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Low-Dose Formalin Treatment for *Gyrodactylus* sp. Infection in Rainbow Trout (*Oncorhynchus Mykiss*) Cultured in a Low-Head Recirculating Aquaculture System in Vietnam

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Abstract

Recirculating aquaculture systems (RAS) for rainbow trout (*Oncorhynchus mykiss*) have been widely implemented worldwide. However, their adoption in Vietnam has occurred only over the past decade and remains limited due to several operational challenges. Among these, disease management is particularly difficult because chemical treatments can disrupt the microbial community within the biofilter. This study assessed the effectiveness of a low-dose formalin treatment for controlling *Gyrodactylus* sp. infection in rainbow trout reared in a recirculating aquaculture system (RAS). During a mortality episode associated with *Gyrodactylus* sp. infestation, formalin was applied at concentrations of 15-20ppm for 10h per day over three consecutive days. Water-quality parameters, parasite infection intensity, and fish mortality were systematically monitored throughout each treatment period. The results indicated that after three days of treatment, fish mortality significantly decreased, while parasite infection dropped 95.3-96.7%. The results also revealed fluctuations in water quality parameters, including temporary increases in COD, N-NH_4^+, and N-NO_2^- concentrations, associated with a decline in nitrifying microbial activity. Nevertheless, these changes remained within acceptable limits and did not cause severe system disruption. The findings demonstrate that low-dose formalin treatments effectively reduce mortality in infected rainbow trout without severely impairing the beneficial microbial community in RAS.

Keywords

Low dose formalin, *Gyrodactylus sp*, rainbow trout, low-head RAS

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Introduction

Currently, rainbow trout (*Oncorhynchus mykiss*) farming in Vietnam is primarily conducted in flow-through systems, in which the supplied water is used only once before being discharged. This method is highly dependent on the availability and quality of incoming water to maintain optimal rearing conditions. However, water shortages during the dry season and flooding during the rainy season pose significant challenges to large-scale commercial operations, as rainbow trout require clean and stable aquatic environments. Recirculating aquaculture systems (RAS) have been shown to be an effective solution for aquaculture, particularly in areas where environmental conditions are unsuitable for traditional systems. RAS technology enables the recycling and reuse of water, supports high-density fish culture, reduces land and water requirements, and is particularly advantageous in water-scarce regions. Over the past few decades, there has been a substantial global increase in aquaculture facilities adopting RAS technology. The adoption of RAS aims to minimize environmental impacts compared to conventional fish farming systems by mastering water management, enhancing environmental biosecurity, and improving feed conversion efficiency (Midilli *et al.*, 2012; Ahmed & Turchini, 2021). The application of RAS has also been shown to improve fish growth rates and disease control (Verdegem *et al.*, 2006; Espinal & Matulić, 2012).

Gyrodactylus sp. is a monogenean ectoparasite commonly found on the skin, fins, and gills of fish. These parasites reproduce rapidly through viviparity (live birth) and are capable of causing epizootics under high-density rearing conditions. Heavy infestations can lead to epithelial damage, hemorrhaging, increased mucus secretion, and immunosuppression in infected fish (Buchmann & Bresciani, 2006; Noga, 2010). In Vietnam, three parasitic species, namely *Gyrodactylus* sp., *Trichodina acuta*, and *Ichthyophthirius multifiliis*, have been identified in rainbow trout reared in RAS facilities in Lam Dong Province (Vo & Tran, 2011). Data from the Coldwater Aquaculture Research Center (2016-2018) indicated that among 1,267 sampled fish, the overall prevalence of monogenean infection was 22.6%, while 18% were infected with *Trichodina*. Mortality rates in rainbow trout farms associated with monogenean infestations were estimated at 20-35% (Chi *et al.*, 2019). *Gyrodactylus* spp. have been reported to infect various freshwater cultured fish species in Vietnam. High prevalences were observed in fingerling grass carp (*Ctenopharyngodon idellus*) in the North (40%) and in *Oreochromis* spp. in the South (45%) (Thuy, 2005; Nguyen & Campet, 2009; Van *et al*., 2015). In contrast, a much lower prevalence (1.3%) was recorded in farmed carp (*Cyprinus centralus*) in the central region (Te *et al*., 2010). Moreover, according to the Vietnam Aquatic Animal Disease Report – 2022, *Gyrodactylus* sp. infection has also been officially reported in rainbow trout farmed in Vietnam (WOAH, 2023). Previous studies have identified two species, *Gyrodactylus taimeni*, and *Gyrodactylus magnus*, in rainbow trout samples collected in Ban Khoang, Sa Pa, Vietnam (Leis *et al*., 2021).

