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Effects of formaldehyde (Parasite-S®) on biofilter nitrification from a cold and a warm freshwater RAS

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Abstract

The effect of Parasite-S® (an aqueous formaldehyde solution) on the nitrification processes of biofilters was evaluated in two recirculating aquaculture systems (RASs). Rearing tanks in the warmwater RAS contained yellow perch (*Perca flavescens*) and grass carp (*Ctenopharyngodon idella*) with an initial weight of 166.8 kg and a mean density of 39.5 kg/m^3. Rearing tanks in the coldwater RAS contained rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*) with an initial weight of 1377.8 kg at a system density of 41.9 kg/m^3. Parasite-S® was administered to the entire system on four consecutive days in both trials to achieve a nominal concentration of 14.8 mg/L formaldehyde (40 mg/L formalin) at the biofilter. Removal efficiencies for total ammonia nitrogen (TAN) and nitrite nitrogen were measured as indicators of biofilter nitrification processes. The active ingredient in Parasite-S®, formaldehyde, was measured until it was below the method detection limit of 0.8 mg/L. TAN volumetric removal rate was significantly decreased in both systems after formaldehyde addition and remained below pre-exposure efficiency in the coldwater RAS. Nitrite nitrogen volumetric removal rate was not significantly different, but the slope and intercepts were less after formaldehyde addition indicating an effect on the nitrifying bacteria. Although removal rates were decreased, no mortality occurred after four consecutive formaldehyde indefinite bath exposures in either system.

KEYWORDS
Paite-S, RAS, biofilter, formaldehyde, nitrification, recirculating aquaculture system

1 | INTRODUCTION

Recirculating aquaculture systems (RASs) are controlled environments used to intensively rear aquatic organisms (Summerfelt et al., 2001; Wik et al., 2009), often in areas where water availability is limited (Badiola et al., 2012; Ebeling et al., 2006) or where biosecurity concerns limit water intake (Avnimelech, 2006). With greater operator control over water quality, an RAS operator can optimize growth conditions for the species being reared (Badiola et al., 2012; Carrera et al., 2013). An

important challenge with RAS is that, unlike flow-through systems, nitrogenous and organic-waste products can accumulate in rearing tanks and degrade water quality. If water quality in the RAS is degraded, the capacity of otherwise healthy fish to resist pathogens may be reduced, allowing obligate or opportunistic pathogens to cause disease, potentially leading to morbidity or mortality. To maintain proper husbandry conditions, an occasional use of chemical disinfectants or chemotherapeutants may be required. Chemical application risks impairing communities of nitrifying bacteria in the biofilters resulting in potential

ammonia or nitrite accumulation. Additionally, elevated ammonia or nitrite nitrogen in RAS can be directly toxic or cause sublethal clinical changes that may regularly affect production (Ciji et al., 2013; Randall & Tsui, 2002; Svobodová et al., 2005).

One critical element to be considered in the optimization of RAS water quality is feeding rate (Hagopian & Riley, 1998). Because fish metabolize protein in feed and release ammonia as a waste product, overfeeding can result in elevated concentrations of ammonia or nitrite nitrogen. Depending on the concentration and duration of the exposure, either compound may cause acute to chronic effects, including death (Noble & Summerfelt, 1996). Aerobic biological nitrification by bacteria is used in most RAS to remove these potentially toxic nitrogenous compounds (Barbu et al., 2008) by oxidizing ammonia to nitrite and eventually to nitrogen gas.

Higher rearing densities in RAS, coupled with the potential for elevated concentrations of nitrogenous wastes, may affect the health and immune response of the aquatic animals in the RAS. Crowding and impaired water quality could simply improve opportunities for pathogen transmission among animals common to the RAS (Yanong, 2003). Alternatively, crowding in excess of preferred densities could alter immune function (Conte, 2004; Small & Bilodeau, 2005; Suomalainen et al., 2005). Either may create conditions favourable for obligate or opportunistic bacterial, viral, fungal and parasitic pathogens (Blancheton et al., 2013; Noble & Summerfelt, 1996; Yanong, 2003) to cause disease. After introduction into the RAS, either from addition of new stock or from replacement water, pathogenic organisms may persist within biofilms (Jacobs & Chenia, 2011; King et al., 2008). This may result in recurring disease, fish mortality and economic losses to the facility.

