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Research article

Response of recirculating saltwater aquariums to long-term formalin treatment

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Abstract
This study documents the degradation of formalin in saltwater recirculating systems and the response of the biological filter during a five-day 25 ppm formalin treatment regimen. A total of nine additions of formalin was administered over the course of five days. The target concentration was 25 ppm and frequent water sampling documented the degradation. Degradation rates changed over time (0.689–7.495 formalin ppm·hr^{-1}) and results revealed frequent periods below therapeutic concentrations. Removal rates were not statistically different in systems with different bacterial diversity or systems with increased fish density. The results suggest the need for frequent testing and follow-up additions during treatment cycles to ensure therapeutic concentrations and prevent recurrence of pathogens.

Introduction

Recirculating aquaria became popular in the 1970s because of their cost-effective reuse of water, and today are standard use for home hobbyists and public aquariums. Recirculating aquaria rely on mechanical and chemical filtration to purify and clarify the water, and biological filters to remove toxic ammonia and nitrite (Colt and Orwicz 1991; Moe 1992). *Nitrobacter* and *Nitrosomonas* are the recognised groups of bacteria found in the biological filter that are most often responsible for the two-step nitrification process of ammonia to nitrate (Burrows and Combs 1968; Spotte 1970; Collins et al. 1975b; Almstrand 2012; Blancheton et al. 2013). In cases where nitrite levels are low, such as recirculating aquariums, *Nitrospira* is more prevalent than *Nitrobacter* (Wagner and Loy 2002; Maixner et al. 2006; Foesel et al. 2008; Pedersen et al. 2010).

Maintaining the function of the biological filter is critical to keep ammonia and nitrite loads low in a recirculating aquarium. Knowing how specific pharmaceutical treatment protocols affect a system is important, as adverse effects could jeopardise animals' lives. The addition of pharmaceuticals can impair the function of the biological filter, particularly when the compounds are added directly to the water (Collins et al. 1975a; Nimenya et al. 1999; Noga 2000). High formalin concentrations, long exposure periods and repeated exposure have been reported to reduce nitrite removal by bacteria (Keck and Blanc 2002; Eiroa et al. 2004; Pedersen et al. 2010); however, these observations are not consistent throughout the literature (Heinen et al. 1995; Pedersen et al. 2010).

Formalin (37–40% formaldehyde, 10–12% methanol and water) has been used in the aquarium and aquaculture industries as a treatment for ectoparasites and fungi for nearly 80 years. A recent study showed that 64% of the zoos and aquariums surveyed use formalin in their quarantine protocols (Hadfield and Clayton 2011). Formalin is added directly to the water and treatment regimens are either high concentration for short durations (<3hrs) or low concentration for a long durations (>24hrs). The necessary therapeutic level for formalin depends greatly on the pathogen being treated and the infected host (Herwig 1979; Noga 2000). The lowest effective concentration published for long-term treatment is 10 ppm; however, many report that higher than 15 ppm is needed (Tieman and Goodwin 2001; Buchmann and Kristensson 2003; Noga 2000). During long-term exposures, formalin concentration degrades over time (Adroer et al. 1990; Wienbeck and Koops 1990; Heinen et al. 1995; Pedersen et al. 2007, 2010). The residence time of formalin in the system varies by concentration, exposure period, treatment frequency and water temperature (Pedersen

et al. 2007, 2010). The exact reaction that causes the degradation remains unclear, but heterotrophic bacteria, fish (Wienbeck and Koops 1990) and proteins and amino acids (Kitamoto and Maeda 1980) are possible contributors. Failure to maintain therapeutic concentration during the entire treatment cycle could lead to decreased treatment efficacy, recurrence of the pathogen, or resistance build up by the pathogen (Kuemmerer 2009).

Very few have studied formalin degradation in saltwater or its effects on saltwater recirculating systems. None of the previous studies have investigated long-term exposure with repeated formalin additions. The purpose of this study was to document the effects of a five-day, 25 ppm formalin treatment on saltwater aquaria and investigate the role of fish density on formalin degradation. The response of the biological filter, changes in degradation rates and the effects of fish biomass on formalin degradation are described.

Methods

This study used two experiments to investigate formalin degradation. The first experiment (single density) explored the effects of formalin on water parameters and the biological filtration, and documents formalin degradation rates. The second experiment (double density) doubled the density of fish to determine the role of biomass in formalin degradation.

Closed recirculating system design

Six closed-loop recirculating systems were used throughout the two experiments. Each 545 L system comprised a pre-filter (50 µm), pleated filter cartridge (20 µm), a biological trickle filter/sump and two 250 L tanks. System flow averaged 53 Lpm resulting in a water turnover time of 10.28 min. Water flow travelled from the tanks through the pre-filter, into the trickle filter/sump, through the pleated filter then returned to the tanks. Artificial seawater was created using Instant Ocean® mixed at a salinity of 32 ppt. Three months before adding fish an established biological filter was created by adding liquid nitrifying bacteria (Fritz-Zyme® Turbo Start 900) to the sump containing 29.5 L of 1 inch bio barrels. The biological filter for all six systems was started from the same bottle of Frit-Zyme®. Ammonium sulphate was added daily until the system could easily convert 3 ppm of ammonium sulphate and produce unionised ammonia and nitrite levels near zero. A supplemental air stone was used in each tank (two per system) to maintain a healthy level of oxygen (>85% saturation) in the water. Water changes and filter changes did not occur during the experiments.

