Aquaculture Studies, 25(5), 254-265, AQUAST2395
http://doi.org/10.4194/AQUAST2395

RESEARCH PAPER

Monitoring Dynamics of Bacterial Pathogens to Improve Disease Preventive Strategies in an Open Aquaculture System with Rainbow Trout (*Oncorhynchus mykiss*)

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How to Cite

Fuentes, J.C., Unger, P., Palm, H. (2025). Monitoring Dynamics of Bacterial Pathogens to Improve Disease Preventive Strategies in an Open Aquaculture System with Rainbow Trout (*Oncorhynchus mykiss*) *Aquaculture Studies*, *25(5), AQUAST2395*. http://doi.org/10.4194/AQUAST2395

Article History
Received 23 January 2025
Accepted 02 May 2025
First Online 12 May 2025

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Keywords
Microorganisms
Bacterial load
Environmental interactions
Disease prevention
Freshwater aquaculture
Pathogens

Abstract

The bacterial community of a flow-through aquaculture farm with cultured rainbow trout in northern Germany was analysed in summer and autumn 2020. Water samples were taken when entering (inflow) and leaving (outflow) the fish raceways. The study was based on bacterial counts to estimate the heterotrophic bacterial load and on isolation of bacterial strains by using selective growth media. Strain identification was performed by sequencing the V3-V4 hypervariable region of the 16S rDNA gene fragment. Heterotrophs abundance showed high variability and temporal variation between sampling points, which was related to water temperature changes. It tended to be higher in the outflow water compared to the inflow water during the warm period, associated with the observation of skin ulcerations, fin necrosis or exophthalmia. The opposite was observed during the cold period. Potentially fish pathogenic bacteria and fungi were detected in both periods regardless of heterotrophic bacterial loads, although fish diseases were only reported during the warm period. The method presented allows the rapid identification of bacterial pathogens that may potentially infect fish, and serves as a guide or indicator to anticipate disease events during the most problematic season of the year.

Introduction

Aquaculture offers a viable solution to secure the demand for aquatic animals and protect the wild fish stocks (Ritchie & Roser, 2021). In 2024, aquaculture production volumes exceeded the wild catches for the first time. Therefore, prioritizing environmentally sustainable practices and promoting regional production is essential for the growth of the aquaculture industry (EC, 2021). In Germany, open aquaculture systems that operate at freshwater environments are located at obtain the water from natural sources such as rivers, lakes or groundwater (Statistisches Bundesamt [Destatis], 2024). Rainbow trout (*Oncorhynchus mykiss*) is one of the mostly produced freshwater species in Germany, and in 2020, this business was responsible for approximately 2,400 employment positions (European Commission et al., 2023). Since 2021, aquaculture in Germany suffers an annual decline of 1.8%, and is still not able to cope with some problems associated with these traditional systems (Statistisches Bundesamt [Destatis], 2025). The limitations that affect Germany aquaculture industry are for instance the increase of energy and fuel prices, market competition (STECF, 2023), new regulations for animal husbandry (Luthman & Robb, 2024) and for environmental conservation (Bostock et al., 2016), which limit the use of antimicrobials or chemicals for disease prevention and treatment.

Semi-intensive open water aquaculture strongly relies on high amounts of water to supply the farmed animals and ensure an adequate oxygenation of the water. This constant need of water means that other biological agents, such as bacteria, parasites and other

animal species, enter the aquaculture system and may interfere with the cultivated fish. One major concern is the occurrence of infections, which can spread rapidly inside the farm when the fish are under stress or are stocked at high densities. Water in the rearing system has a high nutrient load due to farming activities, which favors the growth of heterotrophic opportunistic bacteria and fungi. They also colonize the surfaces (tanks and pipes) from the system by forming biofilm layers that allows a better survival against external factors and persists over longer time span (Wang et al., 2012). Several opportunistic microorganisms such as *Aeromonas* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Vibrio* sp. (Toranzo, 2004), *Acinetobacter* sp. (Kozinska et al., 2014; Zhang et al., 2023), *Staphylococcus* sp. (Çanak & Timur, 2020), and others living in the biofilm and water are capable of infecting rainbow trout and/or other freshwater fish species.

