

**Efficacy of SLICE<sup>®</sup> (0.2% Emamectin Benzoate) to Control an Infestation of *Salmincola californiensis* on Freshwater-Reared Rainbow Trout - REPORT #3**Niccole Wandelaar\*, Jim Bowker, Dan Carty and Wesley Sweet<sup>1</sup>U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program  
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Members of the genus *Salmincola* are parasitic copepods of major concern in wild and cultured salmonids. A species of particular concern in the U.S. is *S. californiensis*. This species is native to North American streams that empty into the northern Pacific Ocean (Kabata 1969), infects all *Oncorhynchus* spp. (Lester and Hayward 2006), and attaches primarily to the gills of fish, causing extensive gill damage. At high levels of infestation, it can also be found on the fish body surface and opercula, and in oral cavities (Sutherland and Wittrock 1985). Infestations of *S. californiensis* can adversely affect the growth, reproduction, and survival of freshwater-reared salmonids (Gall et al. 1972; Kabata and Cousens 1977; Johnson and Heindel 2001). Methods to control infestations have included manual removal of the parasites from fish, use of a surrogate fish host to interrupt the parasite's life cycle, and chemotherapy administered as bath, in-feed, or gavage treatments (Lester and Hayward 2006). Unfortunately, none of the chemotherapeutic treatments tested have been approved by the U.S. Food and Drug Administration (FDA) for use in the U.S.

One of the most promising treatments to control infestations of *S. californiensis* is the use of SLICE<sup>®</sup> (0.2% emamectin benzoate; EB) as an in-feed treatment. SLICE<sup>®</sup> was developed by Intervet/Schering Plough (now Merck) Animal Health Corp. (Roseland, New Jersey USA) to control infestations of sea lice (i.e., the crustacean copepods *Lepeophtheirus salmonis* and *Caligus elongatus*) in seawater-reared Atlantic salmon *Salmo salar*. When SLICE<sup>®</sup>-medicated feed is fed to fish, EB is absorbed from the gut and distributed to a variety of tissues. When sea lice feed on a host fish, EB binds to ion channels of the parasite's nerve cells and disrupts transmission of nerve impulses, thus resulting in paralysis and death (BCCAHS 2007). In addition, EB is metabolized and excreted slowly by fish, resulting in up to 9 weeks of protection from sea lice (Stone et al. 2000). SLICE<sup>®</sup> is approved for use to control sea lice in several countries, including Canada, Great Britain, Norway, and Chile. For these approvals, the recommended treatment regimen is to administer SLICE<sup>®</sup> at 50 µg EB per kg fish per d for seven consecutive days.

In the U.S., SLICE<sup>®</sup> is a candidate for FDA approval to control infestations of *Salmincola* spp. in all freshwater-reared salmonids. To support the approval, we designed and coordinated a field trial to evaluate the efficacy of SLICE<sup>®</sup> to control an infestation of *S. californiensis* on freshwater-reared rainbow trout *O. mykiss*.

**Methods**

The trial was conducted 25 May – 14 July 2011, at Maramec Spring Hatchery (MSH), Missouri Department of Conservation, St. James, Missouri USA. Test fish were adult rainbow trout (mean ±SD weight, 1,474 ± 501 g; mean length, 48 ± 6 cm). SLICE<sup>®</sup>-medicated feed was administered at a target dosage of 50 µg EB per kg of fish per d for 7 d. The trial was conducted under an FDA-approved study protocol. Treatment objectives were to (a) demonstrate a significant difference in mean abundance of adult female *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 used in the evaluation of parasiticides proposed for use in terrestrial animals. This threshold was applied by FDA to the current trial because no standard has been established for evaluating parasiticides proposed for use in aquatic species. As per the FDA-approved study protocol, both objectives had to be met to definitively demonstrate treatment efficacy.

Thirty fish from a reference population of mixed-sex rainbow trout were examined and found to have a *S. californiensis* infestation prevalence of 77% and mean abundance of 6.6 ± 12.6 per fish. Fish from the reference population were impartially netted and randomly allocated to six test tanks (three treated and three control; 20 fish per tank) positioned side-by-side in two rows in the reference population raceway. The tanks were rectangular holding boxes (1,580 L each) with mesh screen on bottom and sides to allow unimpaired water flow. Screen covers were constructed later after fish escapement was suspected. Baseline fish health evaluations were conducted on a sample of 20 fish impartially netted from the reference population. Treatment conditions (treated vs.

