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Aquaculture
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Aquaculture 236 (2004) 67–83
www.elsevier.com/locate/aqua-online

Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand

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Received 16 July 2003; received in revised form 5 January 2004; accepted 3 February 2004

Abstract

This study assessed the efficacy of bath administrations of Aqui-S, formalin and praziquantel against adults and eggs of *Benedenia seriolae* and *Zeuxapta seriolae,* two monogenean ectoparasites of kingfish (*Seriola lalandi lalandi*). Our results showed that 2.5 ppm praziquantel administered for either 24 or 48 h was the most effective treatment for removing *B. seriolae* (>99%). However, *B. seriolae* treated with praziquantel for 24 h deposited viable eggs. Formalin at 250 and 400 ppm for 1 h followed by a 5 min freshwater dip was less effective for removing *B. seriolae* (ca. 80%), but both treatments inhibited the production of eggs by treated parasites. However, a large proportion of *B. seriolae* eggs exposed to either formalin treatment remained viable. Praziquantel (2.5 ppm for either 24 or 48 h) and formalin (400 ppm for 1 h + 5 min f/w dip) were the most effective treatments for removing *Z. seriolae* from the gills of kingfish (>99%) but the parasites thus removed continued to deposit viable eggs. Exposure to the anaesthetic Aqui-S did not significantly increase numbers of *B. seriolae* or *Z. seriolae* removed from the host above those of the control, but it was ineffective in preventing the production of eggs by treated parasites, and did not reduce egg viability. Eggs of *B. seriolae* were not prevented from hatching unless exposed to air for a period greater than 3 h, and *Z. seriolae* eggs were only prevented from hatching if exposed to air for periods greater than 5 h prior to the development of eyespots. These results

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doi:10.1016/j.aquaculture.2004.02.005

indicate that both primary and secondary treatments will be required to successfully control *B. seriolae* and *Z. seriolae* infections in captive kingfish.

Keywords: Aquaculture; Carangidae; Chemotherapy; Disease; Kingfish; Monogenea; Parasites

1. Introduction

In the aquaculture of marine fish, high stocking densities can quickly lead to heavy infestations by monogenean ectoparasites. Various chemicals such as formalin, copper sulphate, praziquantel, potassium permanganate, sodium chloride, sodium peroxycarbonate and trichlofon have been used to control monogeneans (Thoney and Hargis, 1991). However, many of these chemicals are highly toxic to fish (Schmahl and Taraschewski, 1987) and hence may have narrow therapeutic margins. Therefore, it is important to determine the efficacy of potential treatments prior to using them in large-scale commercial aquaculture.

Confinement of broodstock kingfish (*Seriola lalandi lalandi*) at Pah Farm Aquaculture, on Kawau Island (Hauraki Gulf) in northern New Zealand led to the identification of two species of monogenean ectoparasites, *Benedenia seriolae* (Yamaguti, 1934) and *Zeuxapta seriolae* (Yamaguti, 1963) that pose a potential threat to the captive rearing and confinement of kingfish. Epizootics of both *B. seriolae* and *Z. seriolae* infecting the skin and gills, respectively (Sharp et al., 2003), caused disease in broodstock within weeks of the fish being introduced. *B. seriolae* is one of 70 species described in the Family Benedeniiae, many representatives of which are known to be potentially pathogenic, especially in aquaria and under farming conditions (Whittington, 1996). *B. seriolae* attach to the host via a pair of anterior pads and an opisthaptor; the hamuli and marginal hooklets of which pierce the epidermis and penetrate the dermis of the host. The presence of large numbers of *B. seriolae* on broodstock caused considerable irritation to fish, and resulted in the fish 'rubbing' themselves along the bottoms and sides of tanks/cages (Egusa, 1983). This can cause external injuries to the skin that often lead to secondary infection by opportunistic pathogens such as bacteria and/or fungi (Egusa, 1983). Signs of infection by *Z. seriolae* were less obvious as the gills are more difficult to monitor, and cannot be inspected closely without handling the fish. As frequent handling of broodstock fish was discouraged, the detection of *Z. seriolae* initially occurred after observation of behavioral changes associated with advanced infections, including hyperventilation and loss of equilibrium. Heavy infestations of *Z. seriolae* can also lead to anaemia, heavy mucous production and in some instances the loss of respiratory epithelium (Rohde, 1984).

The US Food and Drug Administration (FDA) have approved formalin for use in aquaculture since 1986 for the treatment of finfish and their eggs against external parasites, bacteria and fungal infections (Howe et al., 1995). Praziquantel is an antihelminthic treatment that has been proven effective against digenean and cestode infections in mammals, whilst causing relatively few side effects to the host (Thoney, 1990). More recently, praziquantel has been successfully used to treat infestations of several species of monogenean ectoparasites from fishes (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Thoney, 1990; Kim and Park, 1998; Kim and Cho, 2000; Kim et

al., 2001). Aqui-S is a clove oil-based fish anaesthetic approved for use on food fish in New Zealand and Australia. It is effective at lower temperatures than other anaesthetics such as benzocaine and MS-222, allows a faster recovery and has no withdrawal time (Stehly and Gingerich, 1999). Generally, fish anaesthetics are administered to minimize stress incurred through handling. However, Svendsen and Haug (1991), found that fish anaesthetised in 80 ppm benzocaine caused *Entobdella hippoglossi*, a monogenean skin parasite of the Atlantic halibut, *Hippoglossus hippoglossus*, to rapidly detach. Therefore, in this study, we investigated the performance of Aqui-S not as an anaesthetic, but as a possible treatment for controlling *B. seriolae* and *Z. seriolae*.

