6. Evaluation of treatment methods using sodium percarbonate and formalin on Australian rainbow trout farms

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6.1 Abstract

In aquaculture, effective parasite management often relies on chemical therapy when prevention strategies are ineffective. Fish are often medicated using immersion (bath) treatments. The efficacy of bath treatment relies on maintaining at least the minimum effective dose for the necessary treatment duration. Dose is influenced by the product used, calculation of system volume, application method, flow, mixing, treatment degradation rate and environmental conditions. To maximize efficacy the relationships between system, delivery and effective dose need to be understood or controlled. We tested four application methods using SPC and two application methods using FOR (37% formaldehyde [FA]) in four semi-closed flow-through systems on four Australian freshwater trout farms with different flow and water quality characteristics. Target dose was 64 mg/L SPC and 200 mg/L FOR. Hydrogen peroxide released from SPC was measured photometrically and FA levels were measured colorimetrically. Each application method achieved consistent doses across repeated applications but not all methods resulted in the dose reaching the target concentration in all parts of the system for the whole treatment duration. Eliminating the influence of system variables by creating static baths provides the most stable treatment environment. Where this is not possible, minimising system variables by modifying flow assists in retaining treatment in the system and improving accuracy of delivered doses. Treatment methods must be validated in a system prior to being routinely applied and mechanisms to optimise dose-duration identified and implemented.

6.2 Introduction

Rainbow trout, *Oncorhynchus mykiss* are an ideal species for cultivation and are a key aquaculture species in Victoria, Australia (ABARE, 2012). One factor limiting productivity of the sector is ectoparasitic disease, primarily caused by *Ichthyophthirius multifiliis*. Outbreaks of *I. multifiliis* have a significant negative effect on the host (reviewed by Matthews, 2005), including mortality (Ewing and Kocan, 1992). Management of *I. multifiliis* largely centres on husbandry, including minimising stress, manipulating water velocity in raceways and, when these measures are inadequate, application of strategically timed chemotherapeutants.

Malachite green is an effective treatment for a wide range of ectoparasites but is a potential carcinogen and teratogen and is no longer permitted to be used in food fish aquaculture (Alderman, 1985; Wahli et al., 1993; Meinelt et al., 2009). Sodium percarbonate and FOR are viable alternatives to malachite green for treatment of ectoparasites (Heinecke and Buchmann, 2009). Sodium percarbonate is a granular solid that dissociates in water to release HP, a strong oxidising agent, which is active against ectoparasites over time (Noga 2000; Heinecke and Buchmann, 2009). Sodium percarbonate can be used in Australia pursuant to a minor use permit (PER12944) issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) at doses of up to 100 mg/L, and is currently administered on Australian trout farms at a target dose of 64 mg/L (unpublished observations), which is effective against *I. multifiliis* theronts (Heinecke and Buchmann, 2009). FOR strongly reduces organic compounds (Masters, 2004), cross-links amino groups in proteins (Orlando et al., 1997) and is a general treatment for aquatic ectoparasites (Wise et al., 2004; Rowland et al., 2009). Formalin is typically administered at 200 mg/L for 1 h following Noga

(2000) on Australian trout farms, which is effective against *I. multifiliis* (see Wahli et al., 1993; Lahnsteiner and Weismann, 2007; Heinecke and Buchmann, 2009).

In Australia, rainbow trout are typically cultured in flow-through concrete raceways or earthen ponds, where FOR and SPC have been reported to be ineffective against *I. multifiliis* (pers. comm. E. Meggit, Victorian Trout Grower's Association). The major influences on the efficacy of bath treatments are the distribution of the product in the system and achieving and maintaining the minimum effective concentration for the treatment period (Rach et al., 1997a). Under-dosing and uneven distribution of the product in the system is therefore the most likely cause of low efficacy. The distribution of the dose in aquaculture systems is affected by application method, flow, mixing, degradation rate, environmental and water conditions (Rach and Ramsey, 2000). Understanding how these conditions influence the treatment is critical for achieving the target dose; if one of these variables is altered it may lead to the dose being too low or the exposure too short for appropriate efficacy, or too high or prolonged, with potentially negative effects on the exposed fish.

We designed this study to evaluate FOR and SPC application methods used on four commercial trout farms to determine if the minimum effective dose and desired duration were being achieved throughout each system.