Options are limited to treat diseased fish in a RAS in US aquaculture—when this study was conducted in 2016, only Florfenicol and Chloramine-T were approved for use in RAS. If using Chloramine-T, the biofilter had to be bypassed during treatment and flushing. Formaldehyde, the active ingredient in Parasite-S®, has a broad therapeutic range and a high treatment efficacy against most common parasites found in RAS (Pedersen & Pedersen, 2012). In the United States, no formaldehyde-based products were registered for use in RAS before 2019. Formaldehyde applied to a RAS, unlike applications in flow-through rearing units, would persist until it was either degraded or flushed from the RAS. Residence time of formaldehyde in RAS and the possible negative effects vary depending on how the system is designed and managed (Leal et al., 2018). Applications in RAS have the potential for both fish and the RAS biofilter to experience a long duration (indefinite) exposure. Fish are relatively tolerant of long durations of elevated formaldehyde concentrations (Heinen et al., 1995), thus primary concerns with an indefinite exposure are the potentially negative effect on beneficial nitrifying bacteria, and the subsequent reduced biofilter performance (Colt et al., 2006), resulting in elevated concentrations of nitrogenous waste products. This may be brought about because formaldehyde removal is closely related to biofilter surface area and therefore microbial abundance. Also, decomposition of formaldehyde is significantly dependent on the RAS temperature (Pedersen & Pedersen, 2006; Pedersen

et al., 2007), initial concentration, exposure period and treatment frequency (Knight et al., 2016). Chemical treatments in RAS, therefore, must balance the effects on the pathogen, the animal and the effects to the nitrifying microorganisms. Additional information is needed about formaldehyde degradation, temperature effects and tolerance of ammonia- and nitrite-oxidizing bacteria in biofilters (Pedersen et al., 2010) to allow the use of formaldehyde to be approved by the US Food and Drug Administration (FDA) for the use in RAS for freshwater finfish.

The objective of this study was to determine the effects of formaldehyde on the nitrogen oxidation efficiency of the biofilters of a warmwater and a coldwater RAS. Effects on nitrogen oxidation were evaluated by monitoring total ammonia nitrogen (TAN) and nitrite nitrogen. Specifically, the data were collected to inform the evaluation by the FDA of the use of formaldehyde in freshwater RAS with the goal of informing the drug label decision-making process for formalin. Use of Parasite-S in RAS was approved by the FDA in July 2019, based in part, on the results of this study (Fredricks & Schleis, 2017).

2 | MATERIALS AND METHODS

Before the study was initiated, approval for the methods was provided by the FDA.

2.1 | Test system description

2.1.1 | Warmwater trial

The warmwater trial (temperature range 19.4–19.7°C) was conducted at the US Geological Survey Upper Midwest Environmental Sciences Center (UMESC), La Crosse, Wisconsin, USA. The recirculating system consisted of three rearing tanks [84 cm (height) × 163 cm (diameter); Aquatic Ecosystems, Inc.] each filled with 1365 L of 19.5°C well water. The rearing tanks were attached to a drop-bead Polygeyser® biofilter model DF-6 (Aquaculture Systems Technologies, LLC), which contained about 0.14 m³ of media. Water flowed into the biofilter through a pump that held a basket for solid waste removal. The pump basket was cleaned daily at 0700h to remove collected solid matter. Effluent water from the biofilter travelled through two ultraviolet sterilizers (Sanitron UV Water Purifiers, Atlantic Ultraviolet Corp.) before returning to the rearing tanks. Total system volume was 4200 L. A 5%–5.5% (210–245 L) water replacement rate was used daily. Water was drained from the system through the Polygeyser drain; a flow meter (DJL Hose Bibb Meter, Daniel L. Jerman Co.) was used to add fresh water to bring the RAS back to the appropriate volume.

One rearing tank held grass carp (*Ctenopharyngodon idella*) and two tanks held yellow perch (*Perca flavescens*). The initial weight was 166.8 kg, and the system density was 39.5 kg/m³. Grass carp were fed 312 g of 1.6 mm extruded salmon sinking feed (Skretting USA, Tooele UT) daily, and yellow perch were fed 278 g of 2.5 mm

TABLE 1 Feed analysis provided by manufacturers

Manufacturer	Crude protein (min %)	Crude fibre (max %)	Crude fat (min %)	Phosphorus (Min %)
Skretting (1.6 mm)	45	3	19	1.4
Skretting (2.5 mm)	45	3	16	1.0
Rangen (8 mm)	44	5	15	1.0
Rangen (6.4 mm)	45	<2	16	1.2

extruded classic fry floating feed (Skretting USA, Tooele UT) daily. Feed formulation is found in Table 1. Similar to (Heinen et al., 1995), fish were fed before and during formaldehyde exposure at normal rates. Fish were fed between 1400 and 1500h on non-exposure days and between 1600 and 1700h on exposure days. One-half of the daily ration was fed by hand, and the remainder was placed in a mound on a 12-h belt feeder (Aquatic Ecosystems, Inc.). The feed dropped into the tank 6–8h after the hand feeding.

