# 9. Efficacy of current and alternative bath treatments for *Lepidotrema bidyana* infecting silver perch (*Bidyanus bidyanus*)

James M. Forwood, James O. Harris and Marty R. Deveney.

**Aquaculture (2013) 416-417: 65-71.**

## 9.1 Abstract

*Lepidotrema bidyana*, a gill monogenean, infects silver perch (*Bidyanus bidyanus*) in aquaculture. *Lepidotrema bidyana* is managed using short duration baths during grading, prior to the fish being returned to grow-out ponds. In response to anecdotal reports of poor efficacy, we evaluated the efficacy of treatments used in the silver perch aquaculture industry in Australia: Formalin (FOR), trichlorfon (DEP) and sodium chloride. We also assessed the efficacy of peracetic acid (PAA), chloramine-T (CL-T), praziquantel (PZQ), hydrogen peroxide (HP) and sodium percarbonate (SPC) using a panel of *in vitro* and *in vivo* trials. During *in vitro* trials parasite mortality was calculated as a percent reduction and treatment doses were considered effective when greater than 90% parasite mortality was observed. The minimum effective dose for each treatment after 100 min *in vitro* was 100 mg/L for FOR; 10 g/L for sodium chloride; 20 mg/L for PZQ; 175 mg/L for HP; and 50 mg/L for SPC. Trichlorfon, PAA and CL-T were ineffective *in vitro*. During *in vivo* trials, PZQ administered at 40, 15 and 5 mg/L for 60 min had an efficacy of 77, 68 and 47%, respectively, and surviving worms were predominantly juveniles located at the base of the secondary lamellae. Silver perch treated with HP has a significantly lower intensity of *L. bidyana*, however, fish were highly sensitive to HP, resulting in mortalities. Treatment with FOR, sodium chloride and SPC was ineffective at all doses trialled *in vivo*. These results indicate that the current treatment recommendations are ineffective for managing *L. bidyana* and that PZQ is a potential alternative short duration bath treatment.

## 9.2 Introduction

The cultivation of silver perch (*Bidyanus bidyanus*) is a growing Australian aquaculture industry with a gross production value of AUS $3.4M in 2009-2010 (ABARE, 2012). This fish is also cultivated in Israel (Barki et al., 2000), China and Taiwan (Rowland and Barlow, 1991). Silver perch in Australia are grown predominantly in earthen ponds, but culture in recirculating aquaculture systems (RAS), raceways and cage culture systems is emerging (Rowland, 2009). Parasitic diseases are problematic on silver perch farms (Rowland et al., 2007). The gill monogenean *L. bidyana* (Monopisthocotylea: Diplectanidae) is particularly difficult to manage. Heavy *L. bidyana* infestations cause epithelial hyperplasia (Rowland et al., 2006), reduce appetite and growth, induce stress and facilitate secondary infections (Rowland et al., 2007).

Growers can treat *L. bidyana* with a short duration bath when silver perch are graded, prior to being returned to grow out ponds. Short exposure baths are preferred because they are administered in a reduced volume of water and therefore require less of the product, reducing treatment cost. Fish can be moved out of the treatment system rapidly, eliminating drug residues in the environment, which can lead to prolonged low level exposure and the development of resistance and environmental harm (Noga, 2000). Product disposal can also be controlled, reducing environmental impacts. *Lepidotrema bidyana* infections are currently treated using FOR, DEP or sodium chloride (Read et al., 2007). Formalin (Rowland et al., 2006) and DEP (Landos et al., 2007) are administered as prolonged, dissipative baths in the grow-out ponds and can also be administered as higher dose, short duration baths in tanks (Read et al., 2007). Sodium chloride cannot be administered in ponds because of the large quantities required to achieve target doses and the prolonged environmental

impact of soil salination (Mifsud and Rowland, 2008). There is concern in the Australian industry that the current treatments lack efficacy (M. Landos, pers. comm.) and FOR and DEP have numerous fish health, worker safety and environmental disadvantages (Forwood et al., 2013).