6.3 Materials and methods

6.3.1 Field trials

Treatment application methods were assessed in four semi-closed flow-through systems on four fish farms with different flow and water quality characteristics: Fish farm A, a concrete, low volume, high turnover flow-though system; Fish farm B, a concrete, low volume, low turnover flow-through system; Fish farm C, a concrete,

high volume, low turnover, flow-through system; and Fish Farm D, a low turnover, high volume, flow-through earthen raceway. Flow-though systems on each farm had variable characteristics and were stocked with 1-year old juvenile rainbow trout at different densities, outlined in Tables 6.1 – 6.4. In all experiments, treatments were applied using the standard method used on that farm based on the volume of the system and the residual active compound was measured (SPC was measured as HP; FOR was measured as formaldehyde [FA]). Dose was measured at the inlet, middle and outlet of the system every 15 min until 90 min post 1 h treatments and 60 min post 30 min treatments. The target doses were 64 mg/L SPC (21 mg/L HP) and 200 mg/L FOR (74 mg/L FA). Technical grade SPC (Redox Pty. Ltd.) and FOR (37% FA) (Redox Pty. Ltd.) were used. Fish were observed for adverse reactions during the treatment and dissolved oxygen (DO) was measured in the middle of the raceway at each sample time. Each trial was repeated three times.

6.3.2 Fish Farm A

At Fish Farm A granular SPC was administered for 1 h and FOR for 30 min.

Sodium percarbonate trials

The initial dose of granular SPC was calculated based on the estimated total volume of the system (Table 6.1). During the treatment normal continuous flow was maintained. At the start of the treatment SPC granules were weighed and distributed throughout the raceway by bucket. After the initial dose, additional granular SPC was added evenly throughout the raceway every 5 min for 45 min with the additional doses estimated based on the flow rate (Table 6.1). The raceway was routinely swept with a broom to improve dissolution of granular SPC that accumulated on the bottom of the raceway.

Formalin trials

The initial dose of FOR was calculated based on the total estimated volume of the system (Table 6.1). During the treatment normal flow was maintained. At the start of the treatment, liquid FOR was measured and added by bucket directly to the water throughout the raceway. After the initial dose, additional liquid FOR was added by bucket throughout the raceway every 5 min for 25 min with the additional dose estimated based on the flow rate (Table 6.1).

Table 6.1 Description of the systems at Fish Farm A used in the validation of treatment methods using sodium percarbonate (SPC), measured as hydrogen peroxide (HP) and formalin (FOR) measured as formaldehyde (FA). N/A = Not applicable, N/D = Not determined.

Study system	SPC trials			FOR trials		
	Trial 1 Raceway 12	Trial 2 Raceway 6	Trial 3 Raceway 9	Trial 1 Raceway 12	Trial 2 Raceway 1	Trial 3 Raceway 2
Rearing volume (m^3)	55.8	33.6	34.3	55.8	34.3	57.4
Average depth (m)	1.2	0.72	0.73	1.2	0.73	1.25
In-flow water (L/s)	35	34	34	35	40	40
Fish density (kg/m^3)	32.2	N/D	N/D	32.2	26.8	14.4
Water temp. (°C)	6	5.2	5.4	5.5	9.7	9.8
COD (mg O_2/L)	< 20	< 20	< 20	< 20	< 20	< 20
Aeration	N/A	N/A	N/A	N/A	N/A	N/A
Treatment	SPC	SPC	SPC	FOR	FOR	FOR
Application method	Granular	Granular	Granular	Liquid	Liquid	Liquid
Initial dose	3.57 kg	2.15 kg	2.19 kg	8.04 L	6.8 L	11.48 L
Top ups	0.672 kg	0.65 kg	0.67 kg	2.01 L	2.4 L	2.4 L
Expected concentration of HP or FA (mg/L)	21	21	21	74	74	74

6.3.3 Fish Farm B

At Fish Farm B SPC was administered as a liquid solution and by granular application for 1 h.

Liquid sodium percarbonate trials

The initial dose was calculated based on the total estimated volume of the system (Table 6.2). Sodium percarbonate was added to two 200 L drums of water and left for 15 min to dissolve. Flow was restricted (Table 6.2) and liquid SPC was added into the inlet over 45 min. An additional dose based on the system flow rate (Table 6.2) was

dissolved in a separate 100 L drum of water 25 min after the initial application, the additional dose was added throughout the raceway by bucket. At the completion of the treatment normal flow was resumed.

Granular sodium percarbonate trials

The initial dose of granular SPC was calculated based on the volume of the system (Table 6.2). At the start of the treatment flow was stopped and granular SPC was manually distributed by bucket throughout the raceway. Normal flow was resumed at 40 min and at 45 min an additional dose of 10% of the initial dose was added at the inlet of the raceway.

