ISSN 1393 452X

Caligus

Issue 6, (March 2000)

A newsletter on the biology and control of sea lice distributed free to researchers, aquaculture and fisheries industry, educators, consultants, and management authorities. (http://www.ecoserve.ie/projects/sealice)

This sixth issue of *Caligus* includes:

Page

General

Articles

Reports and News

Future networking between researchers and industry concerning sea-lice biology and control

It was notable that the 4th international conference on sea-lice in Dublin in 1999 had the largest attendance of any sea-lice meeting so far. This indicates that it took 3 years for the sea-lice network to mature. To ensure this momentum was not lost, it was agreed at the conference to form a Steering Group to continue and expand the network. The following volunteered: Ian Davies and David Mackay in Scotland, Myron Roth in Canada, Pelle Kvenseth in Norway, and Mark J. Costello in Ireland. This group has prepared the following report as to how the network may proceed in the future.

The particular activities involved at present are

- 'Caligus' email listserver
- Web site
- Twice a year newsletter 'Caligus'
- Maintenance of a mailing list (for newsletter and future meetings)

All of these activities cost about Ir£10,000 (ca. 12,000 Euro) per year.

Future activities could include:

- 'Fact-sheets on sea-lice biology and control' for fish farm staff and the public
- Educational posters on sea-lice control.

The costs involved are:

1. Time commitment to seek out and send out news items on the listserver.
2. Time to update web site and mailing list.
3. Time to collate material, edit it, do layout of newsletter, and produce and post 250 copies (presently photocopied but could print and have better quality). An additional 200 copies were produced of past newsletters and circulated at related scientific and industry meetings. Thus a typical print run has been 400-500 copies.

The potential sources of funding are:

(1) 'Dissemination' element of EU Research proposals. However this relies on others and their good fortune and so cannot be relied upon.

(2) Application to EU as either a (a) 'Accompanying measure' or (b) Concerted Action. This option will involve some time in preparation and success is not guaranteed, but could provide good independent funding for several years.

(3) Sponsors. This may be possible, and while not secure may usefully maintain funding between research contracts. It could include straightforward advertising.

Any offers of sponsorship?

(4) Subscriptions. While this is unlikely to provide all the funds needed, it may again help keep the network going between major funding sources. What do you think would be a reasonable personal or company subscription? One suggestion received is £15 per person and £50 per company.

The network provides a forum for communication between researchers and industry, and facilitates the exchange of information through publications and meetings. The Steering Group agreed that the strength of the network is that it has included all groups interested in sea-lice biology and control, including researchers, fish farmers, regulators, pharmaceutical company staff, students, anglers, and fishery managers. Information and papers on anything relevant to sea-lice biology and control, including related parasites, chemotherapeutant toxicity, and effects on wild fish have all been made available. The network thus promotes the flow of knowledge, data, and scientific opinion, and encourages individuals to form their own opinions based on the available information.

A name for the network would be desirable: how about “International sea lice network”?

Mark J. Costello,
Co-ordinator of EU Concerted Action project on
sealice biology and control.

A national treatment strategy for control of sea lice on Scottish salmon farms

Gordon H. Rae
Scottish Salmon Growers Association, Perth, Scotland.

At the SSGA Technical Seminar in November 1997, industry scientists reported on significant work regarding a strategic approach to control of sea lice (Wadsworth *et al.* 1998). They have demonstrated that the reproductive capacity of female lice is compromised in the spring, resulting in reduced survival in their offspring. A late winter treatment offensive aimed at lowering the number of adult females to the lowest possible levels can have significant benefits. When data from the 1993 year class was compared with that of the strategically treated 1995 year class, the results showed that they could reduce fish mortalities by up to 83%, reduce fish being downgraded at harvest by 80%, have a 62% reduced mobile louse infestation level and reduce treatments by as much as 46%.

It was suggested that control of sea lice at a national or regional level will be improved if salmon farmers adopt a collaborative policy of co-ordinated treatments much like those that were proposed for the eradication of sheep scab by the Crofters of Skye (WHFP October 1997).

Progress with this initiative depended on the maximum co-operation from all Scottish salmon farmers. In order to initiate improved control of lice, farmers were invited to try out the strategy on a regional basis in the first instance. This involved working with neighbouring salmon farmers to form co-operative groups and all treating together. The way it was achieved is outlined below.

A CODE OF PRACTICE FOR SALMON FARMERS

1. Define the area to be managed. Farmers first identified the area to be managed and to help in this an attempt was made to divide Scotland into manageable hydrographic areas. Twenty-two areas were defined.

2. Identify all the salmon farming operatives in the area and elicit a commitment from them towards the national strategy. Farmers know who their neighbours are. In the case of non-SSGA members, it fell upon the Chairman of the Management Group to identify them and make an approach in order to find out what their views were after seeing the evidence presented.

3. Form a local area Management Group, including veterinarians. A positive response meant that local area Management Groups were formed and of course this included the veterinarians who have responsibility for the fish. Seventeen Groups were formed early in 1998.

Each Management Group had two main tasks to agree and oversee. All lice populations required monitoring at agreed intervals to a standard protocol and regional treatments had to be carried out according to agreements drawn up between neighbouring farms.

4. Agree the monitoring protocol and frequency. Lice numbers on all the farms in one area required regular monitoring. A protocol was suggested and is as follows:

- Starting in February, two pens on each site will be monitored weekly.
- Ten fish should be sampled from each pen and the number of chalimus, mobiles and females with eggs recorded. A useful article on this subject, titled ‘Optimal timing for lice treatments’ was published in Fish Farmer (Treasurer and Grant 1997).
- Results should be audited by neighboring farmers or whomever the Management Group decides.

5. Agree timing and threshold of strategic treatments. It is expected that monitoring will show that the population of lice is low in March and the Management Group will decide when to treat. The main aim of the treatments is to prevent the large settlement of chalimus, which has been shown to occur between weeks 20-25 and the subsequent build up in mobile lice. The idea is, therefore, to treat all the lice in the management area at the agreed time, probably in March, in an attempt to remove all the gravid females. A follow up treatment should then be given as required, to kill any lice who have grown to pre-adult stage. Thereafter lice will be treated in order to keep females with eggs at the lowest possible level to stop recruitment. It is important to synchronise the early treatments.

Some farmers and veterinarians may feel that the level of lice is too low to warrant treatment. However, in order for the strategy to be successful, lice must be treated at the crucial time before numbers build up. Suggested thresholds for mandatory treatment during the crucial period are as follows:

1. For any age of fish - one adult female louse with eggs in the sample of ten fish.
2. For fish in the sea for less than six months - as in 1, <u>or</u> an average of one mobile louse per fish.
3. For growers - as in one, <u>or</u> an average of two mobile lice per fish.

The Management Group may wish to co-ordinate treatments throughout the year but, to be successful, must revert to this code before the Spring louse increase.

6. Carry out the strategic treatments. For those Management Groups organised in time to initiate the strategy in 1998, the only available medicines were Aquagard and Hydrogen Peroxide. Though less than perfect, these medicines were effective on the trial sites and farmers were encouraged to repeat the work using them (provided the farmer is confident of achieving a ‘treatment safe to the fish’).

The success of the Strategy will be improved when new, fully authorised medicines become available. These new bath and in-feed treatments should be used wherever possible. In-feed treatments will be particularly useful for small fish but are unlikely to become available for strategic use until the year 2000.

It is envisaged that, by the winter of 1999-2000, the Strategy will be well established and it will become increasingly effective, as these new, safe and effective medicines become available. The result will be an overall reduction in the numbers of lice around the Scottish coast and farm louse numbers will be at their lowest when wild salmon and sea trout smolts are migrating.

7. Continue monitoring. It is important to continue monitoring in order to keep control of the lice but also to report to the Management Group so that the success of the strategy can be recorded and used to make plans for the following year.

8. Continue consultation with stakeholders. In preparing this Code of Practice SSGA has taken account of constructive comments from salmon farmers, shellfish farmers, Regulatory Authorities, Fisheries Trusts, anglers and fishermen. Management Groups should continue this process in order to take advantage of their collective experience and to take account of their concerns. For example, farmers should be mindful that, due to the presence of juvenile stages of commercial species of fish and shellfish in the plankton, fishermen would like strategic treatments to be complete before week 13 each year.

RESULTS

Results of the first year of co-ordinated treatments were collated in November 1998. Those farmers on the West Coast mainland of Scotland had produced the best data and the results showed variable levels of success. Where the treatments had been successful, the results showed similar reductions in numbers of lice and subsequent treatments to those in the original work and this is reported elsewhere (Wadsworth *et al*, these papers*)*. One area reported a large reduction in treatment costs while another said simply that it had been ‘a good year for lice’, meaning levels of lice remained low. One farmer had to report no success and, on examination, it was found that he only had access to hydrogen peroxide and he was experiencing a reduction in the efficacy of this medicine. This result underlines the need for a range of authorised medicines if the strategy is to be successful.

Access to Authorised Veterinary Medicines. Field studies on a number of candidate products have been completed and applications are now progressing through the formidable EC, UK and Scottish bureaucracies. These are:

European - The European Medicines Evaluation Agency (EMEA). This is an EU agency which is concerned with consumer protection under Regulation (EEC) No 2377/90. Pharmaceutical companies have to submit data so that a Maximum Residue Limit (MRL) can be set.

National - The Veterinary Medicines Directorate (VMD). This is the UK authority and requires access to data on Safety, Quality and Efficacy. It grants Marketing Authorisations (MAs).

Local - The Scottish Environmental Protection Agency (SEPA). This agency superseded the old River Purification Authorities and is concerned with residues and protection of the environment. SEPA has the power to grant Discharge Consents (DCs) to allow actual use of any medicines.

In looking at any list of medicines, the decisions of all three authorities must be taken into account. A negative response from one means the medicine cannot be used and makes positive responses from the others irrelevant. Table 1 shows the position to date.

REFERENCES

Rae, G.H. (1999). Sea lice, medicines and a national strategy for control. *Fish Veterinary Journal* (3) 46-51.

Treasurer, J.W. & Grant, A.N. (1997). Optimal timing for lice treatments. *Fish Farmer* **20**, (6), 20-21.

Nordmo, R. (1993). The veterinary approach to salmon farming in Norway. In: *aquaculture for veterinarians*. ed: L. Brown. Pergamon Press. pp. 179-191.

Wadsworth, S. L., Grant, A.N., & Treasurer, J.W. (1997). A strategic approach to lice control. *Fish Farmer* **21**, (2), 8-9.

West Highland Free Press, 3rd October 1997. *Bid to make Skye scab-free*.

Table 1. State of use of sea lice medicines to July 1999. * Information on Norwegian drugs taken from Nordmo (1993) and given by Mo at an ICES Workshop in Edinburgh November 1996. Annex I = MRL established. Annex II = No MRL required. Annex III = Provisional MRL - more data required. Annex IV = No MRL can be established - cannot be used in food animal. ATX = Animal Test Exemption, allows field trials. ATC = Animal Test Certificate, allows field trials.

MEDICINE	**VMD Market Auth.**	**Reg. 2377/90 EMEA - MRL**	**SEPA CONSENT**	**AVAILABLE IN NORWAY ***	**COMMENTS**
Dichlorvos (Aquagard)	Yes	Not yet	Restricted	Yes	Will be phased out
Azamethiphos (Salmosan)	Yes	Yes Annex I	Consents being granted	Yes Selling well	SSGA Funded Trial Work
Hydrogen Peroxide (Salartect, Paramove)	Yes	Yes Annex II	Yes	Yes	Unsuitable at high summer temperatures
Cypermethrin (Excis)	Yes May 1999	Yes Annex III	Consents being granted	Yes Selling well	SSGA Funded Trial Work
Ivermectin (Ivomec Pre-mix for Pigs)	Yes, not for Fish	Yes, Annex I not for Fish	30+Consents ‘Called-in’	? (Extensive use in Ireland)	Consumer resistance *via* retailers
Emamectin (SLICE)	ATX	Yes Annex I	SEPA approve. No Consents	10 Trials scheduled	SSGA screening project Schering Plough in-feed
Teflubenzuron (Calicide)	Yes (August 1999)	Yes Annex I	SEPA approve. No Consents	General Exemptn. Since Oct 1996	Trouw has developed this in-feed
Diflubenzuron (Lepsidon)	ATC	Yes Annex III	Trials	Yes Authorised	EWOS is developing this in-feed

Measures to control sea lice in Norwegian fish farms

Inger Eithun
Norwegian Animal Health Authority, Statens Dyrehelsetilsyn, PO. 8147 Dep., 0033 Oslo, Norway. email:
inger.eithun@dyrehelsa.no

THE NATIONAL PROGRAM FOR CONTROL OF SEA LICE

Sea lice have been recognised as a health problem in Norwegian fish farms for many years. The situation in fish farms has improved, but during the last years the incidence of sea lice has been recognised as a serious threat to migrating stocks of smolt from Norwegian rivers. In 1997 a national program for control of sea lice was established by The Norwegian Animal Health Authorities, in co-operation with Directorate of Fisheries, Directorate of Nature Management, fish farmers organisation and private fish health services. This has emphasised three important measures:

- routines for counting sea lice in all fish farms
- routines for medical treatments, emphasising on the period before migration of smolts
- use of cleaner fish as a supplement to medical treatment

Six objectives are defined in the program.