Juvenile Florida pompano (*Trachinotus carolinus*) were obtained from a local aquaculture facility, quarantined and introduced into the systems after the biological filter was stable. Fish were allowed to acclimate in the recirculating aquaria for seven weeks before starting the experiment. Each system contained 14 pompano in the "single density experiment" and 28 pompano in the "double density experiment". Average fish body mass was 15.6 g (219 g and 438 g per system for each experiement respectively) during the experiments and each system received 10–12% BW/day Mazuri® gel once daily (26 g and 52 g for each experiment respectively).

Water parameters

Water was monitored for pH and dissolved oxygen (DO) using a portable meter (Hach HQ40D multi-parameter meter) twice daily and once daily for salinity using a refractometer. All systems were maintained within predetermined narrow ranges for each parameter: pH (7.9–8.35), salinity (30–35 ppt), DO (6.6–7.0 $mg \cdot L^{-1}$ or >85% saturation), ammonia (<0.6 $mg \cdot L^{-1}$), and nitrite (<1.5 $mg \cdot L^{-1}$). Alkalinities were measured one day prior to starting the experiment and systems dosed manually into the sump with sodium bicarbonate and/or sodium carbonate to adjust each system to the ideal pH of 8.0–8.2 and alkalinity of 2.5–3.0 Meq. Florida pompano are well documented as having a wide tolerance range for multiple water parameters and the predetermined ranges were well below the LC50 (Moe et al. 1968; Weirich and Riche 2006).

Chemical analysis

Total ammonia as nitrogen (TAN/NH3-N), nitrite (NO2-N) and formaldehyde were tested in both control and treatment systems every three hours or until formalin concentration was zero. All water chemistries were measured using a spectrophotometer (Hach DR 4000). Protocols for testing were outlined by the manufacturer (ammonia-salicylate method 8155, nitrite-diazotisation method 8507, formaldehyde-MBTH method 8110). Hach's formaldehyde concentration program range was 3–500 $\mu g \cdot L^{-1}$, so a 1:50 dilution was performed on water samples from all formalin-treated systems. Unionised ammonia (UIA) levels were calculated from the TAN readings and the formalin concentration was calculated from the formaldehyde readings. Separate glassware was used for each water chemistry parameter and was cleaned using deionised water for ammonia/nitrite tests and chromic acid for formaldehyde tests. A nitric acid wash was used four times during the experiment to reduce buildup in ammonia and nitrite vials.

Formalin treatment

All parasite treatment protocols using formalin specify concentration of formalin and not formaldehyde. Formaldehyde is the active ingredient in formalin (37–40% formaldehyde, 10–12% methanol and water). The equipment used could only measure formaldehyde concentrations; therefore, concentration of formalin was calculated from formaldehyde concentrations:

(1) Formalin concentration (ppm) =
((Formaldehyde x 50)/1000)/0.37

Our experiments followed a five-day low-dose formalin treatment protocol. Target concentration in treatment systems was 25 ppm formalin (Paracide-F®, 37% formaldehyde). Treatment group systems were treated with formalin in the morning (approximately 0800) and if formalin dropped below the target concentration, additional was added in the evening (approximately 1700) to bring the concentration back up to 25 ppm. Formalin was added directly to the tanks with the addition split evenly between the two tanks on each system. Systems were allowed to circulate for 20 minutes (approximately two complete water turnovers) after formalin was added and water was tested to make sure the 25 ppm target was reached. The concentration at 1600 determined the volume of formalin to be added at 1700. The twice-a-day dosing mimics the treatment protocol used by many aquarists, and was selected to evaluate the efficacy of the widely used protocol of dosing at the beginning and end of a shift.

Single density experiment: effects of formalin on water parameters and the biological filtration

Husbandry and water testing

Six recirculating systems were used throughout this experiment; three systems were in the control group and three systems in the treatment group. Water samples were collected before water entered the biological filter at a minimum of every three hours or until the formalin concentration was zero.

