
# Pilot Field Trial to Evaluate SLICE (0.2% Emamectin Benzoate)-Medicated Feed to Reduce a Natural Infestation of Salmincola californiensis in Freshwater-Reared Rainbow Trout

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# Pilot Field Trial to Evaluate SLICE (0.2% Emamectin Benzoate)-Medicated Feed to Reduce a Natural Infestation of *Salmincola californiensis* in Freshwater-Reared Rainbow Trout

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***Abstract***

*Salmincola* spp. infestations can adversely affect freshwater-reared salmonids. Control methods tested to date have had limited success; consequently, we conducted a pilot field trial to evaluate SLICE (0.2% emamectin benzoate, EB)-medicated feed to reduce a natural infestation of *S. californiensis* in freshwater-reared rainbow trout *Oncorhynchus mykiss*. Before the trial started, 96 of 1,500 rainbow trout broodstock held in a flow-through raceway were impartially captured, sedated, uniquely tagged, and returned to the raceway. Pretreatment *S. californiensis* infestation prevalence and intensity (mean ± SD) were 97% and 10.4 ± 7.6 adult female parasites per fish, respectively. Treatment was administered at 50 µg EB·kg fish$^{-1}$·d$^{-1}$ for 7 d. By the end of the trial (43 d posttreatment), infestation prevalence and intensity had decreased to 32% and 1.6 ± 1.1 adult female parasites per fish, respectively. These results suggest SLICE-medicated feed can be used to reduce natural infestations of *S. californiensis* in freshwater-reared rainbow trout.

## INTRODUCTION

Infestations of the ectoparasitic crustacean copepod *Salmincola* spp. can adversely affect growth, reproduction, and survival of freshwater-reared salmonids (Gall et al. 1972; Bell and Margolis 1976; Piasecki et al. 2004). *Salmincola* spp. primarily attach to the gills, opercula, and mouth cavity of fish (Kabata and Cousens 1973), often resulting in severe gill damage and respiratory stress (Sutherland and Wittrock 1985; Mitchum 1995; Duston and Cusack 2002; Roberts et al. 2004). Control methods tested to date have had limited success (Johnson and Heindel 2001; Duston and Cusack 2002; Modin

and Veek 2002; Roberts et al. 2004; Lester and Hayward 2006), and no chemotherapeutic treatments have been approved for use in the U.S.

SLICE (0.2% emamectin benzoate; EB) is an in-feed treatment developed by Intervet/Schering-Plough (now Merck) Animal Health (Roseland, New Jersey) to control infestations of ectoparasitic crustacean sea lice (e.g., *Lepeophtheirus salmonis* and *Caligus elongatus*) in seawater-reared Atlantic salmon *Salmo salar* and trout (Stone et al. 1999, 2000b, 2000c; ISPAH 2003). Sea lice feeding on treated fish ingest EB and eventually become paralyzed and die (BCCAHS 2007). Protection against reinfestation can last up to 9 weeks because EB is slowly metabolized by fish (Stone et al. 2000a). SLICE is safe to both fish and the environment when administered at the standard dosage of 50 µg EB·kg fish$^{-1}$·d$^{-1}$ for 7 d (Roy et al. 2000; ISPAH 2003; BCCAHS 2007) and is approved for sea lice control in Canada, Great Britain, Norway, Chile, and other countries. In the U.S., SLICE is a candidate for U.S. Food and Drug Administration approval for the control of infestations of *Salmincola* spp. in all freshwater-reared salmonids (USFWS 2011). Our objective was to evaluate the efficacy of SLICE, when administered in feed at the standard dosage, to reduce or eliminate a natural infestation of *Salmincola californiensis* in a test population of freshwater-reared rainbow trout *Oncorhynchus mykiss.*

## METHODS

The trial was conducted in 2007 at the Colorado Division of Wildlife, Poudre River State Fish Hatchery, Bellvue, Colorado. The trial comprised 8-d pretreatment, 7-d treatment, and 43-d posttreatment periods. The reference fish population (N = 1,500) was

a mixed-sex cohort of recently spawned, German-strain rainbow trout broodstock (age 2) naturally infested with *S. californiensis*. These fish were held in a concrete raceway (42.5 $m^3$) supplied with Cache la Poudre River water (inflow = 5,400 – 6,400 L/min; temperature = 13°C). Test fish (n = 96; mean weight = 0.83 kg) were impartially captured from the reference population, sedated in a 75-mg/L solution of tricaine methanesulfonate (FINQUEL, Argent Chemical Laboratories, Inc., Redmond, Washington), uniquely tagged with Monel jaw tags (National Band and Tag Co., Newport, Kentucky), and returned to the reference population. Conserving this valuable broodstock population necessitated treating all fish and precluded including a nontreated control group in the trial.