Disease outbreaks in fish farms can result in high economic losses caused by high mortality rates and devaluation of fish with visible skin lesions (Maldonado-Miranda et al., 2022). An effective disease prevention strategy is one of the major challenges of fish farmers, especially in open water intensive systems, where fish might get stressed from environmental changes and are more prone to suffer diseases (Okon et al., 2023). Farmers commonly treat the water with disinfectants as a preventive measure against bacterial and parasitic agents. Other negative impacts associated with hygienic measures and disease treatment practices include the use of antibiotics because it favours the apparition of resistance in bacterial populations. Additionally, the wastewater has a lower quality due to an increased amount of nutrients resulting from the fish metabolism and uneaten feed (Ottinger et al., 2016). The continued discharge of aquaculture effluents into the natural environment could have undesirable effects on the aquatic microbial community. Microbial communities in natural waters are characterized by the environmental conditions that vary temporally and spatially and define each habitat, although their changing patterns are often not fully understood (Atlas 1984; Nogales et al., 2007). The characterization of aquatic communities through metagenomic techniques may potentially become a non-invasive tool for anticipating disease events in the aquaculture sector, although it needs to be further developed. Additionally, this technology is often not accessible to small businesses that need quick and cost-effective solutions.

Early detection of the pathogen and rapid response is key to control a disease outbreack. Therefore, temporal monitoring of open aquaculture systems is necessary to optimize disease control and prevention measures, as well as to mitigate pathogens dispersion to the natural environment. The use of classical microbiological methods in combination with an elaborated sampling plan could serve as an economic and environmentally friendly tool for disease prevention in open water aquaculture systems. The aim of the present study was to test a non-invasive rapid methodology to detect and count potential fish pathogens during significant water temperature changes from an open through-flow aquaculture system with cultured rainbow trout.

Materials and Methods

Location and Description of the Sampling Site

The study was carried out at Forellenzucht Uhthoff GmbH (Figure 1), located at the northern side of Lake Tollense in Neubrandenburg, Mecklenburg-Western Pomerania, Germany. Lake Tollense is considered a Site of Community Importance (SCI) by NATURA 2000 network (Nixdorf et al., 2004), which implies its aquatic fauna and flora conservation. The lake is surrounded by natural forest and agricultural land, with the exception of the northern shore, which is adjacent to the city Neubrandenburg. Freshwater supply of max. 8 $m^3 s^{-1}$ is obtained from the lake Tollense via the Ölmühlenbach Canal, which flows continuously by gravity into the aquaculture raceway system. The fish farm consists of a hatchery and grow out fiberglass tanks and raceways (n=78) since the fish production spans over the entire lifecycle. Each raceway has a volume of $7m^3$ and has a constant renovation of water of several hundred $L \cdot sec^{-1}$. The fish species produced are mainly rainbow trout (*O. mykiss*), arctic charr (*Salvelinus alpinus*) and European sturgeon (*Acipenser sturio*), and from the latter they maintain a broodstock for obtaining eggs thorough the year. The fish farm usually stocks juvenile trout (appr. 50-80g in weight) at late spring. These stocked fish are obtained from polish suppliers. Fish are fattened, harvested during the year, and distributed regionally at sizes of 500 – 1000g. The farm produces around 60 to 80 tons of fish per year and operate with stocking densities of up to 11-14 $kg \cdot m^{-3}$ during warm period. Formalin 10% is commonly used to disinfect equipment and empty raceways. When the water temperature is above 10°C, fish are treated once a week with 13-15% peroxyacetic acid by directly adding the disinfectant into the flow-through raceways. The same treatment is applied for 4 to 5 consecutive days for incoming fish or fish that show disease symptoms. As disease treatment against parasites, the raceways can be separated from the main water flow, and the fish are subjected to bath treatment for 30 minutes with formalin 10%. The treatment against parasites is often applied to *S. alpinus* during autumn season.

Water and Fish Swab Sampling

To assess the problematic months of the year, which was during high water temperatures, the data was obtained from the moment when new fish entered the system until most of the stocked fish were harvested. Water and fish were sampled twice in

summer (22nd of July and 4th of August) and twice in autumn 2020 (14th September and 26th October). Samples are grouped according to the water temperature in; (1) warm period, with water temperatures ranging 17 – 23°C (from June until August), and (2) cold period, with water temperatures below 17°C (September and October). Water temperature and pH were measured inside the aquaculture farm (Table 1). The water parameters from the 9th of June and 29th of July were recorded by the fish farmers. Ten adult rainbow trout were randomly sampled from the raceway tanks at each sampling date, except on the 9th of June and 29th of July. The employers from the farm reported diseased fingerlings in one of the tanks of the installation. Therefore, 10 fingerlings were additionally sampled on the 22nd of July 2020. To minimize the animal stress level, a non-invasive technique using swabs was implemented for the detection of opportunistic microbes from the mucus of the skin.