Bacterial analysis: DNA fingerprinting

DNA fingerprinting was used to describe the bacterial community within the recirculating systems (Jeffreys et al. 1985). 200–500 mL of water was collected from each system daily at 0600. Water was

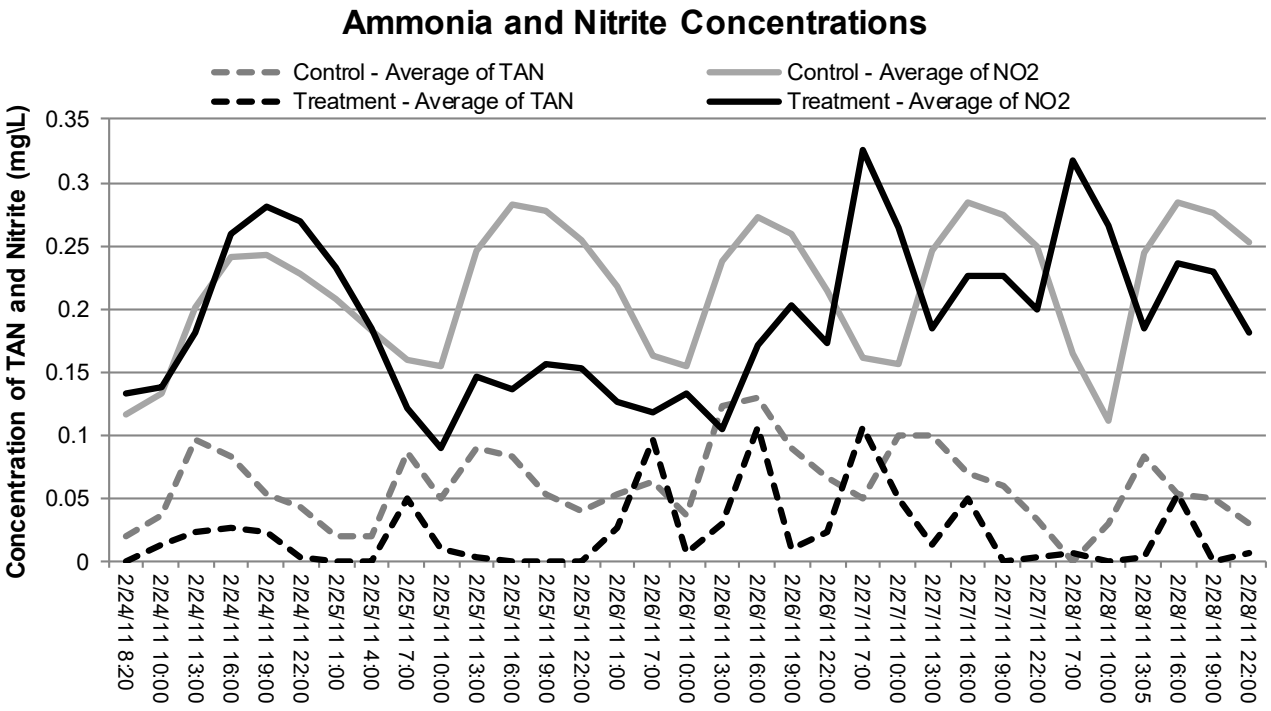

Figure 1. Average concentration of ammonia (TAN) and nitrite in a recirculating system, with and without formalin additions. Concentrations remained low and within normal operating limits.

vacuum filtered onto 0.2 µm supor membrane disc filters using a manifold. Filters were placed in 2 mL cryovials with 1 mL of 70% glycerol and stored at -80°C until analysed. If water could not be filtered immediately samples were kept refrigerated until filtration could occur.

Samples for DNA fingerprinting were sent to the University of Illinois at Urbana-Champaign. Total genomic DNA was extracted by using FastDNA SPIN kit (MP Biomedicals). Automated ribosomal intergenic spacer analysis (ARISA) was used to estimate the bacterial community composition (Fisher and Triplett 1999). Polymerase chain reactions (PCRs) contained 2 µL of extracted DNA and followed the community analysis methodology outlined by Kent et al. (2004). Denaturing capillary electrophoresis was conducted by the Roy J. Carver Biotechnology Center at the University of Illinois.

Double density experiment: impact of fish density on formalin degradation

Husbandry and water testing

Following the single density experiment all fish were consolidated into the three control systems to double the fish density. Unionised ammonia and nitrite concentrations were allowed to stabilise for 14 days before starting the double density experiment; water changes were used to decrease the accumulation of nitrites in the system prior to the start of the experiment. Pleated filters were changed weekly until the start of the experiment but no pleated filter changes occurred during the experiment. Additionally, no water changes were performed during the experiment. Three recirculating systems were used throughout the double density experiment, all were treatment tanks. Each system contained 28 healthy juvenile Florida pompano (438g) and feeding rate was maintained. Water samples were analysed as described above.

Statistical analysis

Over the course of five days, each treatment tank was treated with formalin nine times (Table1). To investigate the change in degradation rate over time, formalin concentrations were plotted against the time passed since formalin addition. Using Microsoft Excel, best-fit linear lines were fit to scatter plots from each tank for each dosing segment and slopes were compared in SPSS. The relationship of system and formalin dose on the degradation slope was examined using ANOVA. To determine appropriate post-hoc tests, Levene's test for variance equality (homogeneity) was used. Games-Howell analysis was used to analyse degradation changes with subsequent doses.

Multivariate analyses were carried out based on bacterial community data generated from ARISA using PRIMER 6 for Windows. The Bray-Curtis similarity coefficient was used to assess the degree of similarity between bacterial community compositions. Non-metric multidimensional scaling (MDS) analysis was applied to identify the patterns among multiple samples. Analysis of similarity (ANOSIM) was used to compare the bacterial community similarity between different days and systems.

