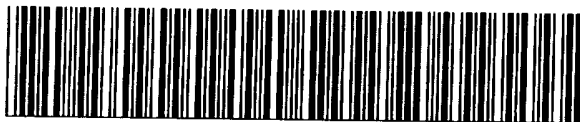


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(54) Title: CONTROL OF SEA LICE IN FISH		
(57) Abstract <p>The use of various pyrethroid compounds is disclosed for the treatment of the immature phases of sea lice infestation in fish, such as salmon, and for the prophylaxis of adult sea lice infestation in such fish. Cypermethrin, high-cis-cypermethrin, lambda-cyhalothrin and deltamethrin are shown to be most effective.</p>		

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CONTROL OF SEA LICE IN FISH

This invention relates to the control of sea lice in fish, such as salmon.

Infestation of salmon with sea lice is a recognised
5 problem. Salmon are infected by free swimming immature sea lice in the copepodid phase of development, which attach themselves to the body of the fish by hooked antennas. Once attached to the fish, the lice develop through four immature
10 chalimus phases before becoming pre-adults about 2-3 weeks after the initial infestation. During the chalimus phases, the lice attach themselves to the fish by frontal filaments, and though they feed on the fish, they do not cause extensive damage. During their final pre-adult and adult
15 phases of development, the lice attach themselves to the body of the fish by suction and graze over its surface, especially the head, back and peri-anal areas, causing extensive damage and ultimately death.

A number of treatments have been used to control sea lice in the past, most notably dichlorvos, azamethiphos and
20 hydrogen peroxide treatment baths. A common feature of these known treatments is that they are effective only against mature lice (i.e. lice in the pre-adult and adult phases), and are ineffective against lice in immature phases of development. Hydrogen peroxide has been suggested by
25 some workers to have a limited effect against immature lice, but this effect is slight compared to its effect on mature lice.

The reason for this selective activity is not known but it is thought likely to be due in some way to the
30 substantial differences in anatomy and metabolism between the immature (copepodid and chalimus) and mature (pre-adult and adult) lice. One theory is that differences in respiration are responsible, but this has not been fully substantiated. Whatever the underlying reasons for the
35 selectivity, it remains an empirical rule that the conventional topical treatments are effective only against mature lice, with the resultant drawback that infested fish require repeated and frequent treatments in order to kill

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the lice as and when they mature.

We have now identified a group of compounds that are effective for controlling immature as well as mature sea lice. The compounds concerned are the pyrethroids, for example Cypermethrin (3-(2,2-Dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano (3-phenoxyphenyl)-methyl ester). We have already suggested the use of these compounds for the control of sea lice in our international patent application, published as WO 92/16106, but it was previously thought that the compounds were effective only for mature lice, and the tests described in our earlier application utilised mature lice exclusively. Because of the known resistance of immature lice to all known conventional bath treatments for mature lice, the identification of a group of compounds which is effective when used topically against both mature and immature lice is surprising. It is also of significant practical importance, in that a single treatment will affect substantially all of the lice present on the fish, whether mature or immature, thereby reducing the frequency with which treatments need to be made. This in turn leads to a saving in labour, a decrease in environmental contamination, and the minimising of stress to the fish.

The present invention therefore provides the use of a pyrethroid compound for the manufacture of a composition for the treatment of the immature phases of sea lice infestation of fish.

Alternatively, the invention provides the use of a pyrethroid compound for the manufacture of a composition for the prophylaxis of pre-adult and adult sea lice infestation of fish.

The invention is hereinafter described by way of example only, with reference to the following experimental trials and the accompanying figures which illustrate the results of the trials.

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TRIAL 1

Trial 1 was carried out at the Marine Harvest International research establishment at Loch Eil, Inverness-shire. The fish used in the trial were mixed sex Atlantic Salmon (Salmo salar), of the "Marine Harvest M6" strain, aged approximately 28 months, and weighing approximately 3kg each.

Preparation

316 study fish were submitted to a pre-study veterinary examination and found to be suitable to participate in the study. The fish were held in a single holding cage in Loch Eil and were fed the same commercial fish feed (Fulmar Expanded Salmon Feed, Super Energy Salmon) for the ten days leading up to the study period, and throughout the duration of the study, therefore ensuring that no acclimatisation problems arose. Feed was thrown onto the surface of the water in the cage at a rate of approximately 0.5% bodyweight daily. On the day prior to treatment 15 fish were examined and counted for major phases of sea lice (chalimus, pre-adult and adult) and the mean counts taken as representative of the pre-treatment levels. The remaining 301 fish were then divided into six similar groups, five containing 50 fish each, and the sixth 51 (the extra fish resulted from a miscount, which was discovered during the trial, but was considered to be insignificant). Care was taken to ensure that all groups were as similar as possible in terms of weight and lice numbers.

Method

Each group of fish was placed in an individual experimental sea cage measuring 5m x 5m x 4m (depth) and constructed of nylon mesh nets. The cages were anchored in Loch Eil and open to the sea. Three of the groups were designated as treatment groups and three as control groups.