Heterotrophic Bacterial Load

For counting the fast-growing and heterotrophic bacteria, namely opportunistic bacteria, the heterotrophic plate count method was used (Gensberger et al., 2015). Samples were taken from the inflow and from the water that collectively leaves the entire system (outflow). At least 1L of water was aseptically collected into sterile glass containers and transported to the facilities at University of Rostock. Serial dilutions (from 10^{-1} to 10^{-4}) of water subsamples (100 µl) were inoculated in triplicates on Plate-Count-Agar (Mibius e.K., Düsseldorf) and incubated for 24 hours at 23°C. A negative control for each dilution group was prepared by inoculating 100 µl autoclaved sterilized water. The total bacterial counts (CFU) were calculated by using the following formula:

$$\text{CFU} = \frac{\text{No. colonies x dilution factor (mL)}}{\text{Volume subsample inoculated (mL)}}$$

A two sample F-test for variances followed by a two-sample t-test assuming equal or unequal variances were calculated for comparing the CFU means between inflow and outflow samples. Differences were accepted as statistically different at P≤0.05. Dissimilarity and contribution percentages were obtained with Primer 7 after performing a Bray-Curtis similarity analysis across age and season groups. Contributions under 70% were cut off the analysis.

Pathogenic Microorganisms' Selection and Identification

The criteria for isolating fast-growing opportunistic fish pathogens was based on a non-invasive sampling method that allowed to identify the strains within 48 to

Figure 1. Picture of the flow-through aquaculture system Forellezucht Uhthoff, Neubrandenburg, Germany (Clols-Fuentes et al., 2024). The overview of the facility with the inflow (left), a closer view of the concrete raceways (middle) and an image of the water discharging from the raceway tanks (right).

Table 1. Information of the sampling dates and the water parameters measured. Two sampling points were considered, water inflow and outflow

	pH		Temperature (ºC)	
Date	Inflow	Outflow	Inflow	Outflow
09/06/2020	7.1	7.6	17.0	17.0
22/07/2020	7.3	7.1	19.0	19.0
29/07/2020	7.6	7.6	23.0	23.0
04/08/2020	7.4	7.4	19.7	19.7
14/09/2020	7.4	7.5	15.5	15.5
26/10/2020	6.9	6.8	12.5	12.5

72 hours, which is commonly used for disease diagnose (Crumlish, 2017). The fish skin-mucus was sampled by swabbing a 3 cm^2 region posterior to the pectoral fin with sterile Amies Agar Gel with Charcoal Transport Swabs and transported in cold conditions to the laboratory. Swabs were streaked aseptically on five different selective media plates, and in triplicates: Columbia Naladixic Acid (CNA) agar, Glutamate-Starch-Phenolred (GSP) medium, Hugh Leifson medium, Thiosulfate-Citrate-Bile Saltes-Sucrose (TCBS) agar and Enriched Cytophaga medium. A negative control group for each type of medium was prepared using sterile swabs. After a 24-48h incubation at 23°C, the colonies were morphologically recognizable. Isolates were processed for molecular analyses based on 16S DNA gene sequencing. DNA was extracted using DNeasy UltraClean Microbial Kit (Qiagen GmbH, Hilden). The V3-V4 hypervariable region of the 16S gene, commonly used for molecular identification in diagnostic studies (Janda and Abbott, 2007) was amplified by RT-PCR from gDNA extracts with universal primers 27F (5´-AGAGTTTGATCMTGGCTCAG- 3´) and 1492R (5´-GGTTACCTTGTTACGACTT– 3´). For each sample and a negative control, RT-PCR reactions were performed with 2.5 µl of forward and reverse primers, 25 µl of Taq PCR Master Mix (Qiagen GmbH, Hilden), 15 µl H_2O and 4 ng gDNA. The amplification protocol was based on an initial activation step followed by 30 cycles of denaturation step at 94°C for 30 sec, annealing step at 57°C for 30 sec and elongation step at 72°C for 1 min. Purification of the PCR products was obtained by using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden). The resulting DNA products were sent to an external laboratory (Microsynth Seqlab GmbH, Germany), which performed a Sanger sequencing of the 16S rRNA gene fragments. The resulting nucleotide segments were aligned by using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI, Bethesda USA) "16S rRNA (Bacteria and Archaea)" database.

To identify which species contribute the most to dissimilarities within groups, we performed a similarity percentages test (SIMPER) in PRIMER-e (Auckland, New Zealand). The SIMPER used two-way Bray-Curtis similarity between the groups, and obtained the average contribution of each specie to the overall dissimilarity between groups. Calculations were usedto construct the multi-dimensional scaling (MDS) graphs, which correlates values between periods and bacterial counts or species. The MDS graph allows to visualise the levels of similarities and distance between samples.

Results

This section contains the representation and analysis of the data obtained after processing the samples, as well as additional information prepared to provide with a more comprehensive understanding of the pathogens currently infecting fish.