In aquaculture, PZQ is delivered both orally and by bath in the treatment of gill and skin monogeneans (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Hirazawa et al., 2000; Kim et al., 2001; Chisholm and Whittington, 2002; Janse and Borgsteede, 2003; Hirazawa et al., 2004; Williams et al., 2007; Forwood et al., 2013). Hydrogen peroxide treatments have been used extensively in aquaculture (Noga, 2000), including against monogeneans (Rach et al., 2000; Ogawa, 2002; Mansell et al., 2005) delivered as liquid HP or as SPC (Buchmann and Kristensson, 2003). Peracetic acid has been identified as a potential chemotherapeutant for aquaculture and is effective against the freshwater ciliate, *Ichthyophthirius multifiliis* (see Rintamäki-Kinnunen et al., 2005a,b; Meinelt et al., 2007a,b; Meinelt et al., 2009; Straus and Meinelt, 2009; Sudová et al., 2010; Picón-Camacho et al., 2012b). Chloramine-T is used widely in aquaculture as a treatment for external bacterial infections (From, 1980; Bullock et al., 1991; Bowker and Erdahl, 1998), protozoa (Cross and Hursey, 1973; Madsen et al., 2000; Harris et al., 2004; Rintamäki-Kinnunen et al., 2005a,b) and monogeneans (Noga, 2000). The use of these products in aquaculture led us to test their efficacy against *L. bidyana*.

The aims of this study were to evaluate the effectiveness of the current treatments and to investigate alternative treatments in a panel of *in vitro* assays and, where effective, using *in vivo* efficacy trials.

## 9.3 Materials and methods

### 9.3.1 Source of fish and treatments

Infected silver perch were obtained from a commercial farm and maintained in a 10 000 L fibreglass recirculation tank at The Flinders University of South Australia (FUSA). On arrival, five fish were euthanased and checked for *L. bidyana* infection as per Forwood et al. (2012). The treatments assessed were FOR (37% formaldehyde reagent, Ajax Finechem ®), DEP (Sigma-Aldrich®, lot no. SZB8021XV), sodium chloride (Merck ®, batch ref. MJ6M562652 during the *in vitro* trials and Oceanpure® during the *in vivo* trials), PAA (Sigma-Aldrich®, lot no. 31996TMV), CL-T trihydrate (Sigma-Aldrich®, lot no. MKBJ5095V), PZQ (Sigma-Aldrich®, lot no. P4668), HP (30% HP, Chem-Supply®, batch ref. (10) 255526) and SPC (Sigma-Aldrich®, lot no. MKBB5394V).

### 9.3.2 Source of parasites for the *in vitro* trial

Parasitized fish were randomly selected from the source population and euthanased with an overdose (40 mL / 1000 L bath) of Aqui-S®. Their gill arches were removed, and separated, and the primary lamellae were clipped, separated by gentle pipetting and immersed in Petri dishes containing filtered, deionized fresh water (DFW). Both adult (200 – 1000 μm) and juvenile (70 – 200 μm) worms (identified by observations of the reproductive systems and the presence of eggs) were used in this study and randomly allocated throughout the treatment groups.

### 9.3.3 *In vitro* trials

Groups of 10 active worms still attached to fragments of gill filaments were rinsed with DFW. All compounds were made to the required concentrations outlined in Table 9.1 in 1 L of DFW. Praziquantel was dissolved in 2 mL of ethanol to facilitate

dissolution, prior to being added to 1 L of DFW. To discount the possible effects of ethanol on *L. bidyana*, additional control wells containing DFW and the corresponding concentration of ethanol were tested. Worms were transferred by pipette to individual wells of a 24 well culture plate (Corning®), rinsed with the designated treatment dose and left in each well containing 2 mL of a designated treatment dose. 10 worms were added to 2 mL of DFW as a control.

Table 9.1: Chemicals and designated concentrations for 100 minute *in vitro* exposure of *Lepidotrema bidyana.* – Higher dose not trialled.

| Chemical treatment | Concentration | | | | | | Units |
|---|---|---|---|---|---|---|---|
| Formalin | 30 | 50 | 75 | 100 | 150 | 250 | mg/L |
| Trichlorfon | 0.2 | 0.5 | 1 | 1.5 | 2.5 | - | mg/L |
| Sodium chloride | 0.5 | 1 | 5 | 7.5 | 10 | - | g/L |
| Peracetic acid | 0.5 | 1 | 2 | 5 | 10 | - | mg/L |
| Chloramine-T | 5 | 10 | 20 | 40 | 60 | - | mg/L |
| Praziquantel | 1 | 5 | 10 | 20 | 40 | - | mg/L |
| Hydrogen peroxide (active) | 100 | 150 | 175 | 225 | 300 | - | mg/L |
| Sodium percarbonate | 5 | 25 | 50 | 100 | 150 | - | mg/L |

Each well was viewed 10, 20, 40, 60, 80 and 100 min after exposure was initiated and the number of dead worms at each time was recorded to generate time concentration toxicity curves. Parasites were considered dead when (normally transparent) parasites became opaque, movement had ceased, lysis was apparent or when a combination of these factors was observed. For each treatment dose three wells and one control well were used, using worms removed from the same host. The experiments were replicated three times.

