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Research Article Araştırma Makalesi

Effects of *Lactococcus lactis* and *Bacillus* sp. on Hatchery Performance of Rainbow Trout (*Oncorhynchus mykiss*) Eggs until Larval Stage

Lactococcus lactis ve *Bacillus* sp.'nin Yumurtadan Larval Döneme Kadar Olan Evrede Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nın Kuluçka Performansına Etkileri

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Abstract: In this study, the effect of *Lactococcus lactis* and *Bacillus* sp. (Gram-positive bacteria) on survival rates of rainbow trout (*Oncorhynchus mykiss*) from egg to larval stage was investigated. In addition, the effects of bacteria on the blue sac syndrome in the fry stage of fish and basic water quality criteria were also noted. Bacteria were applied by immersion method to healthy-eyed eggs during the incubation period. Two bacteria and the control group were examined in a duplicated plan. The highest survival rate was 92.5% observed in the *Bacillus* sp. group (*L. lactis*; 70% and control; 45%). The lowest survival rates in all groups were observed during the alevin stage. It was found that the amount of nitrite in the water decreased in the bacteria-applied groups, and it was determined that the effects of blue sac syndrome (caused by the pathogen *A. hydrophila*) were suppressed in the *Bacillus* sp. group.

Keywords
- Bacteria
- Blue sac fry syndrome
- Hatchery
- Nitrate
- Rainbow trout

Özet: Bu çalışmada *Lactococcus lactis* ve *Bacillus* sp. (Gram-pozitif bakteri) uygulamasının gökkuşağı alabalığının (*Oncorhynchus mykiss*) yumurtadan larva dönemine kadar yaşama oranları araştırıldı. Ayrıca çalışmada balıkların yavru aşamasında mavi kese hastalığı üzerine bakterilerin etkileri ve temel su kalitesi kriterleri de not edilmiştir. Bakteriler kuluçka döneminde sağlıklı gözlü yumurtalara daldırma yöntemiyle uygulandı. İki bakteri ve kontrol grubu, iki tekerrürlü deneme planında incelendi. En yüksek hayatta kalma oranı *Bacillus* sp. grubunda (*L. lactis*; %70 ve kontrol; %45) %92.5 ile gözlendi. Tüm gruplarda en düşük hayatta kalma oranları alevin döneminde gözlendi. Bakteri uygulanan gruplarda sudaki nitrit miktarının azaldığı, *Bacillus* sp. grubunda ise mavi kese sendromunun (patojen etken *A. hydrophila*) etkilerinin baskılandığı belirlendi

Anahtar kelimeler
- Bakteri
- Mavi kese sendromu
- Kuluçkahane
- Nitrat
- Gökkuşağı alabalığı

1. INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is well-known fish species in aquaculture around the world. Broodstock management, nutritional needs, hatchery performances, water quality criteria, nutrient content, and diseases have been investigated in several studies (Atar, et al., 2009; Öztürk and Altinok, 2014). Recently, biotechnological applications such as hybridization, and female, or male stock options for fish have been used successfully in aquaculture systems (Rehman et al., 2022). Besides, there are several studies on health problems in rainbow trout (Öztürk and Altinok, 2014). Diseases caused by bacteria, viruses, and parasites are frequently reported by researchers, especially in intensive breeding systems (Woynarovich et al., 2011).

The highest mortality rates in trout hatcheries were observed in the egg and fry stages. Infertile eggs, water quality problems, and losses caused by pathogens, particularly fungus, have been reported in trout hatcheries (Kayış et al., 2017). Particularly, gas bubble and blue sac fry syndrome in the alevin stage of trout are commonly reported (Kayış et al., 2015; Balta and Dengiz, 2020). Various precautions and applications have been used to prevent these losses (Kayış 2019; Austin et al., 2022). Maintenance of hygiene is among the most important practices. However, the use of some chemicals for disinfection is quite common. For this purpose, formalin has been effectively used from the egg to the fry stage (Barnes et al., 2001). Iodine compounds are other disinfectants that have been recommended for rainbow trout eggs (Goldes and Mead, 1995). Apart from chemicals, other applications have also been used to improve the quality of hatchery water. Ozone and ultraviolet rays have been reported to reduce the pathogenic load of water (Forneris et al., 2003).

Recently, the general use of some probiotic organisms for the welfare of fish has been investigated. Probiotics are beneficial organisms that are known to suppress the harmful microbial flora in the living body and, recently, have been increasingly used in aquaculture due to the negative effects of disinfectants (Kayış, 2019; Austin et al., 2022). Probiotics are used to increase growth performance, improve water quality, suppress bacterial load, improve reproductive performance, and reduce stress, especially by participating in food diets (Cruz et al., 2012). Bacteria such as *Bacillus* sp., *Lactobacillus acidophilus*, and *Lactococcus lactis* are known to be useful in aquaculture, particularly in suppressing pathogens (Yilmaz et al., 2022).