Published studies have concentrated solely on the effectiveness of chemotherapeutants for removal of adult monogeneans from the host fish but this provides only partial information. Monogenean eggs are resistant to chemical treatments (Diggles et al., 1993; Yoshinaga et al., 2000). Therefore, we investigated the effectiveness of Aqui-S, formalin and praziquantel against the eggs of *B. seriolae* and *Z. seriolae*. Hence, this study was separated into three parts: (1) examination of the effectiveness of various bath treatments for removing *B. seriolae* and *Z. seriolae* from their respective microhabitats on kingfish, (2) examination of the effects of treatments on egg production, i.e. the ability of treatments to prevent egg production and/or induce the production of non-viable eggs from treated worms, and (3) examination of the effectiveness of treatments to permeate eggshell membranes and prevent hatching. It was also of interest to assess the ability of eggs to withstand dessication to determine if the eggs of monogeneans remained capable of hatching after various periods out of water.

2. Materials and methods

2.1. Autoinfection of kingfish

Thirty line-caught kingfish (530–755 mm fork length (FL)) were transported from North Cape (34°45′ S–173°19′ E) to Pah Farm Aquaculture on Kawau Island (Hauraki Gulf) in mid May 2000. At the time of capture, FL was measured and coloured tags were inserted at the base of the dorsal fin to allow identification of individual fish. On arrival at Pah Farm Aquaculture kingfish were maintained in an 11,000 l tank at ambient water temperature (14–19 ± 1 °C) for 7 weeks, and fed a mixture of defrosted pilchards and squid every second day. Water supplying the tank was filtered to 50 μm and was isolated from the rest of the facility to prevent contamination occurring in other tanks. During this time the process of autoinfection resulted in monogenean hyperinfections that provided sufficient material for conducting the experiments outlined below.

2.2. Bath treatment protocols to remove B. seriolae and Z. seriolae

Hyper-infected kingfish ($n = 22$; range = 545–755 mm FL) were placed individually in 1500 l plastic tanks containing 400 l of seawater and subjected to one of the following five bath treatments: 8.5 ppm Aqui-S (Fish Transport Systems, Petone, NZ), for 7–10 min (min) (depending on the time required by individual kingfish to show signs of anaesthesia); 250 ppm (F250) or 400 ppm (F400) formalin (40% formaldehyde, Andrew Industries, Auck-

land, NZ) for 1 h followed by a 5 min bath in fresh tap water (f/w); or 2.5 ppm praziquantel powder (Ancare NZ, Auckland, NZ) administered for either 24 (P24) or 48 h (P48). Aqui-S was placed in a mixing container with seawater (500 ml) and agitated until the water turned milky-white. The contents were then poured into the 1500 l tank and mixed using a hand net. Kingfish were considered anaesthetised once equilibrium was lost and swimming had ceased. F250 and F400 were poured directly into the tanks and mixed via aeration and the swimming motion of kingfish. Praziquantel powder was dissolved in 20% glycerol, added to tanks using a 3 ml pipette, and dispersed throughout the treatment tank using a hand net.

After treatment, kingfish were removed, sacrificed by 'iki jime' (brain spiking) and the following information was recorded: FL, tag colour, number of *B. seriolae* and *Z. seriolae*. In order to obtain an accurate count of *Z. seriolae*, the gills were excised and placed under a microscope where each individual filament was examined. Formalin-treated fish (F250 and F400) were placed in the f/w bath for 5 min before being sacrificed. Sediments collected from the bottoms of the 1500 l tanks were siphoned into buckets, left to settle, and the supernatant drawn off until the volume of the sediment was small enough to be examined under a dissecting microscope for parasites dislodged by the treatments. Control fish were maintained in untreated water and were killed by spinal severance and examined for parasites as described above.

2.3. Assessing viability of B. seriolae and Z. seriolae eggs deposited after exposure to treatments

If available, six mature *B. seriolae* and *Z. seriolae* that had remained attached to kingfish after each treatment were removed from the fish and placed individually into

Table 1
Summary statistics of parasites removed from kingfish after treatment with Aqui-S (8.5 ppm), formalin (250 ppm of 400 ppm + 5 min f/w bath) or praziquantel 2.5 ppm (24 h or 48 h)

	Aqui-S	F250	F400	P24	P48	Control
(a) B. seriolae						
NF	4	4	4	3	3	4
TP	2120	1354	2738	930	1137	1963
S	39	1086	2187	922	1137	35
% M	1.8	80.2	79.9	99.0	100	1.8
S.E.	1.34	1.08	5.66	0.47	–	1.21
% R	0.4–5.2	70.6–94.7	59.5–83.8	98.1–99.9	100	0.2–4.4
(b) Z. seriolae						
NF	4	4	4	3	3	4
TP	8487	1946	4800	2577	1216	2678
S	5	959	4780	2577	1215	2
% M	0.1	49.3	99.6	100	99.9	0.1
S.E.	0.1	4	0.8	–	0.1	0.1
% R	0–0.2	31.7–99.5	96.2–99.9	100	99.8–100	0–1

(a) *B. seriolae*; (b) *Z. seriolae*. NF = number of treated fish; TP = total number of parasites (number of parasites on fish + number of parasites in sediment); S = number of parasites found in sediment after exposure; % M = mean percentage of parasites removed from kingfish; S.E. = standard error; % R = range of parasites removed.

culture wells (Costar 3526, 24 cell culture plates) containing fresh seawater filtered to 0.2 μm and incubated at 16 ± 2 °C. Similarly, if available, six mature *B. seriolae* and *Z. seriolae* were also removed from each of the treatment sediments and incubated under the same conditions. Water changes were conducted daily. After 36 h incubation, the worms were removed, measured and the numbers of eggs deposited by individuals were recorded. Eggs were monitored daily for eyespot development and hatching until day 20 or until hatching occurred, whichever came first. If hatching occurred, eggs were left undisturbed for 24 h, after which time the number of larvae were counted, and the percentage viability of eggs deposited by *B. seriolae* and *Z. seriolae* was calculated as $\%V$=(no. eggs hatched/no. eggs deposited) × 100.