2.1.2 | Coldwater trial

The coldwater trial (temperature range 11.1–11.3°C) was conducted at the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility (UWSP-NADF) near Bayfield Wisconsin, USA. The coldwater recirculating system was used as described in Fischer, Held, Hartleb, & Malison (Fischer et al., 2009). Briefly, the system consisted of four rearing tanks [112 cm (height) × 245 cm (diameter); Marine Biotech, Inc.] each filled with 5277 L of 11°C ground water connected to a 122-cm diameter fluidized sand biofilter (CycloBio®, Marine Biotech, Inc.). The sand biofilter had a volume of 5.01 m^3 and contained 1.18 m^3 of sand. Total recirculation system volume was 33,465 L. Freshwater was supplied at a rate of 12 L/min to the system as make-up water, which provided a 1% replacement per pass. About 50% of the volume was replaced each day.

One of the rearing tanks held lake trout (*Salvelinus namaycush*) and the other three rearing tanks held rainbow trout (*Oncorhynchus mykiss*). The initial weight was 1378 kg, and the system density was 41.9 kg/m^3. Lake trout were fed 3.0 kg of a 50:50 mixture of 6.4 mm and 8.0 mm trout feed (Rangen, Inc.) daily. Rainbow trout were fed 10.8 kg of 6.4 mm trout feed (Rangen, Inc.) daily. Fish were offered feed at 0800, 1200 and 1400h by hand. Feed was also placed on a 12-h belt feeder to supplement hand feeding between 1000 and 2000h. Fish were not fasted before or during formaldehyde exposure.

2.2 | Biofilter media inoculation

Before the start of the warmwater trial, 225 ml of Fritzyme® TurboStart® 700 (Fritz Industries, Inc.) was added to the system. The UV sterilizers were unplugged for 5 days after the Fritzyme® was added as recommended by the manufacturer, then the system was allowed to stabilize for 7 weeks. In the coldwater trial, ammonium chloride was added as a nitrogen source to stimulate biofilter function. After nitrification endpoints (ammonia, nitrite and nitrate) had stabilized to

reasonable levels over time (i.e., 11 days), fish were added into the functioning RAS tanks. Fish then provided the nitrogen necessary to maintain biofilter function and ammonium chloride was no longer needed.

2.3 | Water chemistry, total ammonia nitrogen, nitrite nitrogen, alkalinity

Water chemistry (dissolved oxygen, pH and water temperature) was measured daily at 0700h in each rearing tank and at the outflow from the biofilter in both systems. Dissolved oxygen and pH were measured using a Hach HQ40d multimeter with pH (IntelliCAL™ PHC201) and dissolved oxygen probes (IntelliCAL™ LDO101) attached (Hach Company). Total ammonia nitrogen and nitrite nitrogen were measured daily at 0700h in the biofilter inflow and biofilter outflow with an ammonia probe (IntelliCAL™ ISENH3181) attached to a second Hach HQ40d multimeter. Nitrite nitrogen was measured using a LaMotte SMART3 colorimeter and LaMotte test kit 3650-SC; level of detection was 0.02 mg/L (LaMotte Company). In the warmwater trial, alkalinity was measured as mg/L CaCO$_3$ daily in one rearing tank using the pH 4.5 titration method (American Public Health Association (APHA), American Water Works Association, & Water Environment Federation, 2012). In the coldwater trial, alkalinity was measured every other day with a Hach spectrophotometer (model DR3900) and Hach TNT plus™ 870 kit. Alkalinity was maintained between 180 and 220 mg/L as CaCO$_3$ in the warmwater system and between 130 and 190 mg/L in the coldwater system through daily addition of sodium bicarbonate.

