

Efficacy of emamectin benzoate in the control of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout *Oncorhynchus mykiss*

T. Hakalahti^{1,*}, Y. Lankinen², E. T. Valtonen¹

¹Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35 (ya), 40014 Jyväskylä, Finland

²Savon Taimen Oy, 77700 Rautalampi, Finland

ABSTRACT: Efficacy of in-feed treatment with emamectin benzoate (Slice) for the control of ectoparasitic *Argulus coregoni* on rainbow trout *Oncorhynchus mykiss* was tested under laboratory and field conditions. In both experiments fish were fed with fish feed to deliver a therapeutic dose of 0 (control) or 50 µg emamectin benzoate kg⁻¹ d⁻¹ (treatment) for a period of 7 d. After 3 d of challenge with *A. coregoni* in the laboratory, the infestation level in treated fish was lower than that observed in the controls ($p < 0.001$). Efficacy of 100% against newly hatched *A. coregoni* metanauplii and adults and 80% against juveniles was observed. In the field, trial medication was undertaken at 2 sections on a flow-through canal with 1 wk between treatments. Mean infestations of 100 to 200 *A. coregoni* per fish with 100% prevalence was recorded prior to medication. Following the treatment, the mean infestation of *A. coregoni* on fish declined to 31 lice per fish at Section A and 2.5 lice per fish at Section B. Then, after 28 d of treatment, the number of lice per fish was <1 at Section A; in contrast the mean number of *A. coregoni* per fish at the control section was >20. The prevalence of *A. coregoni* remained <50% over a period of 72 d of treatment, but started to increase again thereafter. This suggests that emamectin benzoate concentration in fish remained at a level high enough to kill *A. coregoni* over a period of 9 wk. Emamectin benzoate was very effective in the control of *A. coregoni* infesting trout.

KEY WORDS: Emamectin benzoate · Slice · *Argulus coregoni* · Ectoparasite · Control

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Ectoparasitic fish lice in the genus *Argulus* can create a very serious threat to both farmed and wild fish populations. The seasonal variation of temperature in northern latitudes influences the population dynamics of lice. Highest infestation levels of *A. foliaceus* (L.) and *A. coregoni* (Thorell) are recorded during the warmest months between May and August (Pasternak et al. 2000, Hakalahti & Valtonen 2003). *A. coregoni* over-winters only as eggs in Finland, and the eggs are usually deposited on hard substrates such as stones (Hakalahti et al. 2004b). The hatching of lice eggs starts with a high peak in the spring, when the water temperature exceeds 10°C (Mikheev et al. 2001, Hakalahti & Valtonen 2003). However, the recruitment of *A. coregoni* metanauplii (Hakalahti & Valtonen 2003) and

the hatching pattern, even within cohorts of eggs laid by individual females, is asynchronous and has been shown to extend over several months (Hakalahti et al. 2004a). The attached fish lice pierce the skin of the host by their modified mouthparts and feed on blood while simultaneously releasing toxic anticoagulants into the fish (Stammer 1959, LaMarre & Cochran 1992). Secondary bacterial and fungal infections invade the louse feeding site as the fish lice grow and may weaken the fish further, sometimes leading to mortality (Lester & Roubal 1995).

Severe infestations by *Argulus* species have been reported throughout the world (Shimura 1983, Menezes et al. 1990, Gault et al. 2002, Hakalahti & Valtonen 2003) and control attempts traditionally have been based upon bath immersion treatments using a variety of chemical compounds (e.g. Kabata 1970,

*Email: teihaka@bytl.jyu.fi

Mohan et al. 1986, Singhal et al. 1986, Jafri & Ahmed 1994). Most of the treatments have proven ineffective to some extent, stressful to fish and the environment, and usually require several treatments to eradicate the parasites. Stress faced by the fish during the treatment may impair their immune status, thus rendering them more susceptible to diseases, e.g. metazoan community structure on fish reflects the state of the water ecosystem (Valtonen et al. 1997). The development of treatments administered in feed would avoid the stress associated with bath immersion treatments and could be useful in the control of ectoparasites.

Emamectin benzoate (4"-deoxy-4" epimethylamino-avermectin B₁) is the active ingredient in the in-feed aquaculture parasiticide, Slice (Schering-Plough Animal Health). The efficacy of emamectin benzoate (50 µg kg⁻¹ for 7 d) has been demonstrated against the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on Atlantic salmon *Salmo salar* (Stone et al. 1999, 2000a,b,c, Treasurer et al. 2002), and *Salmincola edwardsii* on brook trout, *Salvelinus fontinalis* (Duston & Cusak 2002). Stone et al. (2000a) ascertained that the duration of efficacy against *L. salmonis* on salmon was up to 10 wk. The mode of action of avermectins is to increase the permeability of chloride ions in the neurons of invertebrate inhibitory synapses, which results in the paralysis and death of ectoparasites infesting fish (Arena et al. 1995, Vassilatis et al. 1997).