2.4. Assessing viability of B. seriolae and Z. seriolae eggs deposited before exposure to treatments

Mature *B. seriolae* (n = 53) and *Z. seriolae* (n = 53) removed from control (non-treated) kingfish were placed in individual culture wells and incubated under the same conditions as above. After 36 h the worms were removed, measured and the numbers of eggs deposited by each worm were recorded. Eggs for each of the treatments (treatments used on eggs were identical to those described for bath treatments of fish) were separated into two groups; the first group were treated prior to eyespot development, whilst the second group were treated 24 h after eyespot

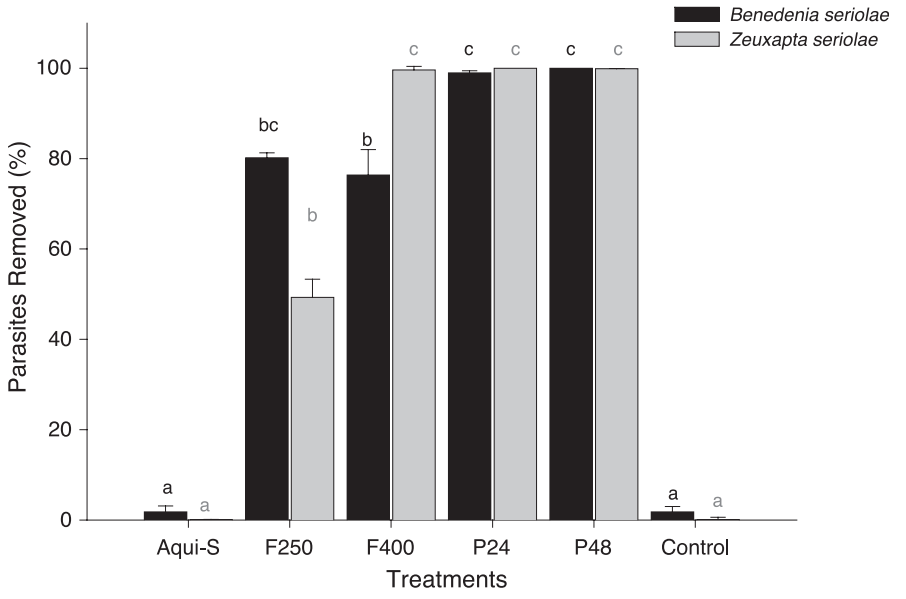

Fig. 1. Percentages of parasites removed from kingfish after treatment with Aqui-S, formalin (+ 5 min f/w bath) and praziquantel. Values are mean percentages ± S.E. Values that are not significantly different (P>0.05) share common superscripts.

development occurred. The effects of air exposure on egg viability was also examined. Untreated mature *B. seriolae* ($n=24$) and *Z. seriolae* ($n=30$) removed from control fish were placed in individual culture wells and allowed to deposit eggs. The eggs were then separated into three treatments; exposure to air for 1 h, 3 h and 5 h. Each of these treatments were also separated into two groups, treated pre- and post-eyespot development as above. After air exposure, the eggs were placed back into filtered seawater. Egg monitoring and water exchanges were conducted daily until day 20 or until hatching occurred, whichever came first. If hatching occurred within 20 days, eggs were left undisturbed for 24 h, after which time the number of hatched eggs were counted and percentage viability was calculated.

2.5. Histological preparation of *B. seriolae* and *Z. seriolae*

Four *B. seriolae* and *Z. seriolae* exposed to 2.5 ppm praziquantel for 24 h were fixed in 10% formalin in seawater and mounted in paraffin wax. Sections (6 μm) were cut and stained using haematoxylin and eosin. Four untreated *B. seriolae* and *Z. seriolae* removed from control fish were also stained and sectioned as controls.

Table 2
Percentage difference of the number of parasites removed from kingfish after treatment with Aqui-S (8.5 ppm), formalin (250 and 400 ppm + 5 min f/w bath) or praziquantel 2.5 ppm (24 and 48 h)

Treatments Compared	% diff	C.I.	*($P<0.05$)
(a) B. seriolae			
Aqui-S:F250	81	16	*
Aqui-S:F400	73	16	*
Aqui-S:P24	96	17	*
Aqui-S:P48	97	17	*
F250:F400	7	16	
F250:P24	15	18	
F250:P48	16	18	
F400:P24	23	18	*
F400:P48	24	18	*
P24:P48	1	19	
(b) Z. seriolae			
Aqui-S:F250	67	30	*
Aqui-S:F400	98	30	*
Aqui-S:P24	100	33	*
Aqui-S:P48	100	33	*
F250:F400	31	30	*
F250:P24	33	33	*
F250:P48	33	33	*
F400:P24	1.6	33	
F400:P48	1.5	33	
P24:P48	0.05	35	

(a) *B. seriolae*; (b) *Z. seriolae*. % diff = percentage difference between treatments; C.I. = 95% confidence interval; *($P<0.05$) = significant difference detected.

2.6. Statistical analysis

Student's *t*-tests and ANOVA were used to detect significant differences between treatments. If a significant difference was detected, Tukey's multiple comparisons tests were used to determine which treatments were significantly different from one another. Data were expressed as the percentage difference ($\%_{diff}$) in the number of animals remaining on kingfish (± 95% confidence interval).

3. Results

3.1. Effectiveness of treatment protocols for removing B. seriolae and Z. seriolae

Mean intensities of *B. seriolae* and *Z. seriolae* on hyper-infected kingfish were 472.61 ± 52.74 standard error (S.E.) ($n = 18$; range = 96–1098) and 1055.78 ± 268.61 S.E. ($n = 18$; range = 96–3892), respectively.

The numbers of *B. seriolae* and *Z. seriolae* found in sediments after treatment with 8.5 ppm of Aqui-S were not significantly different to those found in the sediments of control fish (Table 1a,b; Fig. 1). However, Aqui-S-treated fish retained significantly higher numbers of *B. seriolae* than any of the other treatments (Table 2a). There were no significant differences in numbers of *B. seriolae* between the other treatments, except between F400 and the two praziquantel treatments, although the difference was small (Table 2a). For *Z. seriolae*, Aqui-S was also almost completely ineffective, and F250 less effective than the other treatments (Table 2b). Formalin 400, P24 and P48 were all >99% effective in removing *Z. seriolae* from kingfish. Histological observations of both *B. seriolae* and *Z. seriolae* exposed to P24 showed marked vacuolisation of the tegument.