Lactococcus lactis is a Gram-positive bacterium that is especially used for fermenting dairy products. It has been reported to be abundant in the intestinal microbiota of freshwater fish (Gatesoupe 2008). *Lactococcus* sp. and other lactic acid bacteria of the genus *Leuconostoc* have been reported to be frequently used as probiotics in aquaculture (Merrifield, 2010). Bacteria of the genus *Bacillus*, which are rod-shaped Gram-positive bacteria, are highly resistant to heat. Although they are usually found in soil, they can also be found in air, water, dust, and feces. The vast majority of *Bacillus* sp. do not have pathogenic potential (except for *B. cereus* and *B. anthracis*) and have important microbiological applications (antibiotics, enzymes, toxins, and bioplastics) (Kemmerly and Pankey, 1993; Ahmed et al., 1995).

Therefore, the present study investigated the question “could some bacteria have a positive effect in preventing losses in trout hatchery systems?”. For this purpose, *Lactococcus lactis* and *Bacillus* sp., isolated from trout hatchery systems, were applied to the eggs and the results were evaluated. Also, other two trials were designed to determine probiotic effectiveness against *Aeromonas hydrophila*, which is pathogenic to trout species and a causative agent of blue sac fry syndrome, and on some water quality parameters values such as nitrite, nitrate, ammonia, and phosphate.

2. MATERIAL AND METHODS

2.1. Eggs and potential probiotic bacteria

The rainbow trout (*Oncorhynchus mykiss*) eggs used in this study were obtained from a trout farm at Rize in the Black Sea region of Turkey. To eliminate possible bacterial and fungal pathogens that may have been present in these eggs, 1.65 mg/L formalin was applied to the eggs for 15 min (Barnes et al., 2001). A total of 120 eggs were used for each group of bacteria and control *Lactococcus lactis* (isolated from *Oncorhynchus mykiss*) and *Bacillus* sp. (isolated from *Salmo* sp.) were isolated from the hatcheries of some trout farms in the Eastern Black Sea Region of Turkey (Kayış et al., 2021). For the molecular identification of the bacteria their genomic DNA was obtained by a DNA extraction isolation kit (Qiagen). Specific primers to the 16S rRNA region of eubacteria (27 Fwd 5’-AGA GTT TGA TCC TGG CTC AG-3', 1492 Rev 5’-GTT TAC CTT GTT ACG ACT T-3') were used. PCR reaction is carried out using genomic DNA of bacteria and the primers (Model Px2 ThermoHybrid; Thermo Electron Inc., Waltham, MA, USA). The 1465-bp amplified products were purified with a NucleoSpin PCR purification kit (Macherey-Nagel) and sent for sequencing by double-sided reading (ABI PRISM 310 genetic analyzer, Applied Biosystems). Accession numbers of *Bacillus* sp. and *Lactococcus lactis* are MW295490 and MW295471 respectively in the National Center for Biotechnology Information. The bacteria were previously preserved at –80 °C at the Fish Diseases Laboratory, Faculty of Fisheries of Recep Tayyip Erdogan University. The experiment was designed as a static system, with three groups (control, *Lactococcus lactis*, and *Bacillus* sp.) in duplicates. The eggs were placed in glass containers (20 eggs/500 ml) and sufficient ventilation was provided using an air pump.

2.2. Experiment I (Survival rates of Eggs and Determination of Potential probiotic effects of Bacteria)

The immersion method was used to transmit the bacteria to the eggs. For this, purpose bacteria were transferred to Tryptic Soy Agar (TSA) (Merck 1.05458, Darmstadt, Germany) medium and incubated at 20±2 °C for 24 h. Bacterial colonies were then transferred to 50 ml Tryptic Soy Broth (TSB) (Merck 1.05459, Darmstadt, Germany) medium and the cultures were kept at 20±2 °C for 12 h. This culture was then centrifuged at 9950 g for 5 min and the medium was separated from the bacteria. Sterile Phosphate-Buffered Saline (PBS) (15 mL) was added to the bacteria and the mixture was homogenized using a vortex mixer. About 15 ml of this bacterial suspension was then added to each test container and the bacteria were allowed to contaminate the eggs. To determine the number of bacteria, 10^{-6} and 10^{-7} dilutions were spread on the surface of Plate Count Agar (PCA) (Merck 1.05463, Darmstadt, Germany), and colony counts were performed (APHA 1998; Kayış et al., 2017), and the bacterial count was determined as 4.6×10^{10} and 1.2×10^{10} CFU/ml for *Lactococcus lactis* and *Bacillus* sp., respectively. In this experiment, the temperature of the water was recorded as 14.5±0.6 °C and pH was 6.5±1.3. The eggs reached the stage of the alevin within eight days from the start of the experiment. The alevin phase continued for ten days. After this phase, the trial was conducted for one week. The fish were fed with commercial trout feed during this stage. During this time, the metabolic wastes in the tanks were cleaned and the same amount of fresh water was added instead of the decreased water.