Formalin is a widely used chemical agent for disease control and treatment in hatcheries due to its versatility and effectiveness, particularly in managing diseases infecting skin, fins, and gills (Eric *et al*., 2023). However, exposure to formalin can be lethal to eggs and fry if inappropriate concentrations or dosages are applied. Previous studies have reported that different strains within the same fish species, as well as various developmental stages, may exhibit differing sensitivities to formalin (Fetherman *et al.*, 2023). The effective dosage of formalin depends on both the duration of exposure and the tolerance of the cultured fish species (Tavares, 2021). Typical treatment concentrations range from 125 to 250 ppm for up to one hour (Piper *et al*., 1982), although concentrations as high as 400ppm have been used in acute toxicity tests (Wedemeyer *et al.*, 1971; Howe, 1995). The future use of formalin is expected to decline significantly due to environmental and occupational safety concerns. Residual formalin discharged after treatment can enter receiving waters, where it may harm natural microbial communities (Hohreiter & Rigg, 2001; Masters, 2004; Wooster *et al.*, 2005; EU Biocide Product Directive, 2008). Although numerous studies have investigated the use of less toxic and more environmentally friendly alternatives, no effective replacement for formalin has yet been successfully implemented in RAS. This limitation is primarily due to the reduced efficacy of alternative treatments and their potential negative effects on biofilter performance (Madsen *et al.*, 2000; Schwartz *et al.*, 2000; Rintamaki *et al.*, 2005).

Recent laboratory studies have demonstrated that low concentrations of formaldehyde applied over extended exposure periods can effectively control *Ichthyophthirius multifiliis* and *Gyrodactylus* sp. (Heinecke & Buchmann, 2009). These findings are scientifically significant, as they support the use of lower disinfectant doses that minimize harmful waste generation (Masters, 2004; Gearheart *et al.*, 2006). This approach is particularly suitable for RAS, where water quality is stable and hydraulic retention time is high. However, the long-term and repeated effects of formaldehyde exposure on biofilter performance in RAS remain insufficiently studied. Fredricks *et al.* (2022) applied a commercial formaldehyde solution (Parasite S®) at a concentration of approximately 40 mg L^{-1} (equivalent to ~14.8 mg L^{-1} formaldehyde) for four consecutive days in a RAS, reporting no fish mortality but a temporary reduction in biofilter nitrification efficiency. Pedersen *et al.* (2010) further reported that the formaldehyde removal rate was positively correlated with both treatment frequency and dosage. In systems regularly treated with low-dose formalin, removal rates increased tenfold from 0.19 ± 0.05 to 1.81 ± 0.13 mg $(L\cdot h)^{-1}$. Nitrification performance remained unaffected in systems treated daily with formalin compared to the untreated controls. The relative abundance of ammonia-oxidizing bacteria (AOB) reached 5.4% of the total eubacterial cells, predominantly *Nitrosomonas oligotropha*. Nitrite-oxidizing bacteria (NOB), mainly *Nitrospira* spp., were detected in all biofilm samples at up to 2.9%, whereas *Nitrobacter* spp. were absent.

This study evaluated the effectiveness of formalin treatments for controlling *Gyrodactylus* sp. infections in rainbow trout cultured under intensive conditions in a low-head RAS system in Vietnam.

Materials and Methods

Experimental design and system configuration

The study was carried out from March 2023 to June 2025 to assess the effects of low-dose formalin on rainbow trout (*Oncorhynchus mykiss*) reared in a recirculating aquaculture system (RAS). The study was not designed as a formal experiment but was conducted as an empirical evaluation under real-world farming conditions. Due to the availability of only a single RAS unit, independent triplicate experimental replicates could not be established. Consequently, the assessment was based on three independent treatment events recorded during the monitoring period.