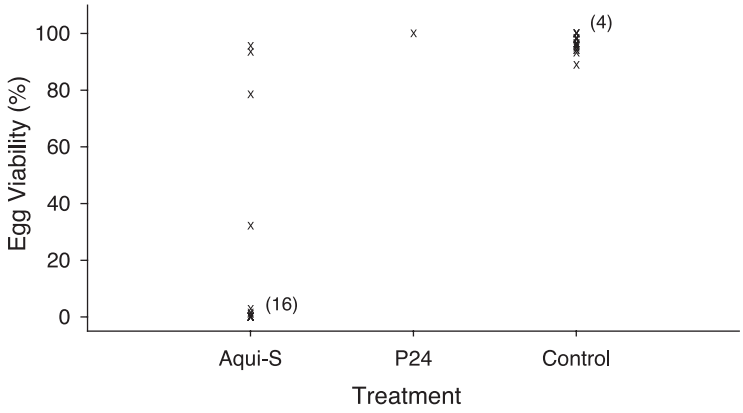

Fig. 2. Percentage hatch rates of eggs deposited by *B. seriolae* remaining attached to kingfish after exposure to Aqui-S and praziquantel. Numbers in brackets represent the numbers of worms within each treatment with 0% hatch (bottom) and 100% hatch (top). Note that no viable eggs were produced by mature *B. seriolae* treated with F250 and F400. P48 was 100% effective in removing *B. seriolae* from kingfish.

Table 3
Summary statistics of eggs deposited by *B. seriolae* that remained attached to kingfish after treatment with Aqui-S (8.5 ppm), formalin (250 or 400 ppm + 5 min f/w bath) or praziquantel 2.5 ppm (24 or 48 h)

	Aqui-S	F250	F400	P24	P48	Control
R	24	24	24	10	0	20
REL	23	0	0	1	0	20
RH	7	–	–	1	–	20
EL	1691	–	–	7	–	3828
EH	347	–	–	7	–	3582
% M	43.6	–	–	100	–	93
% R	1–96.5	–	–	–	–	86.4–100

R = number of worms; REL = number of worms which deposited eggs; RH = number of worms from which hatching occurred; EL = number of eggs deposited; EH = number of eggs hatched; % M = mean percentage viability of eggs in which hatching occurred; % R = range of percentage viability from eggs in which hatching occurred.

3.2. Assessing percentage viability of eggs deposited by treated *B. seriolae* and *Z. seriolae*

Statistical analyses to identify the effects of treatments on egg viability were not conducted for either *B. seriolae* or *Z. seriolae* due to the data being over dispersed. Over dispersion probably resulted from confounding factors within treatments as well as within

Table 4
Summary statistics of eggs deposited by *Z. seriolae* (a) remaining attached to kingfish (b) removed from sediments, after treatment with Aqui-S (8.5 ppm), formalin (250 or 400 ppm + 5 min f/w bath) or praziquantel 2.5 ppm (24 or 48 h)

	Aqui-S	F250	F400	P24	P48	Control
(a) Z. seriolae remaining attached to kingfish						
R	24	20	14	0	1	5
REL	18	16	11	0	1	5
RH	7	10	0	0	0	5
EL	4210	2337	3535	0	39	3005
EH	1674	1083	0	–	–	2977
% M	78.9	95.8	–	–	–	99.4
% R	7.3–100	75–100	–	–	–	97.9–100
(b) Z. seriolae removed from sediments						
R	4	24	24	18	13	5
REL	0	13	8	14	5	5
RH	0	4	7	11	5	5
EL	0	1249	1507	3292	1267	5
EH	–	418	1165	2515	1267	3005
% M	–	68.5	51.5	96.8	100	29.8
S.E.	–	13.5	6.0	1.6	–	99.4
% R	–	72.6–100	46.4–97.0	96.3–100	98.5–100	97.9–100

R = number of worms; REL = number of worms which deposited eggs; RH = number of worms from which egg hatching occurred; EL = number of eggs deposited; EH = number of eggs hatched; % M = mean percentage viability of eggs in which hatching occurred; % R = range of percentage viability from eggs in which hatching occurred.

replicates on the same fish, e.g. the percentage viability of some eggs deposited by some worms from an individual fish were close to 100% whilst others were 0% (Figs. 2–5). Although statistical comparisons could not be made, differences between treatments were observed. These are described below for both *B. seriolae* and *Z. seriolae*.

3.3. Incubation of treated *B. seriolae*

B. seriolae that remained attached to kingfish after treatment with F250 ($n=24$) and F400 ($n=24$) failed to deposit eggs (Table 3). Conversely, 23 of the 24 worms incubated after treatment with Aqui-S deposited eggs, but the majority of eggs were non-viable. Viability of eggs deposited by worms exposed to Aqui-S displayed high variability, ranging between 1.03% and 96% viable (Fig. 2). Variability was greatly reduced in eggs deposited by control *B. seriolae*, which ranged between 86% and 100% viable. Treatment