Results

Effects of formalin on water parameters and the biological filter

Dissolved oxygen in all recirculating systems remained above 86% saturation with an average saturation of 93.6±2.7%. pH in all systems remained stable with an average of 8.03±0.14 and temperature was 25.4±0.45° C. Flow decreased on average by 15±9% over the course of the experiments due to accumulation of organic matter on the pleated filter. This decrease was observed in all systems and mimicked the pattern observed when the systems are under normal operation of weekly filter changes.

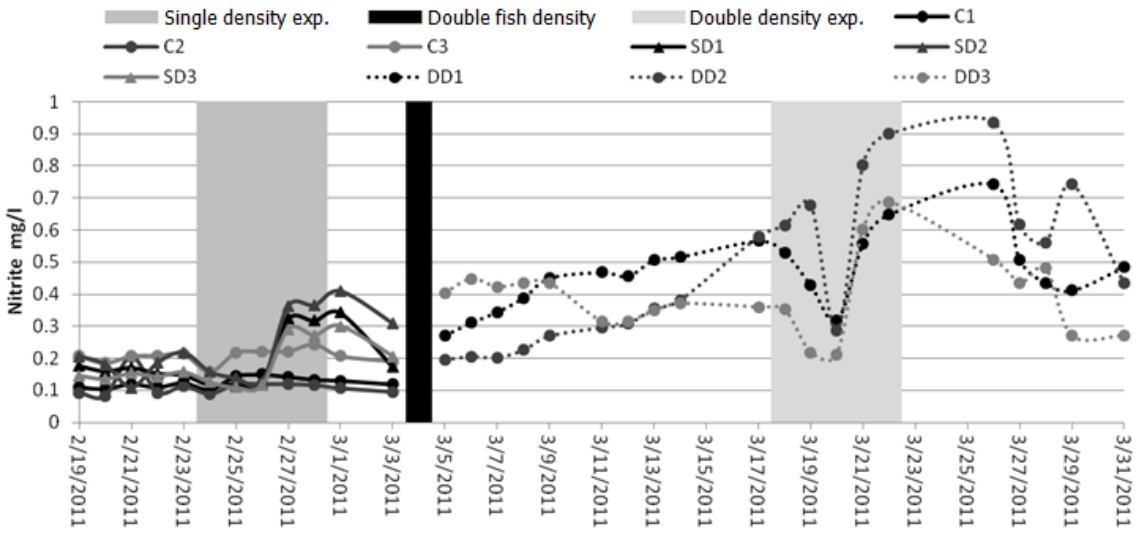

Figure 2. Nitrite concentrations in closed-loop saltwater recirculating systems. Control (C1–3), single density (SD1–3) and double density (DD1–3) nitrite concentrations shown. Grey boxes highlight the experimental periods where formalin was present in the system. Black bar shows the doubling of fish density.

Nitrite concentration was used as an indicator of biological filtration function. A natural cyclical pattern was observed in treatment and control systems during both experiments (Fig. 1). The lowest nitrite concentrations occurred before feedings between 0830 and 1000 and peaked between 1700 and 1800. Throughout the single density experiment, nitrite concentrations remained below 0.4 mg·L^{-1}. During the double density experiment concentration remained below 1.0 mg·L^{-1} (Fig.2).

Throughout the single density experiment TAN and UIA concentrations remained below 0.25 mg·L^{-1} and 0.008 mg·L^{-1} in control systems respectively. Chemical interference between formalin and the salycilate testing method was observed. The

Table 1. Formalin degraded after addition to recirculating systems. Twice daily formalin additions were added to bring the system up to the targeted 25 ppm treatment.

Experiment	Dose number	Dosing time	Formalin added (ppm)	Elapsed time (hrs)	Average degradation rate (ppm·hr^{-1})
Single density	Dose 1	Day 1 0800	25	0	0.657
	Dose 2	Day 2 0800	9–10	24	2.518
	Dose 3	Day 2 1700	13–23	33	3.842
	Dose 4	Day 3 0800	25	48	6.095
	Dose 5	Day 3 1700	25	57	6.268
	Dose 6	Day 4 0800	25	72	4.830
	Dose 7	Day 4 1700	25	81	5.260
	Dose 8	Day 5 0800	25	96	5.048
	Dose 9	Day 5 1700	25	105	5.971
Double density	Dose 1	Day 1 0800	25	0	0.721
	Dose 2	Day 2 0800	10–13	24	1.629
	Dose 3	Day 2 1700	10–13	33	3.998
	Dose 4	Day 3 0800	25	48	7.187
	Dose 5	Day 3 1700	25	57	7.103
	Dose 6	Day 4 0800	25	72	5.612
	Dose 7	Day 4 1700	25	81	5.903
	Dose 8	Day 5 0800	25	96	4.516
	Dose 9	Day 5 1700	25	105	5.022

