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Evaluation of bithionol as a bath treatment for amoebic gill disease caused by *Neoparamoeba* spp.

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Abstract

This study examined the toxicity of bithionol to Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* in fresh- and seawater and the efficacy of bithionol as a 1 h seawater bath treatment for amoebic gill disease (AGD). To examine toxicity, fish were bathed for 1, 3 and 6 h in bithionol, an anti-protozoal at 0, 1, 5, 10, 25 and 35 mg L^{-1} with toxicity determined by time to morbidity. Efficacy was examined by bathing AGD-affected Atlantic salmon and rainbow trout for 1 h at bithionol concentrations of 1–25 mg L^{-1}. Efficacy was determined by examining gill amoeba counts and identifying percent lesioned gill filaments at 1 and 24 h after bath exposure to bithionol. For both species, bithionol was determined to be toxic at 25 and 35 mg L^{-1} exhibiting median lethal times (LT50s) ranging from 21 to 84 min. Morbidity occurred in the 5 and 10 mg L^{-1} treatments, however, due to sampling regime there were not enough fish available to calculate LT50s. Only bithionol at 1 mg L^{-1} was considered non-toxic with no signs of morbidity. Bithionol was more toxic in seawater than freshwater and had no acute effects on gill Na^+/K^+ ATPase and succinic dehydrogenase, or plasma osmolality and chloride concentration. Bithionol at 1 mg L^{-1} reduced percent lesioned gill filaments in Atlantic salmon and rainbow trout by 33 and 27%, respectively, compared to the seawater control. Similarly, numbers of amoeba were reduced by 33 and 43% for Atlantic salmon and rainbow trout, respectively, when compared to the seawater control. Furthermore, bithionol reduced percent lesioned gill filaments as much as did the current industry standard of freshwater. This study demonstrated that a 1 h seawater bath containing 1 mg L^{-1} bithionol could be an improvement to the current method of treatment for AGD-affected Atlantic salmon and rainbow trout.

Keywords: *Salmo salar*; Toxicity; *Oncorhynchus mykiss*; Amoebic gill disease; Efficacy; Bithionol; *Neoparamoeba* spp.; Protozoan parasite

1. Introduction

Aquaculture involves intensive animal husbandry, maintaining large numbers of animals in a relatively limited space, these large numbers and limited space have lead to an increased risk of disease outbreaks (Stoffregen et al., 1996). Parasites are provided with optimal conditions, as culture conditions contribute to rising water temperature and poor water quality thus, leading to significant fish loss (Schmahl et al., 1989). Hence, the ongoing need for control of such diseases is paramount. Recent investigations have focused on antiprotozoals, to keep farmed fish such as rainbow trout, *Oncorhynchus mykiss* (Walbaum) and Atlantic salmon, *Salmo salar* (L.) disease free (Santamarina et al., 1991). Successful elimination of endoparasites, such as nematodes and cestodes, has been achieved using oral administration of drugs including levamisole and praziquantel, whereas bath administration is

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generally used in treating ectoparasites, such as a formalin bath to treat trichodinads and monogeneans (Noga, 2000).

Significantly problematic to Australian salmon aquaculture, amoebic gill disease (AGD) is caused by the protozoan parasite *Neoparamoeba* spp. (Munday et al., 2001). *Neoparamoeba* spp. is a free-living marine amphizoic amoeba, that attaches itself to the gills particularly the secondary lamellae (Adams and Nowak, 2003). It is characterised macroscopically by the presence of raised, white mucoid patches with histological presentation of single or multi-focal epithelial hyperplasia leading to lamellar fusion (Adams et al., 2004; Parsons et al., 2001). Freshwater bathing of affected fish is the current commercial management strategy for AGD; it lowers gross gill lesions, mortalities and is environmentally friendly (Munday and Zilberg, 2003), however, it is labour and cost intensive as well as stressful to the fish (Munday and Zilberg, 2003; Parsons et al., 2001). Attempts to identify potential chemotherapeutic agents have been limited due to either target fish toxicity or the cost of treatment (Alexander, 1991; Howard and Carson, 1994). Toxicity of several compounds to *Neoparamoeba* spp. has been examined *in vitro* including levamisole (Howard and Carson, 1995), chlorine dioxide, chloramine-T, hydrogen peroxide (Powell et al., 2003; Powell and Clark, 2003), amprolium, albendazole, toltrazuril and bithionol (Powell et al., 2003). Howard and Carson (1994), reported that levamisole at concentrations $\geq$10 ppm *in vitro* were lethal to *Neoparamoeba pemaquidensis* and chloramine-T concentration of 25 and 50 ppm effectively reduced amoeba numbers to deionised water equivalents after 2 h (Powell and Clark, 2003). Powell et al. (2003) found amprolium at 1 mg L^{-1} and bithionol at 1 and 10 mg L^{-1} to be amoebicidal *in vitro*. From these studies, it was stated that chloramine-T and bithionol showed promise and would be suitable AGD treatments for *in vivo* examination (Powell and Clark, 2003).