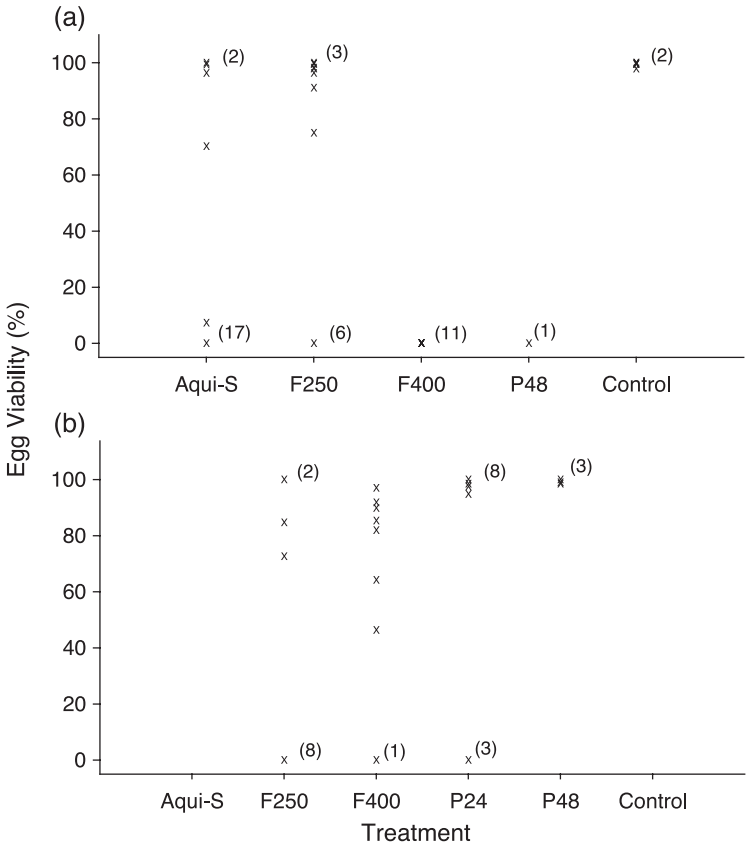

Fig. 3. Percentage hatching rates of eggs deposited by *Z. seriolae* (a) remaining attached to kingfish after exposure to Aqui-S, formalin (+5 min f/w bath), and praziquantel; (b) incubated from sediments. Numbers in brackets represent the numbers of worms within each treatment with 0% hatch (bottom) and 100% hatch (top). P24 was 100% effective in removing *Z. seriolae.*

Table 5
Summary statistics of *B. seriolae* eggs treated with Aqui-S (8.5 ppm), formalin (250 or 400 ppm + 5 min f/w bath), praziquantel 2.5 ppm (24 or 48 h) or exposure to air (1, 3, and 5 h) both pre- and post-eyespot development

	Aqui-S		F250		F400		P24		P48		1 h		3 h		5 h		Control
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
R	5	4	4	5	4	4	5	4	5	5	4	4	4	4	4	4	18
REL	5	4	4	5	4	4	5	4	5	5	4	4	4	4	4	4	18
RH	5	4	2	5	4	4	5	4	5	5	4	4	0	0	0	0	18
EL	104	116	94	89	139	68	254	191	132	119	137	157	236	53	288	53	1853
EH	91	105	30	29	66	15	227	153	121	80	121	100	0	0	0	0	1783
% M	77	88.4	21.1	51	37.6	21.7	88	78.6	91.3	66.5	88.6	80.4	–	–	–	–	96.2
% R	33.3–100	61.9–98.1	40–44.4	23.3–100	11.1–62.5	8.3–33.3	70–98.1	72.7–86.8	88.9–96.8	33.3–100	65.7–100	50–95	–	–	–	–	83.6–100

R = number of worms; REL = number of worms which deposited eggs; RH = number of worms from which hatching occurred; EL = number of eggs deposited; EH = number of eggs hatched; % M = mean percentage viability of eggs in which hatching occurred; % R = range of percentage viability from eggs in which hatching occurred.

with P24 resulted in only one *B. seriolae* individual depositing eggs ($n_{eggs} = 7$), all of which were viable. No *B. seriolae* remained attached after treatment with P48 and no *B. seriolae* incubated from the sediments of any of the treatments, including the control, deposited eggs.

3.4. Incubation of treated Z. seriolae

Many of the *Z. seriolae* that remained attached to kingfish after Aqui-S, F250, F400, P24 and P48 treatments deposited eggs (Table 4a). Eighteen of the twenty-four Aqui-S-treated worms deposited eggs, but the eggs of only seven worms were viable. Percentage viability of these eggs, as observed for *B. seriolae*, were also highly variable (Fig. 3a). Sixteen of the F250-treated worms incubated deposited eggs. However, hatching was

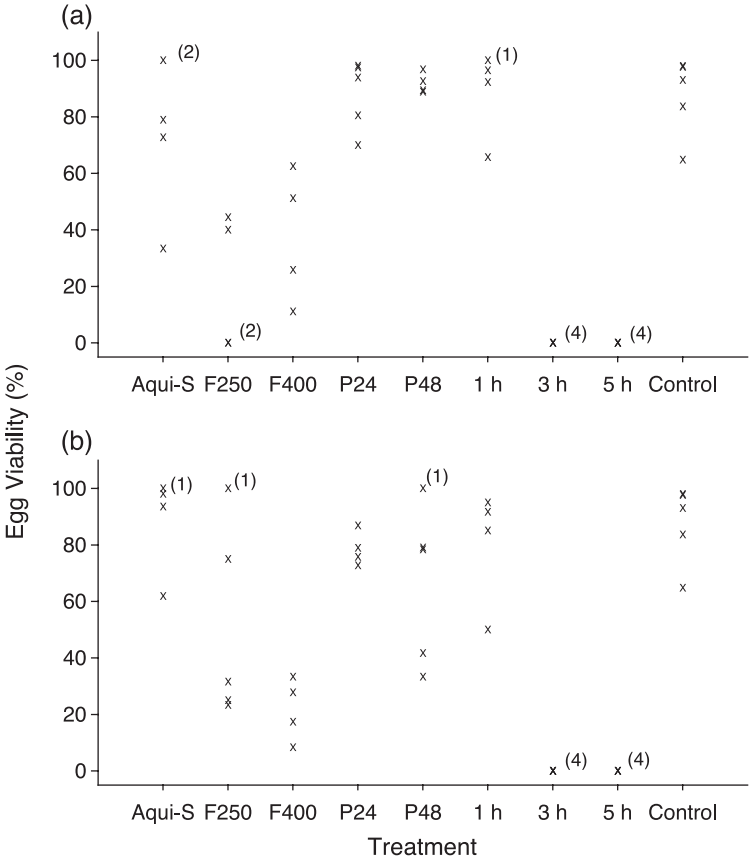

Fig. 4. Percentage hatch rates of *B. seriolae* eggs exposed to treatments Aqui-S, formalin (+5 min f/w bath), praziquantel, 1 h exposure to air, 3 h exposure to air, 5 h exposure to air. (a) Pre-eyespot development and (b) post-eyespot development. Numbers in brackets represent the numbers of worms within each treatment with 0% hatch (bottom) and 100% hatch (top).