The trials reported here evaluated the efficacy of emamectin benzoate for control of *Argulus coregoni* infesting rainbow trout, *Oncorhynchus mykiss* (Walbaum) under controlled laboratory and field conditions. Fish in the field trial were continuously exposed to re-infestation by *A. coregoni* adults and juveniles dispersed from untreated sections of the farm and by metanauplii hatched over several weeks from overwintered eggs, since the hatching pattern of *A. coregoni* eggs is asynchronous (see Mikheev et al. 2001, Hakalahti & Valtonen 2003). Willis & Ling (2003) found that the susceptibility of planktonic marine copepods varies depending on the developmental stage, i.e. nauplii and copepodite EC₅₀-values were significantly lower than those for the adults. Therefore, in the laboratory experiment sensitivity among 3 life-stages (metanauplius, juvenile, adult) of *A. coregoni* to emamectin benzoate was compared.

MATERIALS AND METHODS

Medicated feed. Medicated feed, Slice (Schering Plough Animal Health), was prepared (BioMar AS) by coating the feed pellets with emamectin benzoate and oil, incorporated at a rate of 10 mg emamectin benzoate kg⁻¹ of feed. The basal ration in the field trial fed

to the rainbow trout 2+ yr of age was 7.0 mm feed pellets and was 5.0 mm feed in a laboratory trial fed to fish of 1+ yr of age.

Laboratory experiment. The efficacy of emamectin benzoate in prevention and control of *Argulus coregoni* infestations was tested under laboratory conditions at a commercial fish farm in central Finland in 2002. The experimental challenge of emamectin benzoate-medicated fish with lice was repeated 3 times using 3 ontogenetic stages of *A. coregoni*. The developmental stages of lice used in the experiment were: 24 to 36 h old metanauplii (mean length 0.7 mm, SD ± 0.02), juveniles (3.9 mm, SD ± 0.11) and adults (11.8 mm, SD ± 2.12). *A. coregoni* metanauplii were obtained from egg clutches collected from the earth ponds of the fish farm and stored in water at temperatures below 9°C, to prevent hatching before the experiment started (Hakalahti & Valtonen 2003). At the start of the experiment, hatching of the lice eggs was induced by exposing them to sunlight in the front of the window at room temperature (see Mikheev et al. 2001). Adult parasites were collected from infested rainbow trout *Oncorhynchus mykiss* (total length 30 to 40 cm), and juvenile *A. coregoni* were collected from the flow-through tank (volume 500 l) containing small rainbow trout (80 to 100 g weight), which was established to let *A. coregoni* metanauplii grow to the juvenile stage. Prior to experiments, all lice were pre-starved for a day to stimulate high foraging motivation (see Mikheev et al. 2000).

Prior to the experiment, 100 uninfected rainbow trout (weight range 111 to 263 g) were captured and 50 were randomly allocated into each of 2 established flow-through tanks (volume 500 l). After 24 h of acclimation, fish in the experimental tank were fed with medicated feed for 7 consecutive days, with a nominal dose of 50 µg emamectin benzoate kg⁻¹ d⁻¹. Fish in the control tank were fed an unmedicated feed at rate of 0.5% biomass d⁻¹. New fish were used in each experiment. The water temperature during the medication period was ambient, average daily temperature being 21°C (SD ± 1.3°C) during the metanauplius and adult trial and 23°C (SD ± 0.5°C) during the juvenile trial. Due to the structure of the maintaining tanks, we were not able to collect and estimate the amount of uneaten feed during the medications.

The day following each medication period, both medicated and control fish were randomly allocated into smaller tanks in 45 l of water, without the flow-through system. Each of the 5 replicate tanks contained 5 fish. In these tanks water circulation was provided by using air pumps and air stones, and water was changed every day. After a 12 h acclimation of fish, *Argulus coregoni* were released to all tanks, and, following 3 d challenge period, fish were anesthetized individually, lice were removed, and the efficacy of the medicine was calcu-

lated. The numbers of louse individuals introduced into each experimental tank (5 fish) were either 200 metanauplii, 50 juveniles, or 20 adults, and the efficacy of the medicine was calculated as: %efficacy = $1 - (\text{mean number of lice in control} / \text{mean number of lice in treatment}) \times 100$. After the experiment, fish were released back to their rearing site. In total 3 fish died during exposures to *A. coregoni*.

Field trial. The field trial was carried out at a commercial fish farm in central Finland during the summer 2002. The farm consists of earthen ponds containing Atlantic salmon *Salmo salar*, whitefish *Coregonus lavaretus*, brown trout *S. trutta*, and rainbow trout *Oncorhynchus mykiss* of various age groups. Water flows to the ponds through canals from the nearby lake. The farm also includes a large flow-through canal containing rainbow trout 2+ yr of age. This farming canal, which has a bottom of mud and stones, is about 1 km in length, 8 m wide, and 2 m deep. Bridges and grating divide the canal into 9 interconnected sections (Fig. 1). Section A, where the sampling of this study was started, was the only section containing very heterogeneous fish stock (total length 28.8 to 49.5 cm), transferred from the ponds. The daily water temperatures in the canal in 2002 are represented in Fig. 2.