Objective 1: Regional collaborative groups to plan and co-ordinate measures shall be established.

The objective is considered to have been fulfilled in 1998. There is a certain variation in how well regional activities function, and in the way in which the work has been organised in the different regions.

Objective 2: The occurrence of lice in fish farms raising fish for slaughter shall be documented.

Regional regulations are in force in all the counties involved. The objective has not been achieved with regard to documenting the occurrence of lice, although progress is encouraging. After the regulations entered into force, reporting routines and the quality of the reported data have improved considerably.

Objective 3: The occurrence of lice in wild fish shall be documented

The objective regarding registration of salmon lice in wild fish, as formulated in the established result indicators, has been partly achieved. Progress has been made compared to 1997. This is especially important to enable assessment of the effect that measures implemented in fish farms have on the occurrence of lice in wild fish.

Objective 4: Measures to prevent and combat infestation shall be documented.

The objective has not been fulfilled, though positive progress has been made. In practice, achievement of Objectives 2 and 4 will coincide, as reports contain information both on lice status and on preventive and treatment measures.

Objective 5: Organised de-lousing during the cold periods of the year shall be planned and implemented.

Regional regulations on the control of salmon lice are in force in all relevant counties. As regards the planning and implementation of organised de-lousing in the cold period of the year, the objective has not been fulfilled. De-lousing has been planned and carried out for the winter and spring of 1999 in some important areas. However, the regional working groups must continue and intensify this work.

Objective 6 - Long term objective: The harmful effects of lice on farmed and wild fish shall be reduced to a minimum.

The objective has not been fulfilled. Reference is otherwise made to the reporting of results under Objectives 2 and 3.

Although it is uncertain just how low the incidence of salmon lice in fish farms must be before wild fish are to remain unaffected, the long term goal for control must be that no adult lice are present in fish farms during the period prior to the migration of wild fish to the sea. For control to be successful, systematic efforts must be made over several years, more knowledge must be gained, and progress must be achieved in developing control methods. In addition to active control of salmon lice in fish farms, measures to reduce fish escaping will reduce the harmful affects of salmon lice in farmed and wild fish. There is thus a great need to reduce the amount of fish, which escape from Norwegian sea farms.

Even though the majority of fish-farmers have participated in program on a voluntary basis, the need of regulations soon became obvious, if the long-term objective shall be fulfilled.

Regulations with minimum measures to control sea lice in Norwegian fish farms

As a part of the program, regulations made by the Norwegian Animal Health Authorities have entered into force in 1998. The purpose of these regulations is to lay down minimum measures to reduce the incidence of sea lice. These include minimum measures in all fish farms at sea as follows:

1. Mandatory counting, recording and reporting on the incidence of sea lice.

During the period from 1st March to 15th December, the incidence of lice shall be investigated and recorded at least once a month at a sea temperature of between 4 and 9 °C, measured at a depth of 3 metres. At sea temperatures at or more than 9 °C, measured at a depth of 3 metres, the incidence of lice shall be investigated and recorded at least once a fortnight.

A report of the monthly incidence of the number of adult female sea lice (with and without egg strings), the number of treatments given and the use of wrasse (Labridae) fish, shall be submitted to the District Veterinary Officer by the 15th of the following month.

Sites, which are to be slaughtered out, emptied of fish, within one month are exempted from these requirements.

The Norwegian Animal Health Authority, Central Office, has issued instructions concerning counting and recording of sea lice. When counting lice, at least 20 fish from each of at least two nets are to be examined. One of the nets shall be a permanent reference net, which is counted on each occasion. The net which experience has shown to have the fish with most lice should be chosen as the reference net.

When counting lice, at least the following categories shall be differentiated:

- Adult female lice with and without eggs
- Pre-adult stages and adult male lice
- Chalimus stages

No monitoring is required from 15th December to 1st March at present.

2. Mandatory delousing when maximum limits of sea lice are demonstrated

During the period of 1st March to 1st June, it must not be possible to demonstrate two or more adult female lice (sea lice) per fish in accordance with the instructions for counting and recording lice, in any marine fish farming site for salmon or rainbow trout. Should two or more adult female lice be demonstrated in this period, and the sea temperature is not below 4 °C, treatment against sea lice shall be implemented throughout the site. In the region of Trøndelag the limit is one or more adult female lice.

During the period 1st June to 15th December, it must not be possible to demonstrate five or more adult female lice (sea lice) per fish in accordance with the instructions for counting and recording lice, in any marine fish farming site for salmon or rainbow trout. Should five or more adult female lice be demonstrated in this period, treatment against sea lice shall be implemented throughout the site. In the region of Trøndelag the limit is one or more adult female lice for fish of less than 500 g, and five on larger fish.

3. Administrative fines when regulations are violated

In case of violation of the provisions concerning counting, recording and reporting, the Regional Veterinary Officer, for every new population unit of 10 000 fish, may impose an administrative fine of approximately £100 (Irish).

In case of violation of the provisions concerning mandatory delousing, the Regional Veterinary Officer, for every new population unit of 10 000 fish, may impose an administrative fine of approximately £100 (Irish) per day.

Experiences from implementation and enforcement of the regulations

The regulations have caused a great deal of discussions, but also contributed to a larger focus on the control of sea lice. Since the regulations entered into force, reporting routines and the quality of the reported data have improved considerably. Many people doubt the quality of the data, since the farmers them selves carry out the counting procedures. The District Veterinary Officer can only control the routines when inspecting the farm once, maybe twice a year. Even though the quality of the data may be questioned, we think they give a good indication of the status. The most important thing is to establish procedures for counting sea lice as a standard routine in all fish farms.

There are many different opinions on the limits set for mandatory delousing, and it’s obvious that if all farms acted only on these limits, it would not be satisfactory. The main objective with the regulations is to enable the authorities to act upon those who do not participate on a voluntary basis. In this aspect we feel that they have fulfilled the intention. Use of administrative fines has been warned on a few occasions, but it has not been necessary to effect.

The regulations have only been in force for a year. On the basis of experiences from this year, the Norwegian Animal Authorities will consider adjustments of the regulations concerning lower limits for mandatory delousing, and limits for mandatory delousing during the winter (December - February).

New and more effective medicines have also contributed to a better control of sea lice. And we do think it is possible to reach the objective of no adult lice present in fish farms during the most important period prior to the migration of wild fish to the sea.

The methods to achieve the objectives of the sea lice control programme are reviewed and revised as necessary. For example, it is currently being proposed that

(a) The incidence of lice shall be investigated and recorded at least once a fortnight at a sea temperature at or more than 4 °C, measured at a depth of 3 m.
(b) During the period of 1st December (or 1st November north of Counties Troms and Finnmark) to 1st July, treatment must be carried out if 0.5 or more adult female lice, or in total more than 5 adult female lice and mobile lice, per fish is demonstrated.
(c) During the period of 1st July to 1st December (or 1st November north of Counties Troms and Finnmark) treatment must be carried out if 2 or more adult female lice, or in total more than 10 adult female lice and mobile lice, per fish is demonstrated.

Sealice e-mail discussion group

The Caligus listserver is an e-mail discussion group which enables people interested in lice biology and control to provide, request and discuss information with others in the group. To join the group: (a) send an e-mail to <listserv@listserv.heanet.ie>, (b) leave the ‘subject’ line blank, and (c) type the following command in the main part of the email message: “SUBSCRIBE CALIGUS first-name surname”. Do not include your signature in the message. To leave the list, send the message “SIGNOFF CALIGUS” to <listserv@listserv.heanet.ie>. Sending an e-mail to <CALIGUS@listserv.heanet.ie> will send the message to all subscribers in the group. All e-mails sent to the listserver are archived by month on the website: http://listserv.heanet.ie/caligus.html

Integrated lice management in Mid-Norway

Per Andersen * and Per Gunnar Kvenseth **
* Department of Fisheries, 7770 Flatanger, Norway. Email: peraande@online.no
** KPMG Management Consulting as - Centre for Aquaculture and Fisheries, Sandviksboder 5, N-5035 Bergen, Norway. Email:
pelle.kvenseth@kpmg.no

The scope of this work has been to reduce problems with lice on farmed salmon, by increasing the fish-farmer's awareness about sealice in the farms. The work was carried out in the communities of Flatanger, Namsos and Fosnes in north Trøndelag in mid-west Norway. The most essential elements of the strategy are frequent lice counting, and the use of wrasse to control sealice on small salmon. Lice control requires special attention during wintertime when the lice-levels are usually low. Depending on the lice-levels one or two chemical treatments are carried out during the cold period. To reduce the chances of re-infection of the farmed salmon, neighbouring farms are treated at the same time. Since this work started in 1991 the level of lice on farmed fish has been steadily decreasing, and is now considered to be under control. The average number of gravid female lice has been reduced from 1.4 lice per fish in 1994 to 0.4 lice per fish in 1997.

INTRODUCTION

The experience for this publication comes from the county north Trøndelag in mid-Norway, especially from the communities Flatanger, Namsos and Fosnes. The area encircles the Namsenfjord and the river Namsen, one of the best salmon rivers in Norway.

Background

During the late eighties and the beginning of the nineties salmon lice caused vast problems to salmon farmers in north Trøndelag, especially in Flatanger. One of the reasons has probably been the high and stable salinity (more than 33 ‰) in addition to reduced sensitivity to bath-treatment chemicals (Nuvan® and Neguvon®). During the summer of 1991 we experienced a massive settlement of sealice (*Lepeophtheirus salmonis*) on salmon in all farms in Flatanger and the outer part of Namdalen, as well as in many of the other farms in Trøndelag.

Strategy

During 1991 we were lacking tools to evaluate the lice burden of different farms. In 1991 and in the beginning of 1992 a method for lice counting was developed in co-operation with salmon farmers under practicable farming conditions (Andersen, P.A. 1996).

Lice counting is essential for efficient lice control. Out at the farms it is important to run lice counting, in a feasible way when it is supposed to be run monthly in the winter and bi-weekly in the summer. Results from these countings gave an acceptable basis for actions such as adding more cleaner-fish or starting chemical treatments.

Lice numbers have been registered on individual salmon in all farms in Flatanger since 1992. At counting salmon were sampled from the pens and anaesthetised (Andersen, P.A., 1996). Lice are categorised as follows:

- Adult females, with or without eggstrings;
- Medium sized lice. This also includes adult male lice and pre-adults;
- Chalimus stages. The youngest stages looking like pencil-strokes.

Lice abundance

General requirements have been given from the Norwegian Veterinarian Authorities. These demands are summarised as follows:

- From the 1st March until 1st June the accepted average lice level is a maximum of one adult female lice per salmon;
- In the period from the 1st June until the 15th December, and when the salmon are less than 500 g, the accepted average lice level is a maximum of one adult female lice per salmon;
- In the period from the 1st June until the 15th December, and when the salmon are bigger than 500 g, the accepted average lice level is a maximum of five adult female lice per salmon;
- In the period from the 15th December until the 1st March, there are no restrictions regarding lice numbers.

If the lice abundance exceeds these restrictions, the farm must reduce the lice levels.

Cleaner-fish and Goldsinny

Cleaner-fish have been used in this district since 1992. Goldsinny wrasse are established as a first choice for lice control on all smolts. The use of goldsinny is central in the lice fighting strategy, from smolt release in the spring until the end of the first year. In Trøndelag, goldsinny wrasse are difficult to catch before mid June. By storing locally caught goldsinny wrasse over the winter, it is possible to stock the cleaner-fish simultaneously with the smolt release in the spring. This was done for the 1998 season with good results. Some farms now also run experiments with the bigger ballan wrasse as cleaner-fish on large salmon.

Delousing during winter and spring

We experienced very good results from the winter 1991/1992 when all farmed salmon in a “natural” geographical region were deloused simultaneously in November and January/February with hydrogen peroxide (H_2O_2) (Andersen, P. unpublished). During the summer of 1992 the region had very few lice on the farmed fish. Delousing in the cold part of the year is now included as a part of the strategy to keep the level of lice low during spring and summer. The idea behind delousing during the winter is to break the generation cycle when its reproduction rate is low. If there are no adult lice on the farmed salmon in the spring when the light increases and the temperature rises, this situation will obviously give no or low reproduction of the lice.

Synchronised delousing

The experience from 1991 indicated that a high level of adult lice on one farm gave increased lice settlement on neighbouring farms. As a general rule a synchronised delousing within a geographical area will therefore give a strong positive effect. Usually such delousing action comes naturally when lice are registered on fish simultaneously on all farms within a common "natural" area. We must stress that in our area it is easy to determine what is geographically or hydrographically separated area. We have no indications that neighbouring farms infect each other with sealice during the winter.

Treatment and methods

From our experience there are three conditions in particular that need increased focus regarding a strategy for sealice fighting:

- The use of a totally closed tarpaulin;
- The variation of delousing compounds;
- The use of cleanerfish to fight lice on smolt.