Table 6.2 Description of the flow-through systems at Fish Farm B used in the validation of liquid and granular application methods of sodium percarbonate (SPC), measured as hydrogen peroxide (HP). R = Reduced flow, F = Full flow, N/A = Not applicable

Study system	Liquid application trials			Granular application method		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Type of system	Raceway 2	Raceway 4	Raceway 3	Raceway 4	Raceway 2	Raceway 1
Rearing volume (m^3)	23.2	17.4	21.6	17.4	23.2	18.4
Average depth (m)	0.5	0.5	0.5	0.5	0.5	0.5
In-flow water (L/s)	R 6.4, F 17.5	R 9.8, F 11.8	R 10.3, F 12.7	9.8	10.4	10.3
Fish density (kg/m^3)	29	10.6	13.9	24	16.1	20.2
Water temp. (°C)	6.4	11.2	11.3	6.8	11.4	11.5
COD level (mg O_2/L)	29	< 20	< 20	< 20	< 20	< 20
Aeration	N/A	N/A	N/A	N/A	N/A	N/A
Treatment	SPC	SPC	SPC	SPC	SPC	SPC
Initial dose	1.152	1.152	1.152	1.057	1.482	1.179
Top ups (kg)	0.64	0.64	0.64	0.903	0.148	0.117
Expected concentration of HP (mg/L)	21	21	21	21	21	21

6.3.4 Fish Farm C

At Fish farm C FOR was administered for 1 h. The initial dose was calculated based on half the total volume of the system (Table 6.3). Prior to the treatment the raceway volume was reduced to half, the outlet was blocked, but flow was maintained as normal. The initial dose was mixed in 3 x 20 L buckets of water and administered at

the inlet. Mixing was aided by a paddle wheel placed 2 m from the inlet pushing water against the flow. At 40 min post application normal discharge was resumed.

Table 6.3: Description of the flow-though systems at Fish Farm C used in the validation of application methods of formalin (FOR) measured as formaldehyde (FA).

Study system	Fish farm C		
	Trial 1	Trial 2	Trial 3
Type of system	Pond 2	Pond 5	Pond 3
Rearing volume (m^3)	191	172	152
Average depth (m)	1.3	1.2	1.05
In-flow water (L/s)	7.82	7.82	10.08
Fish density (kg/m^3)	35.6	33.5	30
Water temp. (°C)	6.0	6.1	10.4
COD level (mg O_2/L)	< 20	< 20	< 20
Aeration	Paddle wheel	Paddle wheel	Paddle wheel
Treatment	FOR	FOR	FOR
Application method	Liquid	Liquid	Liquid
Initial dose	20 L	20 L	20 L
Top ups (kg)	-	-	-
Expected concentration of FA (mg/L)	74	74	74

6.3.5 Fish Farm D

At Fish farm D granular SPC was administered for 1 h. The initial dose of granular SPC was calculated based on the total volume of the system (Table 6.4). Normal raceway volume and flow were maintained throughout the treatment. SPC granules were weighed and distributed throughout the raceway by bucket. In the third trial, at the request of the operator, two paddle wheels were added 2 m from the inlet and outlet, the inlet paddle wheel pushing water with the flow and the outlet paddle wheel pushing water against the flow, to aid in mixing and retaining the treatment. The paddle wheels were turned on 15 min after the treatment was initiated and turned off 30 min later.

Table 6.4: Description of the flow-through systems at Fish Farm D used in the validation of granular application of sodium percarbonate (SPC), measured as hydrogen peroxide (HP). N/A = Not applicable, N/D = Not determined.

Study system	Fish Farm D		
	Trial 1	Trial 2	Trial 3
Type of system	Raceway 2	Raceway 2	Raceway 1
Rearing volume (m^3)	221	221	349
Average depth (m)	76.33	76.33	92.7
In-flow water (L/s)	N/D	N/D	N/D
Fish density (kg/m^3)	<1	<1	1
Water temp. (°C)	11.4	12.1	12.6
COD level (mg O_2/L)	32	< 20	30
Aeration	N/A	N/A	Paddle wheel
Treatment	SPC	SPC	SPC
Application method	Granular	Granular	Granular
Initial dose	14.3	14.3	22.34
Top ups (kg)	-	-	-
Expected concentration of HP (mg/L)	21	21	21

6.3.6 Chemical analyses

Residual HP was measured using a Palintest® photometric system as per manufacturer's instructions. Color intensity was measured using a YSI 9300 photometer (YSI Inc.) (±1 mg/L; range 0 – 100 mg/L) within 1 min of mixing. Residual FA was measured using a colorimetric method with test strips and reagent (MQuant™ Product No. 1100360001) (± 10 mg/L; range 10 – 100 mg/L), as per manufacturer's instructions. Both measurement methods were calibrated in a known test solution of HP and FA, prior to and after each trial. Water temperature and dissolved oxygen (mg/L) were measured with a Handy Polaris H01P (OxyGuard®). Organic content in the water was measured as chemical oxygen demand (COD) in mg/L O_2 using the APHA 5220 COD Open Reflux method (Eurofins Environment Testing Australia Pty Ltd).