2.3. Experiment II (Blue sac fry syndrome inhibition trial)

Kayış et al. (2015), stated that *Aeromonas hydrophila* infection is the cause of blue sac fry syndrome disease in trout alevins. To investigate the inhibitory effect of the bacteria against the fish pathogen *Aeromonas hydrophila*, four groups (control, *A. hydrophila*, *A. hydrophila- Lactococcus lactis and A. hydrophila- Bacillus* sp.) of eggs were designed in two repetitions as another independent experiment (Table 1), and the survival rate of the eggs until the fry stage was determined. For this purpose, the bacteria (*Bacillus* sp. and *Lactococcus lactis*) were added to water including eyed trout eggs which were infected with *Aeromonas hydrophila*. At the end of the experiment, it was observed to what extent the blue sac fry syndrome in the alevin was present.

Table 1. Groups in the inhibitory effect of the bacteria against *Aeromonas hydrophila* trial

Groups	Amount of Eggs	Amount of Bacteria
Control (C)	200	(-)
Aeromonas hydrophila (A)	200	(1.6×10^{10})
A. hydrophila- Lactococcus lactis (A-L)	200	(1.6×10^{10})-(4.6×10^{10})
A. hydrophila- Bacillus sp. (A-B)	200	(1.6×10^{10})-(1.2×10^{10})

2.4. Experiment III (Reduction of values of some water parameters)

Another experiment was conducted to observe the reduction of values on some water parameters (ammonia, nitrite, nitrate, and phosphate) by bacteria in water, including fry fish. Three groups; control (C), *Lactococcus lactis* (L), and *Bacillus* spp. (B) were used in this experiment. Fifty fish (average weight of 0.5 g) were placed in each group in duplicate (5-liter tanks). At the beginning of the experiment, the water parameters were measured. Fish were fed commercial trout feed once a day (in the morning, at 2% of body weight) and provided sufficient oxygen (The average amount of dissolved oxygen in the tanks was measured as 9.2±0.9). The bacteria were then added to the groups separately (*Lactococcus lactis* 1.2×10^{11} CFU/mL, *Bacillus* spp. 2.3×10^{10} CFU/mL). Then, on the 13^{th} day, the water parameters were measured again. In both this and the *Aeromonas hydrophila* inhibition trial, the water temperature was recorded as 16.5±0.3 °C and the pH was measured as 6.7±0.8. Water quality parameters were measured by spectrophotometer (Hach DR3900).

2.5. Statistical analysis

The statistical significance of the difference between the water quality values of the groups was carried out with the analysis of variance (ANOVA) in the SigmaPlot 12.0 program. Survival analysis of different groups was carried out with the Kaplan–Meier test.

3. RESULTS

The number of eggs that survive until the hatching stage is given in Figure 1. The group treated with *Bacillus* sp. was observed to have the highest survival rate (92.5%), followed by *Lactococcus lactis* (70%). The control group had the lowest survival rate (45%) (Figures 1, 2).

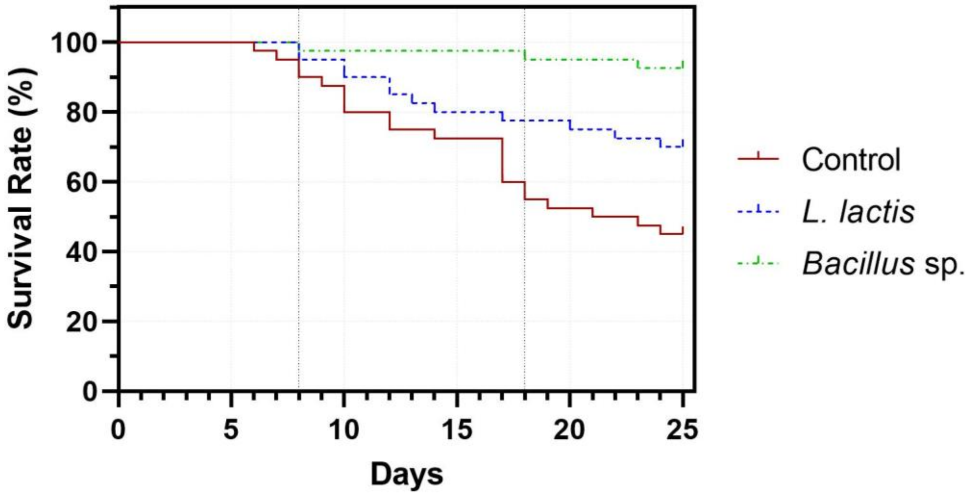

Figure 1. Survival rate of the groups

It was observed that the major losses in all the groups were in the alevin stage. After the alevin stage, the deaths were observed to decrease. No significant pathological finding was observed in any group. During the feeding phase with commercial trout feed, the group treated with *Bacillus* sp. showed more appetite than the other groups.