According to both experience and theory it is a "deadly sin" over time not to follow the recommended concentration or treatment time of the delousing compound. The only technology solving this is the use of a totally closed tarpaulin with a fixed volume. We recommend not using the partly enclosed method with the use of skirt. Whatever delousing compound is used, nobody must be surprised by a rapid development of resistance if a skirt (cage enclosed at sides only) or even totally open cage treatments are used.

The use of only one delousing compound must be avoided. The use of pyrethroids is today dominating the sea lice fighting in Norway. These products are safe chemicals with good effect. To secure effective treatment and avoid development of resistance it would be wise to use delousing compounds from different chemical groups (e.g. hydrogen peroxide and organophosphate).

RESULTS

Figure 1 shows the average number of adult female lice for the 1997 input of smolts in the area of Flatanger, Namsos and Fosnes. The number of plants involved in this is 6, with a total output of 3 million smolts. The data shows that the level of lice is far lower than the recommended levels required by the regulations. The data also shows that the level of lice were very low in the period when the wild salmon and smolt migrate from the rivers out to the sea, in the period April - May.

Figure 2 shows the number of gravid female lice as an average of all counting for every salmon generation since 1994.

The number of treatments is now much lower than before we started this strategy. Chemical delousing of the smolts is now rare. The salmon farmers in Flatanger have during the last 4-5 years deloused their big salmon during the second year at sea 4-5 times, this number also includes winter and spring delousing. As a comparison to the year 1991, the smolt were then deloused on average 9 times in 1991 and the big salmon had to be treated 7 times.

REFERENCES

Andersen, P. 1996, "Registration". In: "*Miljøhåndbok for fiskeoppdrett*" (in Norwegian) by P.Andersen, K.Maroni and P.G.Kvenseth (eds.). Kystnæringens Forlag AS.

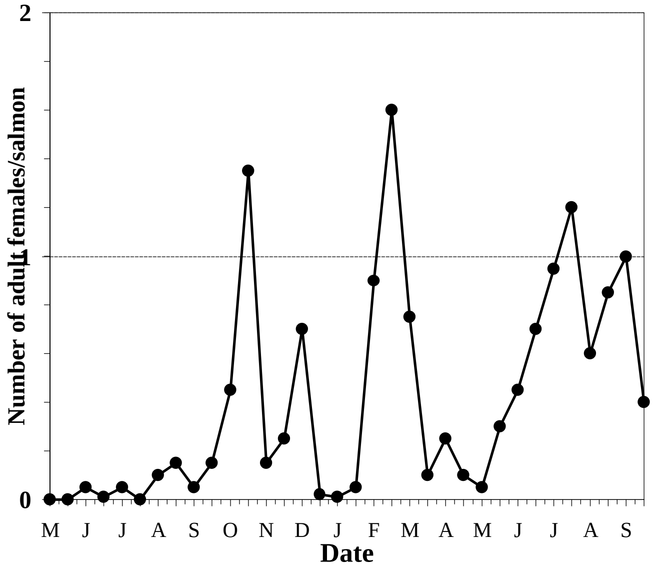

Figure 1. Status of sealice for the 1997 salmon generation in the community of Flatanger, Namsos and Fosnes during 1997 and 1998 (unpublished by Andersen, P.A. and Roasted, S, 1997).

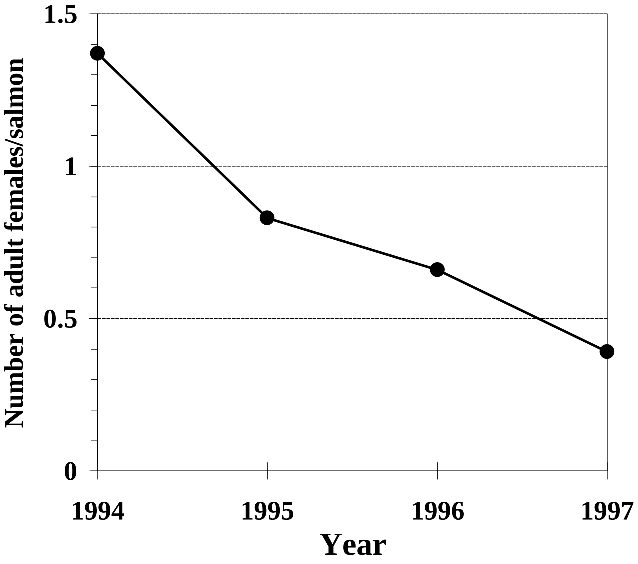

Figure 2. Lice levels counted as gravid females per salmon in mid-Norway, weighted mean as a function of time for the period 1994 - 1997. (unpublished by S. Romstad, 1998).

Wrasse –the video

Anne-Mette Kvenseth * and Per Gunnar Kvenseth**
* University of Bergen, Department of Fisheries and Marine Biology, High Technology Centre, Pob 7800, N-5020 Bergen, Norway.
Email: anne-mette.kvenseth@ifm.uib.no
**KPMG Management Consulting as - Centre for Aquaculture and Fisheries, Sandviksboder 5, N-5035 Bergen, Norway. Email:
pelle.kvenseth@kpmg.no

INTRODUCTION

This manuscript was produced for a video about using wrasse to reduce problems with sealice in seawater farming of Atlantic salmon. The video introduces the use of wrasse, from capture and transport to handling and use in fish farms. To achieve good results with cleaner-fish it is important to understand the biology and behaviour of wrasse. This information is obtained from a combination of results from research and practical experience. The video is intended for both salmon farmers, and people with a more general interest, who want to know more about wrasse and how they can be used to control sealice. It is a way of introducing everyone working in this field to the most environmentally friendly way of reducing sealice problems.

The video can be ordered from Gaia Studio, ruvind@online.no, Fax +47 55 94 64 01. Please specify English or Norwegian text.

Problems with parasites

All living creatures, from bacteria to humans are host to different pathogenic organisms, such as viruses, bacteria and parasites. Under natural conditions the host and parasite live in a balanced relationship. When the local density of one species increases above a certain threshold their numbers will be regulated through natural processes, like starvation or increasing incidents of diseases.

Natural production fluctuates through good and bad years, but for commercial food production a stable high yield is essential. This means that the local density of a species must be increased above the natural density of the species. This makes it easier for pathogenic organisms to spread between individuals of the population, and diseases may become a major problem.

Salmon lice are common parasites of wild Atlantic salmon, and anglers have looked upon lice as a mark of quality because salmon with lice on them are newly returned from sea. In salmon farming however sealice have become a major problem, inducing losses of tens of millions of pounds in every salmon generation.

Natures solution

The parasites themselves however, are also vulnerable and are exposed to attacks from predators and parasites. In agriculture this fact has been known, and exploited, for centuries. Greenhouse farmers use ladybird beetles and parasitic wasps to fight pests. In tropical water, fish and sea-animals go to cleaning stations where different wrasse species remove parasites and skin damage from their body surfaces.

WRASSE

The natural cleaning behaviour of wrasse has been utilised in salmon farming. In Norway wrasse have been used for delousing salmon for more than 20 years, but only since the mid-nineties have they become a commercial alternative. Several million wrasse are used for the purpose of delousing every year. If conditions are favourable for the wrasse, sealice infestations may be kept at a level similar to natural infestations on wild Atlantic salmon (i.e. a few lice per salmon).

Economy

The cost of buying wrasse for a whole generation of salmon is approximately the same as the cost of one chemical delousing, given as a bath or through feed. Chemical delousing however must be repeated, whereas wrasse give the advantage of continuous treatment, as long as they thrive in the pens. Wrasse also have a preference for larger lice, particularly adult females. The production and spread of sealice within the farm, and to neighbouring sites/locations, is thereby further decreased.

Biology

Of the five wrasse species frequently available in northern European waters, goldsinny, corkwing and ballan wrasse seem to have the best delousing potential.

Goldsinny are the smallest, but also the most common species. They are recorded from Morocco, throughout the Mediterranean, to Lofoten in northern Norway. They are easily recognised by the black spots on the dorsal fin and top of the caudal peduncle. Goldsinny work best with smaller salmon from sea-release through the first year in the sea.

Corkwing have a more limited distribution, and are rarely found north of western-Norway. They may be distinguished from ballan wrasse by a black spot on the middle of the caudal peduncle, and are larger than goldsinny. Because of their size corkwing are easier to keep in pens with a wider mesh.

Ballan wrasse are the biggest of the northern wrasse species, and may also be used for human consumption. They are the least common of these three species, and in southern and western Norway a catch of one ballan per ten goldsinny is normal. The ballan wrasse has the best potential for delousing large salmon, second year in sea.

These species are all very territorial at the time of maturation and mating. Keeping them at high densities at this time should be avoided, as attacks and biting may cause extensive skin damage.

Norway represents the northernmost distribution limit for wrasse species, and as the water temperature decreases below 8°C their activity decreases. They gradually stop feeding and reach a state resembling hibernation. In the cold months of the year their effect as delousers may hence be very poor. The ballan wrasse make an exception as they have been found grazing at temperatures as low as 3°C.

CAPTURE AND STORAGE

Within about ten years the wrasse fishery has developed into the best-paid Norwegian fishery, where the fisherman get 300-350 N.Kr. (£ 25-30) per kilo fish. Because of the high profit, wrasse fishing has become a popular summer activity for students as well as for professional fishermen.

Wrasse seem to be particularly sensitive to skin damage, and careful handling of the fish is therefore crucial for the quality, and thereby the delousing effect of the wrasse when they are placed into the salmon pens.

It is important that the fishing gear is adapted for wrasse fishing. Gillnets are not suitable as they will damage the wrasse. Fykenets and eelpots adjusted for a wrasse fishery give the best results. The mesh-size of the fishing gear must be small enough so that wrasse don’t get caught in it, because wrasse that have been entangled in the gear cannot be used. When wrasse get trapped in the mesh this will inevitably cause skin damage and skin lesions over time. This damage may be difficult to notice at an early stage. To ensure careful catching of the fish, special wrasse-pots have been developed.

The pots must be emptied frequently and at least on a daily basis and should be emptied so that the fish remain in water at all times. If they dry out, even for a short while, they may sting each other, which may subsequently cause skin damage. Storage before transport to the fish farm should be of short duration, and sufficient shelter must be provided. Storage in tanks is preferable.

Wrasse are most active in summer and are therefore available for catching then. They are best caught between late April and early May in the southernmost parts of the country, and in July and August further north.

TRANSPORT

Wrasse should be caught locally if possible. This further reduces the possibility of transferring diseases between wrasse populations or between salmon and wrasse. A possible genetic effect on local wrasse populations will also be reduced. In Norway the abundance of wrasse in the northernmost counties is low, and the veterinary authorities therefore allow transport of wrasse from other parts of the country that do not have any fish-farming activity. Wrasse from other counties must obtain a health certificate before transport.

PRACTICAL USE IN FISH-FARMS

Transfer to farms

All handling of wrasse, even distribution within the farm must be performed with thorough attention. The fish must only be kept out of water for the shortest possible time. When transferred from one tank to another only a few fish must be kept in the dipnet. The sharp fin-rays will easily sting other fish. When the fish are being placed in net-pens a bucket with fish and water should be lowered to the water surface and then carefully emptied. When wrasse arrive at the farm one responsible person from the farm must control the quality of the fish. Number, size and species of wrasse should be registered. This control is necessary for the fish farm record and for reporting back to the fisherman.

Distribution in farms

The wrasse should be evenly distributed between different pens, building up the number in each pen gradually. It will normally take too long to build up sufficient wrasse numbers if the pens are stocked individually, which again may lead to high lice levels in some pens.

Delousing in big pens

Wrasse have traditionally been used with best results in smaller pens up to 3-4000 m^3. In recent years the size of these units have increased reaching 15-20.000 m^3. Initially wrasse have produced inefficient results in such large units, but better results may be achieved with the active use of shelters, which are gradually moved to the depth preferred by the salmon.

Dead-fish control and lice-counting

To survey the percentage of wrasse in the pens it is necessary to keep a record of the number of wrasse added, and of wrasse mortalities. When sea temperatures are high dead wrasse disintegrate rapidly, and frequent dead-fish control is necessary.

Even after wrasse are added to the pens it is necessary to survey the number of lice on the salmon. Lice counting will give information on whether the wrasse are present and actively grazing in the pens. The number of wrasse necessary for each pen will depend on the quality of the wrasse, and the number that still are present. Under normal conditions 1-3% actively grazing wrasse to the total number of salmon is sufficient to control sealice infestations. When ballan wrasse are used on larger salmon it is wise to keep the level at about 1%, as a situation where food is scarce may lead to problems with eye damages in the salmon.

Fouling control -net changing and chemical delousing

When using wrasse for delousing it is generally important that no other food is available in the pens. Net fouling makes a good alternative food source for hungry wrasse, and is more easily accessible. Net fouling consists of algae, blue mussels and crustaceans, and the wrasse may prefer this to chasing salmon for a few lice.