SLICE was top-coated with vegetable oil onto Rangen 4-mm, soft-moist trout feed (Rangen, Inc., Buhl, Idaho) at 0.5% SLICE/kg feed (10 mg EB/kg feed) and administered at 0.5% mean fish body weight/d. Medicated feed was administered three times daily in approximately equal portions to maximize feed consumption by test fish. Nonmedicated feed was administered to test fish during the pre-and posttreatment periods.

*Salmincola californiensis* prevalence (proportion of fish infested with one or more of the parasites), mean intensity (mean number of parasites per infested fish), and proportional distribution on fish were assessed following capture and sedation before treatment started and periodically during the posttreatment period. Both the late-stage fourth chalimus female and adult female *S. californiensis* (hereafter collectively referred to as adult females) were counted. Also, fish behavior and changes in the appearances of fish and adult female *S. californiensis* were documented.

## RESULTS

*Salmincola californiensis* infestation prevalence decreased 65%, and mean intensity decreased 85% (from 10.4 ± 7.6 to 1.6 ± 1.1 adult females per infested fish) between the beginning and end of the trial (Table 1). Fish fed aggressively during the treatment period, and no adverse reactions to treatment were observed. Before treatment started, 75.0% of the adult females counted were attached to the gills, 24.5% were attached in the mouth cavity, and 0.5% were attached to other sites (external interdentary angle, underside of the operculum, behind the pectoral fin, or the external subopercle edge). By the end of the trial, 44.0% of the adult females counted were attached to the gills, and 56.0% were attached in the mouth cavity. As the infestation decreased, gross gill morphology of some fish improved as gill arches and primary gill filaments separated and inflammatory exudates diminished. However, fish with initially severe infestations (>10 adult females per gill) had curling and atrophy of the distal edge of the gill opercula that did not resolve by the end of the trial.

Adult female *S. californiensis* changed in appearance between the beginning and end of the trial. Initially, adult females had semi-translucent, white-colored bodies with egg sacs of an opaque buff or buff-yellow color. However, by 8 d posttreatment, most adult females seemed to be decomposing and had bodies of an opaque to translucent buff-yellow color. Moreover, some adult females were attached to the gills or mouth of fish only by a strand of fibrous tissue. By 21 d posttreatment, many of the adult females still attached to fish were obviously dead because only empty or broken exoskeletons were observed.

## DISCUSSION

In our trial, SLICE administered in feed at 50 µg EB·kg fish$^{-1}$·d$^{-1}$ for 7 d reduced—but did not eliminate—a natural infestation of *S. californiensis* in a test population of freshwater-reared rainbow trout. Elimination was unlikely, in part because the initial infestation was relatively severe and fish were continually exposed to reinfestation via the river water supplied to the raceway. It is also possible the posttreatment period was not long enough for maximum treatment efficacy to be observed. For example, Stone et al. (2000c) observed maximum treatment efficacy at 35 – 56 d posttreatment when SLICE-medicated feed was used to control sea lice in Atlantic salmon. Nevertheless, our results are comparable to other studies in which SLICE has been used to control *Salmincola* spp. infestations in freshwater-reared salmonids. Dustan and Cusack (2002) reduced mean abundance of adult female *S. edwardsii* by 58% (from 118 to 49 per fish; 7-d posttreatment period) and 38% (from 56 to 35 per fish; 32-d posttreatment period) in trials conducted with brook trout *Salvelinus fontinalis*. In other trials conducted with rainbow trout, *S. californiensis* infestations were reduced from initial levels of 93 – 100% prevalence and mean abundances of 5.5 – 7.9 live adult females per fish to 15 – 48% prevalence and mean abundances of 1.3 – 3.3 live adult females per fish (Carty et al. 2011; Wandelear et al. 2011a, 2011b). In these latter three trials, posttreatment periods were 30, 42, and 30 d, respectively.