### 9.3.4 *In vivo* trials

Infected silver perch were randomly selected from the source population. Fish were acclimated for 24 h in 50 L aerated holding tanks containing recirculated water covered with black polyurethane mesh and maintained at 18 ± 2 °C (7.5 ± 0.6 mg/L dissolved oxygen). After acclimation, 120 fish were transferred to 50 L treatment

tanks of static, aerated, filtered municipal water (95 mg/L $CaCO_3$ alkalinity, 90 mg/L $CaCO_3$ hardness) containing the designated treatment dose and duration outlined in Table 9.2. Each trial tested the efficacy of one treatment at three doses ($n$ = 10 fish per dose) and a control ($n$ = 10 fish). The designated dose of each treatment was dissolved in a bucket of water, stirred, added to the treatment tank and mixed further. Praziquantel was dissolved in 10 mL of absolute ethanol and SPC was left to dissolve for 30 min prior to mixing. Control tanks underwent the same mixing process but no treatment was added, PZQ controls received the corresponding amount of ethanol without PZQ. Sodium chloride concentrations were measured with a conductivity meter (YSI Inc.) to confirm dose prior to fish being transferred to the bath. Each dose and the controls were replicated three times. Fish were not fed during the experiments.

Table 9.2: Designated dose and duration of chemical treatments and the weight of silver perch ($n$ = 120) during *in vivo* trials.

| Chemical treatment | Concentration | | | Units | Duration (min) | Weight ± SEM g (range) |
|---|---|---|---|---|---|---|
| Praziquantel | 5 | 15 | 40 | mg/L | 60 | 16.3 ± 0.2 (10.5 to 25.1) |
| Hydrogen peroxide | 100 | 150 | 225 | mg/L | 60 | 16.9 ± 0.3 (10.9 to 25.6) |
| Formalin | 125 | 167 | 250 | mg/L | 30 | 44 ± 2.6 (12.1 to 145.5) |
| Sodium chloride | 5 | 10 | 15 | g/L | 60 | 16 ± 0.2 (10.9 to 22.5) |
| Sodium percarbonate | 75 | 100 | 150 | mg/L | 60 | 27.5 ± 1.4 (13.2 to 102.3) |

### 9.3.5 Sampling procedure for *in vivo* trials

During treatment, fish were monitored for signs of toxicity such as impaired swimming performance, colour changes, and loss of equilibrium, and any mortality was recorded. After treatment, fish were returned to the holding tanks, left for 24 h and then euthanased with an overdose (a 40 mL / 1000 L bath) of Aqui-S® and the number of live *L. bidyana* on the first left posterior hemibranch was recorded and an intensity estimate was generated for that fish (Forwood et al., 2012).

### 9.3.6 Statistical analysis

During *in vitro* trials the mortality of *L. bidyana* was calculated as a percent reduction. Prior to analysis, normality of the data was tested using the Shapiro-Wilk test and variances were tested using Levene's test. The data could not be transformed to achieve homoscedasticity therefore non-parametric tests were used. Differences between all treatment groups at the end of the treatment were examined using the Kruskal-Wallis Test. Where overall significant differences were found, a pairwise comparison comparing each treatment dose to the controls was made using a Mann-Whitney *U* Test. A treatment dose was considered effective when there was a greater than 90% parasite mortality and a significant difference ($P < 0.001$) between the treatment and the control. The $LC_{90}$ value for each treatment with 95% confidence intervals (CI) was determined using a probit procedure with $\log_{10}$ function.

During *in vivo* trials the efficacy of each treatment was assessed as a percentage reduction in mean *L. bidyana* numbers, relative to the control groups, and was calculated by adapting the formula of Stone et al. (1999) given below:

$$\% \text{ reduction} = 100 - \left(100 \text{ x } \frac{\text{Mean parasite intensity of each treated replicate}}{\text{Mean parasite intensity of the control replicates}}\right)$$

Intensity data obtained during the FOR and sodium chloride *in vivo* trials were $\log_{10}$ transformed prior to analysis. Differences in the mean intensity of *L. bidyana* between treatment and control groups and differences in the mortality rate between treatment groups were analysed using a 1-way ANOVA. Where significant differences were detected in parasite intensity, post-hoc comparisons were made via Tukey's test. Where significant differences were detected in the mortality rate, post-hoc comparisons between treatment groups and the control groups were made via Dunnett's two tailed t-test. Where data were unable to be transformed to achieve

homoscedasticity, an equivalent non-parametric test (Kruskal-Wallis test) was used. The statistical analysis was performed using IBM SPSS Statistics 19.0 and significance for all *in vivo* tests was judged at $P < 0.05$.

