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Efficacy of SLICE® Premix (0.2% emamectin benzoate) to Control Infestations of *Salmincola* spp. on Freshwater-reared Rainbow Trout

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Copepods are the most numerous of the parasitic crustaceans affecting a wide range of marine and freshwater finfish worldwide (Woo 2006). Species of the genus *Salmincola* are parasitic copepods of major concern in wild and cultured salmonids. The incidence and spread of *Salmincola* spp. can be exacerbated by degraded environmental conditions, e.g., increased water temperatures, high organic loading, low dissolved oxygen, and overcrowding. Such parasitic crustaceans often open portals of entry for secondary, opportunistic pathogens, thereby enhancing morbidity and mortality of fish (Bandilla et al. 2006). Fish heavily infested with *Salmincola* spp. will flash, jump, or rub along hard surfaces as they try to rid themselves of the parasites. Fish may become darker in color, produce excessive mucus, exhibit fatigue in flowing water, and go off feed. As a result, reduced growth and productivity of fish populations may occur.

A *Salmincola* spp. of particular concern to salmonid culturists in the U.S. is *S. californiensis*. This species is native to North American streams, infects all *Oncorhynchus* spp. (Woo 2006), and attaches primarily to the gills of fish, thus causing extensive gill damage. At high levels of infestation, this species can also be found on the fish body surface, opercula, and oral cavities (Sutherland and Wittrock 1985). The life cycle of *S. californiensis* is characterized by three different phases, of which the adult female is the most readily identified. Adult females are macroscopic (2.5 - 8.0 mm total length), pale yellow, have two egg sacs dangling from the body, and develop a bulla on the cephalothorax to attach themselves permanently to a host fish (Woo 2006).

Bath treatments have traditionally been used to control infestations of parasitic crustaceans. However, they can be difficult to apply and stressful to fish (Stone et al. 2000b). Some fish culturists have resorted to manual removal of individual *S. californiensis* from fish with marginal success (Roberts et al. 2004). As a result, a more effective and efficient method needs to be developed to treat large numbers of cultured fish.

SLICE® (0.2% emamectin benzoate; EB) is approved for the control of sea lice (a parasitic marine copepod) in salmonids in the United Kingdom, Europe, Norway, Chile, and Canada. When SLICE® is fed to fish, EB is absorbed from the gut and distributed to a variety of tissues. When sea lice feed on the host fish, EB is taken into the tissues of the parasite, binds to ion channels of nerve cells and disrupts transmission of nerve impulses, resulting in paralysis and death. In addition, EB is metabolized and excreted slowly by the fish resulting in an extended period—up to 9 weeks—of protection from sea lice (Stone et al. 2000a). It has been reported that SLICE® may be similarly effective for the treatment of freshwater copepods. As such, we conducted a trial to evaluate the effectiveness of SLICE®-medicated feed to control infestations of *S. californiensis* on freshwater-reared rainbow trout *Oncorhynchus mykiss*.

Methods

The trial was conducted June 9 - July 27, 2010, at SeaPac of Idaho, Inc., Magic Springs Trout Hatchery (MSH), Hagerman, Idaho USA. Test fish were adult female rainbow trout (mean weight, 399.6 g; mean length, 31.6 cm). SLICE®-medicated feed was administered at a target dosage of 50 µg EB per kg of fish per d for 7 consecutive days. The trial was conducted under a U.S. Food and Drug Administration (FDA)-approved study protocol. Treatment objectives were to (a) demonstrate a significant difference in mean abundance of *S. californiensis* between treated and control groups and (b) achieve a ≥ 90% reduction in mean abundance of *S. californiensis*. The 90% reduction threshold is an FDA standard for establishing the effectiveness of terrestrial animal parasiticides. Because no 'standard' has been established for parasiticides for aquatic species, the terrestrial animal 'standard" became a required component of this trial. Both objectives had to be met to demonstrate treatment efficacy.

The reference population of rainbow trout selected for inclusion in this study had a *S. californiensis* infestation prevalence of 93% and a mean (±SD) copepod abundance of 5.5 ± 4.4 per fish. Fish from the reference population were impartially netted and randomly allocated to ten 114-gal test tanks (five treated and five control; 20 fish per tank). Treatment conditions (treated vs. control) were allocated to tanks with a completely randomized design procedure.