The recirculating aquaculture system (RAS) was built at the Research Center for Coldwater Aquaculture, O Quy Ho ward, Sa Pa commune, Lao Cai province. This is the first low-head recirculating aquaculture system established for cold-water aquaculture in Vietnam. The system was designed and constructed by Arvotec Ltd. (Finland) and consists of 150m^3 of rearing tanks and 48m^3 of biofiltration units. The designed production capacity is approximately 7,500kg of market-size rainbow trout (*Oncorhynchus mykiss*) per year, with a stocking density of 45-55 fish m^{-3} or 52-55 kg m^{-3} at harvest (Chi *et al.*, 2024). The system is supplied with make-up water at a rate of 0.5 L s^{-1}, as specified in the design. The inlet water source originates from a natural spring in the Silver Waterfall, Sa Pa commune, Lao Cai province. The inflow water, which is used to compensate for operational losses, passes through a coarse filtration system.

Water quality parameters were monitored weekly under normal conditions. During disease outbreaks, water samples were collected daily before and after the treatment period. Individuals showing abnormal clinical signs were collected for pathogen examination.

Analytical methods

Water quality parameters

Water temperature, pH, and dissolved oxygen (DO) were measured in situ using a multiparameter probe (YSI Pro 1020, USA). Chemical parameters, namely ammonium (N–

NH_4^+; SMEWW 4500-NH_3 B,F:2017), nitrite ($N–NO_2^-$; SMEWW 4500-NO_2^- B:2017), nitrate ($N–NO_3^-$; SMEWW 4500-NO_3^- E:2017), chemical oxygen demand (COD; SMEWW 5220C:2017), and hydrogen sulfide (H_2S; SMEWW 4500-S^{2-} BD:2017), were analyzed following the standard methods described by Baird and Bridgewater (2017).

Microbial analysis

Total aerobic bacterial counts in the water samples were determined using the standard plate count method on plate count agar (PCA; Merck, Germany). Briefly, 0.1mL of serially diluted samples (prepared with sterile phosphate-buffered saline, PBS, pH 7.4 ± 0.2) was spread on PCA plates and incubated at 28 °C for 24-48h. Colony-forming units were enumerated and expressed as CFU mL^{-1}.

Parasitological examination

Moribund or recently dead fish were collected for parasite detection and identification. Fresh mucus and gill smears were prepared and examined microscopically according to the method of Bui Quang Te (2006). Smears were observed under a Nikon E200LED optical microscope (Nikon, Japan) at 4× and 10× magnification to determine the prevalence of *Gyrodactylus* sp. infections. Fish were sampled only when abnormal behavior or clinical signs were observed. A total of 121 fish were collected over the two culture cycles.

Low-dose formalin treatment during disease outbreaks

The low-dose formalin treatment was applied using the long-bath immersion method, following the approach described by Pedersen *et al.* (2010). When *Gyrodactylus* sp. infections were detected, fish were treated with formaldehyde (Xilong Scientific Co., Ltd; Batch No. 2308092 & 240527A2) at a concentration of 15-20ppm, depending on fish size. Formalin was applied directly into the RAS at the designated low concentration and maintained for 10 hours. After treatment, make-up water was supplied at a rate of 0.5 L s^{-1}, as specified in the RAS design, to dilute and replace the system water gradually. Treatments were performed 2-3 times at 24-hour intervals.

Evaluation of treatment effectiveness

The abundance of *Gyrodactylus* sp. on the skin and gill smears was quantified to compare parasite loads before and after the formalin treatment. Furthermore, fish mortality rates were analyzed prior to and seven days post-treatment to assess the efficacy of the formalin baths in mitigating parasite-associated losses.

