gain of the Control (4.50 g). Fulton's condition factor (K) was also higher in probiotic-treated groups, indicating better body condition. This contrasts with fry-stage results, highlighting the importance of life stage and optimal rearing temperature for determining probiotic effects. These results align with previous studies demonstrating that probiotic supplementation can enhance nutrient utilisation efficiency and promote intestinal health in salmonids, leading to improved growth rates (Ringø et al., 2020; Hai, 2015).

Pathogen monitoring revealed that incoming water contained multiple *Aeromonas* and *Pseudomonas* species. Notably, antibiotic-resistant strains (resistant to florfenicol and oxytetracycline) were detected only in the ProbFeed group at Day 1, but not in the ProbBath or Control. After 14 days, only single susceptible strains were detected in each group. This suggests that probiotic treatments did not increase antimicrobial resistance risk, and that pathogen prevalence decreased over time, possibly due to competitive exclusion or improved host immunity.

Taken together, these findings suggest that ProbBath provides the most consistent benefits across stages, improving both growth and body condition while reducing pathology. ProbFeed, although less effective in parr growth promotion, still conferred significant protection against gill cover necrosis and supported enhanced growth in presmolts. Thus, the efficacy of probiotic delivery routes is context-dependent, with bath administration being particularly advantageous in parr rearing, and feed supplementation showing clearer benefits at the presmolt stage under optimal rearing temperatures.

21. Effects in RAS and smoltification dynamics

In the RAS system, long-term monitoring (six months) revealed significantly slower growth in the ProbFeed group compared to Control, both in length and weight. Although the maximum individual weight occurred in the ProbFeed tank, overall variability was higher, indicating heterogeneous growth responses.

Condition factor analysis showed no statistically significant difference between treatments, suggesting that dietary probiotics did not alter average body condition under RAS conditions.

Smoltification progressed in both groups, with a gradual shift from parr (stage 1) to smolt (stage 3) between December and April. By April, the ProbFeed group had no stage 1 individuals remaining and a higher proportion of stage 3 fish (55% vs. 20% in Control), suggesting faster or more synchronous smoltification. Overally, fish treated with probiotics showed an earlier and more complete transition to the final smoltification stage (stage 3), with over 66% reaching this stage by April. In contrast, the non-probiotic group had a slower progression, with the majority remaining in stage 2 until the final sampling. Results suggest that probiotics may have accelerated or enhanced the smoltification process under the tested conditions. Nevertheless, Fisher's exact test did not reach statistical significance ($p = 0.119$), likely due to sample size. Biologically, this difference is important, as synchronised smoltification is advantageous for migration timing and seawater adaptation and biological trend suggests that probiotic supplementation may accelerate smolt development. Probiotics are known to modulate the gut–brain–endocrine axis, potentially influencing hormonal pathways involved in osmoregulatory preparedness (Schreck et al., 2001; Dimitroglou et al., 2011). Faster and more synchronous smoltification improves downstream migration success, and survival in variable temperature regimes common in the Baltic region.

22. Practical implications

The combined findings highlight that probiotic effectiveness is strongly influenced by the developmental stage of the fish, the applied method, and environmental conditions. At the fry stage, probiotic baths may be safer and more beneficial than by feeding, reducing mortality without growth penalties. However at the presmolt stage, dietary probiotics ingested with feed showed the largest growth benefits. Under thermal stress, dietary probiotics can improve resilience, even if initial growth or survival is compromised.

The performance of probiotic efficiency for Baltic salmon fry and presmolts is strongly influenced by environmental factors, particularly water temperature. Many probiotic strains commonly used in aquaculture, including lactic acid bacteria and Bacillus spp., show optimal metabolic activity and growth at temperatures above 15 °C (Hai, 2015; Merrifield & Carnevali, 2014).

In our FTS trials, water temperatures during the early May fry experiment ranged from 12.2 ± 2.65 °C to 16.3 ± 1.60 °C. This suggests that probiotic activity may have been suboptimal during cooler days, particularly in the first half of May. This limitation may partly explain the less pronounced positive effects observed in the ProbFeed and ProbBath groups under early-season conditions. Conversely, in July, when water temperatures rose to 18 °C (on average) and occasionally exceeded 20 °C, probiotic-fed fish showed improved resilience to thermal stress and lower mortality compared to the Control. This supports the notion that probiotic-mediated benefits are more evident under warmer conditions, likely due to increased metabolic activity of both the probiotic bacteria and the host fish.