Total Viable Bacterial Counts from Water Samples

The quantification from heterotrophic and aerobic bacteria inside the fish farm showed high variability between sampling dates (Figure 2). Water temperature ranged from 12.5°C to 23.0°C and pH from 6.8 to 7.6. Counts obtained from the inflow water samples ranged from 1x10^3 CFU/ml to 3.05x10^3 CFU/ml and from the outflow ranged from 3.3 x 10^{-3} CFU/ml to 6 x 10^2 CFU/ml. The bacterial load tended to be higher at the outflow water compared to the inflow during the warm period, especially in August. The opposite trend was observed during autumn, although no statically significant differences were obtained ($P \leq 0.05$).

Bacterial and Fungal Strains from Fish Swabs

Two fungal species unexpectantly grew on CNA-Agar and Hugh Leifson Agar, which are typically selective mediums for the isolation of the bacterial groups *Staphylococcus*, *Streptococcus* and Enterobacteriaceae. The bacterial and fungal strains that were isolated from the selective media are listed in Table 2. The bibliographic research of each microorganism showed how some of the strains were previously reported as the causative agent of a fish disease and some had probiotic properties, such as *Lactococcus lactiis*.

Potentially pathogenic bacteria and fungi sampled from the skin mucus of the *O. mykiss* were *Pseudomonas* sp., *Pseudomonas fluorescens*, *Acinetobacter* sp., *Aeromonas* sp., *Aeromonas hydrophila*, *Micrococcus endophyticus* and *Candida holmii.* The mentioned microbes were detected at a higher number from fish during warm period compared with the cold period. Species with the highest percentage contribution were *P. fluorescens* and *Pseudomonas* sp. sampled during warm period, followed by *Aeromonas* sp. sampled during warm and cold period. *Acinetobacter* sp. was also only identified at warm period. Several fish showed disease symptoms during the warm period. Skin ulcerations were observed on 5/30 of the sampled fishes (17%) and 6/10 fingerlings (60%) showed other minor symptoms, such as fin necrosis or exophthalmia.

Graphical Representation and Analysis of the Data

A metric MDS was constructed using the software PRIMER-e (version 7) with the data obtained after processing the swab samples. Each point from the metric MDS graph represents the number of colonies from each swab sample obtained from the skin mucus of an individual fish. The graph was produced only with the counts from *Pseudomonas* sp., *P. fluorescens*, *Acinetobacter* sp., *M. endophyticus*, *Aeromonas* sp. and *A. hydrophila*. Vectors representing the contribution of each bacteria species are shown in Figure 3. The total number of fish with pathogens on the skin-mucus was higher during warm period compared to cold period. The microbial community of selected potential