## 9.4 Results

### 9.4.1 *In vitro* trials

Mortality of *L. bidyana* was significantly different between all FOR, sodium chloride, PZQ, HP and SPC treatment groups and controls (Kruskal-Wallis, $P < 0.001$). Formalin, sodium chloride, PZQ, HP and SPC were effective treatments; DEP, PAA and CL-T were ineffective treatments (Fig 1A-H).

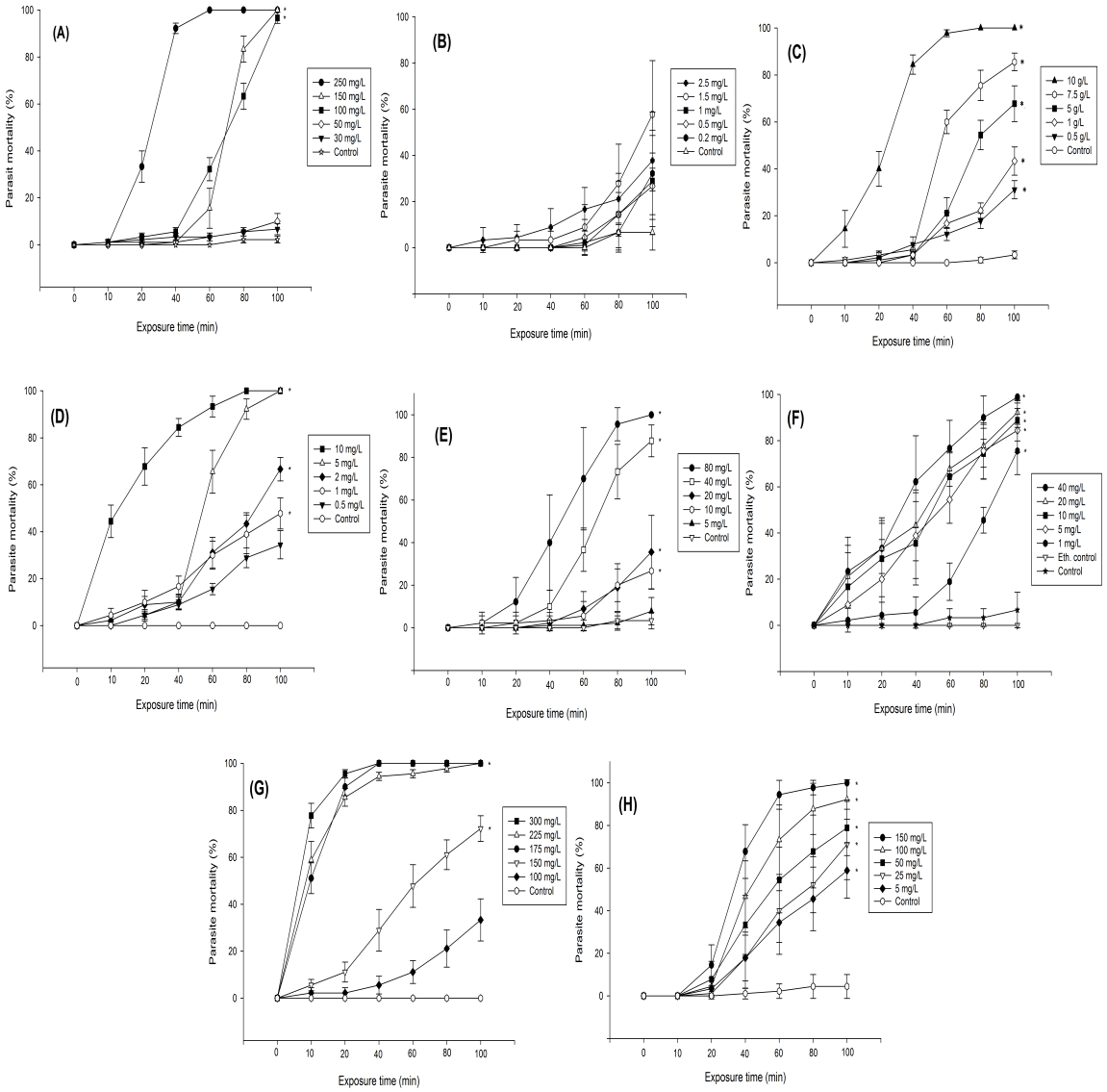


Figure 9 1: Effects of different treatments on *Lepidotrema bidyana*. (A) formalin, (B) trichlorfon, (C) sodium chloride, (D) peracetic acid, (E) chloramine-T, (F) praziquantel, (G) hydrogen peroxide and (H) sodium percarbonate. Values are means and standard error (SEM). Significant differences between treatment groups and the control group were made using a Mann-Whitney *U* Test and are indicated by * ($P < 0.001$). 10 worms in each well of a 24-well plate were used for each treatment dose and exposed for 100 min. Three wells were used for each experimental dose and the experiment was performed three times using different batches of worms.