Samples of *Argulus coregoni* were collected from rainbow trout in 3 separate sections (A, B, and C) of the farming canal that contained infested fish (Fig. 1).

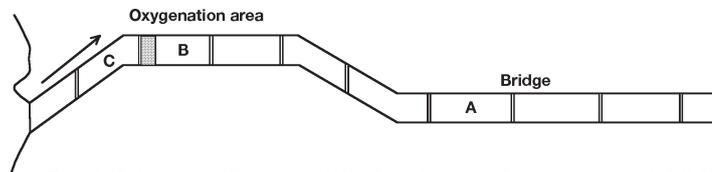


Fig. 1. Schematic diagram of the farming canal at a commercial fish farm in central Finland. *Argulus coregoni* samples were collected from the canal sections A, B, and C. The arrow indicates the direction of water flow. Fish in Section C were untreated, and fish in Sections A and B were treated with emamectin benzoate for *A. coregoni* infestations

Section A contained a stock of about 10 000 fish, Section B contained about 6000 fish and Section C contained about 20 000 fish. Weekly samples were taken from Section A between 14 May and 13 September 2002 (Table 1). Fish in Section A were treated with emamectin benzoate-medicated feed for 7 consecutive days (27 May to 2 June 2002) at a dose of $50 \mu\text{g kg}^{-1} \text{d}^{-1}$. The medicated feed was spread over the whole section area by using a tractor-mounted feed spreader, to facilitate uniform feeding rate among the fish. Samples were taken weekly from Section B between 31 May and 1 July, after which the fish were harvested for food consumption and no further samples were available. This section was treated with emamectin benzoate from 3 to 9 June using the same procedure as in Section A. In both sections individual lice collections were taken from 15 fish before treatment and from either 15, 20, or 50 fish after treatment at every sampling time (see Table 1). All sections of the canal, except the Section C located upstream from Sections A and B (see Fig. 1), were treated with emamectin benzoate. Due to fish management practices, collection of weekly samples from Section C was possible only between 9 and 31 July. Samples obtained from Section C, together with data from an earlier study on population structure and the seasonal cycle of *A. coregoni* undertaken in 1999 and 2001 (Hakalahti & Valtonen 2003), served as a control for the medicated Sections A and B in late summer.

For sampling, the fish were first gathered with a seine net and were then captured individually with a dip net. Fish were anesthetized in a white container with MS-222. Fish skin, gills, and mouth cavities were inspected for argulids. Detached and removed lice were counted and preserved in 70% alcohol for later species identification and measurement in the laboratory. After recovering from narcosis, fish were released back to the same site. The distribution of parasites among individual fish was depicted using the terms prevalence and abundance according to Bush et al. (1997) and calculating the variance to mean ratio (s^2/\bar{x}) of the numbers of *Argulus coregoni* per fish.

RESULTS

Laboratory experiment

Variability within treatments was not found in the mean number of lice per tank of 5 fish in either adult or juvenile *Argulus coregoni* groups (Kruskal-Wallis test: $p > 0.05$). Therefore, data from all tanks within a treatment in the adult and juvenile lice groups were pooled. Three days following the fish lice challenge, the mean number of established

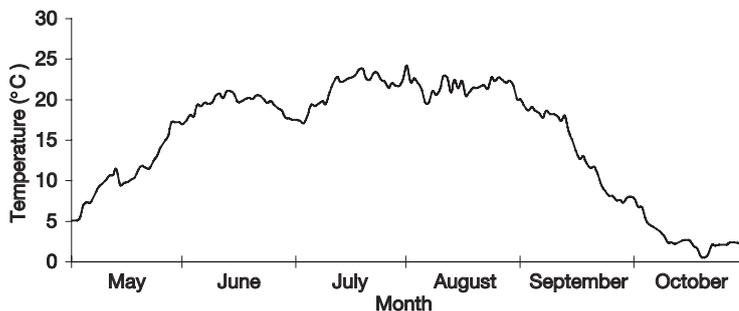


Fig. 2. Daily water temperatures at the fish farm during the open-water period 2002

adult (Mann-Whitney test: $U = 87.5$, $p < 0.001$) and juvenile *A. coregoni* was significantly lower in the medicated group than in their control ($U = 78.0$, $p < 0.001$), and efficacy of 100% and 80% was recorded, respectively (Table 2). After the experiment, only 2 motile adult lice were found in the water, but 5 fish were found infected with 12, 5, 2, 2, or 1 juvenile *A. coregoni*, respectively.

Emamectin benzoate-medicated fish challenged with newly hatched *Argulus coregoni* metanauplii were 100% devoid of lice ($U = 0.0$, $p < 0.001$), showing high efficacy. Variability between the control tanks infected with *A. coregoni* metanauplii was found (Kruskal-Wallis test: $\chi^2 = 16.5$, $p = 0.002$). Three days post-challenge each tank contained 9 (SD ± 2.7), 12 (SD ± 2.6), 23 (SD ± 6.8), 26 (SD ± 11.3), and 13 (SD ± 2.2) established lice per fish. Emamectin benzoate medication was very efficient and lethal to *A. coregoni*.