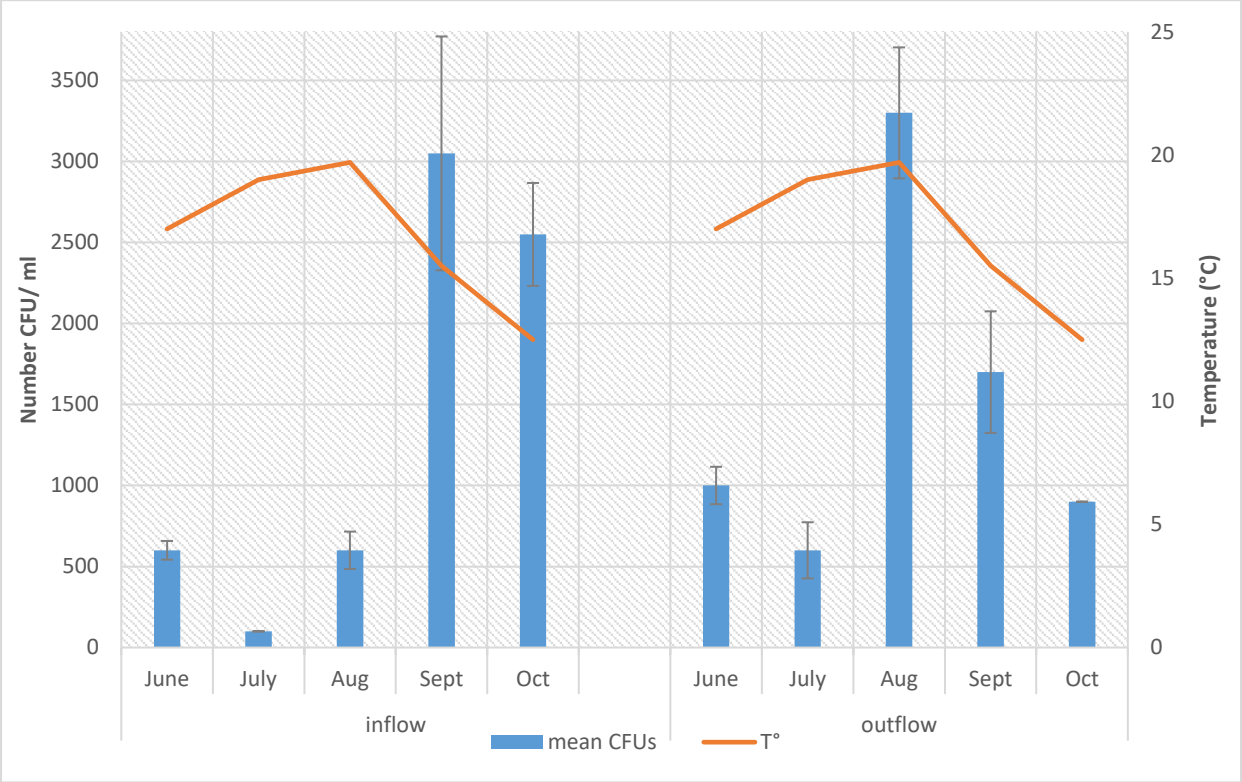

Figure 2. Bar plot representing viable bacterial count medium (CFU/ml) values for water inflow and outflow samples obtained at the fish farm Uhthoff, Neubrandenburg. Standard deviation mean is defined with black lines. Each bar corresponds to the CFU/ml counted at different months of summer and fall season; June, July, August (Aug), September (Sept), and October (Oct).

Table 2. List of bacterial and fungal strains isolated from *O. mykiss* mucus sampled from summer to autumn 2020. The total counts are the sum of the colonies counted during both seasons. Information not available; N/A

Genus/ Species	Reported disease or other properties	Total counts	References
Aeromonas salmonicida subs. *salmonicida*	Furunculosis	24	Connors et al., 2019; Aydoğan et al., 2009
A. hydrophila	Motile Aeromonas septicaemia (MAS)	10	LaPatra et al., 2010; Nya et al., 2009
Acinetobacter iwoffii	Skin ulcerations and internal haemorrhage	11	Zhang et al., 2023;Duman et al., 2020
Aureobasidium pullulans	N/A	5	N/A
Brevundimonas bullata	N/A	9	Zhou et al., 2011
Candida holmii	N/A	2	Seyedmousavi et al., 2018
Chryseobacterium sp.	Skin and tissue ulcerations	15	Bernardet et al., 2005; Loch and Faisal, 2014
Debaryomyces hansenii	N/A, Potential probiotic	1	Morales-Lange et al., 2024; Sanahuja et al., 2023
Lactococcus lactis	N/A, Potential probiotic	10	Yeganeh et al., 2021
Microbacterium oxydans	N/A, Potential probiotic	10	Ringø et al., 2006
Micrococcus endophyticus	N/A	12	N/A
Pedobacter sp.	N/A	1	Zhang et al., 2022
Arthrobacter sp.	Skin ulceration	8	Kämpfer et al., 2020
Pseudomonas fluorescens	Septicaemia	14	Shabana et al., 2022
Pseudomonas sp.	Septicaemia	15	Altinok et al., 2006; Algammal et al., 2020
Skermanella aerolata	N/A	1	N/A
Staphylococcus equorum	zoonosis	1	Oh et al., 2019; Abdel-Gawad et al., 2015
Stenotrophomonas sp.	intussusception syndrome	4	Rosado et al., 2019; Abraham et al., 2016

pathogens was relatively similar during the warm period, because all samples displayed a less dissimilarity distance, in comparison with the samples obtained during autumn (Figure 3).

The composition of the microbial community showed very high dissimilarity percentages when comparing the two age groups and seasons (Table 3). Contribution percentages of the species was higher during warm period compared to cold period, being *Pseudomonas* sp*., P. fluorescens* and *M. endophyticus*, in descending order, the predominant candidates during warm period and *Aeromonas* sp. during autumn (Table 3).

Discussion

This study was conducted before, during and after the most critical season for an open flow-through aquaculture system with *O. mykiss* in northern Germany. To do so, the research was conducted during the months when fish are more prone to develop infections or lesions, particularly in August and early September depending on the climatic conditions of the year. Classical bacteriological methods were used to quantify the heterotrophic bacterial load from the water that enters the system (inflow) and that exits the system (outflow), and to identify potentially pathogenic microorganisms. Methods were chosen due to their simplicity, cost-effectiveness, and quick obtention of results. Owning the increasing efforts to study the infection and transmission biology of fish pathogens in aquaculture environments, the presented data may be applicable in the near future for modelling the fish disease dynamics in cage culture systems (de Blas et al., 2020; Ögüt, 2001).