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The treatment groups were each subjected to treatment with Cypermethrin at a concentration of $100\mu\text{g}/\text{l}$ for one hour. During treatment, the bottoms of the cages were raised to give cage water depths of 2 metres. Impervious tarpaulins were used to surround individually each of the cages, and oxygen was diffused into the cages using a circular diffuser, to maintain an oxygen level of at least $8\text{mg}/\text{l}$ during the time of treatment. Treatment was carried out by measuring an amount of 2.54 wt% stock solution of Cypermethrin calculated to give a final concentration in the cage of $100\mu\text{g}/\text{l}$, mixing this in a bucket of sea water, and then pouring this concentrated mixture into the appropriate cage, at several sites around the cage. After addition of treatment solution to the cages, the tarpaulins remained in place for 1 hour.

The method was repeated with the control cages, but without the addition of Cypermethrin.

Sampling and Measurements

Sampling was carried out by removing 10 fish from each tank, anaesthetizing the fish, and counting the number of chalimus, pre-adult and adult lice on each fish in accordance with the Marine Harvest International Standard Operating Procedure. The fish used for counting were killed, and not returned to the experimental cages. Counts were conducted at the following times:

Day of treatment (Day 1)	:	Approximately 1 hour after removal of the tarpaulin from the cage.
Day 2	:	Approximately 24 hours after removal of the tarpaulins from the cage.
Day 8	:	
Day 15	:	

Results

Table 1 below summarises the results obtained. (Where the number of lice on a fish was greater than 100, it was recorded as >100 and taken to equal 100 during data analysis.) The results are presented graphically in Fig. 1.

Table 1

<u>Treatment</u>	<u>Day</u>	<u>Mean number of lice</u> (Standard deviation in parentheses)		
		<u>Chalimus</u>	<u>Pre-adult</u>	<u>Adult</u>
10 PRE-TREATMENT	0	3.13 (2.42)	4.6 (1.99)	8.6 (2.9)
CYPERMETHRIN	1	2.97 (2.41)	0.3 (0.6)	1.93 (1.48)
CONTROL	1	4.77 (1.89)	3.82 (1.53)	11.4 (4.26)
CYPERMETHRIN	2	3.17 (2.16)	0.23 (0.5)	0.03 (0.18)
15 CONTROL	2	2.2 (2.4)	1.26 (1.31)	7.3 (6.6)
CYPERMETHRIN	8	1.33 (1.19)	2.67 (2.47)	1.5 (1.7)
CONTROL	8	26.6 (16.2)	10.9 (8.9)	9.3 (5.5)
CYPERMETHRIN	15	87.5 (20.6)	77.8 (23.9)	10.5 (7.1)
CONTROL	15	90.3 (17.3)	76.6 (25.5)	22.2 (10.6)

20 Each value = mean of 30 fish; Pre-treatment values = mean of 15 fish

Treatment with Cypermethrin at 100µg/l for 1 hour substantially reduced chalimus, pre-adult and adult numbers of lice on treated fish, as compared to untreated fish. The effect was seen particularly at day 8 (7 days after treatment), but was substantially reduced by day 15. A possible explanation for this lack of difference on day 15 is that reinfection occurred from the untreated to treated fish; a massive sea lice infection of the site noted on day 8 could have contributed to rapid reinfection. When used commercially, all fish would be treated simultaneously, hence all lice would be affected, and so reinfection would not be expected to occur so soon after treatment.

The effect against adult and pre-adult lice was expected from our previous work described in WO 92/16106, but the significantly lower numbers of chalimus lice on

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treated fish 7 days after treatment (day 8) as compared to untreated fish was completely unexpected. The reasons for this effect are unknown, but it is suggested that the treatment killed a proportion of the chalimus lice, and also in some way stopped or delayed maturation of copepodids into chalimus for several days. A second trial (Trial 2) was carried out in order to investigate the effect further:

TRIAL 2

Trial 2 was carried out at the Marine Harvest International Experimental Challenge Unit at Lochailort, Inverness-shire. Two studies were conducted; firstly, a pilot study (control) in which the rate of survival and development of sea lice on fish in a tank environment was investigated; and secondly a trial study to investigate the effect of Cypermethrin.

Method

In both pilot and trial studies, 60 Atlantic Salmon smolts were taken and challenged with a known number of sea lice copepodids. The fish in the pilot study (referred to as GP1) were counted for lice at 10 days, 21 days and 27 days after initial infestation, while those in the trial study (referred to as GP2) were counted for lice at 10 days after infestation (immediately before treatment) and at 20 days and 28 days after infestation. Counting was carried out by killing 10 randomly chosen fish at each count, and examining and identifying stages of lice by use of a microscope. The number of lice in each of the following phases of development was recorded: Copepodid, Chalimus I, Chalimus II, Chalimus III, Chalimus IV, Pre-Adult I, Pre-Adult II and Adult.

Treatment of the fish in trial study GP2 was for one hour with trial solution GPRD01 containing Cypermethrin at a final concentration of 5µg/l of seawater.

Results

The mean total lice counts for the two studies are set out below in Table 2, and are presented graphically in Fig. 2A.