Table 6
Summary statistics of *Z. seriolae* eggs treated with Aqui-S (8.5 ppm), formalin (250 or 400 ppm + 5 min f/w bath), praziquantel 2.5 ppm (24 or 48 h) or exposure to air (1, 3, and 5 h) both pre- and post-eyespot development

	Aqui-S		F250		F400		P24		P48		1 h		3 h		5 h		Control
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
R	5	5	5	4	5	4	5	5	5	5	5	5	5	5	5	5	5
REL	5	5	5	4	5	4	5	5	5	5	5	5	5	5	5	5	5
RH	5	5	5	4	5	4	5	5	5	5	2	5	5	5	0	5	5
EL	425	382	205	247	414	325	760	764	425	409	519	611	797	513	510	535	3005
EH	425	311	138	162	218	268	735	735	425	382	162	584	745	513	0	525	2977
% M	100	81.4	68.5	26.9	51.5	82.9	96.8	96.2	100	93.1	35.6	97.2	90	100	–	98.1	99.4
% R	100	69.2–96	35.1–100	26.9–76.1	36.7–73.1	80.4–85.5	96.3–100	93.2–100	100	89.8–95	0–100	86.1–100	66.7–100	100	–	92.7–100	97.9–100

R = number of worms; REL = number of worms which deposited eggs; RH = number of worms from which hatching occurred; EL = number of eggs deposited; EH = number of eggs hatched; % M = mean percentage viability of eggs in which hatching occurred; % R = range of percentage viability from eggs in which hatching occurred.

recorded in eggs from only 10 of these worms but the percentage viability of these eggs was more consistent ranging between 75% and 100% viable. The majority of worms that remained attached after F400 treatment deposited eggs, but all failed to hatch. Similarly, eggs produced by the only worm remaining attached after treatment with P48, also failed to hatch. No *Z. seriolae* remained attached after treatment with P24. Percentage viability of eggs deposited by control *Z. seriolae* showed virtually no variability ranging between 97% and 100% viable. *Z. seriolae* incubated from the sediments of all treatments except Aqui-S, deposited eggs (Table 4b). High variability was again observed in replicates treated with F250, F400 and P24 (Fig. 3b). However, eggs deposited by worms incubated from P48 sediments showed relatively consistent viabilities ranging between 98% and 100% viability. Worms incubated from Aqui-S and control sediments failed to deposit eggs.

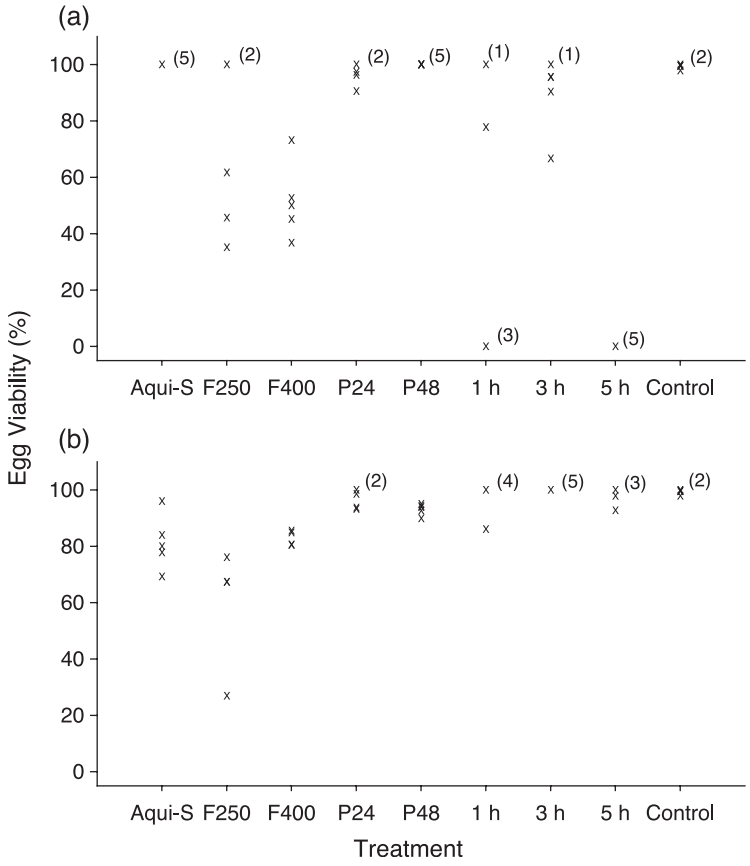

Fig. 5. Percentage hatching rates of *Z. seriolae* eggs exposed to treatments of Aqui-S, formalin (+5 min f/w bath), praziquantel, 1 h exposure to air, 3 h exposure to air, 5 h exposure to air (a) pre-eyespot development and (b) post-eyespot development. Numbers in brackets represent the numbers of worms within each treatment with 0% hatch (bottom) and 100% hatch (top).

3.5. Assessing percentage viability of treated B. seriolae and Z. seriolae eggs

3.5.1. B. seriolae eggs

Hatching occurred in all *B. seriolae* eggs treated with Aqui-S, F400, P24 and P48 (Table 5; Fig. 4). Lowest viability was observed in F250 and F400, whilst hatching rates for P24, P48 and Aqui-S remained at levels close to the controls. Eggs exposed to air both pre- and post-eyespot development failed to hatch when exposure times were longer than 1 h. There was no difference in the effects observed between eggs treated pre- and post-eyespot development for any treatment.