6.3.7 Statistical analyses

Prior to analysis, normality of the data were tested using the Shapiro–Wilk test and variances were tested using Levene's test. Where the data did not satisfy normality, they were log (y+1)-transformed, where y is the measured dose, prior to analysis.

Differences in the measured dose between raceway positions and at each time point were compared using a 2-way ANOVA (Underwood, 1997). Water temperature was included as a covariate (ANCOVA). 95% confidence intervals (CIs) were generated for the mean measured dose at each sample time and position, where the 95% CI did not overlap the target dose the measured dose was regarded as significantly different to the target dose (Zar, 1984). The statistical analyses were performed using IBM SPSS Statistics 20.0 and significance for all tests was judged at $P < 0.05$. All treatment means are reported as mean ± standard deviation (SD) (range).

6.4 Results

6.4.1 Fish Farm A

Sodium percarbonate trials

The mean dose of SPC measured as HP was 8.2 ± 4.1 mg/L (3 – 17 mg/L) at the inlet, 13.2 ± 4.4 mg/L (2 – 22 mg/L) in the middle was and 13.8 ± 5.9 mg/L (3 – 27 mg/L) at the outlet. Measured doses were significantly different between sample times (2-way ANOVA: $F_{4, 29} = 3.129$, $P = 0.030$) and raceway positions (2-way ANOVA: $F_{2, 29} = 8.628$, $P < 0.001$ (Fig 6.1A). There was no interaction between time and position (2-way ANOVA: $F_{8, 29} = 2.227$, $P = 0.055$). There was no significant difference in the measured dose in the middle of the raceway at 1, 15 and 30 min but the dose was significantly lower than the target in all other sections and times (Fig 6.1A). Water temperature did not have a significant effect on dose between trials (ANCOVA: $F_{1, 29} = 1.949$, $P = 0.173$).

Formalin trials

The mean dose of FOR measured as FA was 28.3 ± 19.3 mg/L (10 – 70 mg/L) at the inlet, 34.4 ± 19.9 mg/L (15 – 80 mg/L) in the middle and 35 ± 14.1 mg/L (15 – 50

mg/L) at the outlet. Measured doses were significantly different between sample times (2-way ANOVA: $F_{2, 17} = 8.461$, $P = 0.003$) but not between raceway positions (2-way ANOVA: $F_{2, 17} = 1.524$, $P = 0.246$) (Fig 6.1B). There was no interaction between time and position (2-way ANOVA: $F_{2, 17} = 0.600$, $P = 0.668$). Measured doses were significantly lower compared to the target at each position and time (Fig 6.1B). Water temperature did not have a significant effect on dose between trials (ANCOVA: $F_{1, 17} = 1.154$, $P = 0.298$).

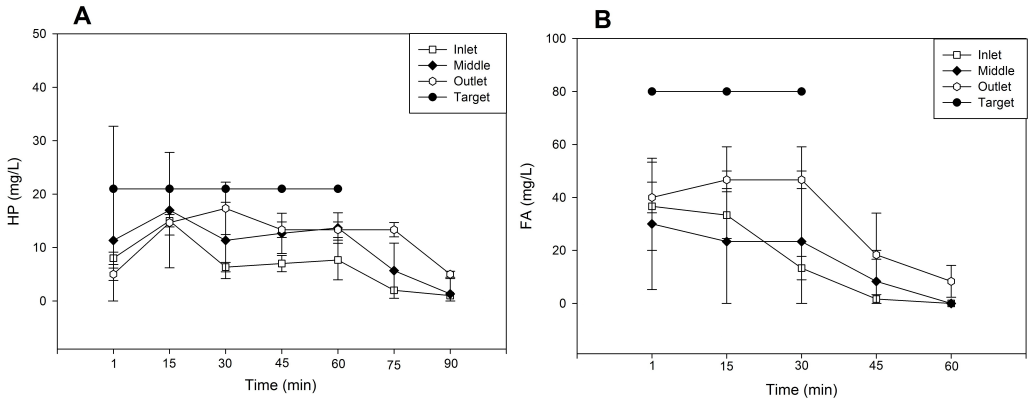

Figure 6.1: Mean doses of hydrogen peroxide (HP) released from sodium percarbonate (SPC) (A) and formaldehyde (FA) from formalin (37% FA) (B) on Fish Farm A. Each trial was repeated three times. Error bars represent 95% CI.