Wrasse may also be used deliberately to reduce problems with fouling, especially when blue mussels are the biggest problem, but when sealice are found simultaneously a poor delousing effect may be expected.

When nets are replaced, or during chemical bath treatments, it is also necessary to consider the behaviour of the wrasse. Salmon will readily swim from one net to another, while the wrasse will try to hide in folds and pockets in the net. If this fact is not considered many wrasse may be lost during such operations.

Use of ballan wrasse

Ballan wrasse, used for delousing salmon on their second year in sea, are large fish and may well reach a size of 30 centimetres. The summer months are used for building up enough energy to last through the winter, and because of this ballan are constantly seeking sufficient feed sources. If ballan are introduced to salmon with few lice, or in a clean net without fouling, this may result in damage to the salmon. Grazing injuries have been experienced on the fins and eyes of the salmon. Injuries however should be observed on free-swimming salmon as the wrasse will readily eat on fresh dead salmon from the bottom of the pens. When using ballan wrasse the fish farm therefore needs equipment for removing the wrasse from the salmon cages if problems are experienced. The ballan may easily be fished out of the cages by removing available shelters and replacing these with baited pots. Crushed crabs or blue mussels will attract the wrasse, which thereafter may be introduced to other cages or stored for later use.

WINTER-STORAGE AND RE-USE OF WRASSE

If wrasse are kept in salmon cages through the winter most of them will die or escape during the cold period. When the temperature decreases below 5ºC the wrasse need a tranquil environment to survive, and such conditions are difficult to obtain in a salmon pen. Bad weather, strong currents and wave movements cause constant disturbance and unfavourable conditions for the wrasse. One way to store the wrasse is in tanks on land. Transfer to closed cages with sufficient shelters is another possibility. These cages are lowered to a depth of about 18-20 m in a calm area. When the temperature increases in the spring the cages are gradually raised to the surface.

The minimum size of wrasse for delousing is 10 cm, but to avoid escapes from a pen with 26 mm meshes the wrasse should be at least 16 to 18 cm.

The natural habitat of wrasse is shore areas where they live in algae, rocks and crevices. When transferred to the netpens the wrasse will continue to slip in and out through narrow passages where possible. A small hole in the net is sufficient for wrasse to escape through.

In the pen the wrasse will attempt to hide, most likely in the dead-fish-collector, or by other structures that give shelter. By being aware of this during daily operations, entrapment and damage to the wrasse may be avoided.

To offer the best possible environment most farmers put shelters or wrasse-houses in the pen. This may include old car tyres, pieces of pipes tied up in bundles, or shredded plastic bags. They all provide resting-places for the wrasse.

The shelters must be constructed and placed so that they do not cause damage to the net, or harm wrasse or salmon. They need to be localised to the depth preferred by the wrasse, which will vary between species, temperature, time of year and the local currents. The shelters must also be easy to clean and maintain.

The best time to put up shelters is before wrasse are transferred to the pen, and to place them near the bottom for the first days. This will reduce the stress on the wrasse and may prevent them from hiding in the dead-fish collector on the bottom. The wrasse will mostly stay in the shelters during the dark hours.

TROUBLESHOOTING

Even if wrasse have been used for delousing in commercial fish farms for several years farmers may sometimes experience poor efficiency. There are several reasons for this and the most important are as follows.

The wrasse are introduced too late

To achieve the best results it is important that lice levels do not build up before the wrasse are introduced. The availability of wrasse however is poor in the spring when the salmon smolts enter sea. Capture of wrasse in autumn and storage through the winter for early introduction in the netpens the following spring is a possibility. Storage in tanks, on land, or in submerged closed cages in the sea may improve survival. The most crucial point is that wrasse are given sufficient shelter and rest through the coldest time in winter. As long as wrasse continue to eat they should be fed. Tanks on the shore are safest, giving the best control and survival. However this demands continuous surveillance.

The wrasse are gone or are impaired

Diseased wrasse are ineffective. The extent of mortality depends on which species is used and how the fish have been handled. The mortality in the pens is normally caused by a combination of injuries caused by mechanical damage and possible outbreaks of disease.

The injuries are almost inevitably caused by development of lesser skin damages inflicted during capture, transport, storage and transfer to fish farms. Stress caused by such operations, and also by unfavourable conditions in the pens, may cause outbreaks of covert infections that fish carry. The importance of careful handling throughout all operations must therefore be stressed.

Wild wrasse may to some extent be susceptible to the bacterial disease atypical furunculosis. The atypical *Aeromonas salmonicida* isolated from wrasse however seems to be a different variant than the bacteria causing furunculosis in salmon, and it has not been possible to transfer this disease to salmon in laboratory experiments. Some of the wild wrasse populations may have a percentage of carriers with covert infections, and stressful conditions may cause outbreaks of the disease. Careless handling or unfavourable conditions in the pen may therefore lead to high mortality in wrasse. Experiments with vaccination of wrasse have been carried out, but a vaccine giving sufficient protection has not yet been found. Examinations of pathogens of natural wrasse populations have so far not exposed viral, bacterial or parasitic diseases that are expected to be harmful to salmon.

FRY-PRODUCTION

All wrasse used for delousing are wild-caught fish. We do not know the size of the natural stocks of wrasse, or to what extent they may be exploited on the longer term. The aquaculture station at Austevoll has initiated projects to survey the populations of the most utilised species, by catching, marking and recapture. The Aquaculture station has also continued work on fry-production of several wrasse species. Ballan wrasse is the species most suited to commercial production. As with most other marine fishes ballan have small eggs and larvae, which makes first-feeding difficult, but after this stage the fry have a fast growth rate and they may reach a favourable size of 10-12 cm only one year after hatching.

CONCLUSION

Wrasse are not a chemical and do not have an instantaneous effect. The wrasse are most effective if they (a) are forced to graze on lice due to a lack of other food sources, (b) are in good health, (c) are kept in suitable conditions and (d) are still present in the netpens. They are more effective when lice reach the mobile stages.

Cell culture bio-assays for potential anti-sea louse chemotherapeutants

J.P.G. Toovey and A.R. Lyndon
Department of Biological Sciences, Heriot-Watt University, Riccarton, Edinburgh, EH14 4AS, UK.
E-mail: J.P.G.Toovey@hw.ac.uk

INTRODUCTION

Novel, putative anti-sea louse treatments are being investigated in increasingly large numbers. *In vivo* testing is, by its nature, expensive and time consuming. A better way to test such chemicals' suitability would be some form of simple and cost-effective screening prior to traditional *in vivo* assays. Cell culture represents a very promising tool for such a task (Austin & Cross, 1998; Toovey & Lyndon, 1998).

The aim of the study was to develop a simple test, which can be used both to determine the effects of potential chemotherapeutants on fish and to determine the effectiveness of potential chemotherapeutants on louse settlement and attachment. The results from both tests can then be used to determine a suitable therapeutic margin for a chemical prior to *in vivo* testing, thus saving time and money by eliminating unsuitable chemicals at an early date and also by providing a preliminary estimate of suitable dosage levels.

METHODS

Primary salmon epidermal cultures were set up and used as the basis for assay. Cultures were grown in six-well plates on an agar surface in supplemented L15 medium for four days to allow establishment of the cells. After this period, the potential chemotherapeutant was added and the cultures grown for a further seven days before being recorded. Growth radius was compared against that of untreated cultures and the occurrence of morphological differences was also noted.

For louse settlement assays, seven days of cell growth preceded removal of half the culture from each well. Louse settlement was then recorded by adding freshly hatched copepodids to the cultures and determining the numbers moving onto the cell-covered portion of the well in a 15 min period. Louse attachment was recorded 1 hour after addition of copepodids and was defined as resistance to removal from the cells by rinsing with a change of seawater (Pasteur pipette).

RESULTS

1. Cell culture growth experiments.

This experiment was conducted with ivermectin and the growth curve in Figure 1 obtained. As can be seen from this, the ivermectin has a similar inhibitory effect on epidermal cell growth as it has on gill arch respiration rates (Toovey *et al*. 1999)

2. Louse settlement and attachment

This experiment has only been performed with untreated cultures to date, giving average settlements of 98% (SD ± 12%) and average attachments of 89% (SD ± 20%) (n = 10 replicates, 5 lice each).

DISCUSSION

The results suggest that this assay system is suitable for testing potential chemotherapeutants prior to *in vivo* studies. Such tests would be useful as they may eliminate unsuitable chemicals without lengthy and expensive trials. The lice attachment test still requires considerable development work, but the initial results suggest that copepodids do settle and attach preferentially on cultured cells. If a putative treatment were to disrupt this reaction *in vitro*, it would seem reasonable to initiate work *in vivo*.

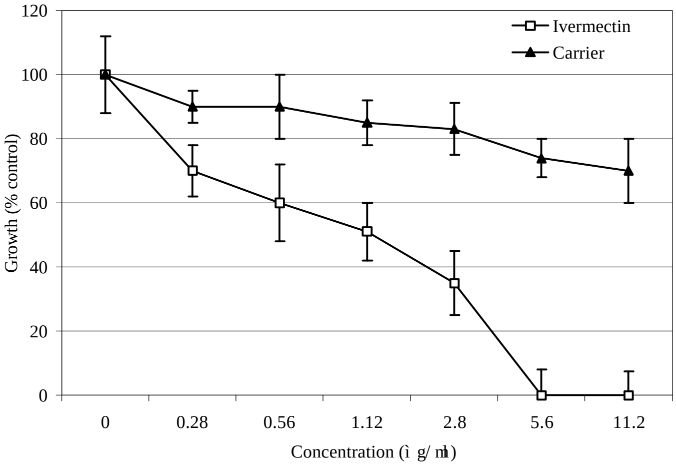

Figure 1. Growth of salmon epidermal culture when ivermectin is applied

REFERENCES

Austin, B. & Cross, N. (1998). *Methods in Cell Science* **19**:317-324.

Toovey, J.P.G. & Lyndon, A.R. (1998). *Proceedings XIX ESCPB Congress*, Turku, Finland. pp 124.

Toovey, J.P.G., Lyndon, A.R. and Duffus, J.H. (1999). *Bulletin of the European Association of Fish Pathologists* **19**:149-152

Chemotherapeutant resistance in sea lice: what is it and what can be done about it?

Gregor J Devine, Ian Denholm and Tor E. Horsberg[1]

Biological and Ecological Chemistry Dept, Institute of Arable Crops Research, Harpenden, Herts AL5 2JQ, UK.
[1]Norwegian School of Veterinary Sciences, Division of Pharmacology and Toxicology. PO Box 8146 Dep, Ullevaalsveien 72, Oslo, Norway.

Based on a paper presented at the 4th International Conference on Sea Lice held at Trinity College, Dublin, Ireland, 28th-30th June 1999.

INTRODUCTION

Throughout Northern Europe, the marine phase of salmon production is seriously affected by attacks from salmon lice, especially *Lepeophtheirus salmonis.* Although the problem is long-standing, the development of sustainable methods of pest management has been unable to keep pace with the intensification of production, leading to excessive but fragile reliance on very few chemotherapeutants. This dependency can be contentious in terms of environmental and human health, and is also threatened by the fact that large-scale reliance on any active ingredient runs the risk of selecting for genetically determined resistance in target organisms. Such threats have so far been most apparent for chemicals used against arthropod pests of agriculture and horticulture, many of which are now highly resistant to a wide range of insecticides and acaricides. Many of the compounds affected by resistance in the crop protection sector are identical or closely related to ones currently employed against sea lice. It is therefore opportune to consider what lessons have been learnt from contending with resistance in crop pests, and their implications for the sustainability of chemotherapeutants used in aquaculture.

In this article we summarise existing knowledge of resistance in sea lice, review what is known about resistance generally and of factors promoting its selection, and consider options for combating resistance that have potential relevance to the salmon production industry.

Current status of chemotherapeutant resistance in sea lice

Since the early 1990s, there have been several reports of treatment failures with the organophosphate (OP) dichlorvos, and more recently with azamethiphos in Norway and Scotland (e.g. Jones et al 1990, Roth et al 1992, S. Wadsworth & J. Treasurer, Marine-Harvest McConnell, pers comm). In 1998, there were two reports of control failure with the pyrethroid deltamethrin in Norway and, in one of these, resistance was confirmed in laboratory assays conducted at the Norwegian School of Veterinary Sciences (T.E Horsberg, unpublished data). In 1999, a further case of pyrethroid resistance was reported in Norway, and there have been anecdotal reports of control difficulties with cypermethrin (another pyrethroid) in Scotland. In Canada, where the only compound licensed for use against sea lice has been azamethiphos, there were reports of treatment failures on the east coast in 1998 (L. Hammell, University of Prince Edward Island, pers comm).

For the most part, it has not been possible to attribute these control difficulties unambiguously to resistance rather than to extenuating factors such as poor or inappropriate application methods. This reflects the lack of research conducted to date on sea lice resistance, and the absence of robust baselines and protocols for resistance detection and monitoring. As a consequence, there is now considerable momentum to develop and standardise such techniques, in order to document and combat this major potential threat to the future of the salmon farming industry.