2.4 | Determination of volumetric removal rates

Volumetric removal rates were calculated for both ammonia and nitrite as an indicator of biofilter function. Volumetric TAN removal (VTR, g/m^3 media/day) = [TAN$_{IN}$−TAN$_{OUT}$] × [Q$_{BF}$/V$_{BF}$]; where TAN$_{IN}$ is the total ammonia nitrogen entering the biofilter (mg/L), TAN$_{OUT}$ is the total ammonia nitrogen in effluent from the biofilter (mg/L), Q$_{BF}$ is the flow rate through the biofilter (m^3 water/day), and V$_{BF}$ is the volume of biofilter media (m^3 media). Because nitrite is produced in the biofilter as ammonia is oxidized, and almost immediately converted to nitrate, apparent volumetric nitrite removal (VNRa) may be near zero, even though nitrite-oxidizing bacteria are functioning. To calculate nitrite volumetric removal rate, the VTR must be included to determine the nitrite conversion activity. Volumetric nitrite removal (VNR, g/m^3 media/day) = VTR + [NO$_{2IN}$ − NO$_{2OUT}$] × [Q$_{BF}$/V$_{BF}$] (Malone & Beecher, 2000).

2.5 | Formaldehyde administration and measurement

The commercially available product Parasite-S® (active ingredient formaldehyde, 37% [w/v]; Western Chemical Company) was applied to the RAS as an indefinite bath exposure on four consecutive days. The target system concentration of 14.8 mg/L formaldehyde (40 mg/L formalin) was determined in laboratory tests (Fredricks et al., 2018) and after consultation with the FDA Center for Veterinary Medicine. An appropriate volume of Parasite-S was weighed out for each rearing tank and simultaneously added to the centre of each rearing tank. The container was rinsed three times and then the water was stirred with a long-handled brush for about 5 min to help mix the chemical into the rearing tank water. Mixing through the system also occurred because water flow processes in the RAS were not altered and the biofilter was not isolated during the formaldehyde application period.

A water sample collected 10 min after addition of formaldehyde from near the centre of each rearing tank was used to determine the 0-h formaldehyde concentration. Water samples for formaldehyde determination were collected again at 4, 8 and 24 h. After the 24-h sample was collected, the next dose of formaldehyde was applied to the rearing tanks. No fresh water was added to the warmwater RAS until after the 24-h sample was collected. In the coldwater RAS, make-up water was supplied continuously at a rate of 12 L/min. These procedures were repeated for four consecutive days.

Formaldehyde concentration was measured with the Spectroquant® Formaldehyde Cell Test Kit (1.14500.0001, EMD Millipore) and the Spectroquant® Move 100 Colorimeter. The method is based on the reaction of formaldehyde with chromotropic acid in sulfuric acid, which forms a violet colour. A standard curve was made from five standards following manufacturer's guidelines and stored as a user-specific method on the meter. Method accuracy was verified daily with well water samples containing 5 mg/L formaldehyde. Method limit of detection was 0.8 mg/L.

2.6 | Data analysis

We used resampling with replacement across different time periods (pre-exposure, exposure and postexposure) and RAS type (coldwater or warmwater) on the volumetric removal rates for TAN and nitrite nitrogen (Ismay & Kim, 2020). For our resampling methods, we randomly re-shuffled our data to estimate the median, and the uncertainty of this distribution allowed us to estimate 95% confidence intervals after resampling 1000 times. This method is also commonly known as bootstrapping. We followed methods described in Ismay and Kim (2020), although Gotelli and Ellison (2013) also provide an overview of bootstrapping from an ecological perspective for resampling and how this method differs from parametric analysis and Bayesian analysis. We used this non-parametric approach due to heteroskedasticity through time.

We estimated the median and 95% confidence interval rather than fitting statistical models due to limits of our system. Specifically, we used only two systems and we did not feel confident making statistical inferences to broader statistical 'populations' given our limited 'sample population'. Estimating effects and the corresponding uncertainty also agrees with current recommendations from organizations such as the American Statistical Association that recommend the avoidance of null-hypothesis testing (NHST) and p-values (Wasserstein et al., 2019). Ho et al. (2019) also provide relevant discussion on the use of point estimates over NHST. Comparing point estimates and their corresponding 95% CIs allows for statistical comparison among locations and time periods (Dushoff et al., 2019). We did not explicitly model day other than as part of aggregating into time periods because we did not have replication to model daily variation. Data were plotted in R using the ggplot2 package (Wickham, 2009).

Half-life of formaldehyde in each system was calculated as:

$$t_{1/2} = t \times \log(2) / \log\left(\frac{C_o}{C_t}\right)$$

where: $t_{1/2}$ is the half-life, t is elapsed time, C_0 is the initial concentration, and C_t is concentration at time t.