5

Table 2A

Lice Numbers	Untreated Group (GP1)	Treated Group (GP2)
Number of copepodids used for challenge	3,500	9,500
10 Post-challenge Count 1 (+10 days)	9.5	29.9 (before treatment)
Post-challenge Count 2	23.5 (+21 days)	14.2 (+20 days)
Post-challenge Count 3	25.7 (+27 days)	14.7 (+28 days)

Tables 2B and 2C below indicate the percentages of total sea lice observed in each developmental phase, the 15 results being represented graphically in Fig. 2B.

Table 2B

Pilot Study GP1 - sea lice development (untreated)

Lice Stage	10 days post infestation	21 days post infestation	27 days post infestation
20 Copepodids	21%		
Chalimus I	79%		
Chalimus II		13%	
Chalimus III		53%	
Chalimus IV		19%	7%
25 PreAdult 1		15%	46%
PreAdult 2			46%
Adult			0.4%

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Table 2C

Trial Study GP2 - seal lice development treated with
GPRD01 10 days after infestation

	Lice Stage	10 days post infestation	20 days post infestation	28 days post infestation
5	Copeopodids	6%		
	Chalimus I	92%	12%	
	Chalimus II	2%	54%	
	Chalimus III		32%	12%
10	Chalimus IV		2%	16%
	PreAdult 1			59%
	PreAdult 2			13%
	Adult			

The results show a marked retardation in lice
15 development when treated with Cypermethrin.

Ambient water temperature during pilot study GP1
(range 8.0 to 10.5°C) was slightly lower than that during
trial study GP2 (range 8.5 to 11.5°C). This difference
would be expected to cause GP2 lice to survive slightly
20 better and develop slightly faster than GP1 lice, with the
result that the differences in lice numbers and growth rates
between treated and untreated populations would be likely to
be ever greater if comparative trials were carried out in
exactly similar conditions.

25 During pilot study GP1 the damage caused to the salmon
by the sea lice was noted to be moderate to severe, and the
extent of the damage was as would be expected in a natural
situation. Some of the untreated fish were suffering with
such severe lesions caused by the sea lice by 28 days after
30 infestation that all remaining fish were culled for welfare
reasons. In contrast, the fish used in trial GP2 had
suffered no significant damage by day 28, when the last fish
were culled for sea lice counting.

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TRIAL 3

Trial 3 was carried out at a seawater site in the Faroe Islands, using hexagonal "Bridgestone" cages having a circumference of approximately 102m and a depth in the order of 10 to 12.5m.

Approximately 200,000 Atlantic Salmon (*Salmo salar*) of average weight 0.96kg were used in the trial, and were divided between two treatment cages (A and B).

On day 0 of the trial, the nets of cage B were raised to a depth shallow enough to allow a tarpaulin to be placed underneath. Oxygen was dispersed into the cage to maintain an oxygen level greater than 7mg/l. The tarpaulin was then used to enclose the cage volume to create a bath for treatment. The actual depth was measured by taking a boat out to the centre of the cage and dropping a weighted rope, which indicated a depth of approximately 4 metres to the tarpaulin.

Cage B was then treated with 700 litres of seawater as a negative control treatment to assess the effect, if any, of the experimental conditions of the fish. The tarpaulin was removed after 60 minutes.

The next day the fish mortalities were recorded and not found to be significantly higher than before the negative control treatment.

On day 1 of the trial, both cages were treated with a dose of 1%w/v cypermethrin, by mixing the required amount (500ml) with 700 litres of seawater and applying, using a pump, to each cage. The final concentration of cypermethrin was found by analysis to be approximately 0.9 $\mu\text{g/l}$ in each cage. The cages remained enclosed for 60 minutes and the oxygen levels were monitored.

On each day on which lice counts were taken, five fish

were removed from each cage, killed by an overdosage of benzocaine and placed in individual labelled polythene bags. The fish were then frozen and stored for lice counting. This was done on day 0 and day 1 (pre-treatment) and on days 5, 2, 8 and 14 (post-treatment).

Results

The culled fish were counted for Lepeophtheirus salmonis sea lice in the following stages of development: Chalimus I-II, Chalimus III-IV, pre-adult, adult, and gravid female. The numbers of Caligus elongatus sea lice were also counted. Mean values are set out in tables 3A and 3B below:

Table 3A (Tank A)

Day	Chalimus I-II	Chalimus III-IV	Pre-Adults	Adults	Gravid females	Caligus
15 1	2.80	2.40	1.40	1.80	1.00	0.60
2	0.20	0.20	0.20	3.40	1.60	0.00
8	0.00	0.60	0.00	1.00	1.00	0.00
15	1.20	0.80	0.20	0.80	0.40	0.00

Table 3B (Tank B)

Day	Chalimus I-II	Chalimus III-IV	Pre-Adults	Adults	Gravid females	Caligus
20 0	1.80	1.20	2.00	2.60	3.40	1.20
1	3.80	2.20	1.60	3.80	3.80	0.80
2	0.20	0.40	0.00	1.40	2.40	0.00
8	0.20	0.00	0.00	0.60	1.20	0.00
25 15	1.40	0.40	0.00	0.40	1.00	0.00

The mean results from tanks A and B are set out in table 3C below, and are illustrated graphically in Fig. 3.