No mortality was observed among *L. bidyana* in the groups incubated in DFW and the corresponding concentration of ethanol. Within 20 min of exposure at 40, 20,

10 and 5 mg/L PZQ *L. bidyana* exhibited an atypical spasmodic movement and affected worms detached from the gill lamellae and died. $LC_{90}$ values at 40, 60, 80 and 100 min for each treatment are reported in Table 9.3.

Table 9.3: $LC_{90}$ values (95% CI) in mg/L against *Lepidotrema bidyana* at various time points during a 100 min exposure period *in vitro*. FOR = formalin, DEP = trichlorfon, NaCl = sodium chloride PAA = peracetic acid, Cl-T = chloramine-T, PZQ = praziquantel, HP = hydrogen peroxide, and SPC = sodium percarbonate. - $LC_{90}$ values were unable to be generated.

| Treatment | Observation time (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 40 | | 60 | | 80 | | 100 | |
| FOR | 288.3 | (234.1 – 429) | 224.2 | (182.5 – 311.9) | 142 | (118.1 – 187.7) | 95.6 | (76.1 – 141) |
| DEP | - | | - | | - | | - | |
| NaCl | 23.1 | (10 – 73.8) | 13.9 | (6.1 – 42.9) | 8.7 | (3.8 – 26.7) | 5.3 | (2.3 – 16) |
| PAA | 34 | (15.6 – 111.3) | 13.4 | (6.9 – 35.5) | 7 | (3.7 – 16.9) | 4.6 | (2.4 – 10.6) |
| Cl-T | 197.8 | (138.9 – 327.8) | 112.9 | (85.8 – 165.4) | 61.7 | (48.4 – 86) | 44.9 | (35.4 – 61.8) |
| PZQ | 836.2 | (386.9 – 2463.2) | 179.7 | (97.2 – 413.7) | 46.7 | (27.5 – 90.3) | 8.1 | (4.5 – 14.9) |
| HP | 235.6 | (212.4 – 264.7) | 212.3 | (191.5 – 238.4) | 189 | (170.4 – 212.1) | 172.9 | (154.7 – 195.5) |
| SPC | 780.5 | (421.8 – 1994) | 260. 5 | (158.7 -534.4) | 138.5 | (87.9 – 257) | 62.4 | (39.4 – 108.2) |

### 9.4.2 *In vivo* trials

Praziquantel had a mean efficacy against *L. bidyana* of 77 ± SEM 4% (range 70 to 81%), 68 ± 3% (62 to 74%) and 47 ± 8% (34 to 62%) in fish bathed in 40, 15 and 5 mg/L PZQ solutions, respectively. Mean intensity of *L. bidyana* was 371.5 ± 32.7 (123 to 823) in the controls and was 86.2 ± 8.5 (8 to 200), 120.2 ± 8.8 (31 to 208) and 198.6 ± 19 (54 to 492) in fish bathed in 40, 15 and 5 mg/L PZQ solutions, respectively, which was significantly different between groups (1-way ANOVA, $F$ = 13.032, $P$ = 0.002, d.f. = 3) (Fig. 9.2A). Worms that were alive post-treatment were predominantly juvenile worms < 100 μm located at the base of the primary lamellae. There were no signs of toxicity in the in the fish post-treatment with 15 and 5 mg PZQ, and impaired swimming performance and increased opercular movement were observed in fish treated with 40 mg/L. There was no significant difference in mortality between treatment and control groups (Kruskal-Wallis, $P$ = 0.532).

Fish treated with HP at all doses had a significantly lower intensity of *L. bidyana* (Fig 9.2B), however, treated fish showed behavioural and physical

abnormalities including loss of equilibrium, skin discoloration and pale gills at all doses trialled. Survival rates of silver perch were 63, 67 and 97% when administered 250, 150 and 100 mg/L HP solutions, respectively. Survival rates in groups treated with 250 mg/L were significantly different to the controls (Dunnett's test, $P = 0.003$), while there were no significant differences in groups treated with 150 and 100 mg/L and controls (Dunnett's test, $P > 0.212$).

Formalin, sodium chloride and SPC were ineffective at all doses, with no significant difference in mean intensity of *L. bidyana* to control groups (Fig 9.2C-E). There were no signs of an adverse reaction to FOR, sodium chloride or SPC in silver perch at any dose trialled and no significant level of mortality ($P > 0.474$). All mortalities prior to and after the sodium chloride treatment procedure showed signs of an oomycete infection.