For practical hatchery application, these findings suggest that the timing of probiotic administration should consider seasonal temperature trends. Probiotic treatments may yield the strongest benefits when water temperatures are consistently above 15 °C, whereas in colder periods, their effectiveness may be limited unless thermotolerant strains are used.

The potential for accelerated smoltification in probiotic-fed fish warrants further investigation, as synchronised seawater readiness could improve post-release survival in stock enhancement programmes.

23. Relevance for flow-through hatchery management

Most probiotic studies in salmonids have been conducted in recirculating aquaculture systems (RAS), with relatively few reports from flow-through hatcheries typical of the Latvia. The present findings suggest that targeted probiotic use under flow-through conditions can confer benefits in both growth and physiological readiness for seawater transfer, without adverse effects on fish health or survival. Given the projected warming trends in Baltic rivers, tools that can improve smolt robustness and susceptibility to disease may become increasingly important for hatchery-based stock enhancement programs.

24. Practical implications and limitations

The trials clearly indicate that the performance of SmartFishery probiotics in Baltic salmon fry and presmolts is strongly influenced by water temperature. Many probiotic strains used in aquaculture, including lactic acid bacteria and Bacillus spp., show optimal metabolic activity above 15 °C (Hai, 2015; Merrifield & Carnevali, 2014). The SmartFishery product manual similarly notes that application is most effective when water temperature exceeds 10 °C.

In our early May fry experiment, water temperatures ranged from 12.2 ± 2.65 °C to 16.3 ± 1.60 °C. Cooler days in the first half of May likely limited probiotic activity, contributing to the modest benefits observed in the ProbFeed and ProbBath groups compared to the Control.

In contrast, during July monitoring—when mean temperatures reached ~18 °C and often exceeded 20 °C—probiotic-fed fish displayed improved resilience to thermal stress and lower mortality than the control group. This supports the conclusion that probiotic-mediated benefits become more pronounced under warmer conditions, likely due to heightened metabolic activity of both the bacteria and the host fish.

The results of this study demonstrate that the performance of probiotic treatments in Baltic salmon fry and presmolts is influenced by environmental factors, particularly the main is water temperature. Many probiotic strains commonly used in aquaculture, including lactic acid bacteria and Bacillus spp., show optimal metabolic activity and growth at temperatures above 15 °C (Hai, 2015; Merrifield & Carnevali, 2014).

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For practical hatchery application, these findings suggest that the timing of probiotic administration should consider seasonal temperature trends. Probiotic treatments may yield the strongest benefits when water temperatures are consistently above 15 °C, whereas in colder periods, their effectiveness may be limited unless thermotolerant strains are used.

The results of this study demonstrate that application of probiotics, whether through feed or bath treatments, significantly improves growth performance and potentially reduces pathogen-associated risks for *Baltic salmon* presmolts. Fish in the ProbFeed group exhibited the highest weight gain (11.26 g) and substantial length increase (0.9 cm) over the 14-day period, approximately double the growth observed in the Control group (4.50 g and 0.55 cm, respectively). Similarly, the ProbBath group achieved a notable improvement in both weight (9.26 g) and length (0.98 cm) compared to the Control, indicating that both probiotic delivery methods enhance somatic growth, with feed and bath based supplementation. These findings align with previous research showing that probiotics such as *Bacillus spp*. and *Lactobacillus spp*. can improve nutrient absorption, enzymatic activity, and feed utilization in fish (Nayak, 2010; Ringø et al., 2010; Dimitroglou et al., 2011).

It is important to note that this experiment was conducted in September—a month typically associated with elevated water temperatures, which are known to increase the metabolic rate of fish but also raise the risk of pathogen proliferation and disease outbreaks in aquaculture systems (Austin & Austin, 2012). Under such high-risk environmental conditions, the observed effectiveness of probiotics in both enhancing growth and managing microbial health is particularly significant. The results suggest that probiotics can serve as a valuable tool in maintaining fish health and improving performance even during biologically stressful periods.