How does resistance arise?

Like all adaptive traits, resistance evolves through a process of natural selection. Genes conferring resistance probably arise repeatedly by mutation, but in the absence of selection will remain at very low frequencies in pest populations. With the onset of exposure to chemical toxins, individuals possessing such genes are selectively favoured and increase in frequency. During the early stages of selection, the number of resistant survivors may be too small to have any discernible impact on the quality of control. If resistance is unchallenged, however, these individuals eventually reach the stage at which control difficulties are readily apparent. The speed at which resistance increases, and the degree of resistance that can be tolerated, depend on many inter-related factors including the nature of damage inflicted by a pest, the potency of resistance mechanisms, the frequency of chemical use, and the biology of the pest itself. For many pests (including sea lice), key aspects of biology such as dispersal capacity and the temporal and spatial stability of populations are still poorly understood. This makes the prediction of resistance risk a complex and difficult task.

What are the mechanisms?

The most important and powerful mechanisms of resistance involve either an increased ability to detoxify a chemical, or a structural alteration to its target site within the pest. Other mechanisms that have been demonstrated or hypothesised include reduced penetration of toxins through the cuticle, and behavioural changes that enable pests to avoid them in the first place. Behavioural resistance in salmon lice is very unlikely due the uniformity of chemical applications and the limited mobility of chalimus and adult stages. Based on experience with other pests, reduced penetration is best considered as a potential ‘modifier’ of increased detoxification or target-site resistance rather than a major mechanism in its own right.

Mechanisms of OP and pyrethroid resistance based on both increased detoxification and altered target sites are well documented for agricultural pests. These are also the most likely to occur in sea lice, and are summarised below.

Increased detoxification of insecticides

Three major detoxification systems have been implicated in causing resistance:

Compounds of many chemical classes are vulnerable to enhanced oxidative breakdown by enzymes known as mixed function oxidases or MFOs. Evidence for this type of resistance may be indirect, based on the ability of known inhibitors of MFOs to overcome resistance when co-applied with toxins in laboratory assays, or direct, based on enzyme binding or metabolism studies.

Enhanced activity of glutathione-S-transferases (GSTs), which assist with cleaving active sites from toxic molecules, is potentially important in resistance to OPs, but for biochemical reasons is unlikely to affect tolerance to pyrethroids.

Many toxins, including most pyrethroids and all OPs, contain ester bonds that are vulnerable to cleavage by esterases, resulting in non-toxic metabolites. Of the three possible detoxification routes, enhanced esterase activity is by far the best-characterised biochemically, and in some cases the esterases involved have been identified, characterised and sequenced at the molecular level.

Alterations to target-sites

Since OPs and pyrethroids attack different sites in the insect nervous system, mechanisms of target site resistance to these classes are obviously distinct.

OPs exert their toxicity by inhibiting the enzyme acetylcholinesterase (AChE), which plays a key role in the transmission of nerve impulses across gaps (‘synapses’) between nerve cells and at neuromuscular junctions. Forms of AChE showing reduced inhibition by OPs have been selected in several arthropod pests of agriculture, livestock and public health. Advances in methodology for characterising resistant AChE now enable this mechanism to be diagnosed rapidly and precisely in individual insects, and offer scope for implicating and monitoring this mechanism in sea lice as well.

Pyrethroids act by binding to and blocking ion channels in nerve cell membranes that are also vital for the transmission of nerve impulses. A mechanism based on the insensitivity of such channels to pyrethroids was first identified in houseflies and termed knockdown resistance or kdr. In recent years, analogous forms of kdr resistance have been confirmed in a diverse range of insect pests. As with insensitive AChE (see above), there are now sophisticated techniques available for diagnosing this type of resistance more accurately than was possible in the past.

Other chemotherapeutants becoming available for sea lice control include the avermectins (also nerve poisons, but acting differently to OPs and pyrethroids) and the chitin synthesis inhibitors (benzoylphenylurea compounds affecting the moulting of immature stages). To date, these have tended to have very specialised uses against other pests, and cases of resistance are therefore less common than for OPs and pyrethroids. The underlying mechanisms are also less well established but are most likely also based on some form of increased detoxification and/or target-site modification.

The phenomenon of cross-resistance

Resistance mechanisms seldom if ever affect just one toxin. For combating resistance, it is therefore important to anticipate how the phenomenon of cross-resistance might influence the effectiveness of management strategies based, for example, on the alternation of products to avoid continuous selection for the same mechanism.

Unfortunately, cross-resistance patterns are inherently difficult to predict in advance, since mechanisms based on both increased detoxification and altered target sites may differ substantially in their specificity. The most commonly encountered patterns of cross-resistance tend to be limited to compounds within the same chemical group (equivalent to the term ‘side-resistance’ as used by parasitologists). However, even these patterns can be idiosyncratic. As an example, OP resistance based on increased detoxification or target-site alteration can be broad-ranging across this group or highly specific to a few chemicals with particular structural similarities. Although the switch by salmon growers from dichlorvos to azamethiphos was not made with resistance management in mind, it may have been a fortuitous one, given the structural dissimilarities between these organophosphates. It is possible that it relieved the selection pressure on lice populations already exhibiting occasional resistance to dichlorvos. Notably, although the two pyrethroids with approval for sea lice control (deltamethrin and cypermethrin) are structurally very similar and almost certain to be subject to cross-resistance.

It is also possible for single mechanisms to confer cross-resistance across chemical groups. For example, mechanisms based on enhanced MFO or esterase activity have the potential to affect both OPs and pyrethroids. Once selected, this mechanism could extend to other unrelated compounds including teflubenzuron and the avermectins, whose molecules are also vulnerable to oxidative attack. As far as cross-resistance is concerned, the only 100% certainty is that we can never be 100% sure, highlighting the importance of detecting and characterising resistance mechanisms at the earliest possible stage. The development and validation of bioassay systems for all existing chemotherapeutants is obviously an accompanying priority.

Factors promoting resistance

Based on experience with agricultural pests, some of the factors that conspire to promote the rapid selection of resistance, and the successive accumulation of resistance mechanisms, are as follows:

- a continuous availability of host plants, enabling pest populations to build up to large numbers and to persist throughout the year
- the relative genetic isolation of populations resulting from a lack of mixing of pests from treated and untreated areas
- lack of untreated ‘refuges’ in which selection for resistance is avoided and susceptible individuals are preserved
- low damage tolerance thresholds, promoting the frequent use of chemicals with little attempt to ascertain through monitoring, whether pest numbers are high enough to warrant treatment.
- a limited diversity of compounds available for inclusion in control strategies, leading to over-reliance on single products or chemical classes.

Protected environments such as glasshouses and greenhouses characteristically exhibit a number of these features, and have historically proved particularly prone to generating resistance problems. Plant hosts are often grown in contiguous cropping cycles, and there is generally little scope for immigration by unselected individuals. Pests are frequently ‘sprayed on sight’ to prevent combat outbreaks of insect-transmitted plant viruses or to avoid cosmetic damage to high value ornamental crops. The number of toxins approved for use in these environments, especially on food crops, is usually very limited, and the occurrence of resistance erodes this choice still further.

To what extent do such considerations apply to the ecology and treatment of sea lice on salmon farms? In some respects this question is difficult to address due to insufficient knowledge of sea lice movement between farms, and of interactions between ‘farmed’ and ‘wild’ lice populations. However, some generalisations are possible on the basis of available information, and do not bode well for maintaining the effectiveness of chemotherapeutants in the long term. Due to their host specificity, the proportion of *L. salmonis* individuals exposed to chemotherapeutants is clearly much greater, and selection therefore much stronger, than for the more ‘polyphagous’ louse, *Caligus elongatus*. As a consequence, concerns over resistance management need to be focussed on the former species. In some areas salmon are grown all year round, and successively from year to year. Even where fallowing occurs, some fallow sites are so close to production ones that the life-cycle of *L. salmonis* may never be effectively broken. We also suspect that salmon lice populations on farms may produce so many nauplii that they overwhelm any immigrants brought in on the currents, thus limiting any dilution by susceptible genotypes. The impact of sea lice on farm productivity, and concerns over the impact of salmon lice on wild fish stocks, encourage salmon farmers to keep lice populations low, and the difficulty in obtaining registration and discharge consents for chemotherapeutants further increases their reliance on single products. While it may be going to extremes to regard many salmon farms as the marine equivalent of glasshouses, there are substantial and worrying parallels between the two production systems.

Approaches to combating resistance

Many of the tactics that have been proposed for combating resistance in agricultural pests are based on complex genetic and/or ecological arguments and beyond the scope of this article; readers seeking further information are referred to papers listed at the end and to references therein. However, one over-riding principle arising from debates in the literature and from practical experience can be summarised neatly as ‘variety is the spice of resistance management’. Rather than relying solely on any single product for pest control, farmers should be encouraged to exploit the maximum diversity of control measures available. Such diversity includes operational diversity, taking full advantage of non-chemical tactics (biological control by natural enemies, physical exclusion of pests, host-free periods etc), and chemical diversity, exploiting a range of products least likely to encounter problems from cross-resistance.

As always, this principle is far easier to extol in theory than to implement in practice, but several tactics, useful in resistance management, have already been partly adopted. These include the complete geographical separation of year classes and the systematic use of fallowing periods or cleaner wrasse. Others, such as the use of enclosed net-pens or land-based fish farms where parasite-free water can be supplied, could be exploited further.

Providing farmers with access to sufficient chemical diversity, and enabling them to exploit this effectively, raises a different set of problems. There has been and still is a marked tendency in the salmon industry to rely on single products. This has not always been under the control of farmers. The difficulty in registering new compounds, and in gaining consents from the authorities for their discharge, has forced this situation upon them. Nonetheless, when a choice has been available, it has often been overlooked (witness the current dependence on pyrethroids in Norway and the UK). A far more desirable approach would be to base sea-lice control on at least two (preferably more) unrelated compounds applied in pre-planned alternations, or conceivably as mixtures of chemotherapeutants. Due to concerns over fish toxicity and the environment, the use of mixtures is probably impractical (as it is in many agricultural systems). Alternating products is therefore the most feasible option. Due to very long withdrawal periods for some compounds, the use of some chemicals will be temporally segregated anyway, and this could assist with resistance management. Since avermectins and benzoylphenylureas have long withdrawal periods and are rarely used on large fish, emamectin benzoate and chitin synthesis inhibitors are likely to be reserved for the control of lice in smolts. Pyrethroids and OPs could be used for larger fish. Both these pairs of compounds have different modes of action, and it is likely that recommendations for resistance management could be built around such combinations.

Ultimately, the key to success will be in implementing and co-ordinating management tactics amid the commercial and socio-economic pressures that drive the use of chemicals by salmon growers. This is an important challenge for the aquaculture industry, various regulatory authorities, the chemotherapeutant industry, and the research community itself. The aquaculture industry must seek to avoid misuse of available products, regulators must be aware of the value of approving new products at a time when there are still effective alternatives available, and chemical manufacturers and distributors should recognise the benefits of greater co-operation between companies. Useful models of such co-operation are provided by the agrochemical industry’s Insecticide Resistance Action Committee (IRAC), which supports work to combat insecticide resistance on a global basis, and the Insecticide Resistance Action Group (IRAG) of the UK, which offers a forum for regulators, industrials, consultants and researchers to review and address problems of resistance in that country. Finally, there is clearly an urgent need to establish more research into the management of resistance in sea lice, both nationally and internationally. The hopeful sign is that following discussions initiated during the Dublin conference in June 1999, attempts to formulate discussion groups and collaborative research programmes are already well underway.

GENERAL FURTHER READING

Byrne, F.J.; Cahill, M.; Denholm, I.; Devonshire, A. L (1994). A biochemical and toxicological study of the role of insensitive acetylcholinesterase in organophosphorus resistant *Bemisia tabaci* (Homoptera, Aleyrodidae) from Israel. *Bulletin of Entomological Research,* **84**: 179-184.

Denholm I, Cahill M, Dennehy TJ, Horowitz AR (1998) Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci. Philosophical Transactions of The Royal Society of London Series B-Biological Sciences* 353, 1757-1767.

Feyereisen, R. (1999) Insect P450 enzymes. *Annual Review of Entomology,* **44**: 507-533.

Field LM, Anderson AP, Denholm I, Foster SP, Harling ZK, Javed N, Martinez-Torres D, Moores G.D, Williamson M.S, Devonshire A.L (1997). Use of biochemical and DNA diagnostics for characterising multiple mechanisms of insecticide resistance in the peach-potato aphid, *Myzus persicae* (Sulzer) *Pesticide Science* **51**: 283-289.

Jones M.W., Sommerville C. & Wootten R. (1992). Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis,* to the organophosphate dichlorvos. *Journal of Fish Diseases* **15**: 197-202.

McKenzie JA (1996). *Ecological and evolutionary aspects of insecticide resistance.* Academic Press, London, UK.

Roth, M., Richards, R.H. and Sommerville, C. (1996). Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquaculture,* **140**: 217-239.

Yu, S. J. (1996). Insect glutathione S-transferases. *Zoological Studies,* **35**: 9-19.