6.4.2 Fish Farm B

Liquid sodium percarbonate trials

The mean dose of SPC measured as HP was 11.1 ± 5.7 mg/L (2 – 19 mg/L) at the inlet, 10.5 ± 5.7 mg/L (2 – 20 mg/L) in the middle and 10.4 ± 6.3 mg/L (1 – 20 mg/L) at the outlet. The measured doses were significantly different between sample times (2-way ANOVA: $F_{4, 29} = 20.145$, $P < 0.001$) but not raceway positions (2-way ANOVA: $F_{2, 29} = 0.219$, $P < 0.804$) (Fig 6.1C). There was no interaction between time and position (2-way ANOVA: $F_{8, 29} = 2.173$, $P = 0.060$). There was no significant

difference in the measured dose in the middle and front of the raceway at 1 min and in the middle section at 15 min but the dose was significantly lower than the target in all other sections and times (Fig 6.2A). Water temperature did not have a significant effect on the dose between trials (ANCOVA: $F_{1, 29} = 1.707$, $P = 0.202$).

Granular sodium percarbonate trials

The mean dose of SPC measured as HP was 15 ± 9.7 mg/L (3 – 29 mg/L) at the inlet, 14.8 ± 7 mg/L (3 – 25 mg/L) in the middle and was 17 ± 12.6 mg/L (0 – 46 mg/L) at the outlet. Doses were significantly different between sample times (2-way ANOVA: $F_{4, 29} = 13.098$, $P < 0.001$) but not between raceway positions (2-way ANOVA: $F_{2, 29} = 1.112$, $P = 0.895$) (Fig 6.2B). There was no interaction between time and position (2-way ANOVA: $F_{8, 29} = 0.350$, $P = 0.938$). There was no significant difference in the measured dose in the front, middle and rear of the raceway at 15, 30 and 45 min but the dose was significantly lower than the target in all other sections and times (Fig 2B). Water temperature did not have a significant effect on dose between trials (ANCOVA: $F_{1, 29} = 0.002$, $P = 0.962$).

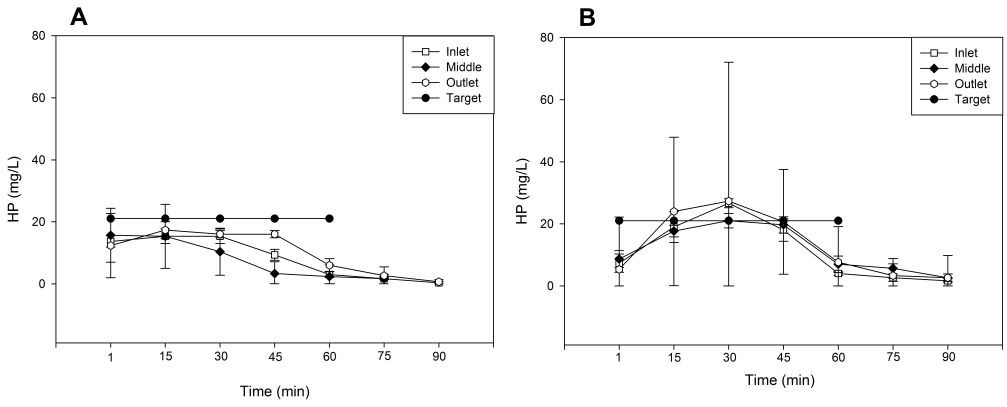

Figure 6.2: Mean dose of hydrogen peroxide (HP) released from sodium percarbonate (SPC) administered by drip feed liquid application with one top up dose at 25 min (A); and by granular application in a static bath, with one top up dose at 45 min (B) on fish farm B. Each trial was repeated three times. Error bars represent 95% CI.

6.4.3 Fish Farm C

The mean dose of FOR measured as FA was 36 ± 22.9 mg/L (0 – 90 mg/L) at the inlet, 25 ± 12.7 mg/L (10 – 60 mg/L) in the middle and was 27.3 ± 14.7 mg/L (0 – 60 mg/L) at the outlet. Measured doses were not significantly different between sample times (2-way ANOVA: $F_{4, 29} = 1.269$, $P = 0.305$) or between raceway positions (2-way ANOVA: $F_{2, 29} = 1.495$, $P = 0.241$) (Fig 6.3). There was no interaction between time and position (2-way ANOVA: $F_{8, 29} = 0.319$, $P = 0.952$). There was no significant difference in the measured dose in the front of the raceway at 1, 15 and 45 min and in the front and rear at 45 min but the dose was significantly lower than the target in all other sections and times (Fig 6.3). Water temperature did not have a significant effect on the dose level between trials (ANCOVA: $F_{1, 29} = 0.992$, $P = 0.345$).