We did not include a nontreated control group of fish in our trial and thus acknowledge the possibility that the *S. californiensis* infestation resolved partly or completely independently of treatment. For example, Carty et al. (2011) observed a

decrease of 16% (from 7.9 to 6.6 per fish) in mean abundance of live adult female *S. californiensis* in a nontreated group of rainbow trout. In contrast, Wandelear et al. (2011a, 2011b) observed increases of 67% (from 7.3 to 12.2 per fish) and 73% (from 5.5 to 9.5 per fish) in mean abundances of live adult female *S. californiensis* in nontreated groups of rainbow trout, and Duston and Cusack (2002) observed increases of 15% (from 109 to 125 per fish) and 23% (from 64 to 79 per fish) in mean abundances of adult female *S. edwardsii* in nontreated groups of brook trout. Also, trials without nontreated groups of fish have been used to demonstrate the efficacy SLICE-medicated feed to control sea lice infestations in Atlantic salmon (Ramstad et al. 2002; Treasurer et al. 2002; Gustafson et al. 2006).

In conclusion, our results suggest SLICE-medicated feed can be used to reduce natural infestations of *S. californiensis* in freshwater-reared rainbow trout without causing adverse effects to the fish. However, we re-emphasize the pilot nature of our work and suggest that comprehensive trials conducted with appropriate nontreated control groups and posttreatment periods longer than 43 d could delineate ranges of infestation reductions attainable and posttreatment times at which maximum treatment efficacy occurs.

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164 **REFERENCES**

165 BCCAHS (British Columbia Center for Aquatic Health Resources). 2007. SLICE:
166 action / use / effects. BCCAHS, Campbell River, British Columbia, Canada.
167 Bell, G. R., and L. Margolis. 1976. The fish health program and occurrence of fish
168 diseases in the Pacific region of Canada. Fish Pathology 10:115-122.
169 Carty, D. G., J. D. Bowker, and N. Wandelear. 2011. The efficacy of SLICE premix
170 (0.2% emamectin benzoate) administered in feed to control *Salmincola* spp. on
171 freshwater-reared rainbow trout *Oncorhynchus mykiss* (SLICE-09-EFF-SAL-02).
172 U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership
173 Program, Bozeman, Montana.
174 Duston, J., and R. R. Cusack. 2002. Emamectin benzoate: an effective in-feed treatment
175 against the gill parasite *Salmincola edwardsii* on brook trout. Aquaculture
176 207:1-9.
177 Gall, G. A. E., E. L. McClendon, and W. E. Schafer. 1972. Evidence on the influence of
178 the copepod (*Salmincola californiensis*) on the reproductive performance of a
179 domesticated strain of rainbow trout (*Salmo gairdneri*). Transactions of the
180 American Fisheries Society 101:345-346.
181 Gustafson, L., S. Ellis, T. Robinson, F. Marenghi, and R. Endris. 2006. Efficacy of
182 emamectin benzoate against sea lice infestations of Atlantic salmon, *Salmo salar*
183 L.: evaluation in the absence of an untreated contemporary control. Journal of
184 Fish Diseases 29:621-627.

ISPAH (Intervet/Schering-Plough Animal Health). 2003. Guidance notes for the use of SLICE in aquaculture: technical report, volume 1. Intervet/Schering-Plough Animal Health, Union, New Jersey.

Johnson, K. A., and J. A. Heindel. 2001. Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. Journal of Fish Diseases 24:197-203.

Kabata, Z., and B. Cousens. 1973. Life cycle of *Salmincola californiensis* (Dana 1852) (Copepoda:Lernaeopodidae). Journal of the Fisheries Research Board of Canada 30:881-903.

Lester, R. J. G., and C. J. Hayward. 2006. Phylum Arthropoda. Pages 466-565 *in* P. T. K. Woo, editor. Fish diseases and disorders, volume 1: protozoan and metazoan infections, 2nd edition. CAB International, Wallingford, Oxfordshire, England.

Mitchum, D. L. 1995. Parasites of fishes in Wyoming. Wyoming Game and Fish Department, Cheyenne.

Modin, J. C., and T. M. Veek. 2002. Biological control of the parasitic copepod *Salmincola californiensis* in a commercial trout hatchery on the lower Merced River, California. North American Journal of Aquaculture 64:122-128.

Piasecki, W., A. E. Goodwin, J. C. Eiras, and B. Nowak. 2004. Importance of Copepoda in freshwater aquaculture. Zoological Studies 43:193-205.