For further information on the activities of the UK Insecticide Resistance Action Group, please contact the Secretary: Dr Ian Denholm, IACR-Rothamsted, Harpenden, Herts. AL5 2JQ, UK (ian.denholm@bbsrc.ac.uk)

A review of field studies on planktonic larvae of *Lepeophtheirus salmonis* on the west coast of Ireland.

Mark Costelloe, John Costelloe, Ger O'Donohoe, Nicola Coghlan and Brendan O'Connor.
Aqua-Fact International Services Ltd., 12 Kilkerrin Park, Galway, Ireland.

An extensive survey on the ecology of the sea louse *Lepeophtheirus salmonis* Krøyer was initiated in 1994 in selected bays in Ireland. This review relates to results from studies on the planktonic larvae carried out in Kilkieran Bay and Killary Harbour where salmon farming is carried out. For a full description of all methods and results of the plankton studies see Costelloe *et al*. (1996, 1998a, b). The data presented in Costelloe *et al*. (1996, 1998a, b) provide the first extensive and detailed empirical data on the densities and distribution of larvae both at fish farms and at distances from farms and ancillary physical oceanographic data. The results show that (1) there was a high retention of larvae within cages reflecting the reduction of water movement caused by the physical barrier of the net and by the fish themselves within the cage. Fouling on the net by (for example) algae or coelenterates may further reduce current flow through the cage. (2) Densities of nauplii and copepodids outside the cages fell by two orders of magnitude within 100m of the cage due to dilution, vertical mixing and natural mortality. Extensive studies in bays with fish farms failed to return figures any higher than 0.2 larvae m^2. The highest densities of larvae were found in inner estuarine areas of bays far away from farms. (3) Regression analysis on the larval densities recorded on a transect from a farm indicated a highly significant relationship ($p < 0.001$) between distance from the farm and the number of larvae picked up in the tows. The models produced by the analysis predict that few larvae would have been found in surface tows taken greater than 2km from the last cage on the farm on the sampling dates. (4) Although low numbers of copepodids were found in most samples (max. $0.28/m^3$, 500m from the farm), the ratio of copepodids to nauplii increased with distance from the farm.

Given the above dispersion patterns and also that the number of nauplii are constantly being reduced due both to development to the copepodid stage and natural mortality, it is not surprising that the ratio of copepodids increased with increasing distance from the farm. The actual numbers of copepodids (max. $0.28/m^3$) were relatively low and the density did not increase with increasing distance.

Sea lice/host relationships have evolved and adapted over millions of years and fish farming has imposed only a recent new dimension to the ecological equation. Ecological and behavioural characteristics of both the host and parasite in natural habitats play major roles in determining the probability of them coming into contact with each other. Lice infections on fish will depend on the number of infectious copepodids within a particular location, the time spent by the fish within this area during periods of available copepodids and the susceptibility of the fish to infection at that particular time. Lice larvae may derive from more than one source, either from wild or from farmed fish.

Salmonid farms normally occur in the outer areas of embayments. In the initial stages of fish production, lice infestations occur from the natural background levels derived from wild fish. After these events, the farm becomes self-infesting with the generation times of the lice being dependent on the prevailing water temperature. The effect that this source of lice has on the water body in which it is located is predominantly related to topography and hydrography. Given the recorded larval densities and the current patterns within the bay, it is likely that the impact of lice from Killary Salmon Farm is mainly confined to the outer harbour areas. A further likely source of lice production occurs in the inner harbour, particularly in the areas close to the river mouths. On their migration from salt to freshwater systems, wild salmonids remain in the inner estuarine areas until conditions are suitable to move into the rivers and thereby provide lice population in this area. In Killary Harbour, wild salmon are present or are caught by netting from mid-March to mid-July. Although the greatest number of fish are caught around June (720 salmon from one boat over a 10 day period), a significant number of salmon are caught in the early part of the season (McDermott *et al.*, 1996). These salmon provide a viable source of infective larvae at much the same time as when salmon and sea trout smolts are running to sea. Salmon smolts are known to swim directly out to the open sea while sea trout smolts are reported to remain close to the mouths of their mother rivers.

The following possible transfer methods of farm-generated larvae to estuaries are suggested: (1) larvae are carried to estuaries from farms where they infect the sea trout. (2) Sea trout swim out to farms, collect lice and swim back to estuary. (3) wild fish pick up larvae at farms and swim to estuaries, the larvae mature and infect the sea trout. (4) Farmed fish, which escape carry adult lice to estuaries where they infect sea trout.

Based on the results presented here on larval densities and physical oceanography, transfer of significant numbers of larvae from the farm studies to the upper parts of estuaries is unlikely. Current speeds are too low and distances are too great for larvae to be able to reach the required locations in a short enough time.

Sea trout are known to make some extensive migrations and can be caught at some distance from their mother rivers. Work on sea trout smolts both in Wales (Moore & Potter, 1994) and Scotland (Johnston *et al.*, 1995) has shown that smolts tend to remain in inner, shallow (< 2 m) waters. It is highly improbable that a population would migrate in any sort of significant numbers to a particular location within a bay and then return to the estuary from where they came.

The possibility that wild fish pick up lice at farms and then swim into the estuary to allow for the infection of sea trout again seems an improbable transfer mechanism, as does the likelihood that farm escapes are a significant vector.

An alternate hypothesis is that it is the population of wild migratory salmon with their own natural loadings of sea lice which acts as the source of larvae not only as the natural background level of lice which initially infects the farm but also for inner estuarine areas: wild salmon return to different systems at different times of the year with their own adult lice load and there may also be a contribution of adult lice from resident trout. Allowing for a figure of 2 gravid female lice per fish with a potential larval output of 600 per louse, a run of 20 salmon could produce 24,000 larval lice. If these fish have to wait in upper estuarine areas for suitable river flow conditions, this figure could multiply rapidly. As at least part of the sea trout population are known to remain in areas close to their mother river and in depths of ca < 2m (Johnston *et al*. 1996), they could be subjected to high levels of sea lice infection. Such phenomena are well recorded off the Canadian west coast (Johnson *et al.* 1996). The data presented by Costelloe *et al*. (1998a, b) clearly show that larval lice are not evenly distributed in bays, which contain fish farms and also have salmonid rivers. The larvae were only recorded close to the aquaculture site and in the upper estuarial locations. The discontinuity in the presence of larvae, the occurrence of nauplii in the upper estuarine areas and the mismatch in timing between the occurrence of larvae in these areas and the presence of ovigerous females on the farmed fish do not support the hypothesis that larvae are carried from the farms to the estuary. These data do however provide support for the hypothesis that wild fish, with their own louse loads, act as the infection source of both the farm and fish in the upper estuary. The corollary to this is that during periods when high numbers of salmon are running, whether from wild stock or from ranching programmes, larval lice numbers can be expected to be most dense.

REFERENCES

Costelloe, M., Costelloe, J. and Roche, N. (1996). Planktonic dispersion of larval salmon-lice, *Lepeophtheirus salmonis*, associated with cultured salmon, *Salmo salar* either from wild or from farmed fish, on the west coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom*, **76**: 141 - 149.

Costelloe, M., Costelloe, J., O'Donohoe, G., Coghlan, N., Oonk, M. and Van der Heijen, Y. (1998a). Planktonic distribution of sea lice larvae, *Lepeophtheirus salmonis*, during the Spring/Summer period in Killary Harbour, west coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom*, **78**: 853 - 874.

Costelloe, M., Costelloe, J., Coughlan, N., O'Donohoe, G. and O'Connor, B. (1998b). Distribution of the larval stages of *Lepeophtheirus salmonis* in three bays on the west coast of Ireland. *ICES Journal of Marine Science*, **55**: 181-187.

Johnson, S.C., Blaylock, R.B., Elphick, J., and Hyatt, K.D. (1996). Disease caused by the salmon louse *Lepeophtheirus salmonis* (Copepoda : Caligidae) in wild sockeye salmon (*Onchyrhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Canadian Journal of Fisheries and Aquatic Science* (in press).

Johnston, A., Walker, A., Urquahart, G. and Thorn, A. (1995). The movements of sea trout smolts, *Salmo trutta* L., in a Scottish sea loch determined by acoustic tracking. *Scottish Fisheries Research Report*, **56**: 1-21.

McDermott, T.J., Willis, S.E. and Mantle, P. (1996). Spring salmon enhancement on the Delphi fishery Ireland. In "Enhancement of Spring salmon". *Proceedings of the Atlantic Salmon Trust*: 98 - 125.

Moore, A. and Potter, E. (1994). The movements of wild sea trout *Salmo trutta* L., smolts through a river estuary. *Fisheries Management and Ecology*, **1**: 1-14.

Recent publications on sealice

Mills, J. (1999). *The bioavailability of cypermethrin (synthetic pyrethroid) to calanoid copepod,* Acartia tonsa *Dana via surface binding of the insecticide to algae: consequences for lethal toxicity.* M.Sc. thesis, University of Stirling, Scotland.

Mustafa, A. & MacKinnon, B. M. (1999). Atlantic salmon, *Salmo salar* L., and Arctic char, *Salvelinus alpinus* (L): comparative correlation between iodine-iodide supplementation, thyroid hormones levels, plasma cortisol levels, and infection intensity with the sea louse, *Caligus elongatus*. *Canadian Journal of Zoology,* **77**: 1092-1101.

Mustafa, A. & MacKinnon, B. M. (1999). Genetic variation in susceptibility of Atlantic salmon to the sea louse, *Caligus elongatus* Nordmann, 1832. *Canadian Journal of Zoology,* **77**: 1332-1335.

Mustafa, A., McWilliams, C., Fernandez, N., Matchett, K., Conboy, G. & Burka, J. (2000). Effects of sea lice (*Lepeophtheirus salmonis* Kroyer, 1837) infestation on macrophage functions in Atlantic salmon (*Salmo salar* L.). *Fish and Shellfish Immunology,* **10**: 47-59.

Pike, A. W. & Wadsworth, S. L. (1999). Sealice on salmonids: their biology and control. *Advances in Parasitology,* **44**: 233-337.

Rae, G. H. (1999). Sea lice, medicines and a national strategy for control. *Fish Veterinary Journal,* **3**: 46-51.

Richmond, J. (1999). *The effect of exogenous application of 20 hydroxyecdysone and methyl farnesoate on the moulting of free-swimming larval stages of the salmon louse, Lepeophtheirus salmonis.* M.Sc. thesis, University of Stirling, Scotland.

Sarusic, G. (1999). Preliminary report of infestation by isopod *Ceratothoa oestroides* (Risso, 1826), in marine cultured fish. *Bulletin of the European Association of Fish Pathologists,* **19**(3): 110-112.

Sayer, M. D. J. (1999). Duration of refuge residence by goldsinny, *Ctenolabrus rupestris*. *Journal of the Marine Biological Association of the United Kingdom,* **79**: 571-572.

SLICE (1999). *SLICE TM. Emamectin. Technical Monograph.* Schering-Plough Animal Health Corporation, Union, New Jersey.

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

Tillett, R., Bull, C. & Lines, J. (1999). An optical method for the detection of sealice. *Aquacultural Engineering,* **21**(1): 33-48.

Toovey, J. P. G., Lyndon, A. R. & Duffus, J. H. (1999). Ivermectin inhibits respiration in isolated rainbow trout (*Oncorhynchus mykiss* Walbaum) gill tissue. *Bulletin of the European Association of Fish Pathologists,* 19**(4)**: 149-152.

Tucker, C. S. (1999). *Larval settlement and epidemiology of* Lepeophtheirus salmonis, *Kroyer, 1837 (Copepoda; Caligidae).* Ph.D. thesis, University of Stirling, Scotland.

Tully, O., Gargan, P., Poole, W. R. & Whelan, K. F. (1999). Spatial and temporal variation in the infestation of sea trout (*Salmo trutta* L.) by the caligid copepod *Lepeophtheirus salmonis* (Kroyer) in relation to sources of infection in Ireland. *Parasitology,* **119**: 41-51.

Sealice 1999
– outcome of the 4th international conference on sealice control

Mark J. Costello,
Ecological Consultancy Services Ltd (EcoServe), 17 Rathfarnham Road, Dublin 6W; www.ecoserve.ie

This report summarises the key points raised by speakers, in poster presentations, and during open forum discussions at the conference. The titles and abstracts of papers presented are available at the website www.ecoserve.ie/projects/sealice.

For the first time, a meeting on sealice control (Box 1) could see light at the end of the tunnel in controlling sealice on salmon farms. The meeting reported the results of co-ordinated measures to control sealice on salmon farms in Ireland and Norway that demonstrated significant reductions in sealice on farmed salmon. Similar measures were being implemented by the Scottish industry, and being developed in Canada. This, the 4th international conference on sealice (Box 2)

- reported the latest results of research related to sealice control,
- described the latest management measures proposed to control sealice in Ireland, Norway, Scotland and Canada,
- enabled researchers, regulators and industry to meet each other so as to develop new ideas and promote co-operation,
- identified key areas for action by researchers, regulators and industry.