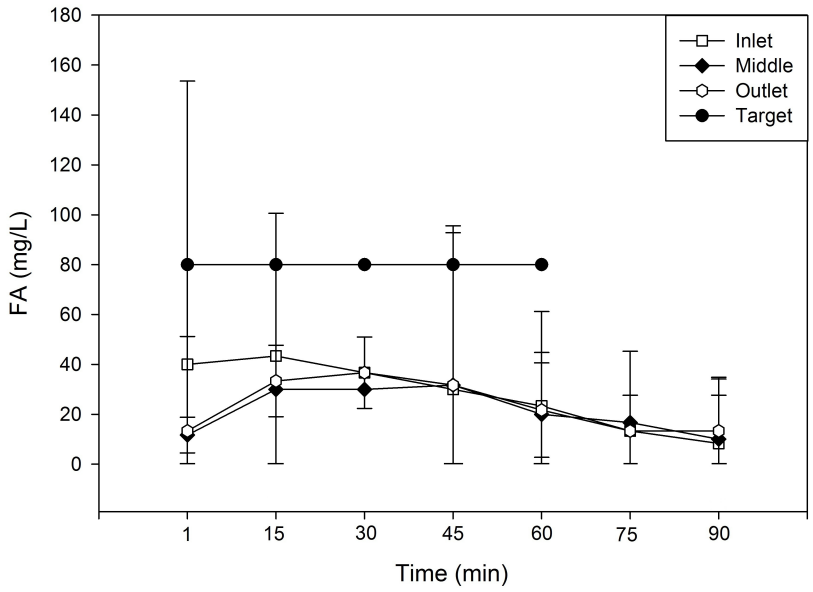

Figure 6.3: Mean doses of formaldehyde (FA) from formalin (37% FA) administered by one dose application into a static bath on Fish Farm C (A). Each trial was repeated three times. Error bars represent 95% CI.

6.4.4 Fish Farm D

The mean dose of SPC measured as HP was 7.9 ± 5.2 mg/L (2 – 18 mg/L) at the inlet, 12.7 ± 6 mg/L (2 – 27 mg/L) in the middle and was 17.2 ± 7 mg/L (1 – 26 mg/L) at the outlet. Measured doses showed high variability and were significantly different between sample times (2-way ANOVA: $F_{4, 29} = 3.609$, $P < 0.017$) and between raceway positions (2-way ANOVA: $F_{2, 29} = 0.219$, $P < 0.001$) (Fig 6.4). There was no interaction between time and position (2-way ANOVA: $F_{8, 29} = 1.627$, $P = 0.160$). There was no significant difference in the measured dose in the front of the raceway at 1 and 15 min; in the middle of the raceway at 1, 15, 30 and 45 min; and in the rear of the raceway at 15, 30, 45 and 60 min, but the dose was significantly lower than the target in all other sections and times (Fig 6.4). Water temperature had a significant effect on the dose between trials (ANCOVA: $F_{1, 29} = 5.597$, $P = 0.025$).

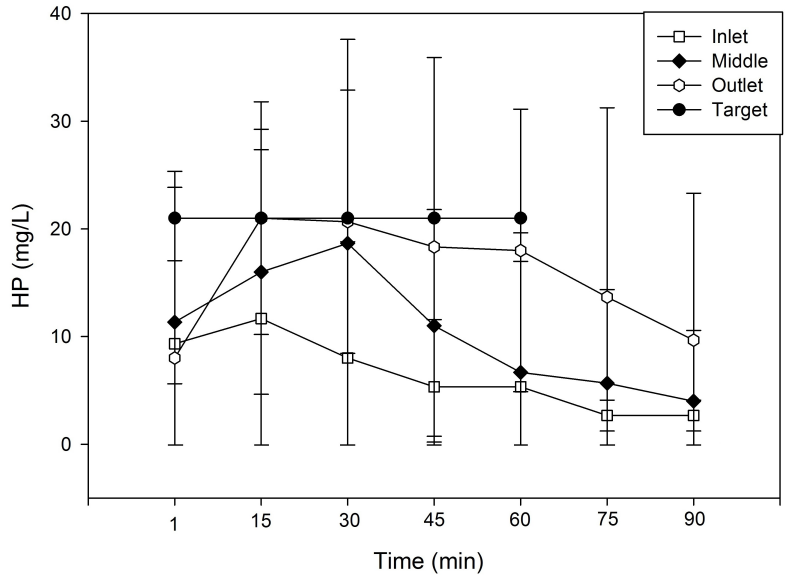

Figure 6.4: Mean doses of hydrogen peroxide (HP) released from sodium percarbonate (SPC) administered by granular application with one dose administered evenly throughout the raceway on Fish Farm D. Each trial was repeated three times. Error bars represent 95% CI.