3 | RESULTS

3.1 | Water chemistry and alkalinity

System water chemistry is summarized in Table 2. Water chemistry in both RAS was stable throughout the trials.

3.2 | Volumetric removal rates

Median VTR was decreased in the coldwater RAS during exposure and postexposure. Median VTR as similar to pre-exposure in the warmwater RAS but decreased during the postexposure period. The 95% confidence intervals overlapped for all time periods (Figure 1). Median VNR were similar across time periods in the coldwater RAS but decreased in the warmwater RAS and the 95% confidence intervals overlapped for all time periods (Figure 2). Variability in the warmwater RAS was greater for both VTR (Figure 1) and VNR (Figure 2) compared with the coldwater RAS. Both VTR and VNR remained below pre-exposure levels in the postexposure period warmwater RAS only.

TABLE 2 Water chemistry for the coldwater and warmwater RAS. Values are the mean ± standard deviation

Water quality parameter	Coldwater RAS	Warmwater RAS
Temperature (°C)	11.3 ± 0.1	19.6 ± 0.12
pH	7.37 ± 0.28	7.56 ± 0.09
Dissolved oxygen (mg/L)	11.4 ± 1.8	5.2 ± 0.9
Alkalinity (mg/L CaCO$_3$)	151 ± 25	185 ± 30

FIGURE 1 Median volumetric TAN removal from a warmwater and a coldwater RAS exposed to formaldehyde for four consecutive days. The red point indicates the median VTR value with the red bars showing the 95% confidence interval. The black points display the observed distribution of the VTR values for each day of analysis.
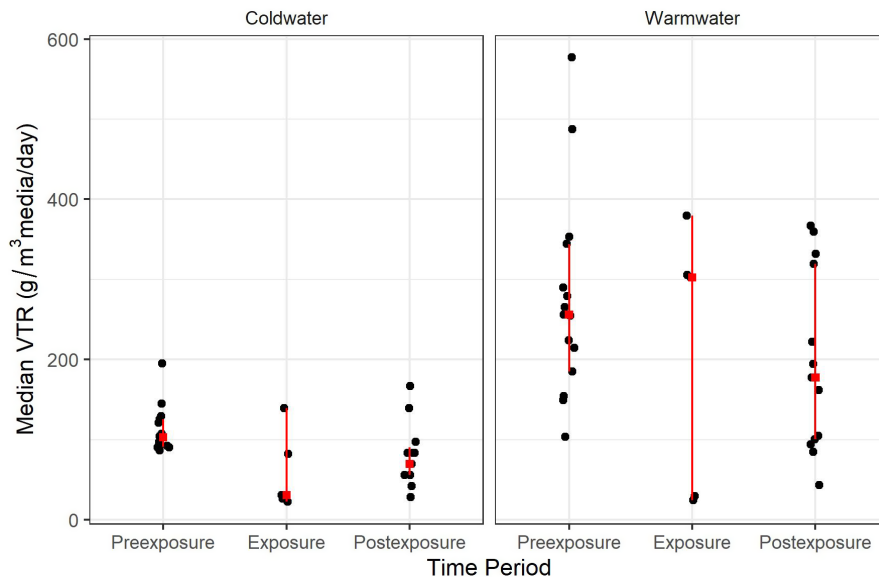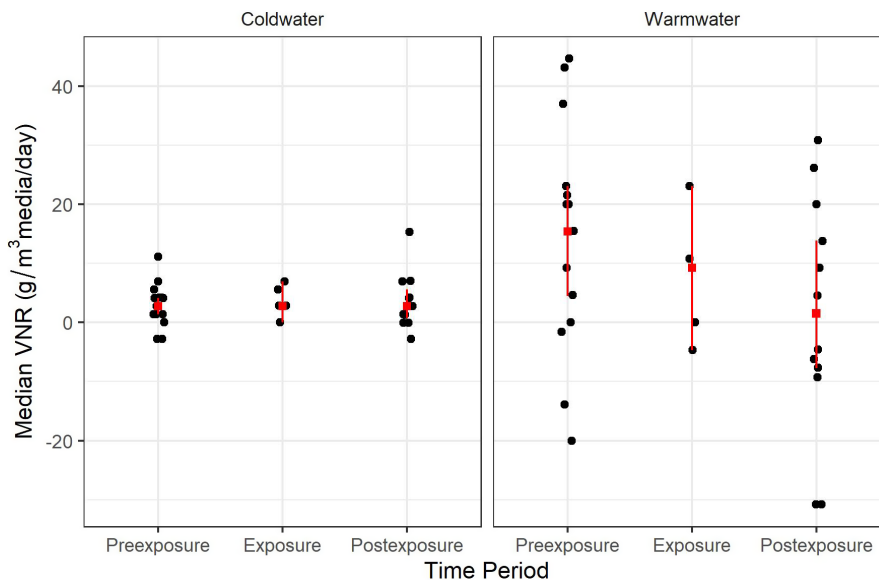