Field trial

Severe *Argulus coregoni* infestations were recorded in May 2002 at the fish farm. Three weekly samples were taken before the emamectin benzoate treatment in Section A of the flow-through canal. At that time the weekly prevalence was 100%, and the mean abundance of *A. coregoni* on fish was 140 (SD ± 274.8) (Table 1). There were no differences in mean numbers of lice on fish in Sections A and B collected before treatment (Mann-Whitney test: $U = 218.0$, $p = 0.369$; Table 1). In samples taken from Section A 4 and 7 d after treatment, the numbers of lice on fish had decreased to 65 (SD ± 83.2 , $U = 78.5$, $p = 0.158$) and 31 (SD ± 54.3 , $U = 53.0$, $p = 0.001$) lice per fish, respectively (Figs. 3 & 4). At the same time (31 May to 3 June), the abundance of lice in Section B, which is connected to Section A and was not treated yet, was

Table 1. *Argulus coregoni* on *Oncorhynchus mykiss*. Number of fish examined (n), mean number of lice at each sampling time at each sampling site (A, B, and C), and the minimum and maximum numbers of lice on fish are given. Dashed lines indicate the beginning of emamectin benzoate treatment of 7 d

Date (d/mo)	Section A				Section B				Section C			
	n	Mean (\pm SD)	Min.	Max.	n	Mean (\pm SD)	Min.	Max.	n	Mean (\pm SD)	Min.	Max.
14/05	15	164 (± 321.6)	15	1315								
21/05	15	155 (± 346.1)	4	1390								
27/05	15	103 (± 108.5)	10	349								
31/05	15	65 (± 83.2)	3	283	15	191 (± 105.5)	8	862				
03/06	20	31 (± 54.3)	0	175	15	112 (± 148.3)	16	589				
06/06	20	7 (± 18.3)	0	82	20	7 (± 9.8)	1	35				
11/06	50	7 (± 19.5)	0	114	50	2.5 (± 16.1)	0	114				
18/06	50	9 (± 30.8)	0	202	50	14 (± 88.2)	0	624				
24/06	50	4 (± 16.1)	0	113	50	1 (± 3.2)	0	22				
01/07	50	0.4 (± 1.18)	0	8	50	3 (± 2.6)	0	11				
09/07	50	0.9 (± 2.71)	0	13					20	24 (± 12.2)	5	53
18/07	50	0.3 (± 0.85)	0	5					20	23 (± 16.7)	3	70
24/07	50	0.3 (± 0.71)	0	3					50	10 (± 9.9)	0	54
31/07	50	0.4 (± 0.66)	0	2					50	2 (± 1.7)	0	6
08/08	50	1 (± 1.8)	0	8								
16/08	50	2 (± 2.2)	0	12								
22/08	50	2 (± 2.2)	0	9								
30/08	50	3 (± 3.8)	0	21								
05/09	50	4 (± 4.1)	0	16								
13/09	50	4 (± 6.5)	0	35								

Table 2. Laboratory experiment on the efficacy of in-feed treatment, emamectin benzoate, against 3 developmental stages of lice *Argulus coregoni* on rainbow trout *Oncorhynchus mykiss*. Fish received feed at a rate to deliver a nominal dose of either 0 (control) or 50 μg emamectin benzoate $\text{kg}^{-1} \text{d}^{-1}$ (medication) for 7 d, after which they were challenged over 3 d with *A. coregoni*. Number of fish in each treatment was 25 (5 tanks each containing 5 fish). n: number of *A. coregoni* per fish

Developmental stage	Medication		Control		Efficacy (%)
	Mean (\pm SD)	Prevalence (%)	Mean (\pm SD)	Prevalence (%)	
Adult (n = 4)	0 (± 0)	0	2 (± 2.1)	71	100
Juvenile (n = 10)	1 (± 2.6)	24	5 (± 4.1)	100	80
Metanauplius (n = 40)	0 (± 0)	0	17 (± 8.9)	100	100