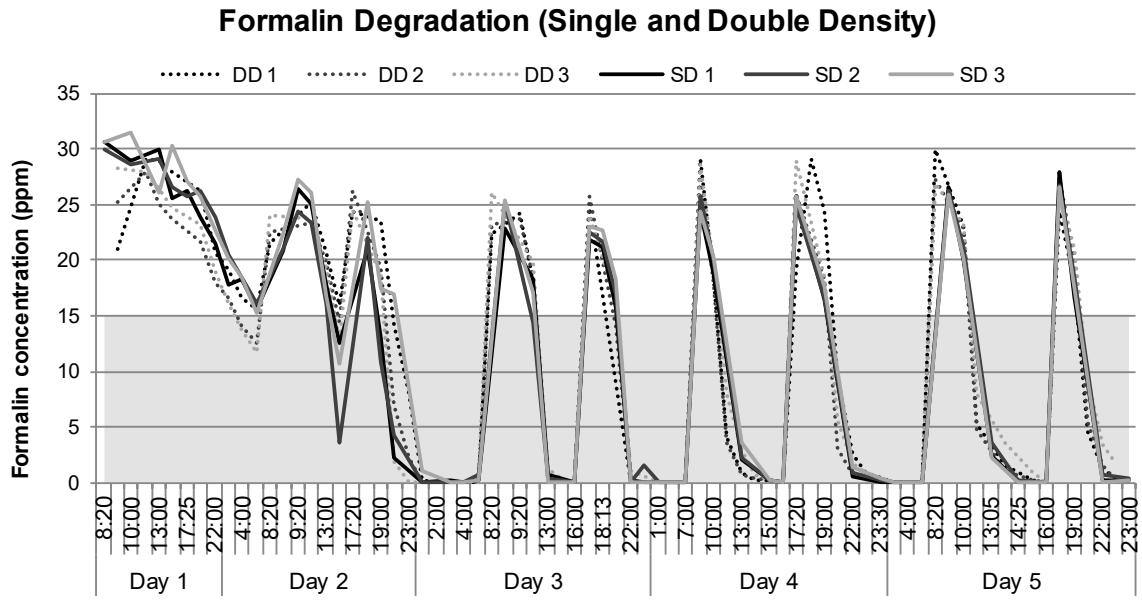

Figure 3. Formalin degradation rates in saltwater aquaria following the addition of formalin in single density (SD1–3) and double density (DD1–3) systems. Formalin added up to twice a day at 0800 and 1700 to achieve 25 ppm formalin concentration. Rate of formalin degradation increased with subsequent formalin additions. The degradation pattern was consistent across systems and across single and double density treatments. Shaded area shows non-therapeutic concentrations.

TAN and UIA concentrations in treatment systems when formalin values fell at or near zero remained below 0.15 mg·L^{-1} and 0.007 mg·L^{-1} respectively. During the double density experiment, TAN and UIA concentrations remained below 0.15 mg·L^{-1} and 0.006 mg·L^{-1} respectively.

Genetic sampling during the single density experiment revealed that bacterial diversity is unique to each biological filter. Significant differences were observed between treatment and control systems (ANOSIM R=0.263, P<0.001). In addition, there were significant differences within the control and treatment groups (treatment: ANOSIM R=0.579, P<0.001; control: ANSOIM R=0.65, P<0.001). Bacterial diversity did not appear to change during the five day experiment.

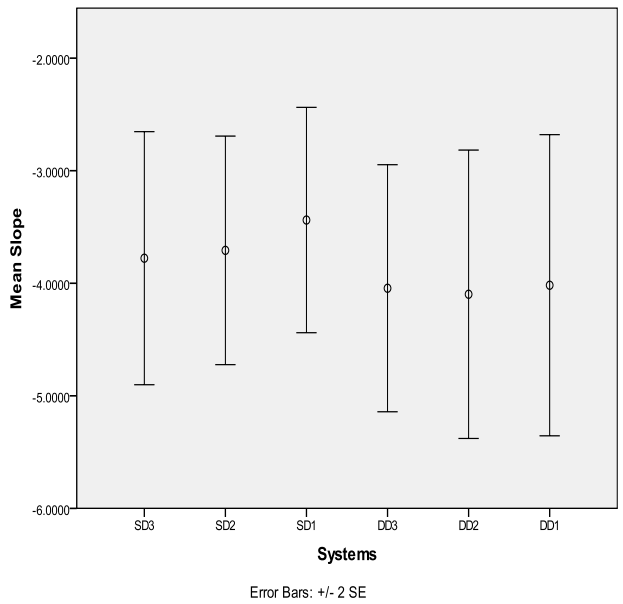

Figure 4. Comparison of slopes (rate) for formalin degradation rates from single density (SD 1–3) and double density (DD 1–3) systems.