The 49-d trial comprised a 12-d acclimation, a 7-d treatment, and a 30-d posttreatment period. During the treatment period, SLICE®-medicated feed was administered to treated tanks, and nonmedicated feed was administered to control tanks. During the acclimation and posttreatment periods, nonmedicated feed was administered to all tanks. During the study, feed was administered at 0.5% mean fish body weight per d, and feed amounts were not adjusted for growth or mortality.

Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily. Feeding behavior was determined based on the relative amount of feed consumed, and values were scored on a 5-point ordinal scale (i.e., 0 = no feed consumed; 4 = 100% of feed consumed). Hardness, alkalinity, and pH of source water were measured at the beginning and at the end of the trial. At the end of the trial, the number of live adult female *S. californiensis* on each fish in each test tank was counted. Feed samples were collected and

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analyzed by Eurofins Scientific Inc., (Portage, Michigan USA) to verify EB concentrations.

Treatment efficacy was assessed at the end of the trial. Mean abundance of *S. californiensis* was compared between treatment groups with a mixed-model, nested analysis of variance (ANOVA; $P < 0.05$). To compensate for parasite counts of zero in some fish, the count for each fish was increased by one and $\log_e$-transformed before analysis. The least squares means from the ANOVA were back-transformed ($e^{\text{treatment group mean}}$) to geometric means, which were used to calculate percent reduction in mean abundance:

$$Percent\ reduction = 100 - \left[100 \times \frac{(geometric\ mean_{treated} - 1)}{(geometric\ mean_{control} - 1)} \right]$$

The PROC GLIMMIX procedure was used to statistically compare mortality between treatment groups ($P < 0.05$).

Results and Discussion

At the end of the trial, mean abundance of *S. californiensis* among fish in treated tanks (3.3 ± 5.4 per fish) was significantly different ($P < 0.001$) from that in control tanks (9.5 ± 8.1 per fish). Additionally, in treated tanks the percent reduction in mean abundance was 79%, and the prevalence (43%) was lower than in control tanks (91%; Table 1).

Mortality occurred in all test tanks; however, mean cumulative mortality of fish in treated tanks (16%; range, 5 - 25%) was not significantly different ($P = 0.2895$) from that in control tanks (10%; range, 0 - 20%). During the treatment period, fish in all test tanks consumed ~ 75% of feed offered. During the posttreatment period, fish ate ~ 100% of feed offered. Throughout the trial, general fish behavior was characterized as normal in all tanks. Mean water temperature and dissolved oxygen concentration were 15.3ºC (range, 15.0 - 15.7ºC) and 8.6 mg per L (range, 7.9 - 9.3 mg per L), respectively. Mean water hardness (236 mg per L as $CaCO_3$), alkalinity (125 mg per L as $CaCO_3$), and pH (8.1) were considered within ranges suitable for rearing rainbow trout at MSH. The analytically verified EB dose administered to fish was 41.8 µg EB per kg fish per d (83.6% of target). No EB was detected in control feed.

Results from this trial indicate that SLICE® administered in feed at 50 µg per kg fish per d for 7 d could be used to control a natural infestation of *S. californiensis* in adult female, freshwater-reared rainbow trout. Mean abundance of the parasite was significantly reduced, but treatment efficacy was not conclusively demonstrated because percent reduction in mean abundance was less than the 90% threshold specified as a treatment objective by the FDA. The posttreatment period in this trial lasted 30 d. However, in a study in which SLICE® was administered in feed at 50 µg per kg fish per d for 7 d to Atlantic salmon *Salmo salar* to control sea lice infestations, maximum treatment efficacy was not observed until 35 - 56 d posttreatment (Stone et al. 2000b). Therefore, we speculate that percent reduction in mean abundance may have reached 90% in our trial had the posttreatment period been longer. Results from the current trial, although not conclusive for treatment efficacy, have been submitted to FDA in support of approval of SLICE® for use in the U.S. to control infestations of *Salmincola* spp. in all freshwater-reared salmonids.

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Table 1. *Salmincola californiensis* infestation levels in the reference fish population at the beginning of the trial and in test fish at the end of the trial.

Group	Prevalence (% of fish infested)	Abundance (number of parasites/fish)		Number of fish evaluated
		Mean (±SD)	Range	
Reference (1 raceway)	93	5.5 ± 4.4	0 – 18	30
Control (5 tanks)	91	9.5 ± 8.1	0 – 29	90
Treated (5 tanks)	43	3.3 ± 5.4	0 – 25	83