Table 3C (Mean of Tanks A & B)

Day	Chalimus I-II	Chalimus III-IV	Pre-Adults	Adults	Gravid females	Caligus
0	1.80	1.20	2.00	2.60	3.40	1.20
1	3.30	2.30	1.50	2.80	2.40	0.70
2	0.20	0.30	0.10	2.40	2.00	0.00
8	0.10	0.30	0.00	0.80	1.10	0.00
15	1.30	0.60	0.10	0.60	0.70	0.00

10 Statistical analysis of results

The lice counts were logarithmically transformed and analysed using parametric statistical methods. In particular, the pre-treatment counts before and after the negative control were analysed by using a two-sample t-test.

15 The results of this test are in Table 3D below:

Table 3D

	Chalimus I-II	Chalimus III-IV	Pre-adults	Adults	Gravid Females	<i>Caligus elongatus</i>
T-value	-2.88	-1.41	0.83	-0.94	0.50	0.88
p-value	0.024	0.200	0.44	0.38	0.64	0.41
significance	Significant	Not significant	Not significant	Not significant	Not significant	Not significant

Significance level = 0.05 (5%)

The negative control did not have a significant effect on the lice numbers of any of the life stages of Lepeoptheirus salmonis or Caligus elongatus, with the exception of the Chalimus I-II stages, this was in fact a 5 statically significant increase in numbers. This may suggest the fish were being continually re-infected with a new generation of lice. (This possible effect was seen between day 8 and 14 post-treatment).

Pre-treatment counts and post-treatment counts were 10 analysed using a randomised block design with cages as blocks. This analysis provided a test for significant differences between counts at different points in time. The results are displayed in Table 3E below:

Table 3E

	Chalimus I- II	Chalimus III-IV	Pre-adults	Adults	Gravid Females	<i>Caligus elongatus</i>
p-value for day	0.001	0.026	0.002	0.239	0.111	0.001
significance	Significant	Significant	Significant	Not significant	Not significant	Significant

15 Significance level = 0.05 (5%).

Caligus elongatus and Lepeoptheirus salmonis phases Chalimus I to IV and pre-adult were significantly reduced using cypermethrin.

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A significant time difference effect was found, so a Newman-Keuls multiple range test was used to make pairwise comparisons between counts at different points in time. The results are as follows:-

5 Chalimus I-II

There were statistically significant differences (at a 5% significance level, i.e. there is only a 5% chance of finding these results in random data) between days 1 & 2; and 1 & 8. This would indicate a reduction in numbers at 10 day 2, and then an increase in lice numbers between days 8 & 14. This is most likely due to a reinfestation of a new generation of lice.

Chalimus III-IV

There were statistically significant differences (5%) 15 between days 1 & 2; 1 & 8; and 1 & 14.

Pre-adults

There were statistically significant differences (5%) between days 1 & 2; 1 & 8; and 1 & 14.

Adults

20 There were statistically significant differences (5%) between days 1 & 2; 1 & 8; and 1 & 14.

Gravid Females

There was no statistically significant reduction in gravid female lice numbers.

25 Caligus elongatus

There were statistically significant differences (5%) between days 1 & 2; 1 & 8; and 1 & 14.

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TRIAL 4Method

Gravid Female lice were collected from Atlantic Salmon and incubated at 14°C for 5 days until the eggs had hatched and developed through the naupilus stages to copepodids. Approximately 100 copepodids were added to each of seven beakers of 500ml seawater. Pyrethroids were added to six beakers at a concentration of 5µg pyrethroid / litre. One beaker was used as a control. All the beakers were maintained at 14°C for one hour.

After one hour the beakers of lice were sieved through 500 µm plankton mesh, and the lice then rinsed in fresh seawater before being placed in clean beakers and seawater.

The lice were then assessed comparing with the control beaker. The following factors were used for assessment:
reaction to light,
reaction to vibration, and
reaction to shadow.

The reaction were classified as Good, Moderate, Poor and None with reference to the control.

Three sub-samples, each of 10 ml, were removed from each beaker. The total number of lice and the number of mobile lice were recorded and displayed as a percentage.

An assessment was made for each beaker at 1 hour, 24 hours and 72 hours.

This experiment was then repeated at a later date, with different copepodids at a higher concentration of 50 µg pyrethroid/ litre. This time the experiment was performed blind, to avoid bias by the observer.