Formalin degradation

The concentration of formalin in treatment tanks decreased over time without assisted chemical or mechanical removal. On day one no additional formalin was added at 1700 because concentrations had not decreased below target concentration. However, by day three the full 25 ppm dose was needed to bring the system up to 25 ppm at 1700 (Table1). The rate of formalin degradation changed over time; this pattern was similar for all systems despite differences in fish density (Figs 3 and 4). Degradation rates were calculated as the water passed through the biological filter and averaged 0.689 ppm·hr^{-1} for dose one and 5.497 ppm·hr^{-1} for dose nine (Table 1). The degradation of formalin per hour was similar between systems and across single and double fish density experiments (Fig. 5). No significant differences were observed between single density and double density degradation rates (Fig. 4). Using degradation rates, the average number of hours spent at therapeutic concentration (formalin concentration ≥15 ppm) was determined (Fig. 6). Water loss due to evaporation was minimal and no water additions were necessary.

Formalin concentrations from each tank during each dosing segment were plotted against time-since-dose in a scatter plot. The slope of the lines from each scatter plot was used to explore the change in formalin degradation over time. In both experiments the degradation began slowly on day 1 and rate of removal increased over the five day experiment. Formalin concentrations regularly decreased from 25 ppm to 0 ppm in five hours and at peak degradation in approximately four hours.

To explore the formalin degradation similarities (Fig. 3) across systems and fish densities, ANOVA was used (Fig. 4). There were no significant differences between systems or fish density (n=17; P=0.96, F=0.195, df=53).

To address the question of whether degradation rates change with subsequent formalin additions, the treatment tanks from the single density and double density experimentswere pooled to increase sample size. This was considered appropriate due to the lack of significance between fish density and degradation (Figs 4 and 5). Comparison between doses showed high significance (P<0.05, F=33.93, df=53) in degradation rates (Fig. 7). Games-

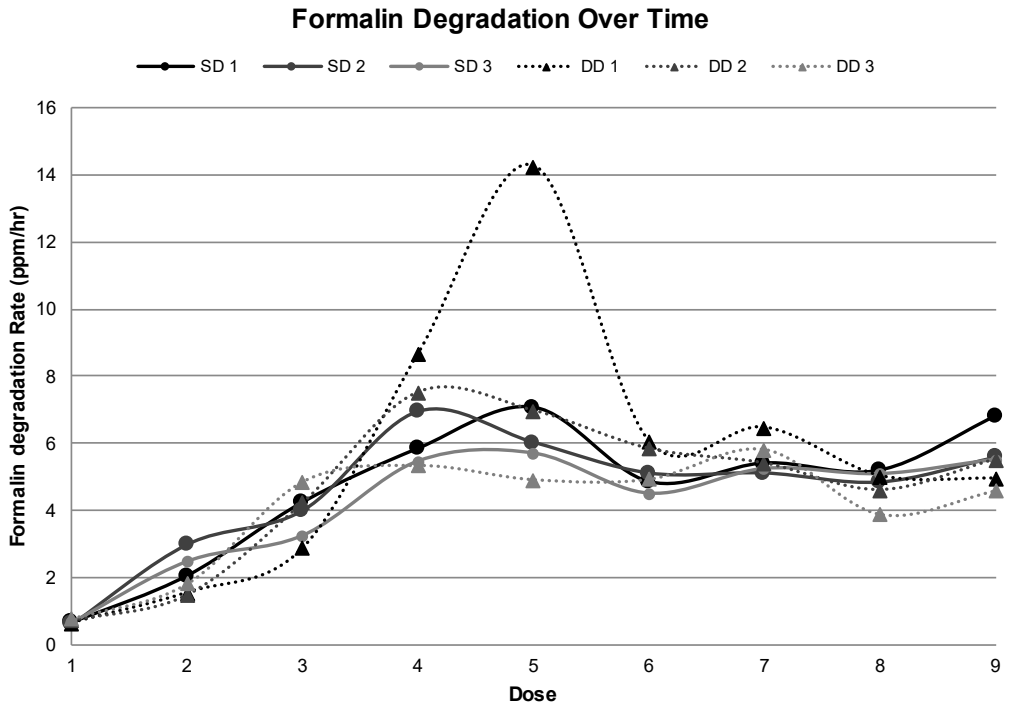

Figure 5. Formalin degradation rates following each of the nine formalin additions in the single density (SD1–3) and double density (DD1–3) experiments.

Howell post hoc analysis showed multiple significant relationships between doses (Table 2).

Discussion

Effects of formalin on nitrification

Prolonged exposure to 25 ppm of formalin was safe for the biological filters under the conditions we tested. The nine additions of formalin during the five-day treatment did not damage the biological filtration process as shown through water quality testing. Heinen et al. (1995) demonstrated that formalin can interfere with ammonia testing methods but does not interact with ammonia itself. Our data support this finding, with ammonia concentrations only detectable after formalin had degraded to low levels. Over the course of our experiment TAN and nitrite levels remained low.

