ABSTRACT
Experiments with a flower extract containing Pyrethrins generally used as an insecticide has been carried out at Austevoll Aquaculture Research Station since 1989. Different types of administration methods have been tested and in this report a bath exposure to the treatment solution has been used.

Dichlorvos (Nuvan®, Ciba Geigy) and Pyrethrins (Py-Sal 25®, Norsk Pyrethrum A/S) were tested as a delousing agent successively one, two and then six times on individually tagged groups of salmon in the line with regular delousing. The delousing was done in accordance with the advises from the producers of the treatment solutions. The numbers of lice were registered between and after delousing. Delousing was carried out for both treatments at different times of the year to see if any difference in susceptibility to treatment was evident.

Overall both treatments gave an adequate delousing effect measured by the decrease in total lice numbers but the ongrowth of lice was more rapid in some of the dichlorvos groups. No certain difference in delousing capacity could be detected between the two treatment methods on mobile fish but Pyrethrins seems to have effect on the chilli.
INTRODUCTION
In Norway two species of ectoparasites usually referred to as salmon- or sea lice, are found on cultivated salmon \textit{(Salmo salar)} with up to 100\% prevalence. They are both copepoda of the family Caligidae eating mucus, skin and blood of their host, thus creating lesions, osmoregulatory problems and secondary infections (Brandal et al., 1976; Wootten et al., 1982; Pike, 1989). The predominating species in Norwegian waters is \textit{Lepeophtheirus salmonis} while \textit{Caligus elongatus} so far occurs in smaller numbers and hence causes less damage. \textit{C. elongatus} is not considered very host specific and has been reported on some 80 fish species (Kabata, 1979, Johnson and Albright, 1991). \textit{L. salmonis}, however, is believed to be host specific to salmonids. Bruno and Stone, (1990) reported findings of preadult \textit{L. salmonis} on saithe \textit{(Pollachius virens L.)} kept close to salmon cages but only \textit{C. elongatus} was found on saithe kept in cages away from salmon farms. No younger stages of \textit{L. salmonis} have been found on saithe and so far salmonids seem to be the only species were the louse can live their total life cycle. The use of the term salmon lice is therefore identical with \textit{L. salmonis} in this article.

The work on sea- and salmon lice at Austevoll Aquaculture Research Station started in 1987 and with full-time studies from 1989 with regular registrations of lice all year round. \textit{C. elongatus} have in this time become more regular and more numerous especially the last two years. However when delousing the cause is always large numbers of \textit{L. salmonis}.

Salmon lice are recognised as one of the major loss factors in salmon farming today. Calculation of the possible size of the loss to the industry varies but including chemicals for delousing, man hours for delousing, weight loss from stress, possible loss of fish and other the sum approach about 500 mill NOK (£ 50 - 60 mill) a year in Norway alone. Lice are also a major problem in other salmon producing countries like Ireland, Scotland and the Faroe Islands.

So far only chemotherapeutic treatments have been available against salmon lice and the most used is dichlorvos in the form of Nuvan® or Aquagard® (both Ciba-Geigy) or thriclorfon (Neguvon®, Bayer) which was introduced as the first method of chemical delousing by Brandal and Egidius, (1979). Both compounds are acetyl cholinesterase (AChE) inhibitors and as such nerve poisons (Salte et al., 1987, Hoy et al., 1991).

Pyrethrum is an extract from a chrysanthemum flower \textit{(Chrysanthemum cinerariaefolium)}, grown commercially on the high plains of Kenya. Extracts from this or related plant species containing Pyrethrins as the active component have been used in insecticides by the industrialised world for more than 150 years and the effect of Pyrethrum on mammals and environment is well documented (e.g. Casida, 1973). In the autumn of 1989 a new administration principle for using Pyrethrins to combat sea lice was tested for the first time (Jakobsen and Holm, 1990, Boxaspen and Holm, 1991). The experiment made use of Pyrethrum dissolved in oil instead of adding a synthetic emulsifier to make a water based Pyrethrum solution. The water solubility of the salmon mucus apparently leaves the fish unharmed while the salmon lice with a lipid membrane in the exoskeleton, are selectively penetrated. Pyrethrum was selected as a possible candidate for improved chemotherapy because of the already existing legislation for use in food industry (as an insecticide), the rapid breakdown (Maciver, 1962, Otieno & Pattenden, 1979) and the documented low mammalian toxicity (Barthel, 1973, Griffin, 1973). The retention time for salmon after treatment with the commercial delousing agent Py-Sal 25 containing Pyrethrins is set to 7 days by Norwegian Medicines Control Authority.
MATERIALS AND METHODS
All experiments were done at Austevoll Aquaculture Research Station on salmon held in sea cages and naturally infested with salmon lice.

Chemical compounds:
Dichlorvos was obtained as the commercial delousing agent Nuvan® from Medisinaldepotet, Norway and Py-Sal 25® containing pyrethrins, obtained from Norsk Pyrethrum A/S, Norway.

Counting of lice:
The salmon were anaesthetised by Metomidate (Mattson and Riple, 1989). This was done in 30 to 100 l tanks depending on salmon numbers and size. The total numbers of mobile lice were counted by visual registration in four groups, divided into grown and preadult of both sexes on individually tagged fish before treatment. The day after treatment the procedure for counting was repeated to verify any delousing effect.