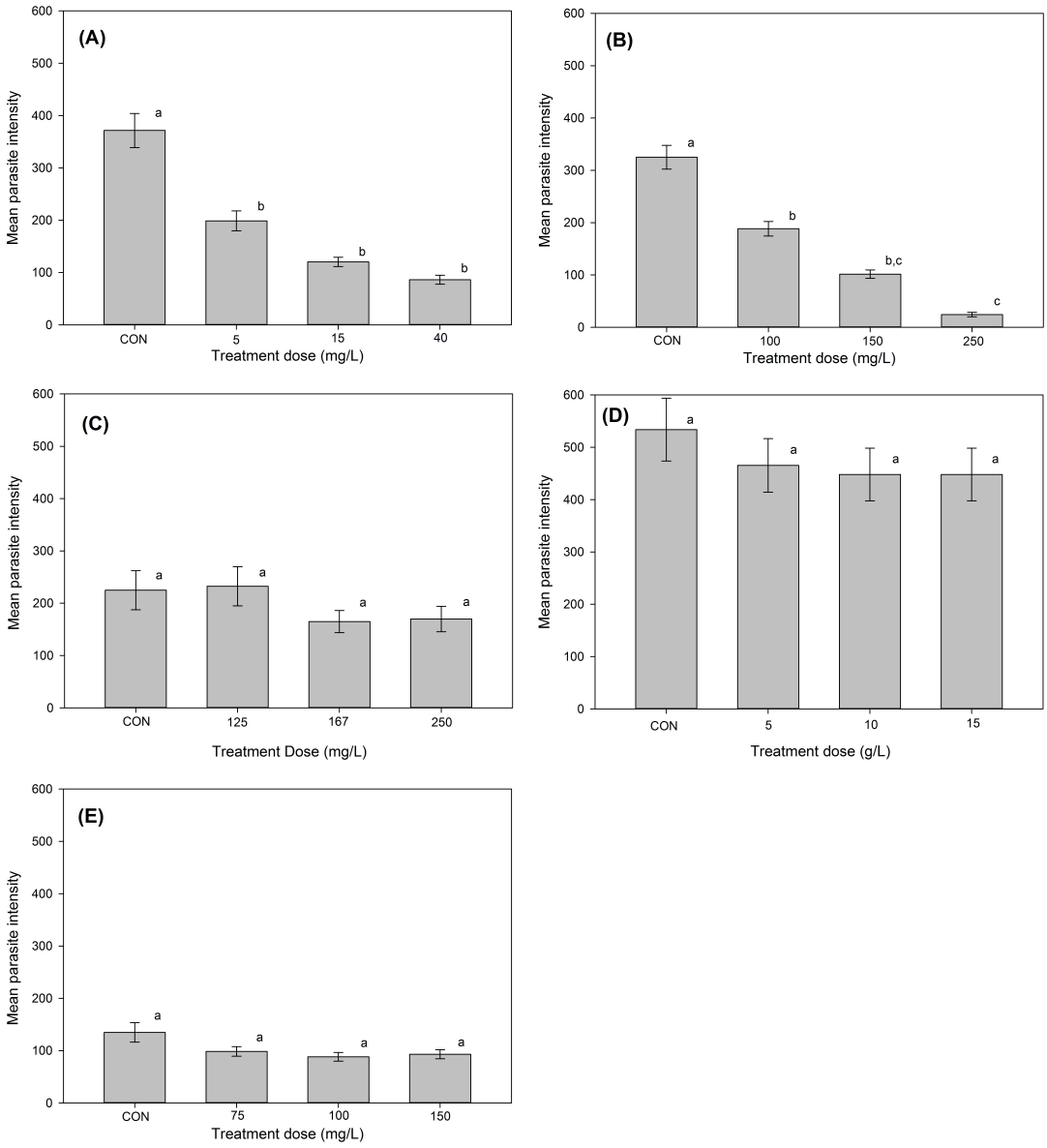


Figure 9.2: Mean intensity of *Lepidotrema bidyana* remaining after bath treatment with praziquantel for 60 min (A); hydrogen peroxide for 60 min (B); formalin for 30 min (C); sodium chloride for 60 min (D); sodium percarbonate for 60 min (E). Different super scripts represent significant differences between treatments analysed by Tukey's test ($P < 0.05$). Error bars represent the Standard Error of the Mean (SEM). CON = control.