Nitrite concentrations in the treatment tanks were higher than in control tanks at the end of the experiment, but these concentrations were safe for long-term fish holding and more than two orders of magnitude lower than lethal levels (Weirich and Riche 2006). Similar increases in nitrites were observed in fresh and saltwater systems after exposure to high formalin concentrations or multiple formalin additions (Heinen et al. 1995; Keck and Blanc 2002; Pedersen et al. 2010). Heinen et al. (1995)

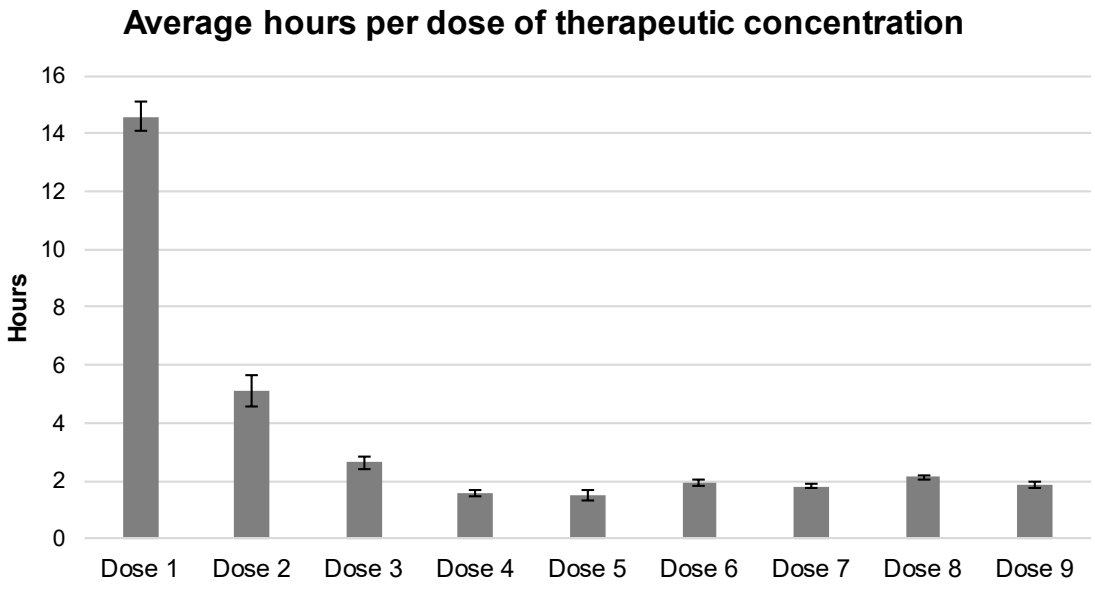

Figure 6. Average number of hours spent above 15 ppm formalin (therapeutic concentration) for each dose of formalin throughout the course of the 5-day experiment.

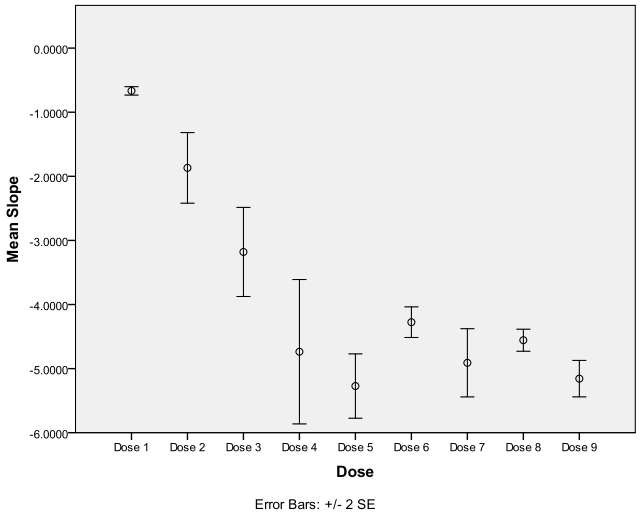

Figure 7. Comparison of slopes of formalin degradation in recirculating systems after each of nine formalin additions. Single density and double density degradation data combined because density was shown not to effect degradation (see Figure 4).

found that biological filters repeatedly exposed to formalin had reduced tolerance to formalin concentrations with subsequent exposures, and claimed that biological filters can become damaged with repeated exposure to high concentrations of formalin. This suggests that treatment frequency can have an impact and is important to monitor. These results are especially important for the aquarium and aquaculture industry because systems are rarely sterilised and restarted after each therapeutic treatment, and it is common practice for persistent pathogens to receive more than one treatment, especially in quarantine systems (Madsen et al. 2000; Hadfield and Clayton 2011).

Formalin degradation and possible factors responsible

Formalin was shown to degrade in these tropical saltwater recirculating aquariums. In our experiments formalin degradation rates were an order of magnitude higher on day three when compared to day one. These degradation rates were higher but comparable to those found in freshwater studies, where degradation rates increased one half to a full order of magnitude from initial dose to end of experiment (Pedersen et al. 2010). In our experiments, the rate of formalin removal was initially slow, increased quickly, and then plateaued. Similarly, Pedersen et al. (2010) demonstrated that the rate of formalin removal increased with dosing frequency. The primary concern around formalin degrading over the course of a treatment cycle is the amount of time that the treatment is not therapeutic. During our five-day, 25 ppm experiment, several hours were spent below 15 ppm formalin (Fig. 6). Possible influences on formalin degradation include evaporation, dilution due to water exchange, aeration, ingestion/digestion by fish, organic matter, shifting bacterial populations adjusting to formalin exposure, and bacterial digestion.