Bithionol has been examined *in vitro* and *in vivo* as a bath treatment for other salmonid parasites, such as *Gyrodactylus* sp. and *Ichthyobodo necator*. Santamarina et al. (1991) observed limited toxicity and complete *in vitro* efficacy against *Gyrodactylus* sp. in rainbow trout at 12.5 mg L^{-1}, with a minimum 20 mg L^{-1} reported as efficacious *in vivo*. Tojo et al. (1994) stated that bithionol was efficacious *in vivo* against *I. necator* in rainbow trout at 25 mg L^{-1} for a 3 h bath on two consecutive days, higher concentrations exhibited some mortality. Finally, Madsen et al. (2000) determined that bithionol at 0.1 mg L^{-1} was an effective treatment against trichodiniasis in European eels *Anguilla anguilla*, but found bithionol to have a relatively narrow therapeutic index.

This study aimed to determine the efficacy of bithionol as a bath treatment for AGD. The first objective was to evaluate the toxicity of bithionol administered via a bath treatment to Atlantic salmon and rainbow trout held in either fresh- or seawater using concentrations between 1 and 35 mg L^{-1}. The second objective was to evaluate the efficacy of bithionol at 1 to 25 mg L^{-1} as a bath treatment for AGD-affected Atlantic salmon and rainbow trout.

2. Materials and methods

2.1. Toxicity study

2.1.1. Fish husbandry

Juvenile diploid mixed sex rainbow trout (RBT), with a mean ($\pm$standard error of the mean (S.E.M.)) mass of 74.5 g ($\pm$1.0) and a mean ($\pm$S.E.M.) fork length of 18.5 cm ($\pm$0.1) ($N = 234$) were obtained from Sevrup Fisheries (Tasmania, Australia). Atlantic salmon (AS) diploid mixed sex spring smolts, with a mean ($\pm$S.E.M.) mass of 74.1 g ($\pm$0.9) and a mean ($\pm$S.E.M.) fork length of 18.8 cm ($\pm$0.1) ($N = 234$) were obtained from SALTAS salmon hatchery (Tasmania, Australia). Both groups of fish were maintained at the University of Tasmania Aquaculture Centre for a minimum of three weeks prior to experimental use. Fish were housed in two 3000 L Rathburn tanks with recirculated water and an individual biofilter system. Half of each species were housed in freshwater (de-chlorinated municipal source, mean $\pm$ S.E.M. 15.5 $\pm$ 1.0 °C) and the other half of each species were maintained in seawater (35‰, 1 μm filtered, mean $\pm$ S.E.M. 15.5 $\pm$ 1.0 °C). The tanks received constant aeration and oxygen levels were monitored daily using a Handy Gamma Oxy Guard (Birkerød, Denmark). Fish were fed twice daily to satiation and feed was withheld one day prior to bath administration. Immediately prior to bath treatment, nine fish were sampled as pre-experimental controls (see below).

2.1.2. Bath administration

Bithionol concentrations of 0, 1, 5, 10, 25 and 35 mg L^{-1} were examined for Atlantic salmon and rainbow trout toxicity using maximum 6 h bath duration. Toxicity was examined in both freshwater (fw) (municipal source, 15.5 °C, pH 7) and seawater (sw) (35‰, 15.5 °C, pH 8.2). Baths were conducted in triplicate with six fish in 20 L plastic tubs at mean ($\pm$S.E.M.) stocking densities ranging between 19.8 ($\pm$1.2) and 24.4