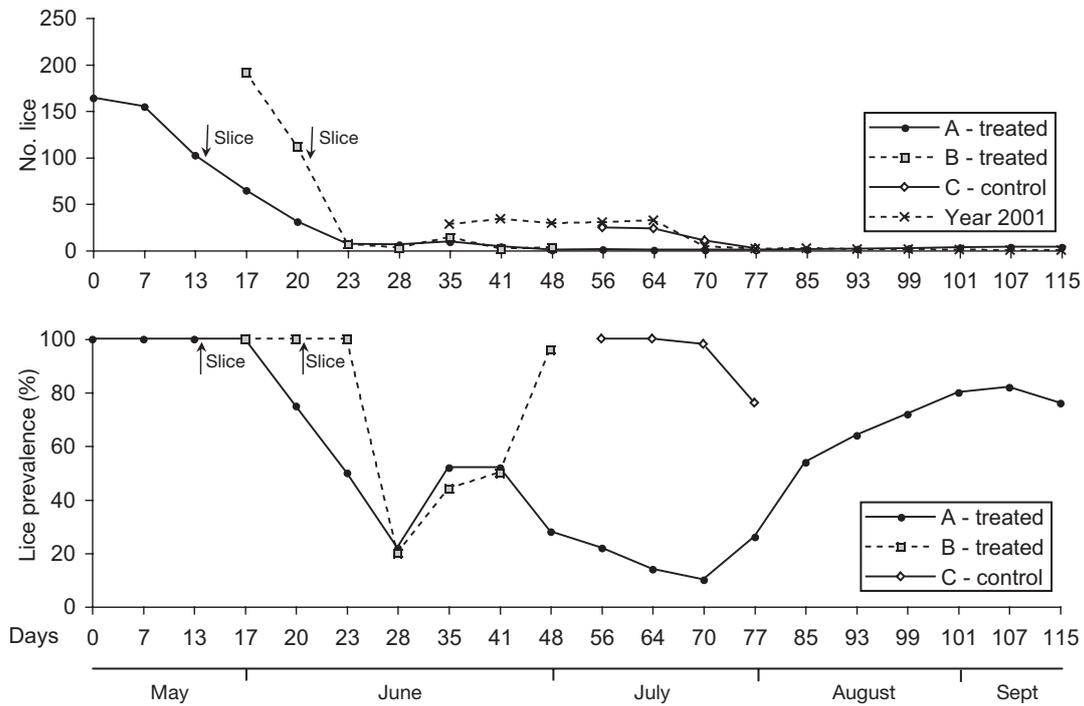


Fig. 3. Prevalence and mean number of *Argulus coregoni* infesting rainbow trout, *Oncorhynchus mykiss*. The field trial of emamectin benzoate was conducted on a commercial fish farm in central Finland in 2002. Average number (upper panel) and prevalence (lower panel) of lice on fish following treatment with 50 µg emamectin benzoate kg⁻¹ d⁻¹ for 7 consecutive days. Treatments in Sections A and B are depicted with arrows. *Argulus* infestation data for 2001 were obtained from Hakalahti & Valtonen (2003)

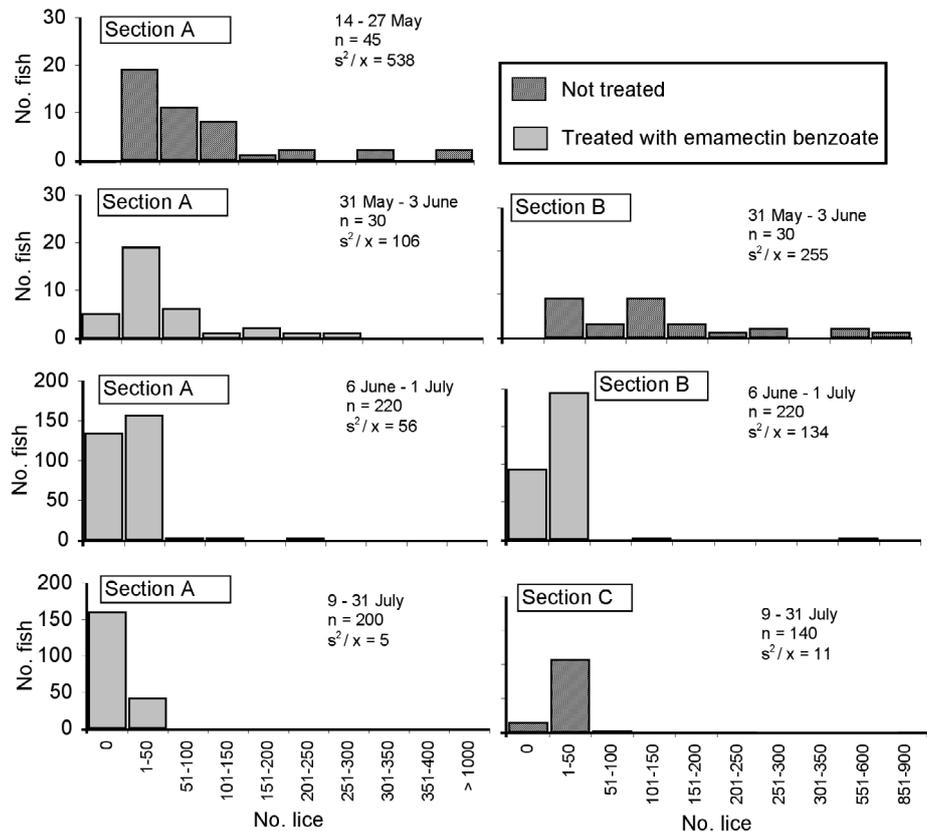


Fig. 4. Field trial of emamectin benzoate against *Argulus coregoni* infesting rainbow trout *Oncorhynchus mykiss*, conducted on a commercial fish farm in central Finland in 2002. Frequency distributions of the numbers of lice on fish at 3 sampling sites of the flow-through canal. Section A was treated from 27 May to 2 June, and Section B was treated from 3 to 9 June with 50 µg emamectin benzoate kg⁻¹ d⁻¹ for 7 d. The number of fish examined and the variance to mean ratio of number of *A. coregoni* attached on each fish are indicated in the legends

notably higher than in Section A, which was treated ($U = 218.0$, $p < 0.001$). The same decline in *A. coregoni* numbers was seen in Section B after treatment with emamectin benzoate from 3 June onwards. Within 3 d after treatment, the mean number of lice per fish had decreased from 151 (SD \pm 196.5) to 7 (SD \pm 9.8) ($U = 13.0$, $p < 0.001$).