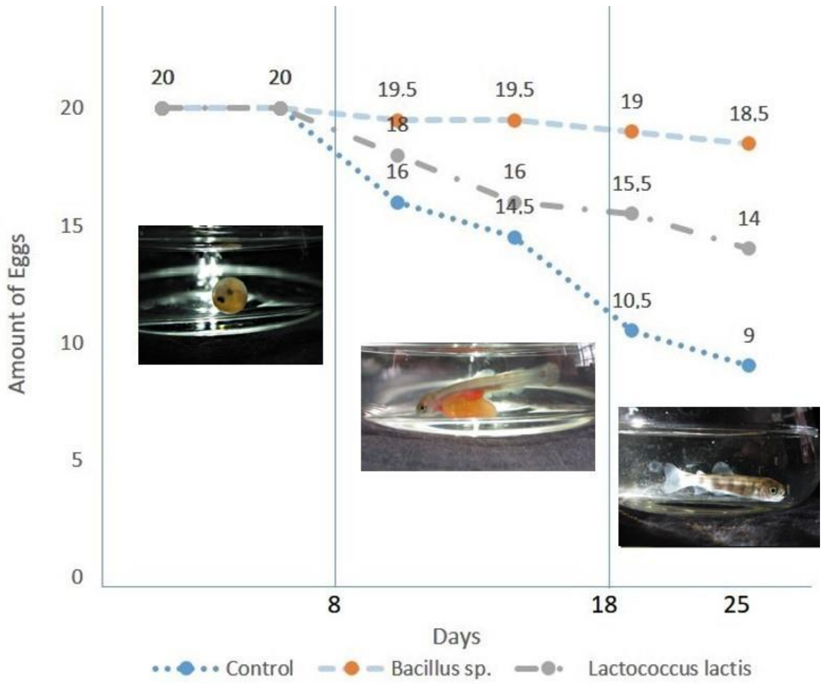

Figure 2. Mortalities of the fish during the experiment (I).

Regarding the inhibitory effect of bacteria against *Aeromonas hydrophila* (Blue sac fry syndrome), the survival rate was recorded as 80% in the control group (C), 79% in the *A. hydrophila* (A) group, 80% in the *A. hydrophila-Lactococcus lactis* (A-L) group, and 77% in the *A. hydrophila-Bacillus* sp. (A-B) group. Also, the Blue Sac Fry Syndrome was observed in 3% of trout in the (A) and (A-L) groups in the alevin stage. This symptom was not observed in groups (B) and (C) (Figure 3).

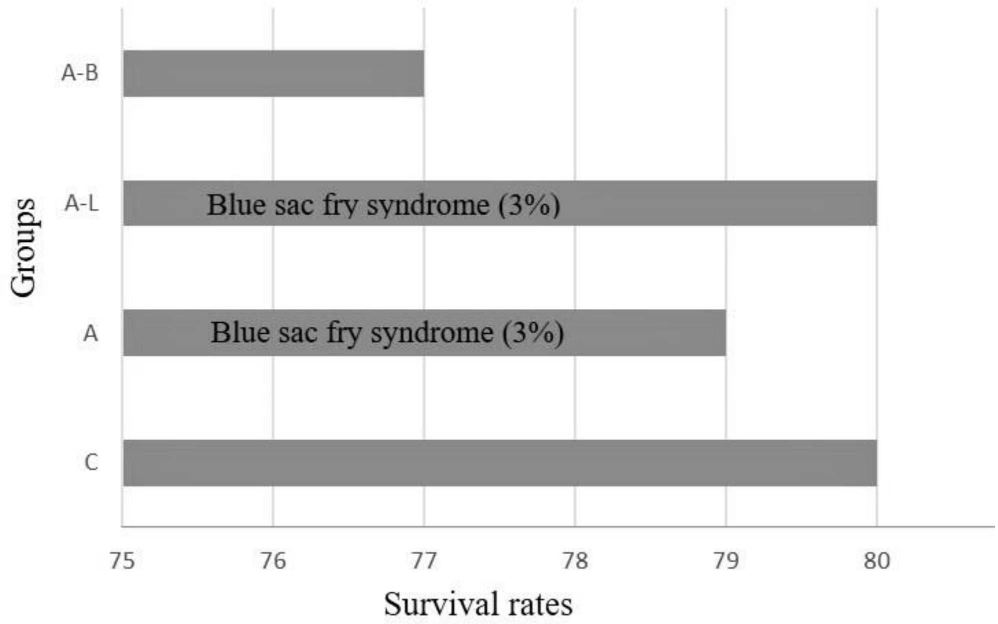

Figure 3. Result of *Aeromonas hydrophila*/Blue sac fry syndrome trial (Experiment II). Control (C), *A. hydrophila* (A), *A. hydrophila- Lactococcus lactis* (A-L), *A. hydrophila- Bacillus* spp. (A-B).