3.5.2. Z. seriolae eggs

Hatching occurred in all *Z. seriolae* eggs exposed to Aqui-S, F250, F400, P24 and P48, both at pre- and post-eyespot development (Table 6; Fig. 5). Lowest viability was observed in F250 (both pre- and post-eyespot development), and F400 (pre-eyespot development), whilst Aqui-S, P48, P24 and F400 (post-eyespot development) remained at levels close to the controls. Eggs exposed to air remained viable if exposure occurred 1, 3, or 5 h post-eyespot development and 3 h pre-eyespot development, but were non-viable if exposed to air for 5 h pre-eyespot development.

4. Discussion

Generally, fish anaesthetics are administered to minimize stresses during handling. However, Svendsen and Haug (1991), observed rapid detachment of *E. hippoglossi* from the skin of Atlantic halibut (*H. hippoglossus*) when fish were anaesthetized in 80 ppm benzocaine. In the present study, Aqui-S failed to remove significant numbers of parasites of either study species. Although some *B. seriolae* were found in Aqui-S sediment samples, these were probably removed as a result of net abrasion rather than as an effect of treatment with Aqui-S. This was suggested by the presence of similar numbers of *B. seriolae* in control sediments. Similarly, some *Z. seriolae* were also found in the sediments of Aqui-S-treated fish but again these numbers were not significantly higher than observed in the control.

Statistical analysis was not done on the effects of treatments on egg viability due to extreme dispersion in the data that probably resulted from confounding factors within treatments as well as between worms from the same fish. Possible causes of confounding include: (1) some worms selected for incubation may not have been mature. Although larger individuals were preferentially selected, some sexually immature individuals (which will not produce eggs, regardless of treatment) may also have been incubated, (2) mechanical damage, especially to *Z. seriolae*, may have occurred during the removal of some parasites from kingfish, (3) some individuals may have been collected whilst in a state of ‘recovery or rest’ from a previous period of egg output, (4) there may have been treatment effects to which some worms were more susceptible than others, (5) dissolved oxygen levels may have been lower in some culture wells than in others, and (6) although water was filtered to 0.2 μm the occurrence of bacterial infections in some wells cannot be excluded.

As with most chemical compounds, susceptibility is not only dependent on concentration and/or exposure time but also on species. Varying the concentration and exposure time of formalin has been investigated against many species of monogeneans. A 1-h bath at 250 ppm eliminated all *Neobenedenia melleni* from the Atlantic spadefish (*Chaetodipterus faber*). However, this same treatment eliminated only some *Psuedodactylogyrus* sp. from the gills of eels and was ineffective against *Microcotyle hiatulae* on the gills of *Tautoga onitis* (Thoney and Hargis, 1991). Our study found that F250 and F400 were reasonably effective for removing *B. seriolae* and *Z. seriolae*. For *Z. seriolae*, F400 was significantly more effective than F250 (99% and 49% of parasites removed, respectively). For *B. seriolae*, F250 and F400 removed ca. 80%. The reason why a dose-dependent response to formalin treatment was not observed in *B. seriolae* is not entirely clear. However, it was noted that a proportion of moribund worms exposed to formalin/freshwater treatments remained on the fish solely due to firm penetration of their hamuli into the fish dermis. F400 was 100% effective for preventing the production of viable eggs from mature *B. seriolae* and *Z. seriolae* that remained attached to the host. This indicated that worms which remained attached after the F400 treatment had been killed by the treatments but had remained attached due to either hamuli penetration of the dermis (*B. seriolae*), or entrapment in the increased mucous produced by the gills in response to the irritation of the formalin treatment (*Z. seriolae*). However, *Z. seriolae* which detached from the host during formalin treatments at both 250 and 400 ppm (thus escaping the 5 min freshwater bath) still deposited viable eggs. Neither F250 nor F400 prevented hatching when in direct contact with eggs of both *B. seriolae* and *Z. seriolae*. In fact, the only egg treatment trialled which killed 100% of eggs was air exposure. However, although our study found hatching was prevented in *B. seriolae* eggs exposed to air for 3 h or greater, hatching was only prevented in *Z. seriolae* eggs that were exposed to air for 5 h or greater prior to the development of eyespots. These data provide further proof that monogenean eggs are resistant to physical and chemical treatments (Diggles et al., 1993; Yoshinaga et al., 2000).

Praziquantel, at 2.5 ppm for 24 or 48 h, was highly effective for removing *B. seriolae* from kingfish (>99%, respectively). Praziquantel also effectively prevented the production of eggs by mature *B. seriolae* incubated from the sediments. However, this study indicates that a bath of 2.5 ppm praziquantel for more than 24 h may be more appropriate, although only one *B. seriolae* produced eggs while remaining attached to kingfish after 24 h, these eggs were 100% viable. An investigation on the efficacy of praziquantel at varying concentrations over different exposure times was conducted by Schmahl and Taraschewski (1987) on *Gyrodactylus aculeate*, a monogenean skin parasite of sticklebacks (*Gasterrosteus aculeatus*). Their study concluded that the intensity of vacuolisation was dependant on exposure time rather than on drug concentration. Mitchell (1995) also found treatment duration to be the most important factor determining the efficacy of praziquantel against larval digenetic trematodes. This is supported in the present study by the complete absence of *B. seriolae* on the skin of kingfish at 48 h, and the inability of *B. seriolae* incubated from 48 h sediments to deposit eggs. Furthermore, longer duration baths allow use of lower drug concentrations, which improves the economics of praziquantel administration, which is important bearing in mind the high cost of the drug. Unfortunately, praziquantel did not significantly reduce the viability of eggs. *B. seriolae*

eggs exposed to 2.5 ppm praziquantel for 24 or 48 h had viabilities >66%. These results are similar to those of Thoney (1990) who found that 72% of the eggs of *Benedeniella posterocolpa*, a monogenean skin parasite of the cownose ray (*Rhinoptera bonasus*) were viable after direct contact with 20 ppm praziquantel for 24 h.