The sampled aquaculture system exemplifies the fluctuations of the heterotrophic bacterial load in relationship to water temperature (Figure 2). Two phases were observed in bacterial growth dynamics. On the one hand, from June to August, the bacterial counts of the inflow water tended to be lower than of the outflow water. Changes in water quality and its bacterial community have been documented when aquaculture activities depend on the natural environments (Dunne et al., 2021; Zhang et al., 2020). The enrichment of the water nutrients, with higher load of particulate organic matter, nitrogen, and phosphate, is often associated with fish feeding events and the fish metabolism originated from farming activities (Shireman & Cichra, 1994). A previous study on the water microbiome from

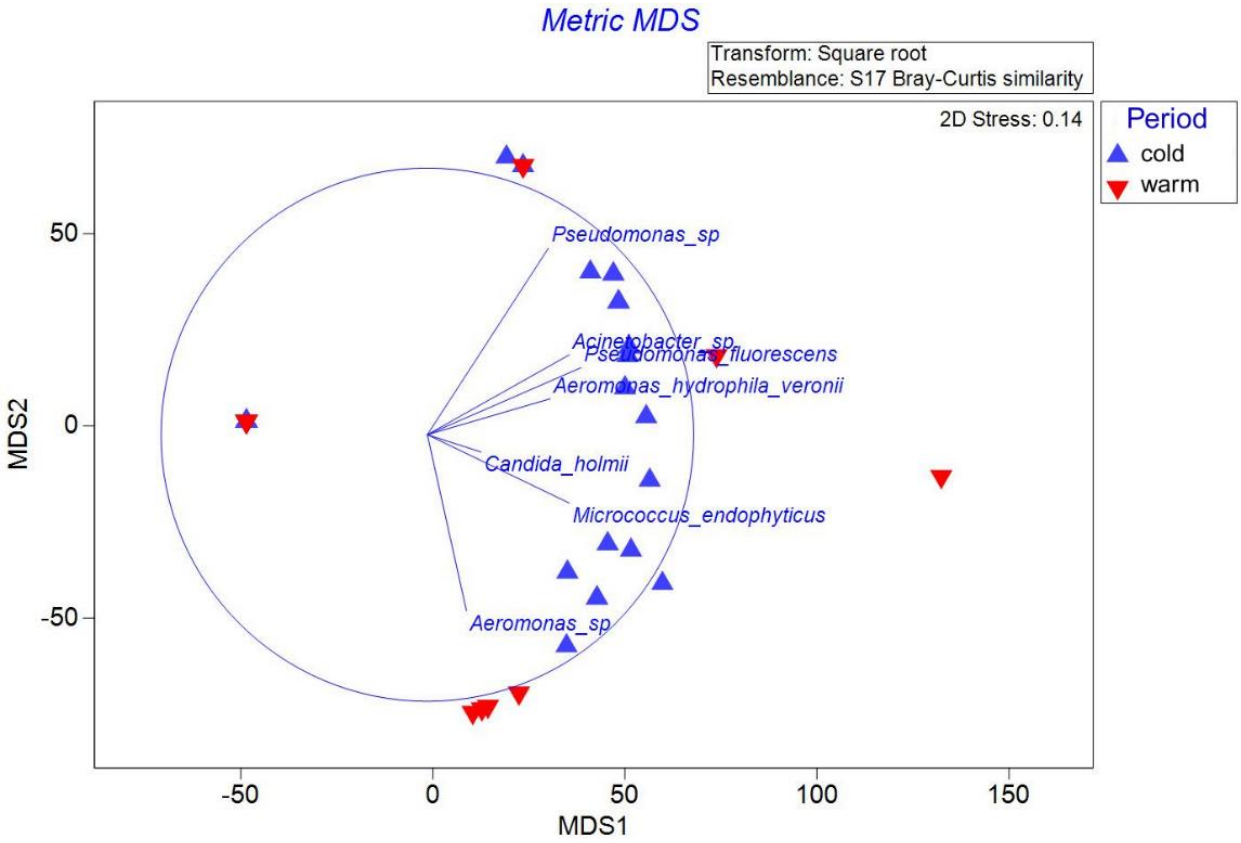

Figure 3. Metric Multidimensional scaling (mMDS) plot of mucosal bacteria and fungi detected on fish from Neubrandenburg. Each point represents one fish in which pathogenic microorganisms were identified. Blue points belong to the cold period, and red points to the warm period. The plot indicates simple matching similarity between samples as ordinated with dimensions k=2 and stress=0,14. Vectors are calculated based on Pearson correlation between samples. They represent the contribution percentage of each species (Pseudomonas sp., P. fluorescens, Acinetobacter sp., M. endophyticus, Aeromonas sp. and A. hydrophila) in all samples.