The conference was the largest ever on sealice, with 120 delegates from 11 countries; Norway, Scotland, England, Canada, USA, Chile, The Netherlands, Spain, Switzerland, Sweden and Ireland. Many of delegates remarked that they had learned something new and valuable at the meeting. This included the little known fact that the first attempt at growing salmon in sea cages was in Dublin Bay in 1862 (see book by Wilkins 1989, Aquaculture in Victorian Ireland).

The conference was the 3rd European meeting held as part of a project part-funded under the European Union FAIR (aquaculture) research programme (Box 3). This project has provided networking support for researchers, and improved access to information for industry, regulators and the public. Testimony to the success of the project is that delegates agreed to expand the network beyond Europe. To achieve this, an International Steering Group was established to plan the way forward. Several funding options were discussed, including linking with large research projects and obtaining subscriptions and sponsorship, but the issue of a funding mechanism remained unresolved.

The conference was sponsored by the EU FAIR programme, Schering-Plough Animal Health, Trouw Aquaculture, Bord Iascaigh Mhara (Irish Sea Fisheries Board), Bord Failte Convention Bureau (Irish Tourist Board), and the Marine Institute of Ireland.

The conference proceedings are being published in a special issue of *Aquaculture Research* and the sea-lice newsletter, *Caligus*.

Box 1.

Sealice are the most commercially damaging parasite on salmon farms in the world, and cost Norwegian and Scottish industries over 35 mECU each in 1996 and 1997. They kill salmon, reduce fish growth, facilitate disease transmission, damage salmon and reduce marketability, cross-infect wild fish and other salmon farms, and the chemicals used to control lice are a concern to the public because of potential environmental impacts and tissue residues. The control of sealice is thus a priority for both salmon farmers and aquaculture regulators. The large number of papers being presented at this conference demonstrated the efforts of all concerned to deal with the sealice problem.

Box 2.

Previous conferences on sealice or related to sealice control

	Dates	Location	Proceedings
1st	3-4 September 1992	Paris	Boxshall G.A. & Defaye D. (eds.). 1993. *Pathogens of wild and farmed fish: sea lice.* Ellis Horwood Ltd., London
2nd	6-9 November 1997	Trondheim	Report in *Caligus No. 2*
3rd	22-24 July 1998	Amsterdam	In journal *Contributions to Zoology*
4th	28-30 June 1999	Dublin	In journal *Aquaculture Research* and *Caligus No. 6*
In addition there has been one conference on the use of cleaner-fish to control sealice:			
	6-7 October 1994	Oban	Sayer M.D.J., Treasurer J.W. & Costello M.J. (eds.) 1996. *Wrasse biology and use in aquaculture.* Blackwell Scientific Publications, Oxford, 283 pp.

Box 3.

The EU FAIR project which initiated the conference was entitled *"Biology and management in the control of lice on fish farms"* (FAIR CT96-1615). It was managed by Mark J. Costello (Ecological Consultancy Services Ltd, Dublin), Geoffrey A. Boxshall (Natural History Museum, London), Kjell Maroni (KPMG, Trondheim, Norway), Per Gunnar Kvenseth (KPMG Trondheim and previously HydroSeafood Mowi a/s, Bergen, Norway), Andrew Barbour (HydroSeafood Mowi a/s), and Carmel Mothersill (Dublin Institute of Technology, Ireland).

Actions and outcomes of the EU project which has led to the holding of this conference

• provided a World Wide Web site	*http://www.ecoserve.ie/projects/sealice* which has received almost 2000 visits
• established an e-mail network	over 180 people subscribe to <Caligus@listserv.heanet.ie>
• published a newsletter	5 issues of *Caligus* newsletter sent to an average of 250 persons per issue
• established register of persons interested in lice control	about 240 persons on current mailing list from 24 countries, of whom 33 % are from industry
• hosted research-industry workshops and scientific conferences	In Trondheim 1997, Amsterdam 1998, and Dublin 1999
• produced a computerised list of scientific literature relevant to sealice control	bibliography of over 580 publications compiled in database, published in *Caligus*, and on web site

INTEGRATED LICE CONTROL

A common strategic to lice control was evolving in each country. This integrated farm practices, including stocking and harvesting times, and husbandry techniques, with measures to minimise the occurrence of lice and treatments to control infestations. The control measures involved:

⇒ fallowing sites, and single year class stocking to avoid transfer of lice between smolts (salmon first transferred to sea cages) and salmon near harvest size

⇒ stocking cages with cleaner-fish which preferentially eat the larger egg-bearing sealice where wrasse species were sufficiently available

⇒ co-ordinating and optimising the timing of chemical treatments and assess treatment success

⇒ leadership by state agencies in co-ordinating measures and fostering better control practices on farms

This integrated approach was similar to the development of Integrated Pest Management in agriculture where management measures were shown to be an essential and cost effective step in pest control. Difficulties in implementing IPM in salmon farms included the costs of fallowing sites, early harvesting, co-ordinating control, and costs and availability of treatments. There was optimism that IPM may be more successful in aquaculture than land-based agriculture due to the smaller size and better communication within the industry. In fact, good IPM was ideally suited to quality management schemes and was an integral part of codes of practice being developed by the industry. It also had parallel benefits in the control of other diseases and parasites on farms.

Specific, recent developments in IPM for sea lice control discussed at the meeting included the following:

Winter treatments

Results of treating lice, specifically egg-bearing (ovigerous) females, over winter showed significantly reduced infestations the following summer, and also reduced the risk of sealice transfer to migrating sea trout in spring. There was agreement that treating lice over winter was worthwhile although lice populations do not increase significantly at winter temperatures. Studies in Scotland recommended a winter level of not more than 0.5 egg-bearing lice per salmon.

Regulatory measures

While well-managed farms can voluntarily control sealice, and cooperate with other farms and authorities in doing so, regulatory control of lice is appearing in some countries. In Norway, it was decided to formally regulate lice control, and introduce fines for (the few) farmers who failed to control lice adequately. In Ireland, one farm had a formal agreement with a local wild fishery owner to keep lice below a certain level, and the aquaculture licences of some farms require lice control. Sampling of sea trout and arctic charr in two marine and freshwater (estuarine) sites in Norway found higher levels of lice infestation at the sites near fish farms. The data suggested that some heavily infected fish die at sea and do not return to the estuary. Regulatory authorities were thus placing increased emphasis on lice control on farms, both for the benefit of the farms and to reduce the risk of infestations of wild fish.

Use of cleaner-fish

The surprising discovery that cleaner-fish (wrasse) could effectively control lice on salmon farms in Norway in 1988, had now led to 4 million wrasse being used to control sealice in 80 % of the farms in Norway in 1998. The use of wrasse significantly reduces, and sometimes eliminates, the need to use of chemicals. Wrasse preferentially eat the larger sealice, and a typical sign of cleaner-fish control is the absence of egg-bearing and fewer adult lice than in cages without cleaner-fish.

Wrasse are particularly abundant in Norway and studies had found no impacts of fishing on wild stocks. Problems in the effective use of wrasse related to poor capture, handling and maintenance. Preliminary studies indicated that more research into wrasse husbandry requirements and behaviour would improve their effectiveness as cleaner-fish.

A major limitation on the use of cleaner-fish was that the smaller species (goldsinny, rockcook, corkwing) were only successful in cleaning salmon in their 1st year at sea. Norwegian studies had now discovered the larger ballan wrasse could control lice on 2nd sea-year (up to 7 Kg) salmon, but that more careful management of the wrasse densities was required. Some ballan ate up to 150 lice per day, and in aquaria ate 50 lice in 2 hours.

A concern over the use of wild wrasse has been the potential introduction of diseases into salmon cages. Wild wrasse have been found to be infected by a form of furunculosis but this does not infect salmon (even when injected). However, salmon furunculosis is pathogenic to wrasse. Attempts to transfer ISA from salmon to wrasse, and from wrasse to salmon, even by injection, failed. All known diseases and parasites of wrasse are not directly transmissible to salmon. To minimise the potential for disease transfer, wrasse are preferentially collected close to a farm, or from areas without farms. To certify the disease free status of wrasse, cultured wrasse may be required.

Successful commercial trials and use of cleaner-fish has been conducted in Ireland, Scotland, and Shetland. Until the ISA virus in Scotland, the industry there also used cleaner-fish widely. In contrast to chemotherapeutants, there were no corporate sponsors for the development of wrasse for lice control. However, as the experience has shown, they can play a valuable role on lice control. With the increasing cost of, and occurrence of lice resistance to, chemotherapeutants, it is likely that the use of wrasse may become more popular in future. To this end, preliminary studies on the potential of commercial scale culture of certified disease-free wrasse had been undertaken in Norway and Scotland.

EDUCATION AND TRAINING

Continuing education of farm management and staff, and local veterinarians and regulatory authorities, was essential for effective lice control. Not only was it necessary to understand lice biology, but staff needed to use standard and reliable methods for counting sealice, and be able to use control measures from cleaner-fish to chemicals in the most effective and safe manner.

AVAILABILITY OF CHEMOTHERAPEUTANTS

For over 10 years the only chemical used to control sealice was dichlorvos and a closely related compound, trichlorfon. New options slowly appeared in the early 1990’s and now a range of products was at an advanced stage of development (Box 4). There had been successful commercial trials of several chemicals added to fish feed (‘in-feed’ treatments) and new bath treatments. Some of these chemicals have different modes of toxicity, which could be used to reduce the risk of lice developing multiple resistance. The increased variety of chemical treatments was welcomed because previous meetings had concluded that no one treatment could ever provide universal control of sealice. The approval of additional chemotherapeutants for sealice control would decrease the use of chemicals, which may be used in the absence of, approved options.

Once a compound was found to be efficacious in lice control and not harmful to the salmon, there was still a complexity of regulations regarding tissue residues (maximum residue limits, MRL) and environmental impacts to be addressed. Regulators and pharmaceutical companies had to act in a transparent and defensible manner to demonstrate careful consideration of public and environmental health issues. As part of regulatory approval, studies were conducted to determine MRL and environmental threats before the general use of the compounds by industry.

The restricted or lack of release of information on these topics fuelled public concerns with negative consequences for the image of the industry. Public concerns could delay or prevent regulatory approval. The approach should be to fully inform the public. Given the availability of information and willingness of a manufacturer to get a chemotherapeutant licensed, there remained scope for better co-ordination of the regulatory processes within and between countries.

Resistance to chemotherapeutants

The development of resistance by some populations of sealice to 3 chemicals (dichlorvos, azamethiphos and hydrogen peroxide) was described, and compared to the development of resistance in agricultural pests. The reality of lice being able to rapidly (1-2 years) develop resistance, retain this resistance over years, and develop cross- and multiple- resistance to chemotherapeutants was fully appreciated for the first time at this conference. It seemed likely that there are populations of lice already developing resistance to other chemotherapeutants. The management of sealice control needed to consider routine monitoring for resistance to chemotherapeutants, as already occurs for antibiotics in aquaculture and pesticides in agriculture. Specific plans to avoid the development of resistance should be produced. However, implementing them may be difficult without strict veterinary and regulatory control because it was tempting for a farmer to treat with the latest and most effective treatment until it no longer worked.

Box 4.

Chemotherapeutants used in the control of sealice.

Active ingredient	Trade name
Dichlorvos	Aquagard, Nuvan,
Azamethiphos	Salmosan
Hydrogen peroxide	Salartect, Paramove
Cypermethrin	Excis
Deltamethrin	Alpha max Vet
Pyrethrum	Py-Sal
Ivermectin	Ivomec Pre-mix for pigs
Emamectin	SLICE
Teflubenzuron	Calicide, Ektobann
Diflubenzuron	Lepsidon

ROLE OF LICE AND HOST BIOLOGY

In parallel with the need to develop methods to treat lice on farms today, is a range of studies on lice biology and related aspects of salmon biology and behaviour. Better understanding of the fundamentals of lice growth, reproduction, dispersal, host location, diet, and host impacts, as well as of salmon behaviour in sea cages and the wild, may provide new ways to interrupt the infestation cycle and reduce impacts on the host.

Studies suggested that the distribution of salmon in cages affected their rate of infection by sealice larvae, and that artificial light may influence lice burdens by affecting lice and/or salmon distribution. The manner of feeding salmon also affected their distribution in the cage. Better understanding of these factors may result in husbandry measures that reduce lice levels. Another simple method of reducing lice levels is to trap lice dislodged off salmon during size grading, and special grading machines have been designed for this.

An exciting new discovery in lice biology reported at the conference was that adult male lice can detect and will swim towards the odour of salmon in the water. It would thus appear that chemical as well as physical cues might be important in lice finding their hosts in the sea. Previous beliefs considered the role of chemical cues to be unlikely.

New findings on the susceptibility of salmon to sealice showed that different strains of salmon had different responses to sealice, that more lice infected immuno-suppressed salmon, and that even low levels of 3 adult lice per fish had detectable impacts on salmon health. There may also be differences in biology between lice populations, and the first studies producing genetic markers for sealice were also reported at the conference.