## 9.5 Discussion

We found that FOR required 250 mg/L to be effective against *L. bidyana* within 40 min of exposure *in vitro* and required a longer exposure period to be effective at 150

and 100 mg/L. Formalin showed similar results *in vivo* and was ineffective against *L. bidyana* at all doses trialled, including the current recommended regime for *L. bidyana* of 150 mg/L FOR for 30 min (Read et al., 2007). We observed no negative effects on silver perch at doses of up to 250 mg/L FOR. The current recommended regime is unlikely to effectively control *L. bidyana* infections, but elevating the dose or extending the treatment period may increase the negative effects of FOR on fish (Wedemeyer and Yasutake, 1974; Ross et al., 1985; Reardon and Harrell, 1990; Jørgensen and Buchmann, 2007). Lowering the dose and extending the treatment period may improve efficacy but may not be logistically feasible during grading. Applying repeated treatments at the recommended dose and duration is unlikely to achieve 90% efficacy.

Sodium chloride was effective *in vitro* against *L. bidyana* at 10 g/L and the 60 min $LC_{90}$ was 13.9 g/L against *L. bidyana*, which suggests that the current recommended dose of 15 g/L for 60 min (Read et al., 2007) is likely to be effective. Sodium chloride, however, was ineffective at all doses *in vivo.* The efficacies obtained include some negative values, showing that the treatment groups had higher parasite intensity than the controls, which reflects the variation in intensity between individuals in natural infections (Poulin, 2007), but this variation was too low to create significant differences between treatment and controls. Schelkle et al. (2011) reported approximately 80% efficacy for sodium chloride against, *Gyrodactylus turnbulli* and 40% efficacy for *Gyrodactylus bullatarudis*, both skin monogeneans, when administered at 15 g/L for 15 min. Gill monogeneans, however, may be protected from treatments by withdrawing into the inter-lamellar spaces (Thoney and Hargis, 1991; Chisholm and Whittington, 2002) where they may be protected by mucus (Shephard, 1994). Increased salinity causes an increase in fish mucus

production (Wells and Cone, 1990), which may offer further protection from the external environment, limiting the effectiveness of topical treatments.

The efficacy of sodium chloride could be increased by increasing bath duration, which may also facilitate reduced mucus production as mucus cells become depleted (Wells and Cone, 1990), ameliorating the influence of mucus on efficacy. Silver perch tolerate 12 g/L salinity for 7 days (Guo et al., 1995) and we observed no negative effects in fish undergoing treatment. There were, however, some mortalities but these were associated with an oomycete infection, which were probably facilitated by the high *L. bidyana* intensity and handling stress, rather than the sodium chloride treatment. An extended treatment period is unlikely to cause mortality, but may decrease growth and cause stress, negating the effect of decreasing parasite loads. Increasing the dose could also improve efficacy, but exceeding 15 g/L is not logistically feasible due to the large quantities of salt required for treatment and increased environmental impacts or costs of disposal.

Trichlorfon provided low *in vitro* efficacy against *L. bidyana*. Trichlorfon did not significantly affect parasite mortality at any trialled dose and parasite mortality was variable between doses. Ineffectiveness (Buchmann et al., 1987; Székely and Molnár, 1987) and resistance (Umeda et al., 2006) to DEP have been observed in other species of monogeneans. Given that DEP was effective against *L. bidyana* (see Landos et al., 2007), our observations may indicate that resistance to the drug has emerged. There are also negative effects on the host associated with treatment; DEP causes hypertrophy and hyperplasia of epithelial cells in gill lamellae (Capinpin, 1995) and a reduction in AChE activity (Guimarães et al., 2007), which can result in host mortality (Salte et al., 1987; Horsberg et al., 1989). Organophosphate exposure also has sub-lethal effects on fish kidney tissue and renal function (Veiga et al., 2002).

Due to the adverse environmental effects DEP cannot be used in areas that are used as water supplies or drinking places for animals (Schlotfeldt et al., 1995). These factors combine to make DEP a poor choice of treatment for *L. bidyana.*