Results

1 HOUR	5 µG PYRETHROID / LITRE				50 µG PYRETHROID / LITRE			
	LIGHT	VIBRATION	SHADOW	% MOBILE	LIGHT	VIBRATION	SHADOW	% MOBILE
Control	Good	Good	Good	90	Good	Good	Good	80.6
Flumethrin	Moderate	Good	Moderate	93	None	None	None	0
Permethrin	Moderate	Moderate	Moderate	86	Moderate	Moderate	Moderate	36.4
Bifenthrin	Moderate	Good	Good	90	N/A	N/A	N/A	N/A
High-cis-cypermethrin	None	None	None	0	None	None	None	0
Cypermethrin	None	None	None	0	None	None	None	0
Deltamethrin	None	None	None	0	Good	Moderate	Good	60
Trebon	N/A	N/A	N/A	N/A	Good	Good	Good	61.1
Lambda-cyhalothrin	N/A	N/A	N/A	N/A	None	None	None	0

24 HOURS	5 µG PYRETHROID / LITRE				50 µG PYRETHROID / LITRE			
	LIGHT	VIBRATION	SHADOW	% MOBILE	LIGHT	VIBRATION	SHADOW	% MOBILE
Control	Moderate	Good	Good	93	Good	Good	Good	100
Flumethrin	Good	Good	Good	92	Good	Good	Good	18
Permethrin	Good	Good	Good	94	Moderate	Good	Moderate	87
Bifenthrin	Moderate	Good	Good	78	N/A	N/A	N/A	N/A
High-cis-cypermethrin	None	Poor	Poor	6	None	None	None	3
Cypermethrin	None	Poor	Poor	12	None	None	None	0
Deltamethrin	None	Poor	Poor	23	None	None	None	16
Trebon	N/A	N/A	N/A	N/A	Poor	Poor	Moderate	63
Lambda-cyhalothrin	N/A	N/A	N/A	N/A	None	None	None	19

72 HOURS	5 µG PYRETHROID / LITRE				50 µG PYRETHROID / LITRE			
	LIGHT	VIBRATION	SHADOW	% MOBILE	LIGHT	VIBRATION	SHADOW	% MOBILE
Control	Moderate	Good	Good	83	Good	Good	Good	74
Flumethrin	Moderate	Good	Good	92	Poor	Moderate	Moderate	33
Permethrin	Moderate	Good	Good	80	None	None	None	0
Bifenthrin	Moderate	Good	Good	89	N/A	N/A	N/A	N/A
High-cis-cypermethrin	None	Poor	Poor	0	None	None	None	0
Cypermethrin	None	Moderate	Moderate	19	None	None	None	0
Deltamethrin	None	None	None	11	None	None	None	0
Trebon	N/A	N/A	N/A	N/A	None	None	None	28
Lambda-cyhalothrin	N/A	N/A	N/A	N/A	None	None	None	0

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The results show that all pyrethroids appeared to have had some effect on the sea lice, although the results indicate a varying level of potency between different pyrethroids. Of the formulations tested lambda-5 cyhalothrin, high-cis-cypermethrin, cypermethrin and deltamethrin were the most efficacious.

It is a possibility that efficacy will vary with formulation as well as with pyrethroid. It was noted in the second experiment that the deltamethrin formulation did not mix well and formed oily droplets in the beaker. The pyrethroid formulations which mixed well and formed emulsions appeared to be effective immediately against the sea lice.

The bifenthrin was replaced in the second experiment with trebon and lambda-cyhalothrin. This was simply due to availability of the compounds.

Efficacy was assessed on the basis of decreased mobility of the lice, but there is no way of assessing if the lice are dead or merely immobile. It can be assumed, however, that if the immobile lice do not recover, they will not find a host, and will therefore die.

Conclusions

The results from Trial 1 show that Cypermethrin at a concentration of 100µg/l substantially reduces not only the number of pre-adult and adult lice on the infested fish, as was expected, but also, surprisingly, the number of immature lice. Trial 2 shows that this effect is still marked when the concentration of Cypermethrin is reduced to 5µg/l, and furthermore that the effect of the Cypermethrin is not only to reduce chalimus numbers by killing a portion thereof, but also to prolong development times of those which survive, thus delaying maturation to the most damaging

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pre-adult and adult phases. It is also thought that there may be an effect in reducing the feeding rate of the lice, thus reducing the damage caused by each individual surviving louse.

5 Trial 3 demonstrates that the effect of cypermethrin is still statistically significant, even at concentrations as low as $0.9\mu\text{g}/\text{l}$, and furthermore that it is effective not only against immature phases of Lepeoptheirus salmonis, but also against Caligus elongatus. It is anticipated that
10 Cypermethrin will be effective in concentrations ranging from $0.05\mu\text{g}/\text{l}$ to $500\mu\text{g}/\text{l}$, the concentration preferably being in the range $0.5\mu\text{g}/\text{l}$ to $100\mu\text{g}/\text{l}$.

Trial 4 demonstrates the efficacy of other pyrethroid compounds, in particular lambda-cyhalothrin, high-cis-
15 cypermethrin and deltamethrin.

It should be noted that the term "sea lice" is a general one, which applies to a number of different species of parasite, both of seawater and fresh water fish. It has been found in the past that effective treatments for one
20 species are generally effective against other species also, and the invention therefore extends to the treatment of immature sea lice in general, regardless of species, and regardless of whether the infestations are of seawater or fresh water fish.