The prevalence of *Argulus coregoni* on fish in Section A decreased during the 14 d period after medication ending on 11 June, increased slightly between 18 and 24 June, but decreased thereafter (Fig. 3). From 57 d after treatment, the prevalence of lice gradually increased. However, >1 mo post-treatment (between 9 and 31 July), the abundance of lice in Section A was significantly lower than in the control Section C sampled simultaneously ($U = 2389.0$, $p < 0.001$). At that time, an average of 0.5 (SD \pm 1.51) and 11 (SD \pm 13.1) lice per fish were collected from Sections A and C, respectively. Although the prevalence of lice increased towards autumn, the mean number of *A. coregoni* infesting fish in Section A remained <5 lice per fish up to 13 September, when the sampling ended (Table 1).

Before the medication, the variance to mean ratio (s^2/\bar{x}) of the number of lice per fish in Section A was 538, indicating very high aggregation of lice populations among individual fish (Fig. 4). The fish length in Section A was not correlated with the number of *Argulus coregoni* on fish (Pearson correlation coefficient: $r = 0.220$, $p = 0.151$). In samples collected 4 and 7 d after the 7 d medication period, the variance to mean ratio of lice had decreased to 106. At the same time the variance to mean ratio was 255 in Section B, which was still untreated.

DISCUSSION

The field and laboratory experiments presented here proved that emamectin benzoate is effective in the control of *Argulus coregoni* on rainbow trout, *Oncorhynchus mykiss*. A 100% efficacy was achieved against newly hatched *A. coregoni* metanauplii and adults in the laboratory experiment. Although most emamectin benzoate-medicated fish were devoid of juvenile *A. coregoni* as well, a slightly lower total efficacy of 80% was recorded for them. This may imply differences in susceptibility between the life-stages of *A. coregoni*, as has been observed in some plankton copepods (e.g. Medina et al. 2002, Willis & Ling 2003). Observed differences in lice mortality could also be due to differences in feed intake between the individual fish during medication. The temperature during the juvenile trial was higher, about 23°C, compared to the water temperature of 21°C during the adult and metanauplius trial. High temperature coupled with

handling stress may have reduced feed intake by certain fish individuals in the laboratory; thus, the actual medication doses were not able to be determined. During the field trial, medications were performed in early summer, when the water temperature increased from 15.4 to 20.4°C. Results, which will be discussed later, showed that fish with the highest parasite burdens at the fish farm lost their juvenile *A. coregoni* due to medication.

The seasonal pattern of *Argulus coregoni* population dynamics after application of emamectin benzoate in 2002 was markedly different from that reported in our previous study undertaken in 1999 and 2001 (Hakalahti & Valtonen 2003). In both studies, the first *A. coregoni* metanauplii hatched from over-wintered eggs were observed on fish when water temperature exceeded 10°C. Infestation levels of 100 to 200 *A. coregoni* per fish were recorded in weekly samples taken before medication (14 May to 3 June), whereas, in all samples collected in 2001, <40 lice per fish were found (see Hakalahti & Valtonen 2003). *Argulus* were not controlled extensively in 2001, which explains the much higher infection level observed in 2002. The over-wintering *A. coregoni* eggs laid in 2001 accumulated on the bottom of the canal and started to hatch the following spring. *A. coregoni* females lay approximately 317 eggs (Hakalahti et al. 2004a). Following the emamectin benzoate treatment in 2002, the numbers of *A. coregoni* in the medicated Section A of the canal declined, but remained at the same level in sections of the canal (Section B) that had not been treated yet. A more rapid decrease in *A. coregoni* numbers followed medication undertaken a week later in Section B, when the water temperature was higher. Temperature can affect the onset of maximum efficacy of emamectin benzoate, e.g. maximum efficacy against *Lepeophtheirus salmonis* in Atlantic salmon *Salmo salar* was achieved more rapidly at temperatures of 13.0 to 15.5°C than at temperatures of 7.2 to 8.5°C (Stone et al. 2000c). In the present study an average of 31 lice per fish were recorded in Section A and 2.5 lice per fish in Section B at the end of the emamectin benzoate medication period of 7 d, when the water temperatures in Sections A and B were 18.0 and 20.4°C, respectively. However, the resulting efficacy was the same in both sections: within 3 d after treatment the number of lice per fish in Section A had decreased to 7.