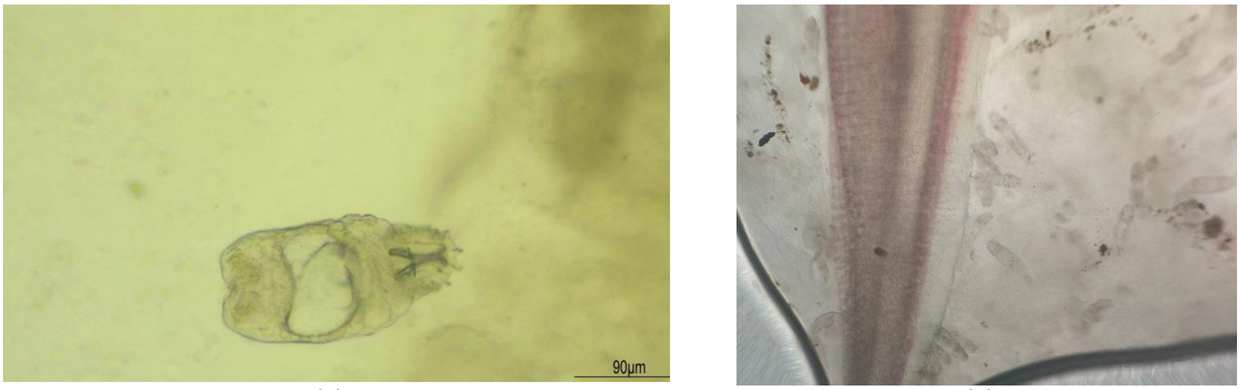
Statistical analysis

Differences between the pre-treatment and post-treatment groups were analyzed using SPSS version 23. Data were first tested for normality and homogeneity of variance. When the data met the assumptions of normal distribution, a T-test was applied; otherwise, nonparametric tests were used for statistical analysis. Differences in parasite density on rainbow trout among treatment times were analyzed using the Kruskal–Wallis test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Identification of parasitic agents causing disease in rainbow trout cultured in recirculating aquaculture systems

A total of 121 rainbow trout (*O. mykiss*) showing weak swimming behavior and moribund conditions, were collected over 27 months from the experimental grow-out trials conducted in the RAS for parasitological examination. During the experiment, the primary ectoparasite associated with fish mortality in the RAS-cultured rainbow trout was identified as *Gyrodactylus* sp. (Monogenea), which was predominantly found on the skin, gills, and fins, and caused chronic epithelial damage (**Figure 1**). Disease outbreaks were commonly observed after the addition of new water from natural streams, particularly during the rainy season, suggesting a potential infection risk from an unsterilized water source. The infection frequency increased markedly following heavy rainfall or periods of high water inflow, indicating that natural water sources constitute a major risk factor for parasite transmission to RAS.



(A) (B)

Figure 1. *Gyrodactylus* sp. parasitizing the (A) skin and (B) gills of rainbow trout (*O. mykiss*) cultured in a low-head RAS.

Mortality rate and effectiveness of the low-dose formalin treatments

Between 2023 and 2025, three outbreaks of *Gyrodactylus* sp. parasitic infection were documented in experimental batches of rainbow trout reared in a recirculating aquaculture system (RAS). The outbreaks differed in their timing and the size of affected fish. The body weight of infected fish ranged from fingerling (40.4 g) to adult size (1000g) (**Table 1**). Adult fish were treated at a higher dose of 20 mg L^{-1}, whereas smaller fish were treated with 15 mg L^{-1}.

Parasite infection outbreaks coincided with the rainy and flood season (June to August), a period during which fish are most susceptible to disease. In Vietnam, water used for rainbow trout farming primarily originates from mountain streams, which carries a high risk of parasite contamination, especially during the rainy season. Within RAS facilities, the use of chemical antiparasitic treatments such as formalin has proven effective in disease control; however, improper application can pose risks to water quality, biofilter performance, and fish health. Therefore, the management of incoming water quality is a key preventive measure. Moreover, stable and low water temperatures, such as those provided by groundwater sources, can help limit parasite population dynamics, thereby reducing the likelihood of disease outbreaks compared with systems relying on river water, which is subject to greater temperature fluctuations (Kunze *et al.*, 2009).

The results of this study demonstrated that the use of low-dose formalin (15-20 mg L^{-1}) through a prolonged bath treatment is an effective and safe method for controlling *Gyrodactylus* sp. infections in rainbow trout cultured in a RAS. Applying formalin at the early onset of infection symptoms significantly reduced mortality rates. Specifically, mortality decreased from 6.7% in 2023 (**Table 1**). In 2024, treatment was applied at an early stage of the outbreak, when mortality reached approximately 2%, resulting in improved effectiveness and reduced losses. Similarly, in 2025, the mortality rate declined to only 0.8%. These findings indicate that rainbow trout can be infected by *Gyrodactylus* sp. at various growth stages, ranging from a small size (42 g $fish^{-1}$) to market size (around 1 kg $fish^{-1}$). Treatment efficacy was enhanced when formalin was applied in a repeated regimen (2-3 applications at 24-hour intervals), which provided better control over parasite prevalence on the fish. In addition to reducing mortality, the parasite load on the skin and gills declined markedly within a few hours after the first treatment. Furthermore, early detection of parasitic infection and timely intervention were critical factors contributing to treatment success, as fish mortality ceased after two treatment applications.