In the water criteria trial, both Gram-positive bacteria reduced the amount of nitrite in the water. Moreover, the nitrate content was observed to be lower in the *Bacillus* group compared to the other groups (Figure 4). In this trial, fish deaths started on the 11th day in the control and *Lactococcus lactis* groups. While all fish in the control group died by the 13th day, the percentage mortality in the *Lactococcus lactis* and *Bacillus* sp. groups was 52% and 68%, respectively (Figure 5).

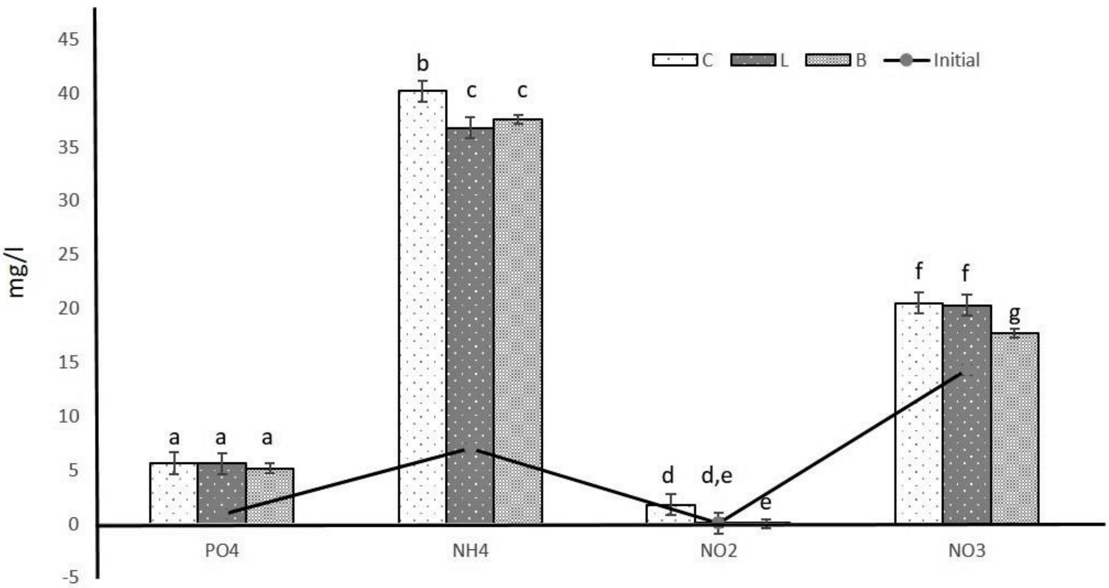

Figure 4. Some water criteria in reduction of some water parameters trial (Experiment III). Control (C), *Lactococcus lactis* (L) *Bacillus* sp. (B).

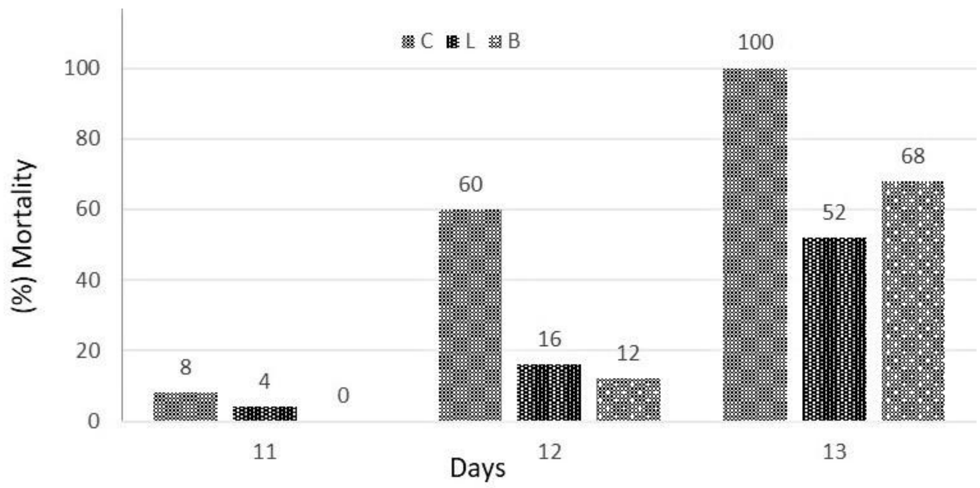

Figure 5. Fish mortalities (%) in the water criteria trial (Experiment III). Control (C), *Lactococcus lactis* (L) *Bacillus* sp. (B).

4. DISCUSSION

The use of probiotics stands out in all live production sectors in the world (Austin et al., 2022). For example, *Carnobacterium* spp. has been reported to be effective against some fish pathogens (*Aeromonas hydrophila, A. salmonicida, Flavobacterium psychrophilum, Photobacterium damselae*, and *Vibrio* spp.) in rainbow trout (Robertson et al., 2000). Probiotics some times are used to improve the water quality in fish farms and aquarium units. In this context, bacteria of the genus *Bacillus* are widely used. Therefore, the benefits of *Bacillus* sp. and *Lactococcus lactis* isolated from trout hatchery systems were investigated to the survival rate of trout eggs in the present study. The results indicated that *Bacillus* sp. and *L. lactis* had a positive effect on the survival rate of trout eggs.