The effects of praziquantel at a cellular level have also been investigated on several species of monogenean gill parasites (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Kim et al., 2001). These studies showed that treatment with praziquantel caused severe contractions and irreversible vacuolisation of the monogenean tegument, and that the degree of tegument damage was more dependent on the length of exposure rather than chemical concentration (Mitchell, 1995). Our study found no significant differences between the efficacy of F400, P24 or P48 for removing *Z. seriolae* from kingfish. All *Z. seriolae* incubated from P24 and P48 sediments produced viable eggs, and hatching was not prevented in *Z. seriolae* eggs after direct contact with either P24 or P48. Although, through the process of autoinfection, *B. seriolae* and *Z. seriolae* were allowed to reach significant intensities (in order to enable experimentation), this would be an artificial scenario under natural aquaculture practices. The combined use of quarantine procedures, disease management practices, good husbandry techniques, and chemotherapy should prevent monogeneans from reaching such high numbers. Our data suggests that both a primary and a secondary treatment are required to successfully control infections of *B. seriolae* and *Z. seriolae* in captive kingfish. Primary treatments will enable the removal of adult worms, whilst a secondary treatment would be required to remove immature worms that have hatched either from eggs present in the system during the primary treatment, or from any eggs subsequently deposited by treated worms detached from the host. To ensure complete disruption of the lifecycle of both parasites, the secondary treatment would need to be appropriately timed after all residual eggs have hatched but before the next generation of worms matured.

Acknowledgements

We thank Dave Shields (Moana Pacific Fisheries), Andrew Steele, Steve Pether and Simon Angley (Pah Farm Aquaculture), Colin Harvey (Ancare New Zealand), Steve and Julie Haddock (Zambucca Charters), Bob Creese and John Montgomery (University of Auckland).

References

Diggles, B.K., Roubal, F.R., Lester, R.J.G., 1993. The influence of formalin, benzocaine and hyposalinity on the fecundity and viability of *Polylabroides multispinosus* (Monogenea: Microcotylidae) parasitic on the gills of *Acanthopagrus australis* (Pisces: Sparidae). Int. J. Parasitol. 23 (7), 877–884.

Egusa, S., 1983. Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a review. Rapp. P.-V. Reun.-Cons. Int. Explor. Mer. 182, 10–18.

Howe, G.E., Marking, L., Schreier, T., 1995. Efficacy and toxicity of formalin solutions containing paraformaldehyde for fish and egg treatments. Prog. Fish-Cult. 57, 147–152.

Kim, K.H., Cho, J.B., 2000. Treatment of *Microcotyle sebastis* (Monogenea: Polyopisthocotylea) infestation with

praziquantel in an experimental cage simulation commercial rockfish *Sebastes schlegeli* culture conditions. Dis. Aquat. Org. 40, 229–231.

Kim, K. H., Park, II, Jee, B.Y., 1998. Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* (Monogenea) infestation of cultured rockfish (*Sebastes schlegeli*). Fish Pathol. 33, 467–471.

Kim, K.H., Kim, C.S., Kim, J.W., 2001. Depletion of praziquantel in plasma and muscle tissue of cultured rockfish *Sebastes schlegeli* after oral and bath treatment. Dis. Aquat. Org. 45, 203–207.

Mitchell, A.J., 1995. The importance of treatment duration for praziquantel used against larval digenetic trematodes in sunshine bass. J. Aquat. Anim. Health 7 (4), 327–330.

Rohde, K., 1984. Diseases caused by metazoans: Helminths. In: Kinne, O. (Ed.), Diseases of Marine Animals. vol. 4(1), pp. 193–320.

Schmahl, G., Mehlhorn, H., 1985. Treatment of fish parasites: 1. Praziquantel effective against monogenea *Dactylogyrus vastator*, *Dactylogyrus extensus*, *Diplozoon paradoxum*. Z. Parasitenkd. 71 (6), 727–738.

Schmahl, G., Taraschewski, H., 1987. Treatment of fish parasites: 2. Effects of praziquantel niclosamide, levamisole, hydrochloride and metrifonate on monogenea *Gyrodactylus aculeati*. *Diplozoon paradoxum*. Parasitol. Res. 73 (4), 341–351.

Sharp, N.J., Poortenaar, C.W., Diggles, B.K., Willis, T.J., 2003. Metazoan parasites of yellowtail kingfish, *Seriola lalandi*, in New Zealand: prevalence, intensity, and site preference. N.Z. J. Mar. Freshw. Res. 37, 273–282.

Stehly, G.R., Gingerich, W.H., 1999. Evaluation of AQUI-STM (efficacy and minimum toxic concentration) as a fish anaesthetic/sedative for public aquaculture in the United States. Aquacult. Res. 30 (5), 365–372.

Svendsen, Y.S., Haug, T., 1991. Effectiveness of formalin, benzocaine and hypo- and hypersaline exposures against adults and eggs of *Entobdella hippoglossi* (Muller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). Laboratory studies. Aquaculture 94, 279–289.

Thoney, D.A., 1990. The effects of trichlorfon praziquantel and copper sulphate on various stages of the monogenean *Benedeniella posterocolpa* a skin parasite of the cownose ray *Rhinoptera bonasus* Mitchill. J. Fish Dis. 13 (5), 385–390.

Thoney, D.A., Hargis, W.J., 1991. Monogenea (Platyhelthminthes) as hazards for fish in confinement. Annu. Rev. Fish Dis., 133–153.

Whittington, I., 1996. Benedeniine capsalid monogeneans from Australian fishes: pathogenic species, site specificity and camouflage. J. Helminthol. 70, 177–184.

Yamaguti, S., 1934. Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes. Jpn. J. Zool. 5 (3), 249–541.

Yamaguti, S., 1963. Systema Helminthum IV. Monogenea and Aspidocotylea. Interscience Publ., New York-London-Sydney.

Yoshinaga, T., Segawa, I., Kamaishi, T., Sorimachi, M., 2000. Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. Fish Pathol. 35, 85–88.