Although the seasonal dynamics of *Argulus coregoni* populations are temperature dependent and variability between years occurs (Hakalahti & Valtonen 2003), they cannot explain the sharp decrease in lice numbers following the treatment. At the end of the medication period in Section A (3 June), the largest recorded *A. coregoni* were only 3 mm in length. Females of *A. coregoni* start to lay eggs upon reaching about 8 mm in

length (Hakalahti et al. 2004a). In 2001 females started to lay eggs from early July onwards, and the egg laying period extended over 3.5 mo (Hakalahti & Valtonen 2003, Hakalahti et al. 2004b). In the present study, <1 *A. coregoni* per fish was found in July, and the numbers remained low up to the end of September. That low abundance was recorded only at the end of August in 2001, when most females had finished their egg laying and were dying (Hakalahti & Valtonen 2003). The notable decrease in *A. coregoni* numbers observed in late July in untreated fish (Section C) of the present study is in accordance with the earlier study on the population dynamics.

The numbers of *Argulus coregoni* per fish showed an aggregated distribution, the variance to mean ratio (s^2/\bar{x}) being 538 and 255 in 2 sampling sections of the canal, before the emamectin benzoate medication. The variance to mean ratio was tremendously decreased after treatment, suggesting that fish with high parasite loads had either died or recovered from *Argulus* sp. infestation. No adverse mortality due to lice infestation was observed among treated fish following medication, suggesting that the fish received an effective dose of emamectin benzoate. It must be pointed out that treatments were performed in early summer, soon after the majority of the *A. coregoni* population of that year had recruited on fish and lice were still small in size (Mikheev et al. 2001, Hakalahti & Valtonen 2003). Growing *Argulus* spp. juveniles may expose fish to fungal and bacterial infections by irritating the skin that can affect the fish's feeding and can sometimes lead to death of the host (Lester & Roubal 1995).

In a field trial, where fish were continuously exposed to *Argulus coregoni* recruited from untreated sections of the canal or hatched from eggs on the canal bottom (Hakalahti et al. 2004b), the duration of efficacy of emamectin benzoate extended well beyond the medication period. Although the prevalence of *A. coregoni* increased from 20% to >50% 21 to 28 d following medication, the decreasing trend in the prevalence of lice thereafter suggests that *A. coregoni* were not able to survive on medicated fish. About 9 wk from the start of the application of emamectin benzoate, the prevalence of lice on fish started to increase steadily, suggesting that the efficacy of the medicine was ceasing. Due to its extended duration of efficacy, emamectin benzoate has been used as a preventative treatment for salmon smolts before the transfer to sea (Stone et al. 2002), when high-order efficacy against sea lice *Lepeophtheirus salmonis*, was still achieved 10 wk after treatment (Stone et al. 2000a).

In conclusion, emamectin benzoate was very efficient for the control of *Argulus coregoni* on rainbow trout. By medicating the fish, which initially had high

A. coregoni infestations, we were able to prevent fish mortality and break the reproductive cycle of the parasite. Due to the strategy of *A. coregoni* to overwinter only as eggs, the mean lice infestation levels on fish would have decreased towards the end of the open-water period without control (Mikheev et al. 2001, Hakalahti & Valtonen 2003). In that case, presumably, the majority of fish would have been killed by *Argulus* and the lice egg stock on the bottom of the canal would have increased heavily. Eggs of *A. coregoni* can remain viable buried in sediments for at least 2 yr, and retain the ability to hatch throughout an extended period (Mikheev et al. 2001). At this particular fish farm, a single emamectin benzoate treatment administered in-feed in early summer proved to be an effective control strategy, due to extended residence of the medicine in fish tissues. However, to overcome such *A. coregoni* mass infestation as in the present case, application of the medication should be administered over several years and preferably in all ponds of the farm simultaneously. The medication should be given in early May, prior to the spring peak of *A. coregoni* metanauplii hatching. Present results suggest a need for another treatment after 9 wk. However, the duration of efficacy of emamectin benzoate in the control of lice should be studied experimentally.

Acknowledgements. We thank H. Häkkinen and M. Bandilla for assistance during the experiments. We also thank R. Endris for editorial assistance and for helpful comments. The study was funded by the Graduate School for Biological Interactions, the Foundation for Research of Natural Resources in Finland, SUNARE—project of the Academy of Finland, Savon Taimen Oy and Schering-Plough Animal Health.

LITERATURE CITED

- Arena JP, Liu KK, Paresse PS, Frazier EG, Cully DF, Mrozik H, Schaeffer JM (1995) The mechanism of action of avermectins in *Caenorhabditis elegans*, correlation between activation of glutamate-sensitive chloride current membrane binding and biological activity. *J Parasitol* 81: 286–294
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms. *Margolis et al. revisited. Parasitology* 83:575–583
- Duston J, Cusack RR (2002) Emamectin benzoate: an effective in-feed treatment against the gill parasite *Salmincola edwardsii* on brook trout. *Aquaculture* 207:1–9
- Gault NFS, Kilpatrick DJ, Steward MT (2002) Biological control of the fish louse in a rainbow trout fishery. *J Fish Biol* 60: 226–237
- Hakalahti T, Valtonen ET (2003) Population structure and recruitment of the ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura) on a fish farm. *Parasitology* 127: 79–85
- Hakalahti T, Häkkinen H, Valtonen ET (2004a) Ectoparasitic *Argulus coregoni* (Crustacea: Branchiura) hedge their bets — studies on egg hatching dynamics. *Oikos* (in press)