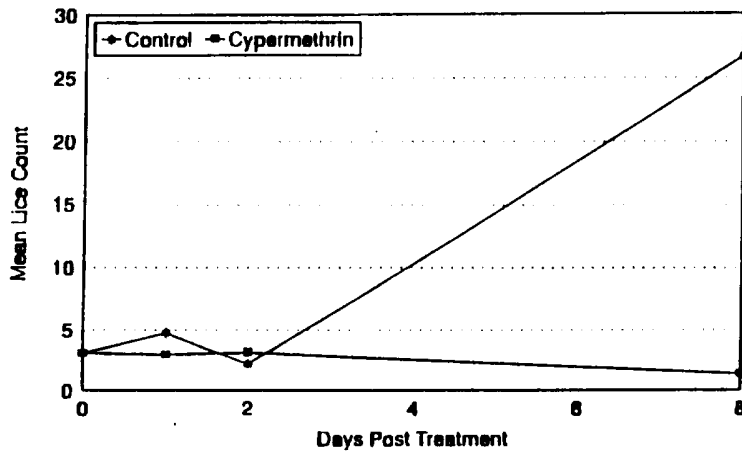
- 18 -

CLAIMS

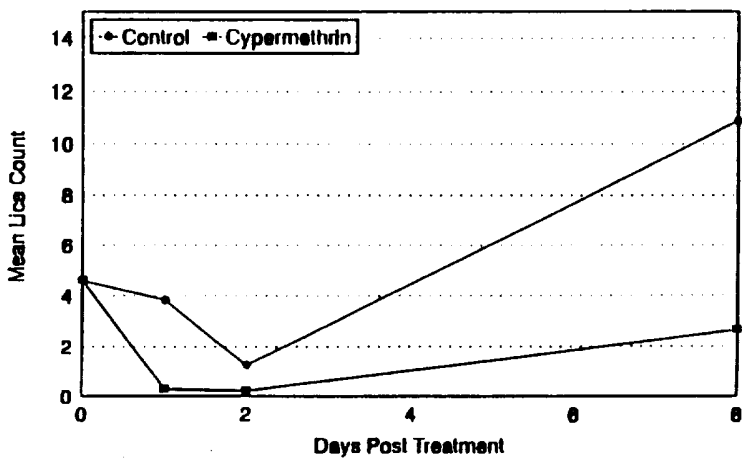
1. The use of a pyrethroid compound for the manufacture of a composition for the treatment of the immature phases of sea lice infestation of fish.
- 5 2. The use of a pyrethroid compound for the manufacture of a composition for the prophylaxis of pre-adult and adult sea lice infestation of fish.
3. The use according to claim 1 or claim 2, wherein the pyrethroid compound is Cypermethrin, high-cis-10 cypermethrin, lambda-cyhalothrin or deltamethrin.
4. The use according to any of claims 1 to 3, wherein the composition is adapted for topical administration to the fish.
5. The use according to claim 4, wherein the 15 composition comprises a solution or suspension of the pyrethroid compound.
6. The use according to claims 3 and 5, wherein the solution or suspension contains the pyrethroid compound at a concentration of between 0.05 μ g/l and 500 μ g/l.
- 20 7. The use according to claim 6, wherein the concentration is between 0.5 μ g/l and 100 μ g/l.