the same farm also showed modulations between the inflow water entering the system and the water effluent (Clols-Fuentes et al., 2024). This study reported at a specific time point an increase on heterotrophic groups at the effluent compared to inflow water, as well as a higher proportion of *Pseudomonas* sp. These observations concur with the results obtained in the present study, where the microbial pathogens were more commonly detected during the warm period (Table 3). In total, seven potentially pathogenic microorganisms were identified at the warm period, and only three at the cold period (Figure 3). Pathogenic microbes are also known as opportunistic bacteria because of their highly efficiency on populate resource-rich environments, and most commonly use C and N sources (Sigee, 2004). Most of the documented fish pathogens benefit from higher temperatures and nutrients, since they have an heterotrophic metabolism (Duman et al., 2020; Kämpfer et al., 2020; Zhao et al., 2020; Tello-Martin et al., 2018; Vadstein et al., 2018). Additionally, the raceways have large surface areas where pathogens can colonize and form a stable biofilm layer that resists unfavourable conditions and persists over longer time spans (Schoina et al., 2022). The genus *Pseudomonas* was one of the predominant species encountered, with contribution percentages of 82% in the cold period and 27.64% in the warm period (Table 3). A high occurrence of *Acinetobacter* sp., which causes infections to various fish species including rainbow trout (González-Palacios, 2020; Gram, 1999), was also detected during the warm period consequently, it is being suggested that the increase of water temperature (during the warm period) and nutrients inside the production unit may promote the growth of the heterotrophic and pathogenic microbes.

During a second phase between September and October, the effluent water had less heterotrophic bacteria compared with the inflow water. High bacterial loads in the inflow at the end of the summer and beginning autumn could be caused through high amounts of decomposing bacteria that decay biomass from algae blooms and plant growth during the year (Sharma et al., 2019). During the studied months, disinfection treatments are routinely applied in the water to eliminate pathogenic agents. These treatments may have resulted in the reduction of the parasitical and bacterial load after being applied consecutively, as we observed during the months of September and October (Figure 2). Despite disinfection measures, the fish remained susceptible to multiple infections.

The risk of infection in aquaculture is mostly attributed to different factors, such as high stocking densities, poor water quality, or unpredictable temperature variations (Clark et al., 2021). Firstly, intensive farming systems stock fish at higher densities, so fish are continuously in contact with each other. Thus, high stocking density and the coinfection with other pathogens such as parasites, is a factor that can magnify and accelerate infections rates (Okon et al., 2023). A seasonal trend was observed in a parasitological study from the same aquaculture facility with *O. mykiss* (Unger et al., 2022). Fish were infested with nine different metazoan parasite species during summer, while in April and November of the same year only four metazoan parasite species were found in the same batch of fish. Secondly, the toxicity of PAA and formalin is higher at low alkaline waters and low temperatures (Kunigk et al., 2001), which could act as a stressor that compromises the fish immune refenses against pathogens (Mota el al., 2022; Straus et al., 2018; Pedersen et al., 2007; Chinabut et al., 1988). It could be questioned if this disinfection might be required at lower temperatures to avoid negative effects onto the adult and/or juvenile fish health. Additionally, the reduction of heterotrophs at the outflow and disinfectant residuals could affect the microbial community from the natural water body by altering the nutrient cycle and/or promoting the apparition of pathogens that are resistant to treatments with different substances and drugs (Rozman et al., 2021; Sahulka et al., 2021).

In aquaculture, the water temperature is a decisive factor that interacts with the levels of dissolved oxygen (DO), pH, nutrients cycle, the bacterial community's composition, and the fish physiological stability. Higher water temperature reduces the DO levels (Rajesh & Rehana, 2022), which in combination cause physiological stresses on the fish (Walakira et al., 2014).

The higher the heterotrophic bacterial load, the less DO is available since these bacteria also consume oxygen. A higher bacterial load combined with a low DO and high ammonia values are often attributed to lower fish survival rates and a higher risk to experience disease

Table 3. Contribution (Contr%) percentages of the potentially pathogenic bacterial and fungal species sampled from the fish mucus of the skin surface