FIGURE 2 Apparent volumetric nitrite nitrogen removal in a warmwater and a coldwater RAS exposed to formaldehyde for four consecutive days. The red point indicates the median VNR value with the red bars showing the 95% confidence interval. The black points display the observed distribution of the VNR values for each day of analysis.

TABLE 3 Formaldehyde concentrations at time 0 (the time when formaldehyde was added) during the warmwater trial for yellow perch and grass carp. Target concentration was 14.8 mg/L

	Formaldehyde Concentration (mg/L)[a]			
Location	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Rearing tank 1	13.0	14.5	13.7	13.5
Rearing tank 2	13.5	13.7	14.5	12.7
Rearing tank 3	13.7	14.5	14.0	12.0
Biofilter inflow	13.5	14.2	14.2	12.5
Biofilter outflow	13.2	14.2	13.0	12.7
Overall mean (n = 5)	13.4±0.2	14.2±0.3	13.9±0.5	12.7±0.5

[a]Formaldehyde concentrations were determined from samples collected 10 min after dosing to allow for mixing through the system.

3.3 | Formaldehyde concentrations

The target exposure concentration for both trials was 14.8 mg/L formaldehyde. In the warmwater trial, actual formaldehyde concentration was below target on all days but within 15% of the target (Table 3). In the coldwater trial, actual formaldehyde concentrations were above target concentration on all days, but within 25% of target (Table 4). No mean is reported for Day 2 of coldwater trial because only one tank was analysed for formaldehyde due to a sampling oversight.

Mean half-life for formaldehyde in the warmwater RAS was 6.45 h, and it was 6.93 h in the coldwater trial. In the warmwater trial, formaldehyde was at or below detection limit on all days, except the first day, 24 h after addition (Figure 3). In the coldwater trial, formaldehyde was below the detection limit only on Day 4 (Figure 3). In both trials, the amount of formaldehyde at 24 h was greatest on the first day of exposure and decreased each day afterwards.

4 | DISCUSSION

4.1 | Effects on biofilter nitrification processes

The reported effects of formaldehyde on nitrification processes of RAS are variable. Low dose (9.25 mg/L formaldehyde), short duration exposures did not significantly impair nitrification in a

TABLE 4 Formaldehyde concentrations at time 0 (the time when formaldehyde was added) during the coldwater trial for rainbow trout and lake trout. Target concentration was 14.8 mg/L

Location	Formaldehyde Concentration (mg/L)			
	Day 1	Day 2[a]	Day 3	Day 4
Rearing tank 1	19.5		20.5	19.0
Rearing tank 2	18.0	20.2	20.0	18.2
Rearing tank 3	17.3		17.3	18.2
Rearing tank 4	14.8		17.3	16.5
Biofilter inflow	15.8		16.5	11.4
Biofilter outflow	16.8		16.6	11.9
Overall mean ($n = 6$)	17.0 ± 1.7		18.0 ± 1.8	15.9 ± 3.4

Note: Formaldehyde concentrations were determined from samples collected 10 min after dosing to allow for mixing through the system.
[a]A formaldehyde sample was only collected from rearing tank 2 on Day 2 due to a sampling oversight.

small freshwater RAS containing rainbow trout even though the abundance of ammonia-oxidizing bacteria (AOB) and NOB (nitrite-oxidizing bacteria) were reduced in the presence of formaldehyde (Pedersen et al., 2010). However, low doses of formaldehyde were reported to destroy nitrifying bacteria in another study (Burrows & Combs, 1968). Single, indefinite exposures to formaldehyde ranging from 5.5 to 44.4 ppm did not impair biofilter function, and 120 ppm formaldehyde disappeared in 11 h at 17 ± 1°C in a fluidized bed-sand biofilter RAS containing rainbow trout. However, an indefinite exposure at 25.9 ppm formaldehyde after several other doses resulted in elevated nitrite nitrogen, but not TAN, for 9 days, indicating repeated formaldehyde exposure can cause sufficient cumulative damage to NOB to cause biofilter impairment (Heinen et al., 1995). Schwartz et al. (2000) found that a single indefinite 111 ppm formaldehyde exposure did not significantly impair ambient TAN removal efficiency in a small-scale coldwater RAS.