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Chalimus



Pre-Adult



Adult

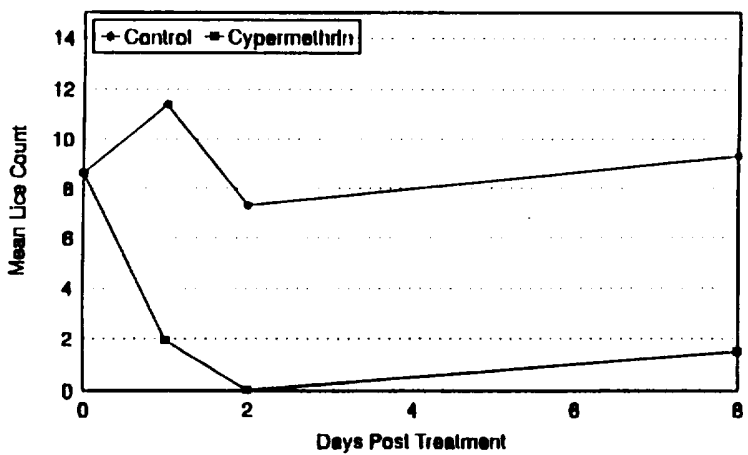


FIG. 1

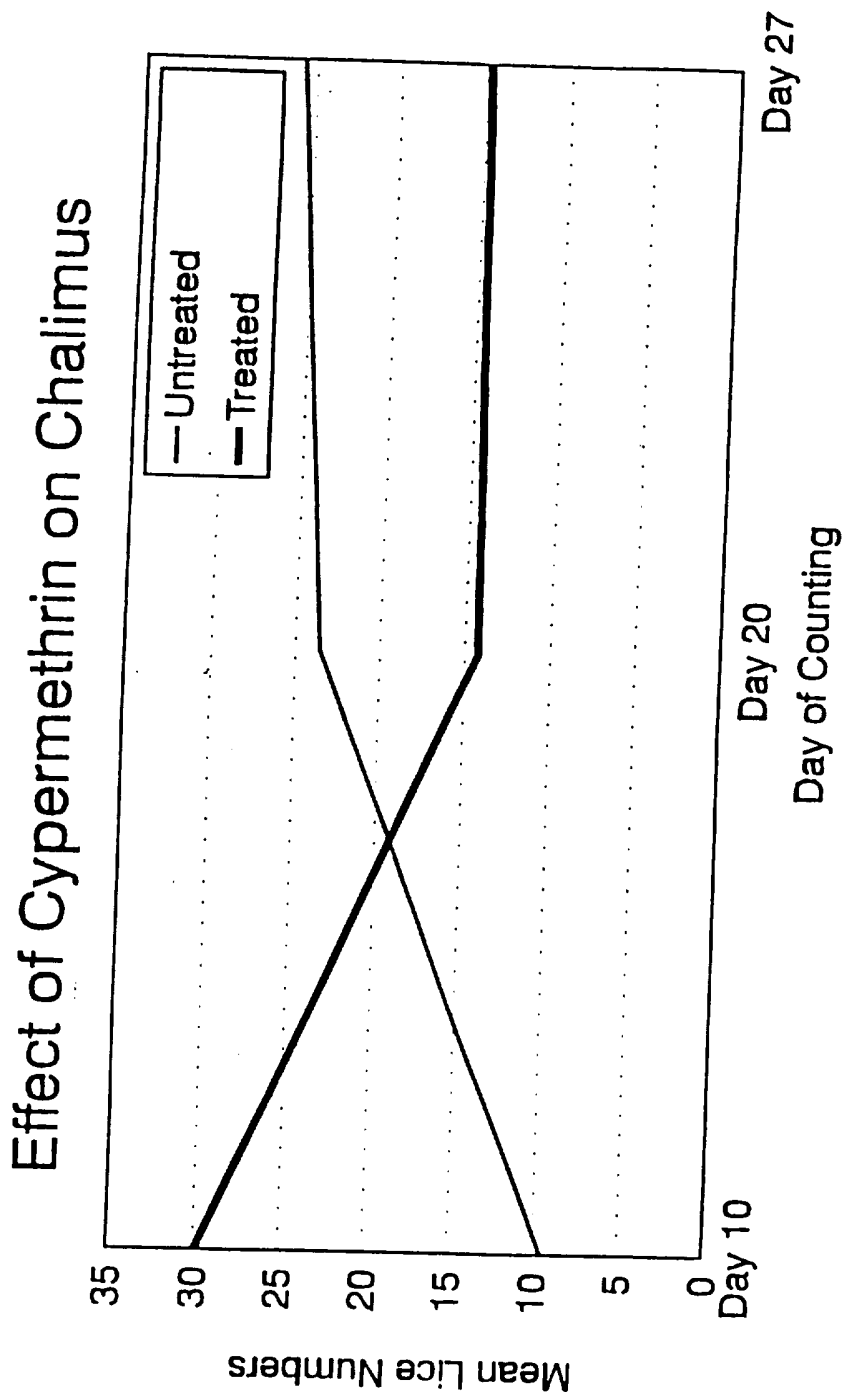
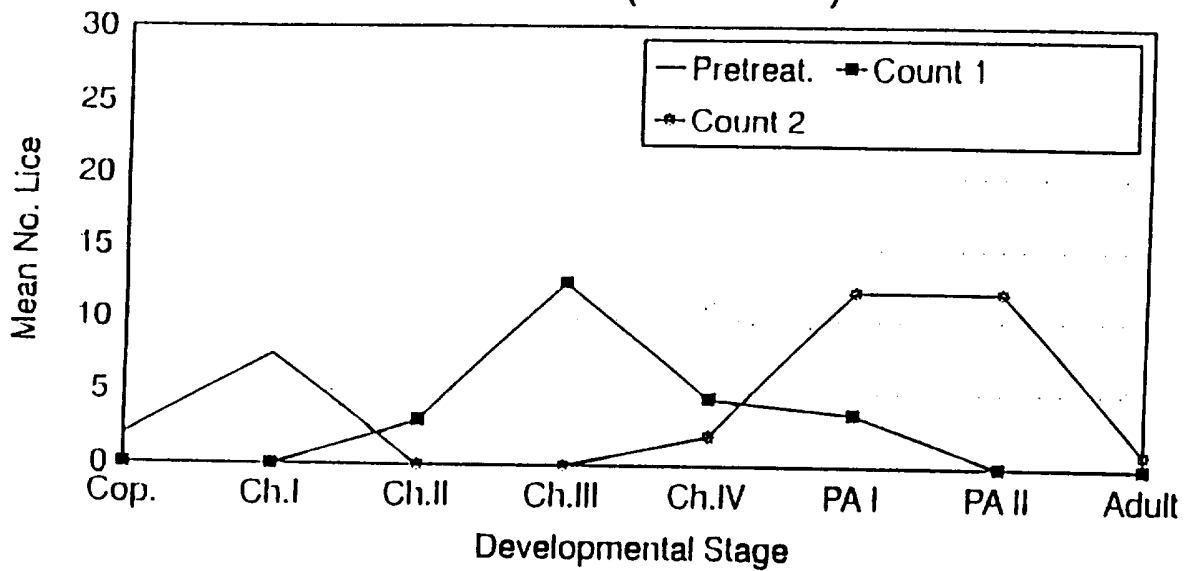