Evaporation and water exchange are factors of system design. They can be controlled for during the treatment process and do not need to alter formalin concentration. Previous studies demonstrated that reactions between formalin and ammonia or CO_2, or increased aeration, did not alter formalin concentration (Wienbeck and Koops 1990; Heinen et al. 1995).

Since established formalin treatment protocols do not account for fish density, one might assume biomass does not have an impact on formalin degradation. Wienbeck and Koops (1990) reported that the presence of fish contributed to the degradation of formalin. However, it is important to point out that their experiments did not account for all variables and lacked replication; additionally, they were unable to duplicate the results in a controlled recirculating system. If fish biomass and their associated organic matter (i.e. waste and food) did reduced formalin retention, we should have seen increased degradation during our second experiment when fish density was doubled. This was not observed in our experiment. The degradation rates of formalin in systems with doubled bioload (fish density, waste and food) were not statistically different from single density systems. It is possible that the reduction of formalin observed by Wienbeck and Koops (1990) was the result of attached biofilm on the tanks and the bacteria-rich sludge in the commercial system, rather than fish in the system. Given the shortcomings of their experiment and our results, data suggest that fish biomass, waste, and food do not affect formalin retention. However, it is important to point out that our double density systems were much lower than the density of commercial aquaculture systems, and thus our results may not apply at the large scale.

Microbial digestion is the leading explanation for formalin degradation with several studies demonstrating formalin neutralisation via bacteria-rich reactors and activated sludge (Adroer et al. 1990; Wienbeck and Koops 1990; Oliveira et al. 2004). Numerous genera of bacteria have been shown to utilise formaldehyde and methanol as energy sources (Adroer et al. 1990; Kaszycki and Koloczek 2000; Hidalgo et al. 2002; Oliveira et al. 2004). The replication systems in our study had similar temperature and salinity to one another, but statistically different bacterial diversity. Even with different bacterial communities, formalin degradation was similar across systems (Figs 3 and 6). Such bacterial diversity further supports the idea that multiple species of bacteria are capable of degrading formalin. Degradation of formalin has been shown to increase with repeated formalin additions and is thought to be the result of increased bacterial activity and/or population growth (Dickerson and Heukelekian 1950; Kaszycki and Koloczek 2000; Pedersen et al. 2007) . It is unclear if the relationship of degradation rate to dosing frequency is linear or logarithmic and additional research in this area would be useful.

Many of the potential factors influencing formalin degradation could be further influenced by abiotic factors (i.e. temperature,

Table 2. Games-Howell statistical test results. Comparison of slopes for degradation rate following each formalin addition during the five-day treatment regimen (see Figure 7). Statistically significant differences between slopes show change in degradation rates between doses. Comparison includes data from single and double density experiments.

Formalin dose *x*	Slope of degradation rate at dose *x* significantly different (P<0.05) from dose #:
Dose 1	3, 4, 5, 6, 7, 8, 9
Dose 2	4, 5, 6, 7, 8, 9
Dose 3	1, 5, 7, 9
Dose 4	1, 2
Dose 5	1, 2, 3
Dose 6	1, 2, 9
Dose 7	1, 2, 3
Dose 8	1, 2
Dose 9	1, 2, 3, 6

pH, salinity, etc.) and are important to consider before and during formalin treatments. Additonally, warmer water temperatures have been shown to decrease formalin retention (Pedersen et al. 2007, 2010). The increased removal due to temperature could be linked to metabolic rates, as several microbes have been shown to use formalin as a carbon energy source (Adroer et al. 1990; Kaszycki and Koloczek 2000; Hidalgo et al. 2002; Oliveira et al. 2004), but metabolic rates have not yet been studied. A different study showed that after a period without formalin in the system, the degradation rate upon re-exposure was as fast as or faster than the rate of the previous exposure, suggesting that systems can develop a memory to drug exposure (Pedersen et al. 2010). The authors argue that the bacteria utilising formalin are dynamic and can readily use other compounds in the system for energy when formalin is not available. Understanding how a system responds to formalin after a period without exposure is important for the aquarium industry because parasites can recur.

Conclusion

Under the conditions we tested, five-day 25 ppm formalin treatments were safe to use in these tropical saltwater aquariums and did not impair the biological filter. However, microbial diversity influences the nitrification process and the bacterial composition of each system is unique; therefore, monitoring biological filtration during prolonged treatments is a good idea. This study showed that formalin can degrade quickly and therapeutic levels might only be achieved for four hours in a 24-hour period. The need to monitor formalin concentrations during treatment periods is apparent. Such reduction in concentration could lead to decreased treatment efficacy and recurrence of the pathogen. Since formalin degradation rates are dynamic, dosing frequency will probably need to increase over the course of the treatment in order to maintain therapeutic concentrations.

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