Temporary effects on nitrification have been previously reported during indefinite exposures (Keck & Blanc, 2002). In this study, a nominal concentration of 14.8 mg/L formaldehyde applied as an indefinite exposure on four consecutive days resulted in increased variability in median VTR and VNR for the warmwater RAS and median VTR and VNR deceased during the postexposure period. Taken together, these results indicate a negative effect on AOB in the warmwater RAS. The effects on NOB in the warmwater RAS are not as easy to determine. If ammonia conversion to nitrite was not occurring normally, less substrate would be available for NOB to process, and VNR could be normal.

There appears to be less of an effect on AOB and NOB function in the coldwater system after four consecutive exposures to formaldehyde. The median VTR and VNR were similar across exposure periods and variability was not as great as with the warmwater RAS.

Keck and Blanc (2002) reported that NOB bacteria were more sensitive to formaldehyde. They used a marine RAS, but it is unknown whether different bacterial species were in their system
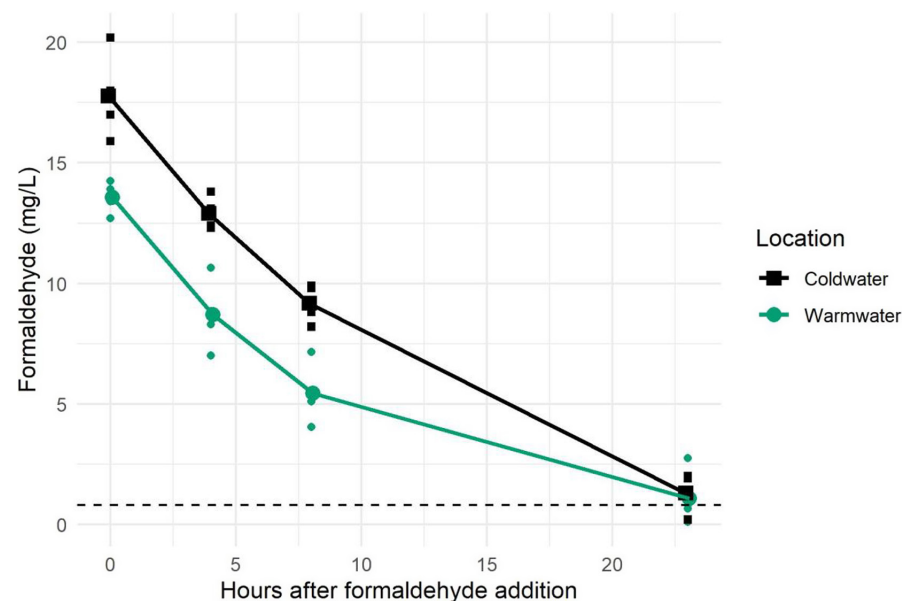

FIGURE 3 Formaldehyde concentrations in a coldwater RAS (■) and warmwater RAS (●). Large symbols represent the system mean ($n = 5$ for warmwater RAS, $n = 6$ for coldwater RAS). Smaller symbols are the individual data points. Vertical lines represent the standard deviation. Dashed horizontal line represents limit of detection (0.8 mg/L).

compared the freshwater system used in this study. Inhibition of or reduction in abundance of NOB in RAS is concerning because mortality can occur, especially in salmonids, with nitrite nitrogen greater than 0.15 mg/L (Liao & Mayo, 1974; Smith & Williams, 1974). Nitrite is oxidized to nitrate by NOB nearly as quickly as it is formed so if NOB were negatively affected, VNR would be expected to decrease. In our study, NOB were negatively affected in the warmwater RAS but did not appear to show adverse effects in function in the coldwater RAS. The median VTR was similar in pre-exposure and exposure time periods, but VNR declined during exposure in the warmwater RAS and median VNR was similar across exposure periods in the coldwater RAS.

One difference in our study and hatchery practice is that many producers would take their fish off feed during treatment. This would reduce the amount of ammonia produced by the fish and reduce the amount of ammonia nitrogen the biofilter would have to process. Our methods were designed to represent a worse-case scenario. If effects were negative on the nitrifying bacteria, they were more likely to be seen under the worse-case scenario. Minimizing ammonia production by withholding feed may not have allowed us to detect changes in nitrifying bacterial function.