Ramstad, A., D. J. Colquhoun, R. Nordmo, I. H. Sutherland, and R. Simmons. 2002. Field trials in Norway with SLICE (0.2% emamectin benzoate) for the oral

treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 50:29-33.

Roberts, R. J., K. A. Johnson, and M. T. Casten. 2004. Control of *Salmincola californiensis* (Copepoda:Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. Journal of Fish Diseases 27:73-79.

Roy, W. J., H. Rodger, I. H. Sutherland, and K. J. Varma. 2000. Tolerance of Atlantic salmon *Salmo salar* L. and rainbow trout *Oncorhynchus mykiss* (Walbaum) to emamectin benzoate, a new orally administered treatment of sea lice. Aquaculture 184:19-29.

Stone, J., I. H. Sutherland, C. Sommerville, R. H. Richards, and K. J. Varma. 1999. The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Kròyer), infestations in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 22:261-270.

Stone, J., I. H. Sutherland, C. S. Sommerville, R. H. Richards, and R. G. Endris. 2000a. The duration of efficacy following oral treatment with emamectin benzoate against sea lice, *Lepeophtheirus salmonis* (Kròyer), in Atlantic salmon, *Salmo salar*. Journal of Fish Diseases 23:185-192.

Stone, J., I. H. Sutherland, C. S. Sommerville, R. H. Richards, and K. J. Varma. 2000b. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeophtheirus salmonis* (Kròyer) and *Caligus elongatus* Nordmann, infestations in Atlantic salmon *Salmo salar* L. Aquaculture 186:205-219.

Stone, J., I. H. Sutherland, C. S. Sommerville, R. H. Richards, and K. J. Varma. 2000c. Commercial trials using emamectin benzoate to control sea lice *Lepeophtheirus salmonis* infestations in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 41:141-149.

Sutherland, D. R., and D. D. Wittrock. 1985. Effects of *Salmincola californiensis* (Copepoda:Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 63:2893-2901.

Treasurer, J. W., C. Wallace, and G. Dear. 2002. Control of sea lice on farmed Atlantic salmon *S. salar* L. with the oral treatment emamectin benzoate (SLICE). Bulletin of the European Association of Fish Pathologists 22:375-380.

USFWS (United States Fish and Wildlife Service). 2011. Study protocol for a compassionate aquaculture investigational new animal drug (INAD) exemption for SLICE (emamectin benzoate): INAD #11-370. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana.

Wandelear, N., J. D. Bowker, and D. G. Carty. 2011a. The efficacy of SLICE premix (0.2% emamectin benzoate) administered in feed to control *Salmincola* spp. on freshwater-reared rainbow trout *Oncorhynchus mykiss* (SLICE-09-EFF-SAL-03). U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana.

Wandelear, N., D. G. Carty, and J. D. Bowker. 2011b. The efficacy of SLICE premix (0.2% emamectin benzoate) administered in feed to control *Salmincola* spp. on freshwater-reared rainbow trout *Oncorhynchus mykiss* (SLICE-09-EFF-SAL-01).

252 U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership
253 Program, Bozeman, Montana.

254 TABLE 1. Infestation prevalence, intensity, and distribution of adult female *Salmincola californiensis* in a test population of

255 freshwater-reared rainbow trout in response to treatment with SLICE at 50 µg emamectin benzoate·kg fish$^{-1}$·d$^{-1}$ for 7 d.

| Trial day | Number of fish examined | Number of *S. californiensis* counted | Number of fish infested with ≥1 *S. californiensis* | *S. californiensis* infestation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Prevalence (%) | Intensity (mean ± SD) | Distribution on fish (%) | | |
| | | | | | | Gills | Mouth | Other |
| 8 d pretreatment | 96 | 963 | 93 | 97 | 10.4 ± 7.6 | 75.2 | 24.5 | 0.5 |
| 8 d posttreatment | 96 | 945 | 94 | 98 | 10.1 ± 7.9 | 73.4 | 26.6 | 0.0 |
| 21 d posttreatment | 96 | 475 | 87 | 91 | 5.5 ± 4.6 | 60.0 | 40.0 | 0.0 |
| 43 d posttreatment | 96 | 50 | 31 | 32 | 1.6 ± 1.1 | 44.0 | 56.0 | 0.0 |

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