	Contribution (%)					
Species	Adult	Fingerling	Contr% across age	Summer	Fall	Contr% across seasons
Pseudomonas sp.	35.98		13.60	27.64	82.00	30.35
Pseudomonas fluorescens	22.70		15.69	22.53	0	16.51
Acinetobacter sp.	13.98			14.71	5	15.56
Aeromonas sp.		31.46	26.11	10.29	85	16.25
Aeromonas hydrophila				14.71	0	
Micrococcus endophytics		50.30	23.66	17.61	0	
Average dissimilarity			**90.17**			**95.62**
Average similarity	17.02	23.24		25.72	4.41	

outbreaks (Gamoori et al., 2023; Hossain et al., 2021). Rainbow trout tolerate narrow ranges of dissolved oxygen and temperature, so DO levels below 5.0-6.0 g/L and temperature above 24°C are critical for normal metabolic activities (Stiller et al., 2017). Under such conditions the fish are more vulnerable to opportunistic infections, which can rapidly spread and affect a large part of the fish population (Manchanayake et al., 2023; Mramba and Kahindi, 2023). Similarly, in another case from trout raised in a river, bacterial pathogens were also reported during summer (Delghandi et al., 2020). The authors suggested, that temperature might act as a main stressor factor that can lead to subsequent disease outbreaks. At the presented study, more juvenile fish (around 60%) had disease symptoms compared with the adults. The use of disinfectants in the water to reduce the bacterial load might be effective for adult fish, but could impair juveniles. Early stages of development are more susceptible when exposed to chemicals than adults (Mohammed, 2013), so disease preventive treatments must be adjusted to each fish age, species and season. Juveniles showed some body lesions that could be attributed to an infection with *Aeromonas* sp. or *Micrococcus endophytics*. It is of great difficulty to link a specific disease to the symptomatic fishes reported, since several opportunistic bacteria were simultaneously isolated from the samples. Nevertheless, the information obtained in this study could serve as a guide to identify the bacterial species with the highest likelihood of causing infections and to define the time periods when disease events are most likely to occur.

Finally, the findings may provide valuable insights with implications for environmental monitoring and ecosystem conservation. Fish farms act as reservoirs for different pathogenic agents, that can be transferred to the wild fauna and the natural environment (Muziasari et al., 2017). In most of the intensified systems it has been reported a negative impact to the microbiome, fauna and flora of the natural reservoir where wastewater is discharged (Marmen et al., 2021). In other cases, an impact attributed to the farming activities was very low and seasonal variations were the main drivers defining the water microbiome dynamics (Marmen et al., 2021). Indeed, the potential impacts that freshwater aquaculture systems have to the microbiome of the natural reservoirs are difficult to establish, and depend on the nature of the water body. Since some microbial taxonomic groups tended to change in abundance between inflow and outflow, but the differences were not significative ($P \leq 0.05$) (Clols-Fuentes et al., 2024), it is suggested that aquaculture activities had minimal impact on the water microbiome's functionality. However, further investigations of the fauna and microbial community from the studied region should be considered to determine if such aquaculture activities damage the surrounding ecosystem. Integrating the presented bacteriological monitoring into the farm´s preventive strategy plan, could aid in anticipating major disease events and contribute to reduce and adapt water disinfection treatments.

Conclusion

Our results showed that the heterotrophic bacterial load, presence of pathogens and apparition of diseased fish followed a seasonal pattern in relationship to water temperature fluctuations. Changes in bacterial abundances and pathogenic community inside the fish farm were notably more pronounced during the warm period. It is suggested that the water source, the water temperature and disease controlling measures of the farm could have decisive effects onto fish health. Frequent microbial monitoring can support the farmers understanding of the natural bacterial fluctuations and threads to their fish, and coping with their efforts to reduce chemical/disinfectants input while improving disease preventive strategies.

Ethical Statement

The work presented is original, and its research is conducted in a way that respects the rights, dignity, and welfare of the participants. Fish were manipulated according to the German regulations for animal protection, namely "Animal Welfare Act in the version published on 18 May 2006 (Federal Law Gazette I p. 1206, 1313), last amended by Article 2 (20) of the Act of 20 December 2022 (Federal Law Gazette I p. 2752)."

Funding Information

This work was supported by the European Fisheries Fund (EMFF) and the Ministry of Agriculture and Environment from Mecklenburg-Western Pomerania as part of the project Hygiene Management and Health Concept for surface water-dependent partial circulation systems in M-V (project grant number MV-II. 12-LM-03).

Author Contribution

Conceptualization, J.C.F, P.U. and H. P.; methodology, J.C.F.; software, J.C.F.; validation, P.U. and H.P.; formal analysis, J.C.F. and P.U.; investigation, J.C.F.; resources, P.U. and H.P.; data curation P.U.; writing – original draft preparation, J.C.F.; writing – review and editing, P.U. and H.P.; visualization, J.C.F.; supervision, P.U.; project administration, P.U.; funding acquisition, P.U. and H.P. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dr. Regina Dibbert for its technical and educational support for acquiring the mentioned laboratory methodology. We appreciate the support of Mr. Uhthoff for being in closely cooperation with the investigations conducted at his company.

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