Praziquantel was effective *in vitro* against *L. bidyana* at 20 and 40 mg/L. Doses of 5 mg/L and above caused all *L. bidyana* to display atypical spasmodic movement and detach from the gills of silver perch *in vitro*, with subsequent mortality. Detachment was also observed in *Dactylogyrus extensus*, which, when treated with 1.5 mg/L PZQ for 20 min, displayed vacuolisation of the peduncle, lost its marginal hooklets and, in most cases, its hamuli, resulting in detachment from the gills (Schmahl and Mehlhorn, 1985). These results suggested that PZQ would be effective when applied as a short duration bath. Praziquantel significantly reduced the intensity of *L. bidyana* when applied at 40, 15 and 5 mg/L for 60 min, however, it was ineffective at removing juvenile *L. bidyana* from the base of the primary lamellae, decreasing efficacy. Monocotylid monogeneans infecting giant shovel nose rays, *Rhinobatos typus*, showed a similar effect (Chisholm and Whittington, 2002). To remove all worms from *R. typus* required repeated long duration baths of PZQ (Chisholm and Whittington, 2002); a similar method (Forwood et al., 2013) obtained 99% and 84% efficacies against adult and juvenile *L. bidyana*, respectively. Exposure duration may be more important than dose in PZQ bath treatments (Thoney and Hargis, 1991; Mitchell, 1995), however, higher dose, short duration baths can significantly reduce the intensity of *L. bidyana*. 15 mg/L is not, however, significantly more effective than 40 mg/L, suggesting that dose-efficacy plateaus rapidly. Silver perch also tolerate high dose short duration baths, unlike *R. typus* (see Chisholm and Whittington, 2002).

Hydrogen peroxide at 175 mg/L and greater was effective against *L. bidyana* *in vitro* and the 60 min $LC_{90}$ was 212.3 mg/L. Hydrogen peroxide, when administered *in vivo* at 225 mg/L effectively controlled *L. bidyana*, resulting in a mean efficacy of 93%, however, this dose significantly reduced host survival. The margin of safety for HP varies between species of fish and is proportional to water temperature (Rach et al., 1997b). High organic loads and low pH increase the oxidation rate of HP, causing dose time relationships to fluctuate with water conditions (Bishop et al., 1968). At high doses, HP causes epithelial lifting and gill necrosis (Johnson et al., 1993; Kiemer and Black, 1997; Tort et al., 2002). There are also security concerns and occupational health and safety hazards associated with handling HP (Eul et al., 2001). The temperature-dose curve and safety margin for HP treatments of silver perch are unknown, however, these results demonstrate that silver perch are sensitive to HP.

We also trialled SPC which is a safer alternative to HP, SPC has a wider host safety margin and lesser occupational health and safety risk profile (Buchmann et al., 2003). Sodium percarbonate gradually dissociates in water into sodium carbonate and HP, avoiding the rapid rise in concentration associated with adding liquid HP, and stabilising the pH of aquaculture systems (Buchmann et al., 2003). Sodium percarbonate was effective *in vitro* against *L. bidyana* at 150 mg/L, but had low efficacy and was ineffective at all doses *in vivo*. Sodium percarbonate at 80 mg/L for 18 h had 100% efficacy against *Gyrodactylus derjavini* infecting rainbow trout (Buchmann and Kristensson, 2003), indicating that longer duration baths may be more effective but further work is required to identify treatment regimes that increase efficacy against *L. bidyana*.

Peracetic acid was effective against *L. bidyana* at 5 mg/L *in vitro* but this dose exceeds the current recommendation of 0.2 to 2 mg/L in fresh water flow through

systems (Pedersen et al., 2009). Chloramine-T was effective at 40 mg/L *in vitro*, which also exceeds the recommended dose in freshwater fish of 12 to 20 mg/L for 60 min (Meinertz et al., 2004). Based on the previous recommended dose ranges, using these products, as short duration baths to obtain 90% efficacy against *L. bidyana* is likely to require a dose that will exceed the safety margin in silver perch.

## 9.6 Conclusions

Our results show that current short duration bath treatment regimes provide low efficacy against *L. bidyana*, facilitating rapid reinfection, and are ineffective for managing *L. bidyana* in aquaculture. Praziquantel was the only safe alternative treatment against *L. bidyana* and can be administered as a short duration bath. Effective management of *L. bidyana* using PZQ in 60 min baths will require detailed knowledge of larval and juvenile development at different water temperatures. This will allow repeat treatments to be strategically timed to kill newly attached larvae and to target juvenile worms that evaded the first treatment. Praziquantel use in aquaculture is constrained by the cost of treatment. Formalin, sodium chloride, PAA and SPC may have greater efficacy when administered in longer duration baths and could be investigated if the longer duration baths become amenable to the industry.

Finalisation of the World Association for the Advancement of Veterinary Parasitology (WAAVP) *Guidelines for testing the efficacy of ectoparasiticides for fish* will provide a stable basis for the design and analysis of trials such as this and will improve comparability of results between trials.

## 9.7 Acknowledgments

We are grateful to Dr Matt Landos and to the Scifleet family who run the Pioneer Fish Farm in Gloucester, NSW for providing silver perch to work with. This work was

supported by internal funds from the School of Biological Sciences, Flinders University and was done under approval from the Flinders University Animal Welfare Committee (Permit E354) and the Australian Pesticides and Veterinary Medicines Authority (APVMA) (Permit PER 7250).