Egg deaths in freshwater hatchery systems are reported because of fungal infestations (Thoen et al., 2011). However, some studies show that bacteria in trout hatchery systems can also cause the deaths of fish (Declercq et al., 2013; Yardımcı and Turgay, 2021). Bacterial contamination studies generally have been conducted on trout eggs to determine the negative effects of various bacteria. These studies have reported that egg mortality rates increased and reached high levels during the alevin phase (Kayış et al., 2017). Similarly, in this study, it was observed that mortality rates increased during the alevin phase. However, it was observed that these deaths were significantly prevented using *Bacillus* sp. in the aquatic systems. This is an important finding, and it may be advisable to use bacteria of the genus *Bacillus*, which have probiotic properties, during the egg stage.

In the present study, the difference in survival rates of fish in *Bacillus* sp. and *Lactococcus lactis* groups can be discussed. It is thought that especially water quality criteria may affect these rates. Water temperature and pH are important factors in the reproduction of bacteria in aquatic systems. Other factors such as adherence to the surface and organic load are also important. These criteria are necessary for the reproduction of bacteria as well as enzyme activities. The optimum temperature for the activation of enzymes of *Bacillus subtilis* is 37 °C, while the optimum pH is reported as 5.5 (Sneath, 1986). For *Lactococcus lactis*, the optimum temperature is 30-40°C (Chen at. al., 2015). These values are higher than those recorded in this experiment. For the incubation of trout eggs, the optimum water temperature is 12 °C or lower, although the temperatures in the experiment were tolerable for the eggs. The difference in survival rate between the use of *Bacillus* sp. and *Lactococcus lactis* group may be explained by the optimum temperature values of the bacteria.

The blue sac fry syndrome in fish is explained by different reasons. Among those, physicochemical parameters and chemical contamination of the water are well-known. Temperature, pH, nitrogen compounds and some dissolved gasses and xenobiotics (retene (7-isopropyl-1-methylphenanthrene) are the most important reasons that can cause blue sac disease on alevins (Noga, 2010; Brzuzan et al., 2007). However, recently, there have been reports that this symptom is caused by bacteria. It has been stated that *Aeromonas hydrophila* causes this symptom in the alevin stage of trout (Kayış et al., 2015). This study confirms the previous reports on the blue sac symptom. The blue sac syndrome was observed in all the fish groups infected with *A. hydrophila*, except for those given *Bacillus* sp. as a probiotic (Figure 2). This result revealed the preventive effect of *Bacillus* sp. against the blue sac fry syndrome in trout alevins. It is reported that *Bacillus* spp. produces some metabolites with antibiotic properties. Due to this feature, these bacteria are used against many pathogenic bacteria (Miljakovic et al., 2020). Details of the reasons and mechanism of this positive effect (inhibition of blue sac fry syndrome/*Aeromonas hydrophila*) can be investigated in future studies.

It is known that some bacteria improve some water quality criteria. It has also been reported that these bacteria suppress pathogens in fishponds. These positive effects are also tried to be evaluated in terms of aquaculture (Padmavathi et al., 2012). In a study, positive effects of *Bacillus subtilis* and *Lactobacillus acidophilus* on growth performance and pond water quality of Nile tilapia (*Oreochromis niloticus*) fingerlings were reported. (Khalafalla et al., 2020). Similarly, Hlordzi et al. (2020), stated that *Bacillu*s species showed great success in maintaining water quality at a low cost in aquaculture. The present study determined that the nitrite content in water was significantly decreased, especially in the fish groups treated with *Bacillus* sp. and *Lactococcus lactis*. This situation was found to support the study of Korenekova et al. (2004) and Usharani et al. (2017), on the reducing effect of *L. lactis* bacteria on nitrate levels in milk and *Bacillus* sp. in wastewater respectively. This finding is particularly important, as nitrite is known to cause brown blood disease caused by nitrite in fish. The survival rates of the fish in this trial support this finding (Figure 4).

5. CONCLUSION

The beneficial effects of *Bacillus* sp. and *Lactococcus lactis* have been demonstrated in the present study, especially in preventing deaths, blue sac fry syndrome of trout eggs and fry, and reducing the nitrite level of hatchery water. Based on these results, we can recommend that farmers use the bacteria in trout hatchery systems.

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CONFLICT OF INTEREST

Example: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: ŞK, EP; Literature: EP; ŞK; Methodology: ŞK, EP; Performing the experiment: ŞK, EP, ZZİ, EA, MM; Data analysis: EP, ŞK; Manuscript writing: ŞK; Supervision: ŞK. All authors approved the final draft.

ETHICAL STATEMENTS

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Recep Tayyip Erdoğan University (Date: 27.01.2016, No: 2016/10).