In terms of pathogen management, initial screenings revealed the presence of multiple bacterial pathogens—including *Aeromonas salmonicida*, *A. veronii*, and *Pseudomonas spp*.—in both the Control and probiotic-treated groups, underscoring the natural pathogen pressure in hatchery environments with open water systems (Austin & Austin, 2012). Notably, antibiotic-resistant strains, particularly against Florfenicol and Oxytetracycline, were detected only in the early stages of the experiment. Whereas no antibiotic resistance was detected in isolates from the ProbBath group at any time, suggesting that immersion-based probiotic treatments may contribute to suppressing the proliferation or transmission of resistant bacterial strains. This observation is supported by studies showing that certain probiotics can inhibit pathogen colonization through competitive exclusion and the production of antimicrobial compounds (Verschuere et al., 2000; Balcázar et al., 2006). After 14 days, each group harbored only a single bacterial species, and none displayed antimicrobial resistance, indicating a possible stabilizing effect of probiotics on microbial communities (Vadstein et al., 2018). These findings suggest that while both probiotic strategies improve growth and potentially modulate pathogen presence, bath application may offer an added advantage in mitigating antimicrobial resistance risks.

These findings are in agreement with descriptions provided by BalticProbiotics, whose SMART FISHERY probiotic line has been scientifically validated by research at the Latvia University of Agriculture. According to their data, SMART FISHERY formulations reduce the spread of multiple harmful pathogens, including *Aeromonas hydrophila*, *E. coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Pasteurella multocida*, and *Mannheimia haemolytica*. Our results support this claim, showing that probiotic applications, especially via bath treatments, not only improved growth performance, but also limited the presence and antibiotic resistance of key aquatic pathogens. Thus, the use of such probiotics represents a promising approach to sustainable aquaculture through microbial modulation and pathogen suppression.

6. Conclusions

This study evaluated the effects of dietary (ProbFeed) and bath-based (ProbBath) probiotic applications on Baltic salmon (*Salmo sala*r) fry, parr, and presmolts reared under both flow-through (FTS) and recirculating (RAS) systems. The results show that probiotic efficacy is strongly influenced by life stage, application method, and environmental temperature.

Fry stage – Probiotic baths reduced mortality compared to the control without impairing growth, suggesting a protective effect during the vulnerable post-yolk transition. In contrast, continuous dietary probiotics initially increased mortality and reduced growth, although performance improved under higher late-season temperatures.

Thermal resilience – Under summer thermal stress (summer 2025, mean 18 °C, peaks > 20 °C), probiotic-fed fry exhibited lower mortality and greater stability in feeding activity compared to the control. This supports the role of probiotics in enhancing resilience during high-temperature periods.

Presmolts health – Both delivery methods significantly improved growth compared to control, with dietary supplementation producing the largest gains in weight and length. Fulton's condition factor (K) was also higher in probiotic-treated groups.

Pathogen management – Pathogenic *Aeromonas* and *Pseudomonas* species were detected at trial onset, with antibiotic-resistant strains present only early in the ProbFeed group. After 14 days, pathogen diversity and resistance declined in all treatments, with no resistant isolates detected in the ProbBath group, suggesting possible benefits in reducing antimicrobial resistance risk.

RAS performance – Over six months, dietary probiotics did not improve average growth or condition factor, but fish displayed faster and more complete smoltification, with 55 % reaching stage 3 by April compared to 20 % in the Control. Although statistical significance was not reached, the trend suggests potential for accelerating seawater readiness.

Temperature dependency – Many probiotic strains used in aquaculture, including lactic acid bacteria and *Bacillus spp*., show optimal metabolic activity above 15 °C (Hai, 2015; Merrifield &

Carnevali, 2014). In our trials, cooler early-May temperatures (12.2–16.3 °C) may have limited probiotic effects, while July's warmer conditions enhanced benefits.

Effect on pathologies occurrence – The study demonstrates that probiotic administration exerts both life stage- and route-dependent effects in salmonids. At the parr stage, bath-applied probiotics (ProbBath) significantly enhanced growth performance (length, weight, condition factor), whereas feed-applied probiotics (ProbFeed) did not improve growth but still reduced the prevalence of gill cover necrosis. At the presmolt stage, both probiotic treatments improved growth relative to Control, with ProbFeed achieving the highest weight gain and ProbBath slightly lower, but both approximately doubling the Control growth. Importantly, probiotic treatments also reduced gill cover necrosis without increasing antimicrobial resistance risks.

Life stages – taken together, results highlight that bath administration provides the most consistent benefits for fry and early parr, while feed supplementation can be more effective at the presmolt stage. Optimizing probiotic delivery strategies according to developmental stage and rearing conditions may therefore represent a valuable approach to improving fish health and production outcomes in aquaculture.

8. References

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