- Hakalahti T, Pasternak AF, Valtonen ET (2004b) Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* 128:655–660
- Jafri SIH, Ahmed SS (1994) Some observations on mortality in major carps due to fish lice and their chemical control. *Pak J Zool* 26:274–276
- Kabata Z (1970) Diseases of fishes. Book I. Crustacea as enemies of fishes. THF Publications, Neptune City, NJ
- LaMarre E, Cochran PA (1992) Lack of host species selection by the exotic parasitic crustacean, *Argulus japonicus*. *J Freshw Ecol* 7:77–80
- Lester RJG, Roubal FR (1995) Phylum Arthropoda. In: Woo PTK (ed) Fish diseases and disorders, Vol 1. Protozoan and metazoan infection. Cab International, Wallingford
- Medina M, Barata C, Telfer T, Baird DJ (2002) Age- and sex-related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa* Dana. *Arch Environ Contam Toxicol* 42:17–22
- Menezes J, Ramos MA, Pereira TG, Moreira Da Silva A (1990) Rainbow trout culture failure in a small lake as a result of massive parasitosis related to careless fish introductions. *Aquaculture* 89:123–126
- Mikheev VN, Mikheev AV, Pasternak AF, Valtonen ET (2000) Light-mediated host searching strategies in a fish ectoparasite, *Argulus foliaceus* L. (Crustacea: Branchiura): the role of vision and selectivity. *Parasitology* 120:409–416
- Mikheev VN, Pasternak AF, Valtonen ET, Lankinen Y (2001) Spatial distribution and hatching of overwintered eggs in a fish ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura). *Dis Aq Org* 46:123–128
- Mohan CV, Ramaiah N, Shanbhogue SL (1986) Effects of some therapeutics on fish ectoparasites. *Environ Ecol* 4: 98–100
- Pasternak AF, Mikheev VN, Valtonen ET (2000) Life history characteristics of *Argulus foliaceus* L (Crustacea: Branchiura) populations in central Finland. *Ann Zool Fenn* 37: 25–35
- Shimura S (1983) Seasonal occurrence, sex ratio and site preference of *Argulus coregoni* Thorell (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan. *Parasitology* 86:537–552
- Singhal RN, Jeet S, Davies RW (1986) Chemotherapy of 6 ectoparasitic diseases of cultured fish. *Aquaculture* 54: 165–171
- Stammer HJ (1959) Beiträge zur Morphologie, Biologie und Bekämpfung der Karpfenläuse. *Z Parasitenkd* 19:135–208
- Stone J, Sutherland IH, Sommerville C, Richards RH, Varma KJ (1999) The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer), infestations in Atlantic salmon, *Salmo salar* L. *J Fish Dis* 22:261–270
- Stone J, Sutherland IH, Sommerville C, Richards RH, Endris RG (2000a) The duration of efficacy following oral treatment with emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis* (Krøyer), in Atlantic salmon *Salmo salar* L. *J Fish Dis* 23:185–192
- Stone J, Sutherland IH, Sommerville C, Richards RH, Varma KJ (2000b) Commercial trials using emamectin benzoate to control sea lice *Lepeophtheirus salmonis* in Atlantic salmon *Salmo salar*. *Dis Aquat Org* 41:141–149
- Stone J, Sutherland IH, Sommerville C, Richards RH, Varma KJ (2000c) Field trials to evaluate the efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordman, infestations in Atlantic salmon, *Salmo salar*. *Aquaculture* 186:205–219
- Stone J, Roy WJ, Sutherland IH, Ferguson HW, Sommerville C, Endris R (2002) Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, *Salmo salar* L., smolts in freshwater, as a preventative treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Krøyer). *Aquaculture* 210:21–34
- Treasurer JW, Wallace C, Dear G (2002) Control of sea lice on farmed Atlantic salmon *S. salar* L. with the oral treatment emamectin benzoate (SLICE). *Bull Eur Assoc Fish Pathol* 22:375–380
- Valtonen ET, Holmes JC, Koskivaara M (1997) Eutrophication, pollution and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in 4 lakes in central Finland. *Can J Fish Aquat Sci* 54:572–585
- Vassilatis DK, Elliston KO, Pares PS, Hamelin, M, Arena JP, Schaeffer JM, van der Ploeg LH, Cully DF (1997) Evolutionary relationship of the ligand-gated ion channels and the avermectin sensitive, glutamate-gated chloride channels. *J Mol Evol* 44:501–508
- Willis KJ, Ling N (2003) The toxicity of emamectin benzoate, an aquaculture pesticide, to planktonic marine copepods. *Aquaculture* 221:289–297

Editorial responsibility: Wolfgang Körting,
Hannover, Germany

Submitted: March 3, 2004; Accepted: May 10, 2004
Proofs received from author(s): August 12, 2004