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

REFERENCES

Ahmed, R., Sankar-Mistry, P., Jackson, S., Ackermann, H. W., & Kasatiya, S. S. (1995). *Bacillus cereus* phage typing as an epidemiological tool in outbreaks of food poisoning. *Journal of Clinical Microbiology, 33,* 636-640. https://doi.org/10.1128/jcm.33.3.636-640.1995

Allen, T., Khoni, N. S., Dunphy, B., Kinge, A. R. E., & Court, N. B. (2017). Efficacy and safety of Specific Conjugate Particle (SCP)-Doxorubicin in Patients with Soft Tissue Sarcoma, a Randomized Clinical Study. *Journal of Vaccine Immunotechnology*, *3*(1), 5.

American Public Health Association (APHA) (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. American Water Works Association and Water Environmental Federation, Washington DC.

Atar, H.H., Bekcan S., & Dogankaya L. (2009). Effects of Different Hormones on Sex Reversal of Rainbow Trout (*Oncorhynchus mykiss* Walbaum) and Production of All-Female Populations. *Biotechnology & Biotechnological Equipment*, *23*(4), 1509-1514. https://doi.org/10.2478/V10133-009-0002-X

Austin, B., Lawrence A.L., Can., E., Carboni, C., Crockett, J., Demirtaş Erol, N., Dias Schleder, D., Jatobá, A., Kayış, Ş., Karacalar, U., Kizak, V., Kop, A., Thompson, K., Mendez Ruiz, C. A., Serdar, O., Seyhaneyildiz Can,S., Watts, S., & Yücel Gier, G. (2022). Selected topics in sustainable aquaculture research: Current and future focus. *Sustainable Aquatic Research, 1*(2), 74-122. https://doi.org/10.5281/zenodo.7032804

Balta, F., & Dengiz Balta, Z. (2020). A Study on Gas Bubble Disease and Treatment Observed in Rainbow Trout (*Oncorhynchus mykiss*) Fry. *Journal of Anatolian Environmental and Animal Sciences, 5*(1). https://doi.org/100-105.10.35229/jaes.706925

Barnes, M. E., Sayler, W. A., & Cordes, R. J. (2001). Use of formalin treatments during incubation of eyed eggs of brown trout. *North American Journal of Aquaculture*, *63*, 333-337.

Brzuzan, P., Wozny M., Dobosz, S., Kuzminski, H., Łuczynski, M. K., & Gora M. (2007) Blue sac disease in larval White fish, Coregonus lavaretus (L.): pathological changes in mRNA levels of CYP1A, ERa, and p53. *Journal of Fish Diseases, 30*, 169–173.

Chen J., Shen, J., Hellgren, L. I., Jensen, P. R., & Solem, C. (2015). Adaptation of *Lactococcus lactis* to high growth temperature leads to a dramatic increase in acidification rate. *Scientific Reports*, *5*, 14199. https://10.1038/srep14199 https://doi.org/10.1577/1548-8454(2001)063<0333:UOFTDI>2.0.CO;2

Cruz, P. M., Ibáñez, A. L., Hermosillo, O. A. M., & Ramírez Saad, H. C. (2012). Use of probiotics in aquaculture. *International Scholarly Research Network Microbiology,* 916845. https://doi.org/10.5402/2012/916845

Declercq, A.M., Haesebrouck, F., Broeck, W.V.D., Bossier, P., & Decostere, A., (2013). Columnaris disease in fish: A review with emphasis on bacterium-host interactions. *Veterinary Resource, 44*, 27.

Forneris, G., Bellardi, S., Palmegiano, G. B., Saroglia, M., Sicuro, B., Gasco, L., & Zoccarato, I. (2003). The use of ozone in trout hatchery to reduce saprolegniasis incidence. *Aquaculture, 221*(1-4), 157-166. https://doi.org/10.1016/S0044-8486(02)00518-5

Gatesoupe, F. J. (2008). Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *Journal of Molecular Microbiology and Biotechnology, 14*, 107-114. https://doi.org/10.1159/000106089

Goldes, S.A., & Mead, S.L. (1995). Efficacy of Iodophor Disinfection against Egg Surface-Associated Infectious Hematopoietic Necrosis Virus. *Progressive Fish-Culturist*. 57(1), 26-29. https://doi.org/10.1577/1548-8640(1995)057<0026:AEOIDA>2.3.CO;2

Hlordzi, V., Kuebutornye, F.K.A., Afriyie, G., Abarike, E.D., Lu, Yishan, Chi, S., & Anokyewaa, A. (2020). The use of *Bacillus* species in maintenance of water quality in aquaculture: A review. *Aquaculture reports,* 18, 1005003. https://doi.org/10.1016/j.aqrep.2020.100503

Kayış, Ş., Er, A., Yilmaz, C., Duzgun, A., Kose, O., & Kurtoglu, İ. Z. (2015). *Aeromonas hydrophila* as a causative agent of blue sac fry syndrome in different trout species. *Journal of Fish Diseases*, *38*, 1069-1071. https://doi.org/10.1111/jfd.12326

Kayış, Ş., Yilmaz, C., & Er, A. (2017). Pathogenic effects of some common bacteria on trout in hatchery systems. *Bulletin of the European Association of Fish Pathologists, 37*, 244-252.