4.2 | Formaldehyde degradation

Formaldehyde did not accumulate during the four consecutive day exposure in either system in this study, and the amount formaldehyde remaining at 24 h was less each treatment day, similar to reports for both fresh- and saltwater RAS (Knight et al., 2016; Pedersen et al., 2010, 2007). The mostly likely cause of the increased formaldehyde removal was microbial action (Eiroa et al., 2004; Knight et al., 2016; Pedersen & Pedersen, 2006; Pedersen et al., 2007). Some strains of bacteria are known to detoxify formaldehyde and can use formaldehyde for growth (Chongcharoen et al., 2005; Glancer-Šoljan & Dragičević, 2001; Oliveira et al., 2004). The extent to which bacteria contributed to the decrease in formaldehyde concentration in our study is unknown.

The half-lives of formaldehyde reported (6.4 h for the warmwater RAS, 6.9 h for the coldwater RAS) differ from those reported by Pedersen and Pedersen (2006). They found that water temperature had a significant effect on formaldehyde removal and reported half-lives of 5 and 9.5 h for RAS operating at 14.5 and 10°C respectively. We would expect that the half-life of the warmwater system in this study, which was operated at 19°C, to be significantly less than that of the coldwater system operated at 11°C. Dilution of formaldehyde in the coldwater RAS mostly likely accounts for the difference we observed between systems in this study and for the differences compared with the Pedersen and Pedersen (2006) study. In the warmwater RAS, 5% of the system volume was replaced each day and in the coldwater RAS, about 50% of the volume was replaced each day. The RAS used by Pedersen and Pedersen (2006) had a daily water replacement rate of 10%.

4.3 | Protection of nitrifying bacteria from formaldehyde

Although we observed effects on biofilter nitrification processes, formaldehyde can be administered to treat fish in RAS. Currently label language recommends bypassing the biofilter, if possible, to protect the nitrifying bacteria. Another protective action is that the treated tank could be isolated from the system and water flushed from the system before flow to the biofilter is re-established. This is not ideal because it requires the RAS operator to have sufficient temperature acclimated water on hand. If the rate of decomposition in the isolated tank and dilution by the system water after system flow is re-established results in concentrations that are not harmful to nitrifying bacteria, it could minimize the amount of make-up water required. Another option is to isolate the biofilter by allowing treated water to circulate among the rearing tanks if the RAS design permits this flow path. This is not ideal because the biofilter could harbour the infectious agent (Noble & Summerfelt, 1996).

5 | CONCLUSIONS

Nitrification processes in a warmwater RAS at 19°C decreased after four consecutive exposures to formaldehyde. VTR and VNR remained below pre-exposure levels after formaldehyde exposure stopped. AOB appeared to be more affected in the coldwater RAS compared with the warmwater RAS but data indicate that recovery occurs after formaldehyde exposure ends. It is not clear if the inhibition was due to the higher concentration of formaldehyde (18.5 mg/L), the colder water temperature, or a combination of both. Formaldehyde did not accumulate in either system and was undetectable at 24 h after application. Fish in both systems tolerated four consecutive indefinite exposures to 14.8–18.5 mg/L; no mortality occurred in either system during or after formaldehyde exposure. Current label language states to use caution when applying Parasite-S in RAS and to consider bypassing the biofilter, if possible.

CONFLICT OF INTEREST
The authors declare they have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS
Fredricks, Schleis, and Smerud performed the study and wrote drafts of the manuscript. Gaikowski designed the study and critically reviewed drafts of the manuscript. Erickson and Herbert performed

the stastical analysis, wrote the data analyisis section and prepared the figures. Fischer, Holmes, and Hartleb performed rearing of test fish and established the RAS system at UWSP-NADF, and provided critical reviews of the manuscript.

DATA AVAILABILTY STATEMENT

Data for the field trails can be found at https://doi.org/10.5066/F7R78CDT (Fredricks & Schleis, 2017). Code to recreate our results may be found at https://doi.org/10.5066/P9N4XR2Z (Erickson et al., 2022).

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All animal procedures for protocol AEH-11-RASFRM-01 were reviewed and approved by the US Geological Survey's Upper Midwest Environmental Sciences Center Institutional Animal Care and Use Committee.

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