6.4.5 Dissolved oxygen

During SPC trials on Farm A the mean dissolved oxygen (DO) was 13.6 ± 4.9 mg/L (9.9 – 26.5 mg/L) and was 9.4 ± 0.3 (9.1 – 10.1 mg/L) in FOR trials; mean DO in liquid SPC trials on farm B was 4.8 ± 1.6 mg/L (2.8 – 6.5 mg/L) and 7.8 ± 2.5 mg/L (4.8 – 11.4 mg/L) in granular SPC trials; mean DO in FOR trials on farm C were 8.9 ± 1.7 mg/L (6.5 – 11 mg/L); and mean DO in SPC trials on farm D were 11.1 ± 3.3 mg/L (7.7 – 19.6 mg/L) (Table 6.5).

Table 6.5: Mean ± SD (range) dissolved oxygen levels during the treatment period using sodium percarbonate (SPC), measured as hydrogen peroxide (HP) and formalin (FOR) measured as formaldehyde (FA) on Australian trout farms.

Farm	Treatment	Dissolved oxygen (mg/L)			
		Trial 1	Trial 2	Trial 3	Total
Farm A	SPC	18.8 ± 5.7 (11.1 – 26.5)	10.7 ± 0.6 (10.1 – 11.4)	11.3 ± 0.49(9.9 – 12.1)	13.6 ± 4.9 (9.9 – 26.5)
	FOR	9.7 ± 0.3 (9.5 – 10.1)	9.4 ± 0.3 (9.1 – 9.7)	9.2 ± 0.06 (9.2 – 9.3)	9.4 ± 0.3 (9.1 – 10.1)
Farm B	SPC (liquid)	4.5 ± 1.6 (2.8 – 6.3)	7.8 ± 1.2 (6.5 – 9)	7.9 ± 0.6 (7.3 – 8.6)	6.7 ± 2 (2.8 – 9)
	SPC (granular)	11 ± 0.4 (10.6 – 11.4)	6.3 ± 1 (5.4 – 7.9)	6.1 ± 1.1 (4.8 – 7.4)	7.8 ± 2.5 (4.8 – 11.4)
Farm C	FOR	10.7 ± 0.2 (10.4 – 11)	9.2 ± 0.2 (8.9 – 9.4)	6.8 ± 0.2 (6.5 – 7.1)	8.9 ± 1.7 (6.5 – 11)
Farm D	SPC	10.7 ± 5 (7.7 – 19.6)	11.3 ± 2.3 (10 – 15.3)	11.3 ± 2.7 (9.3 – 15.9)	11.1 ± 3.3 (7.7 – 19.6)

6.5 Discussion

No application method achieved the target dose in all sections of the raceway or over the entire treatment period. In SPC trials the most spatially and temporally stable treatment was granular SPC applied to a static bath on farm B. The slowly dissipating single application of SPC on Farm D also provided a temporally consistent dose but the concentration of product was substantially below the target at the inlet and middle sections of the raceway. On farm D paddle wheels were added to the raceway in the third trial, causing the HP concentration to remain higher for longer in this trial (Rach and Ramsay, 2000) and increasing variability between trials. Liquid application of SPC on farm B also provided a spatially and temporally consistent dose throughout

the raceway but the dose was substantially lower than the target. Farm A was treated by continual addition of SPC under normal flow. This method provided a spatially consistent but highly temporally variable dose and was below the target dose in all sections and at most times due to dilution of the product and poor matching of product addition with flow rate. Rach et al. (1997a) reported under-dosing in incubators using liquid HP, caused by dilution of the product as it moved through the system. Rach and Ramsay (2000) also noted that dilution and variable flow patterns caused under-dosing when applying liquid HP to incoming water in connected flow-through raceways.