Kayış, Ş. (2019). Analysis of Fish Health Status in Terms of Sustainability of Aquaculture in Turkey-A SWOT Analysis. *Aquaculture Studies, 19*, 65-76. https://doi.org/10.4194/2618-6381-v19_1_07

Kayış, Ş., Soyköse, G., İpek, Z. Z., & Er, A. (2021). Determination of Bacterial Contamination and Antibiotic Resistance of the Bacteria in the Some Trout Farm Hatcheries in the Eastern Black Sea Region of Turkey. *Journal of Limnology and Freshwater Fisheries Research*, *7*(2), 101–107. https://doi.org/10.17216/limnofish.827718

Kemmerly, S. A., & Pankey, G. A. (1993). Oral ciprofloxacin therapy for *Bacillus cereus* wound infection and bacteremia. *Clinical Infectious Diseases, 16*, 189. https://doi.org/10.1093/clinids/16.1.189

Korenekova, B., Skalicka, M., Kottferova, J., & Korenek, M. (2004). Effect of addition of nitrate or nitrite on *Lactococcus lactis* dairy culture. *Arch Lebensmittelhyg, 55*(2), 42-44.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., Castex, M., & Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture, 302*, 1-18. https://doi.org/10.1016/j.aquaculture.2010.02.007

Miljakovi´c, D., Marinkovi´c J., & Baleševi´c-Tubi´c, S. (2020). The Significance of *Bacillus* spp. in Disease Suppression and Growth Promotion of Field and Vegetable Crops. *Microorganisms,* 8, 1037; https://doi.org/10.3390/microorganisms8071037

Noga, E. J. (2010). Fish Disease –Diagnosis and Treatment, 2ndedn. Wiley-Blackwell, 536 pp. Hoboken, New Jersey.

Öztürk, R. Ç., & Altinok, I., (2014). Bacterial and Viral Fish Diseases in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences, 14,* 275-297.

Padmavathi, P., Sunitha, K., & Veeraiah, K. (2012). Efficacy of probiotics in improving water quality and bacterial flora in fishponds. *African Journal of Microbiology Research, 6.* https://doi.org/7471-7478.10.5897/AJMR12.496

Rehman, S. M., Aleem, M. T., Umar, M., Khan, A., Jamil, M., Rehman, T., Ijaz, A., Hussain J., & Abbas, R. Z. (2022). Advancement in Aquaculture System by Applying Biotechnological Tools, Animal Health Prespective (pp.259) Edition: 1stPublisher: Unique Scientific Publishers, https://doi.org/10.47278/book.ahp/2022.3

Robertson, P. A. W., O'Dowd, C., Burrells, C., Williams, P., & Austin, B. (2000). Use of *Carnobacterium* sp. as a probiotic for atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 185*, 235-243. https://doi.org/10.1016/S0044-8486(99)00349-X

Sneath, P. H. A. (1986). Section 13. Endospore-forming Grampositive rods and cocci. In: *Bergey's Manual of Systematic Bacteriology* Vol 2, 9th edn. Sneath P. H. A, Mair N. S., Sharpe M. E. and Holt J. G. (eds), pp. 1104-1139. Williams & Wilkins, Baltimore, MD.

Thoen, E. 1, Evensen, Ø., & Skaar, I. (2011). Pathogenicity of *Saprolegnias* pp. to Atlantic salmon, *Salmo salar* L., eggs, *Journal of Fish Diseases*, 34, 601–608. https://doi.org/10.1111/j.1365-2761.2011.01273.x

Usharani, K., Sruthilaya, K., & Divya, K. (2017). Determination of nitrate utilization efficiency of selective strain of *Bacillus* sp. isolated from Eutrophic Lake, Theerthamkara, Kasaragod, Kerala, *Pollution*, *3*(1), 55-67. https://doi.org/10.7508/pj.2017.01.007

Woynarovich, A., Hoitsy, G., & Moth-Poulsen, T. (2011). Small-scale rainbow trout farming. FAO Fisheries and Aquaculture Technical Paper No. 561. Rome, FAO.

Yardımcı, R.E., & Turgay, E. (2021). Diagnosis of *Aeromonas sobria* and *Saprolegnia* sp. co-infection in rainbow trout fry (*Oncorhynchus mykiss*). *Aquatic Research*, 4(1), 65-72. https://doi.org/10.3153/AR21006

Yılmaz, S., Yılmaz, E., Dawood, M. A. O., Ringo, E., Ahmadifar, E., & Abdel-Latif, H. M. R. (2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. *Aquaculture*. *547*, 737514. https://doi.org/10.1016/j.aquaculture.2021.737514