Parasite density before and after the formalin treatment

Microscopic examination showed a significant reduction in *Gyrodactylus* sp. density following low-dose formalin treatment. Parasite loads decreased markedly after 3 hours of exposure and continued to decline further after 10 hours compared with pre-treatment levels

Table 1. Mortality rates and effectiveness of the formalin treatments in rainbow trout infected with *Gyrodactylus* sp. in a RAS (2023-2025)

Year	Outbreak period	Average fish weight (g $fish^{-1}$)	Total stock (fish)	Number of deaths (fish)	Mortality rate (%)	Formalin dose (mL m^{-3})	Number of treatments	Post-treatment outcome
2023	August	700.2 ± 5.6	6,750	450	6.7	15	3 times (every 24h)	Mortality ceased after 3 days
2024	July	40.4 ± 3.2	7,500	152	2.0	15	3 times (every 24h)	Mortality ceased after 3 days
2025	June	1.000 ± 121	6,535	55	0.8	20	3 times (every 24 h)	Mortality ceased after 2 days

Note: The fish weight data are presented as mean ± SD.

(P <0.05; **Table 2**). These results indicate that *Gyrodactylus* sp. is highly sensitive to formalin, with parasite abundance significantly reduced after 10 hours of exposure.

Variation of the environmental parameters

The water quality parameters, namely temperature, DO, pH, COD, $N–NH_4^+$, $N–NO_2^-$, H_2S, $P–PO_4^{3-}$, and $N–NO_3^-$, showed slight variations but remained within acceptable limits throughout the treatment process for *Gyrodactylus* sp. infection in rainbow trout cultured in the RAS (**Table 3**). The analysis of the water quality parameters before and after the low-dose formalin treatment showed that most indicators remained stable and within acceptable limits for rainbow trout culture. DO, temperature, pH, and $N–NO_3^-$ did not change significantly (P >0.05), indicating that the formalin applications did not adversely affect basic water conditions. However, COD, ammonium ($N–NH_4^+$), and nitrite ($N–NO_2^-$) levels increased significantly after treatment (P < 0.05), suggesting a temporary rise in organic load and nitrogenous compounds following formalin exposure. Total aerobic bacterial counts decreased significantly (P <0.05), likely due to the disinfectant effect of formalin. The results indicate that low-dose formalin (15-20 mg L^{-1}) caused fluctuations in water quality, which may have temporarily influenced the performance of beneficial bacteria involved in nitrogen and organic matter processing. However, these effects were not detrimental to the overall stability of the RAS environment. Low-dose formalin applications in recirculating aquaculture systems have been shown to be a feasible treatment strategy as they exert minimal effects on nitrification processes. Because formaldehyde is gradually degraded by microorganisms, factors such as biofilter surface area, hydraulic retention time, and temperature can be used to predict its removal dynamics (Pedersen *et al.*, 2010).

Rowland *et al.* (2006) reported that exposure to formalin concentrations ≥ 30 mg L^{-1} at 24.1-26.9°C resulted in a sharp decrease in dissolved oxygen (DO) levels, from 10.1-11.9 mg L^{-1} down to 3.0-3.3 mg L^{-1} and 1.2-1.7 mg L^{-1} within 36-42 hours of treatment. Additionally, the pH level declined from 7.2 to 8.4 to 6.3-6.7 within 36 hours, and turbidity decreased after 48 hours. In our study, formalin concentrations ranging from 15 to 20ppm did not have adverse effects on the water quality parameters. This finding indicates that the concentration of formalin plays a crucial role in determining treatment effectiveness. Previous studies have reported that formalin exposure caused severe stress in silver perch (*Bidyanus bidyanus* Mitchell) (Rowlan *et al.*, 2006). However, in the present study, no significant adverse effects of formalin at concentrations of 15-20ppm were observed in rainbow trout. This may be attributed to the adequate oxygen supply maintained continuously throughout the treatment process, which helped minimize stress responses in the fish.

One of the major concerns regarding the use of chemical treatments in RAS is their potential impact on beneficial microorganisms in the biofilter, particularly those involved in the nitrification process, which is critical for nitrogen waste management. In this study, the total bacterial counts slightly decreased after the formalin treatment; however, the difference was

Table 2. Parasite density on rainbow trout before and after the low-dose formalin treatment

Observation time	Average parasite density (parasites per microscopic field)		
	2023	2024	2025
Before treatment	$150^a \pm 45$	$125^a \pm 26$	$120^a \pm 15$
After 3 hours of treatment	$42^b \pm 8$	$28^b \pm 6$	$20^b \pm 3$
After 10 hours of treatment	$7^c \pm 3$	$5^c \pm 3$	$4^c \pm 2$

Note: Data are presented as mean ± SD. Different superscript letters represent statistically significant differences of means within the same column at P <0.05.