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control) were allocated to tanks with a completely randomized design procedure.

The 50-d trial comprised 1-d acclimation, 7-d treatment, and 42-d posttreatment periods. During the treatment period, SLICE<sup>®</sup>-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 1.0% mean fish body weight/d, and feed amounts were not adjusted for growth or mortality.

Mortality, general fish behavior, fish 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). Water hardness, alkalinity, and pH of source water were measured at the beginning and end of the trial. Feed samples were collected and shipped to Eurofins Scientific Inc., (Portage, Michigan USA) to verify EB concentrations. At the end of the trial, the number of live *S. californiensis* on each fish in each test tank was counted.

Mean abundance of *S. californiensis* was compared between treatment groups with a mixed-model, nested analysis of variance (ANOVA;  $P < 0.05$ ; SYSTAT 12). To compensate for parasite counts of zero, 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:

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

Mortality of fish was compared between treatment groups with a general linear mixed model (GLIMMIX, logit link;  $P < 0.05$ ; SAS 9.2).

## Results and Discussion

At the end of the trial, mean abundance of *S. californiensis* among fish in treated tanks ( $1.3 \pm 3.8$  per fish) was significantly different ( $P = 0.017$ ) from that in control tanks ( $12.5 \pm 22.9$  per fish), and percent reduction in mean abundance was 90%. Prevalence of *S. californiensis* had decreased (mean, 26%; range, 18 - 32%) in treated tanks while remaining near the pretreatment level (mean, 74%; range, 56 - 92%) in control tanks (Table 1).

Also, at the end of the trial, mean cumulative mortality of fish in treated tanks (1.7%; range, 0 - 5%) was not significantly different ( $P = 0.8952$ ) from that in control tanks (2.4%; range, 0 - 7%). To account for fish escapement in some tanks during

the posttreatment period, the total number of fish remaining in tanks at the end of the trial was used for statistical analysis.

During the treatment period, fish in all test tanks ate ~ 50% of feed offered. During the posttreatment period, fish ate > 75% of feed offered. Throughout the trial, general fish behavior was characterized as normal in all tanks.

Mean water temperature and dissolved oxygen concentration were  $14.1 \pm 0.4^\circ\text{C}$  (range,  $13.5 - 14.9^\circ\text{C}$ ) and  $8.7 \pm 0.5$  mg per L (range, 8.0 - 9.5 mg per L), respectively. Mean water hardness (160 mg per L as  $\text{CaCO}_3$ ), alkalinity (157.5 mg per L as  $\text{CaCO}_3$ ), and pH (7.3) were within ranges suitable for rearing rainbow trout at MSH.

The analytically verified EB dose administered to fish was  $44 \mu\text{g}$  EB per kg fish per d (88% of target). No EB was detected in control feed.

Results from this trial clearly indicate that SLICE<sup>®</sup> administered in feed at a target dosage of  $50 \mu\text{g}$  EB per kg fish per d for 7 d is efficacious for the control of a natural infestation of *S. californiensis* in adult, freshwater-reared rainbow trout. Treatment significantly reduced mean abundance of the parasite, reduced prevalence, and resulted in a 90% reduction in mean abundance. Results from this trial have been submitted to FDA in support of approval of SLICE<sup>®</sup> for use in the U.S. to control infestations of *Salmincola* spp. in all freshwater-reared salmonids

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**Table 1.** Trial 1—*Salmincola californiensis* infestation levels in the rainbow trout reference population at the beginning of the trial and in rainbow trout control and treated groups at the end of the trial.

Group	Prevalence <sup>a</sup> (% of fish infested)	Abundance (number of parasites per fish)		Total number of fish examined
		Mean ± SD	Range	
Reference	77	6.6 ± 12.6	0 – 51	30
Control	74	12.5 ± 22.9	0 – 121	42
Treated	26	1.3 ± 3.8	0 – 20	51

<sup>a</sup> Infestation prevalence in the reference population was based on a single 30-fish sample, whereas infestation prevalence in the treated and control groups are means based on three tanks per treatment group.