Treatment procedure:
Nuvan
After counting of lice the fish were revived with running water in a tank of 1 m³. The volume of sea water in the tank was then reduced to 800 l and 2 ml of Nuvan was added. The fish were deloused in this solution under constant aeration for 40 minutes before running water washed out the treatment solution.

Pyrethrins
One litre of Py-Sal 25 mixture was placed into a small sink of 25 cm x 15 cm x 5 cm and the anaesthetised fish was turned for 4 to 6 seconds as shown in Fig. 1. Then the fish were revived in a tank of 1 m³ with running water similar to the fish in the Nuvan treatment.

Fig. 1 Principle for bath treatment with Pyrethrin solution.
Experimental design:
The comparative treatments were done on a total of five groups of salmon. They were either treated one (I), two (II) or six times (III) successively.

Parallel delousing with the two treatments were conducted as outlined in Table I for single treatments.

Table I  No. and size of salmon in single treatments.

<table>
<thead>
<tr>
<th>Time</th>
<th>No. Pyrethrin group</th>
<th>Size (cm±st.dev)</th>
<th>No. Dichlorvos group</th>
<th>Size (cm±st.dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1991</td>
<td>21</td>
<td>(36.4 ±2.1)</td>
<td>28</td>
<td>(37.7 cm ± 3.5)</td>
</tr>
<tr>
<td>August 1991</td>
<td>30</td>
<td>(28.9 ± 1.7)</td>
<td>30</td>
<td>(28.6 cm ± 1.8)</td>
</tr>
<tr>
<td>February 1992</td>
<td>19</td>
<td></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

II: Two successively treatments.
From end of April to beginning of June 1991 the development of salmon lice on two groups of salmon taken from the same pen (each group containing 25 fish) were followed through two necessary delousings with dichlorvos and pyrethrins respectively. On Day 10 after 1st treatment an additional counting was done to establish the ongrowth of lice.

III: Six successively treatments.
At 21st of August 1992 two groups of lice free salmon (each group containing 27 fish) were transferred to sea cages. From September same year to March 1993 they were followed through a total of six delousing procedures. The fish were kept in separate sea cages throughout the experimental period thus getting naturally infested with lice.

All results were compared with a Student's t-test for significance of differences.

RESULTS AND DISCUSSION

I
All single treatments gave a significant decrease in lice numbers on the fish (Table II). The lower effect of delousing in February in both groups might be explained by the lower temperature.
Table II  Lice numbers before and after single treatments with Pyrethrins or Dichlorvos given as total no. of lice (± st. dev).

<table>
<thead>
<tr>
<th>Time</th>
<th>Pyrethrins start</th>
<th>Pyrethrins end</th>
<th>Delousing (%)</th>
<th>Dichlorvos start</th>
<th>Dichlorvos end</th>
<th>Delousing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1991</td>
<td>69.6 (± 13.5)</td>
<td>1.5 (± 1.3)</td>
<td>97.8</td>
<td>66.1 (±12.9)</td>
<td>1.3 (± 1.2)</td>
<td>98.0</td>
</tr>
<tr>
<td>August 1991</td>
<td>19.8 (± 5.4)</td>
<td>3.5 (± 3.2)</td>
<td>82.3</td>
<td>23.2 (± 6.6)</td>
<td>1.7 (± 1.1)</td>
<td>92.7</td>
</tr>
<tr>
<td>February 1992</td>
<td>10.9 (± 5.2)</td>
<td>4.3 (± 3.7)</td>
<td>60.6</td>
<td>10.9 (± 5.2)</td>
<td>3.7 (± 2.1)</td>
<td>66.1</td>
</tr>
</tbody>
</table>

Delousing in both groups gave a significant decrease in numbers of lice (Fig. 2). Before the two delousings the lice numbers in the Dichlorvos group were significantly higher than in the Pyrethrin group (p_dich=0.02/p_pyr=0.024). The day after treatment the opposite relationship were found (p_dich=0.024/p_pyr=0.001). The Dichlorvos treatment thus gave a better delousing. At Day 10 after the first delousing the Pyrethrin group had a significantly lower count of lice (p=0.016) as was the case before the second delousing. Thus the ongrowth of lice in the Pyrethrin group was slower than in the Dichlorvos group. This effect can be explained by Pyrethrins affecting the chalimus stages of the lice and thus decimate the transition to pre- and adult lice.

Fig. 2  Development of lice numbers with two successive treatments. De, marks date of delousing. (--- Dichlorvos, —— Pyrethrins).
III

All six points of treatment for both groups of fish gave significant delousing (Fig. 3). There was no difference in lice numbers when the treatments started but before delousing 2 and 4 the Dichlorvos group had a significantly higher number of lice. After delousing 2, 3, 4 and 6 the Pyrethrin had a significantly lower number of lice. This again indicates that Pyrethrins might affect the chalimus stages of the salmon lice.

The settlement of copepodids on the fish come in pulses (pers.obs.) and copepodids settled after one of the points of treatment will make an even ongrowth in both groups. Chalimus already present on the fish will be exposed to the treatments and thus give an uneven pattern of ongrowth if influenced by the treatments. This seems to be the case after treatment 1, 3 and possibly 5 in the Pyrethrin group. This will be further studied.

Fig. 3 Development of lice numbers with six successive treatments.

(—) Dichlorvos, (—) Pyrethrins).

REFERENCES


Mattson N. and T.H. Riple, 1989, Metomidate, a better anaesthetic for cod (Gadus morhua) in comparison with benzocaine, MS-222, chlorbutanol and phenoxyethanol, Aquaculture, 83, 89-94.