Table 3. Environmental parameters pre- and post-treatment with low-dose formalin

Parameters	Unit	Pre treatment	Post treatment
DO	mg L^{-1}	$6.32^a \pm 0.13$	$6.21^a \pm 0.30$
Temperature	°C	$18.15^a \pm 0.10$	$18.22^a \pm 0.32$
pH		$7.41^a \pm 0.10$	$7.12^a \pm 0.28$
COD	mg L^{-1}	$1.2^a \pm 0.05$	$4.8^b \pm 0.13$
$N–NH_4^+$	mg L^{-1}	$0.713^a \pm 0.003$	$1.33^b \pm 0.01$
$N–NO_2^-$	mg L^{-1}	$0.031^a \pm 0.001$	$0.129^b \pm 0.045$
H_2S	mg L^{-1}	ND	ND
$P–PO_4^{3-}$	mg L^{-1}	0.049 ± 0.01	0.048 ± 0.02
$N–NO_3^-$	mg L^{-1}	$3.66^a \pm 0.04$	$4.08^a \pm 0.01$
Total aerobic bacteria	CFU mL^{-1}	$3.9 \pm 0.52^a \times 10^4$	$1.8 \pm 0.56^b \times 10^4$

Note: Data are presented as mean ± SD, ND: not detected. Different superscript letters represent statistically significant differences of means within the same row at P <0.05.

not statistically significant, suggesting that biofilter microbial activity was not substantially affected. Pedersen *et al.* (2010) reported that under steady-state conditions, formaldehyde removal rates varied between 5-25 mg $(m^2 \cdot h)^{-1}$. Fluctuations in biofilter performance reflect the adaptation of formaldehyde-degrading microorganisms, which can persist within the biofilm and utilize other organic substrates. Formaldehyde has no clear effect on ammonia-oxidizing bacteria (AOB), but it may inhibit nitrite oxidation at concentrations $\geq$ 40-60 mg L^{-1}. Repeated treatments can reduce nitrification efficiency, leading to elevated nitrite levels; therefore, nitrite concentrations should be closely monitored in RAS during multiple or prolonged formalin applications (Kech & Blanc, 2002). Fredricks *et al*. (2022) reported that under a formalin treatment of 40 mg L^{-1}, the volumetric TAN removal rate in a cold-water RAS decreased significantly and remained below pre-exposure levels, while nitrite removal was largely unaffected. However, changes in the slope and intercept indicated an impact on nitrifying bacteria. Despite the reduced removal rates, no fish mortality was observed after four consecutive indefinite-bath treatments.

A key finding of this study is the critical role of the make-up water as a potential vector for pathogen introduction. In Vietnam, most aquaculture facilities, especially in the northern mountainous and Central Highlands regions, use surface water from streams, reservoirs, or rivers that is not disinfected by UV sterilization or biofiltration. In contrast, modern RAS facilities in Europe typically utilize deep groundwater, which provides stable temperatures and low pathogen loads. In Vietnam, surface water sources are highly influenced by weather fluctuations, especially following heavy rainfall, and can introduce parasite pathogens such as *Gyrodactylus* spp. and *Ichthyophthirius* spp. through mud, wild animals, or nearby farms. These factors likely contributed to the disease outbreaks observed in this study.

Therefore, pre-treatment of make-up water using UV sterilization, sand filtration, or mild chlorination should be considered mandatory, particularly for commercial rainbow trout farming in cold-water regions. Moreover, introducing infected juvenile fish from traditional earthen ponds into modern RAS poses significant risks, as pathogens can spread rapidly within the closed system due to (1) difficulties in control once infection occurs, and (2) losses in both production efficiency and treatment cost. As emphasized by Katrina *et al.* (2022), the use of pathogen-free broodstock and clean, stable water sources are essential to optimize production efficiency, extend grow-out periods, and minimize the need for chemical treatments.

Conclusions

The application of low-dose formalin (15-20ppm) can effectively reduce *Gyrodactylus* sp. infection in cultured rainbow trout reared in recirculating aquaculture systems (RAS) without adversely affecting the water quality parameters, particularly the performance of the biofilter system.

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