Most SPC treatments we monitored failed to meet the target dose, but dose at the inlet was often lower than at the outlet, particularly at the end of the treatment period. Rach et al. (1997a) and Rach and Ramsay (2000) observed that HP doses were higher at the inlet and decreased as the product moved through the system due to dilution. This difference is likely to be due to incoming water flushing the solid SPC through the system in our study, diluting it more at the inlet and concentrating it at the outlet over the treatment period. Granular SPC treatments often failed to reach the target dose within 15 min of application; dissociation and release of HP from the product is gradual. Mixing SPC with water prior to application facilitates the release of HP, decreasing the time between adding the product and the HP concentration reaching the target dose. If this is impractical, treatments could be managed by assuming that the start of the exposure is when the product reaches the target dose, rather than when the product is added. In liquid SPC trials, consistent under-dosing was probably largely due to addition of the product to the system by gravity driven flow at too low a rate (Rach and Ramsay, 2000), particularly on farm A which had the greatest flow (34 - 35 L/s) and required constant addition of SPC to compensate for

the diluting effect of incoming water and loss of product at the discharge point. This can be addressed by increasing the initial dose of SPC and/or by monitoring the dose during the treatment and adding additional product when required. Buchmann et al. (2003) suggested that SPC may display reduced efficacy in organically loaded systems and that higher doses would be required in these conditions to achieve adequate efficacy. We found no evidence that using SPC in our higher organic load systems reduced the amount of available HP in the system during treatment. Monitoring dose throughout treatment should also adequately address this problem.

During FOR trials on farms A and C, product was consistently distributed throughout the raceway, but the dose was significantly lower than the target. On farm C, FA doses failed to meet targets probably because of an underestimation of system volume combined with FA being bound to organic material in the water (Pedersen et al., 2007). On farm A, FA doses failed to reach the target because of the high flow rate (34 - 35 L/s) removing the product from the system more quickly and continuously than additional product was applied. Each system has different water chemistry and environmental factors; measuring the concentration of FA after initiating treatment and adjusting dose appropriately is necessary to accurately achieve the target dose. Limiting or stopping flow provided a more consistent distribution of the product in the system than application during normal or slowed flow. Limited flow or static baths limit the effect of dilution and dose can be maintained by monitoring during the treatment and adjusting as required. Formaldehyde concentrations rapidly decreased in the system once the treatment was completed. We did not assess the concentration of FOR in discharge water, but all farms achieved the ten-fold dilution required by APVMA PER14489. Masters (2004)

outlined neutralisation methods that could be employed on farms whose infrastructure and/or water supplies are inadequate to achieve the required dilution.

The addition of SPC or FA into aquaculture systems can influence oxygen availability. During SPC treatments there were significant increases and decreases in DO depending on the application method. In trial 1 on farm A and all trials on farm D there were increases in DO. When HP oxidises organic material, oxygen is liberated (Pedersen et al., 2006) and in high organic load systems increased DO during SPC treatment is common (Buchmann and Kristensson, 2003). We observed this on farm D, which had the highest COD levels and consistently displayed elevated DO during treatment. Elevated DO needs to be managed because prolonged exposure can result in fish mortalities (Pedersen et al., 2006). Across all SPC trials the DO peaked at 26.5 mg/L but reduced to normal (~10.7 mg/L) within 60 min. Exposure to DO in this elevated range for this duration is unlikely to negatively affect rainbow trout (Edsall and Smith, 1990). Fish undergoing SPC treatment did not display any signs of adverse effects and there was no associated mortality. Formalin treatments can reduce DO in aquaculture systems (Rowland et al., 2006), but in our FOR trials DO was stable and adequate to maintain rainbow trout.

Reducing the flow to facilitate a more spatially and temporally consistent dose during liquid SPC treatment on farm B caused fish to use the available oxygen causing a hazardous drop in DO. The reduced DO led to the abandonment of the treatment and normal flow of water into the system was resumed to avoid hypoxia in treatment fish. When reducing or stopping flow for treatment, adequate supplementary oxygenation must be used to maintain DO, particularly during FOR treatments in which the product also depletes DO (Noga, 2000).

When the application method was consistent, variability in the measured dose of both products between repeated applications was low, and using consistent methodology should provide a predictable dose. We confirmed that static baths largely eliminate the factors that increase spatial and temporal variability in dose and are therefore the preferred bath treatment method (Noga, 2000). Where static baths are not logistically feasible, such as in systems where flow cannot be stopped, mechanical mixing devices can aid mixing and maintenance of dose. Each treatment approach must be validated and each treatment should be monitored to ensure that the target dose is being reached and maintained but not exceeded throughout the system. Dose adjustment can then be employed in an informed manner to optimise efficacy. Consideration should be made of changing application method, including altering infrastructure where appropriate, if treatments cannot be consistently managed in existing facilities, and these recommendations should be taken into account when designing new farms.

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