FIG. 2A

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Trial GP1 (Untreated)



Trial GP2 (Treated)

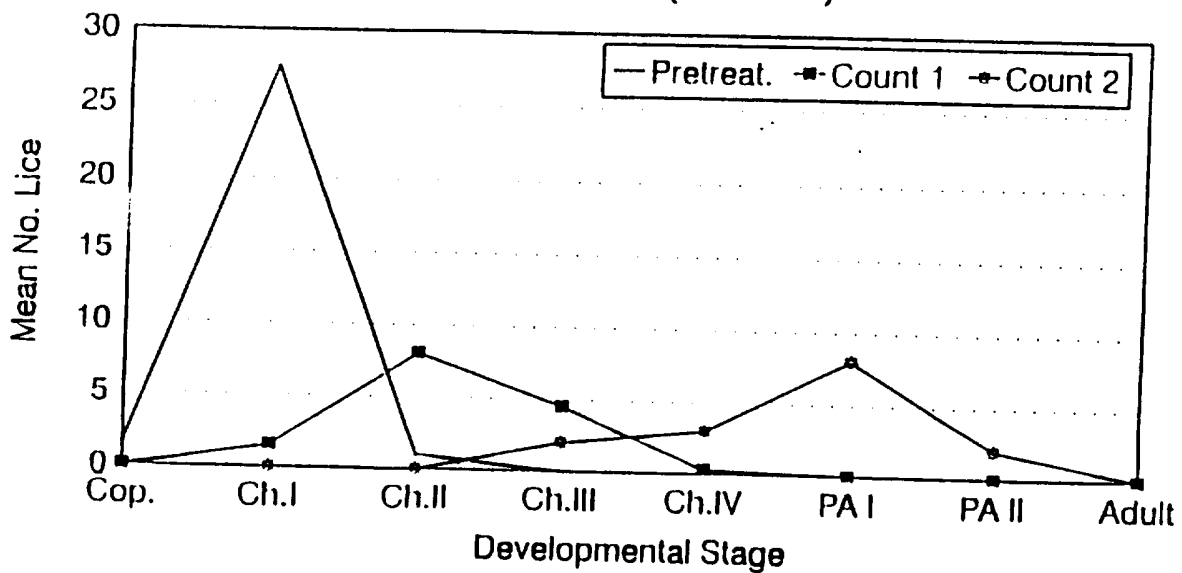


FIG. 2B

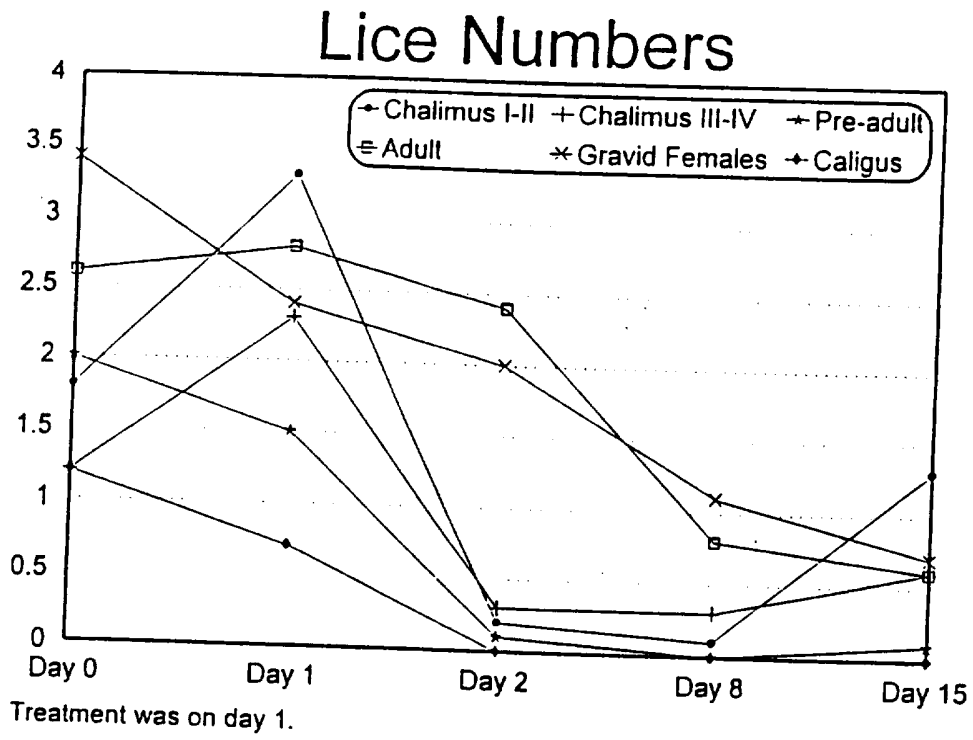


FIG. 3