FUTURE RESEARCH

Further research was required to assess what levels of lice should be a trigger for treatment. Laboratory studies indicated lice cause significant stress and harm to salmon at levels below which visually apparent skin damage has occurred.

There was general agreement in the frequency of monitoring sealice (fortnightly in summer), that generally at least 15 fish per cage should be counted, and that at least the pre-adult, adult, and egg-bearing lice should be recorded. A concern raised was that if it was required to keep the level of lice below a certain level, that an unreasonable number of fish may need to be counted to detect this level with any accuracy. At present different monitoring programmes require sampling of 15 to 30 salmon per cage, in at least 2 cages per cage block. The issue of the best sampling approach still needed further study to address the statistical requirements and practical limitations.

There was recognition that not only was the average number of lice per fish important in maintaining the lice population, but the total size of the lice population on the farm, i.e. the average number multiplied by the total number of fish. Maintaining the average lice burden per fish could prevent significant damage to the farmed salmon. However, to reduce the viability of the lice population on the farm, and reduce the risks of transfer of lice to adjacent farms and wild fish, would require the total number of lice to be taken into account. Lice control now needed to address the threats not only to farmed fish, but also to wild fish.

A key problem in knowledge of lice biology is the few studies that have been conducted. There is minimal replication of observations in different countries and conditions, such that early mistakes and miss-interpretations may have gone unnoticed. There is also considerable citation of unpublished ‘grey’ literature that has not been peer-reviewed and is difficult to get hold of. In order to model lice population dynamics in particular, more information is needed on lice longevity, generation time, body size variation, egg production rates, effects of temperature, host and salinity, seasonal dynamics on fish in both farms and the wild.

A particular difficulty in developing methods to assess the environmental risk of new pesticides to marine life, was the lack of information on what the ‘natural’ dynamics of the fauna and flora around fish farms was. Such information would greatly facilitate the design of ecotoxicological experiments and monitoring of impacts.

In Norway, an important additional source of lice was from escaped farm fish that typically had lice burdens higher than wild fish. A preliminary budget of lice levels on wild, escaped and farm fish illustrated the need for a quantitative approach to understanding the dynamics of lice populations, and the importance of escapees, wild fish and farmed fish in perpetuating infestations.

Ultimately, integrated lice control would need to incorporate economic aspects of treatment costs into the long-term viability of the farm. At present, lice control was necessary at almost any cost. However, as lice management became more effective, the cost effectiveness of longer-term options, such as fallowing entire bays, could be evaluated.

SUMMARY

- effective lice control now demonstrated on many farms.
- further research to define what an acceptable level of lice is on a farm was required.
- lice control needed to address the risks to wild as well as farm fish.
- several new compounds are being evaluated for lice control, which may lead to better use of Integrated Pest Management.
- lice could rapidly develop resistance to chemotherapeutants.
- plans to avoid the development of resistance to chemotherapeutants were required.
- assessment of potential and actual impacts of chemotherapeutants on marine life was compromised by insufficient understanding of the dynamics and ecology or fauna and flora around fish farms
- submission of studies on safe levels of chemotherapeutants in fish tissues and for the environment was necessary for regulatory approval, and prior publication is desirable to allay public concerns.
- better co-ordination of regulatory systems for chemotherapeutants within and between countries was needed.

ACKNOWLEDGEMENTS

Myron Roth, Ian Davies and John Smith are thanked for their helpful suggestions on a first draft of this report. However, its content is the authors responsibility and can not be considered to represent the views of any particular delegate of the conference.

Progress with publication of the conference proceedings

Some papers based on presentations given at the sea lice conference were submitted for publication in this newsletter, or in a special issue of *Aquaculture Research*. The 10 papers accepted so far following external peer-review are listed below. An additional seven papers are being reviewed and may be published in this and subsequent issues of *Aquaculture Research*.

Branson, E. et al. Efficacy of teflubenzuron (Calicide) for the treatment of sea lice (*L. salmonis*) infestations of farmed Atlantic salmon

Davies, I & Rodger, G.K. A review of the use of ivermectin as a treatment for sea lice (*L. salmonis* and *Caligus elongatus*) infestation in farmed Atlantic salmon.

Finstad, B, Bjorn, P.A., Grimnes & A., Hvidsten, N.A. Laboratory and field investigations of salmon lice (*L. salmonis*) infestation on Atlantic salmon postsmolts.

Heuch, P.A. *et al.* Egg production in salmon lice (*L. salmonis*) in relation to temperature and origin.

Lyndon, A. & Toovey, J.P.G. The effects of feeding regimes on sea louse (*L. salmonis*) infestation on farmed Atlantic salmon.

Nolan, D.V. *et al.* Development of microsatellite PCR typing methodology for the sea louse, *L. salmonis.*

Nolan, D.T. *et al.* Juvenile *L. salmonis* affect the skin and gills of rainbow trout *Oncorhynchus mykiss* and the host response to a handling procedure.

Poole, R. *et al.* Modelling the effects of capture and sea lice infestation (*L. salmonis*) on the cortisol stress response in trout.

Treasurer, J.W. *et al*. Resistance of sea lice, *L. salmonis*, to hydrogen peroxide on farmed Atlantic salmon.

Tully, O. *et al*. Variation in sensitivity of sea lice (*L. salmonis*) to dichlorvos on Irish salmon farms in 1991-1992.

A standard protocol for lice sampling on salmon farms

Per Gunnar Kvenseth and Per Andersen[1]
KPMG Center for Aquaculture and Fisheries and [1]Aquaculture Consultancy

This paper was drafted following discussions at the 1997 workshop on sealice as part of the EU FAIR Concerted Action project entitled "Biology and management in the control of lice on fish farms", Trondheim 6 - 8 November 1997. This protocol for lice sampling is proposed to further the development of a standardised EU salmon farming method, to be used by all salmon producing fish farmers. It is not intended as a method for research purposes. For the latter, it is agreed that the registration method has to be adjusted in accordance with the purpose of the investigation.

Important questions to evaluate before sampling

Before the sampling of lice it is important to be aware of the following questions:

- Which species and stages of lice are supposed to be present?
- How many fish and cages are to be sampled?
- How often will the sampling be done?
- Will it be of use to register other damages to the fish?

The person responsible for the registration has to be trained to identify the different species (*Lepeophtheirus salmonis* and *Caligus elongatus*), and separate between the different stages.

The questions on how many and how often means that the more often you sample the fish, the less fish you have to sample each time. Many farmers advise is that it is more important to sample a few fish often (every week), than many hundreds of fish each month.

It is recommended that notes are taken about other damage to the fish on an individual basis (e.g. spinal damage, mouth deformations, gill cover damage, winter wounds, snout wounds, cataract, maturation etc.).

Subsamples for lice counting in a salmon pen

To get an unbiased representative sample of fish you need a special knotless net (smooth in order to avoid damage to the fish), called a sampling net (orkastnot). This net is used to partition the cage. It should have such a length that it will reach over the whole pen, and to a depth corresponding to the depth of the lifted pen. This separates off part of the fish in the pen and concentrates them so they are more easily captured by hand or dip net. It is also important that the leadrope at the bottom of the sampling net is heavy enough so that the fish cannot escape. Use of feed to attract the fish into the sampling net may give false results, as it will tend to attract the hungriest fish and the fish in best condition.

Netting

To handle the fish carefully it is important to use a knotless dip net. To get the correct measurements, it is important to get a representative sample. When netting it is important to get a vertical cross-section of the fish. The net should be brought deep into the sampling pen and raised. Avoid sampling the fish at the surface. Bring the fish (1-3 at a time), carefully over to a bath with anaesthetic added to seawater. The bath should hold at least 80 - 100 litres and be painted preferably in white so that the lice that fall off can be seen. It is not advisable have more than 5 - 8 fish in the bath simultaneously.

Anaesthetic

The fish must be anaesthetised before lice counting can start. Several types of anaesthetic are available. Good results have been attained previously with the use of benzocaine, 5% in propylene glycol, 10 ml to 10 litre of seawater. However Benzocaine does not have a maximum residue limit (MRL) and from 1 January 2000 is no longer allowed to be used on fish for human consumption in the EU. We therefore recommend the use of metacain for the anaesthetic of salmon in production. Remember to always use good rubber gloves. Anaesthetise the fish so that they are calm, but be careful not to leave them in the anaesthetic for too long. Lift the fish out of the water like treating a baby, do not lift the fish with a tough grip around the tail. If you do so the fish may panic. The seawater in the anaesthetic bath should be oxygenated or changed often to avoid mortality from lack of oxygen. Remember to check the anaesthetic bath for lice when you have finished counting a pen, and add the number of lice you find to the fish sampled in proportion to each fish's lice load.

Number of fish to be counted

It is recommended that lice are counted on at least 15 fish (preferably 20) in every second pen spread around the farm. For most of the farms it is recommended that lice counting is undertaken in one fixed pen at every counting, and an additional count in the 3-4 pens which seem to have the heaviest lice burden. If for some reason you want to only count a few fish from each pen, this can be compensated for by frequent sampling (weekly).

Counting frequency

One counting day a month is suggested as a reasonable frequency for lice counting, but this should be increased when the temperature is high (weekly counts are recommended when temperature is above 12 (C). In the spring when increasing light and temperature speeds up the development time for the lice, the counting must be on a weekly basis.

Counting methods

It is easiest to count the lice before weighing the fish and making other evaluations. Hold the fish over the anaesthetic bath that it will only fall into the bath if you let it go by mistake. A good alternative is to place the fish on a table covered with a rubber cloth (e.g. neoprene).

Counting procedure

To ensure a good basis for decision-making and to evaluate the necessity for delousing, it is important to distinguish between four different lice categories. This procedure can be aided by the use of plastic covered illustrations of the different lifestages of the lice, which are produced by several feed companies.

Category A: Reproductive "cod lice" - (*Caligus elongatus*), covers both males and females. Maximum 10 mm long - usually much paler than the traditional salmon lice, moves easily around on the fish, with or without touching it.

Category B: Reproductive "salmon lice" (Lepeophtheirus salmonis)
Length 18 -25 mm. Easy to separate from other lice and other stages. Females with two long egg strings. Important to count - gives information about the reproduction potential from the farm. Reproductive lice are often found at the operculum, at the back of the fish, behind the adipose fin and anal fin in addition to the tail.

Category C: "Mobile" lice (pre-adults, males) of both species (difficult to separate species at this stage). They will all be rather loosely attached to the fish. If you are uncertain, touch the lice with your finger, and they will be loose.

Category D: Larval stage (chalimus stage) - looks like short pencil stroke. These are smaller than category C. If you touch them with your finger they will be attached to the fish. This stage is often found on the belly side, or under fins (pelvic and pectoral), fin basis etc.

If you prefer to reduce the number of categories to three, you can combine categories A + B. Category D will always be underestimated. It is difficult to find (and count) all the chalimus stages, but it still gives a picture of the situation as long as you do the counting in a standardised way each time.

Registrations of the counting results

Use a standard form to write down the results of the count, fish by fish. Several feed companies produce very good forms for this use, on waterproof paper. A soft pencil is best suited for writing on these. When a pen (cage) is sampled, summarise all lice in each category and divide by number of fish counted from the pen. The number you get (average) can be compared with the previous count results. Keep a record of the count on each fish so you have information on how variable the lice infestation is. Sometimes one fish may have more lice than others or several fish may have no lice. For an overview, it is recommended that you put the data into a computerised worksheet (Excel, Lotus 123 etc.) and graph the development of lice over time.

A lice sampling protocol developed as part of a research project on cleaner-fish technology

Mark J. Costello, Ecological Consultancy Services Ltd (EcoServe)

This protocol is based on a 1991 report to the European Commission as part of the FAR project on ‘Cleaner-fish Technology’ (1991-1993). It was developed and used by researchers at Trinity College Dublin, University of Aberdeen, University College Galway and Fanad Fisheries Limited. The following persons assisted in its development: M. J. Costello; W. Darwall; A. Pike; O. Tully; D. McCorry; S. Lysaght; S. Deady.

EQUIPMENT

- Two large holding bins (e.g. plastic dustbin),
- measuring board,
- benzocaine anaesthetic (40 g l^{-1} acetone),
- specimen tubes with 70 % ethanol,
- plastic sheet,
- 0.5 mm mesh sieve,
- blunt forceps,
- waterproof writing pad, recording forms and pencil.

PROCEDURE

Preparation

⇒ Half-fill bin with about 55 litres of seawater, and add 3-4 capfuls (ca. 60-80 ml) of benzocaine.
⇒ Lay out the plastic sheet to lie anaesthetised fish on.

Fish capture

- Abnormal or moribund fish are not sampled as they often have abnormally high numbers of lice.
- At least 15 fish are sampled per cage. Statistical analysis has shown that 15 fish will provide a standard error of about 20 % of the mean number mobile (pre-adult and adult) lice per fish.
- Throw a handful of food into the cage and remove fish by a knotless handnet.

Handling fish

- If possible, fish should be starved for 12 hours before being anaesthetised. The effects of exposure to rapid change in temperature during sampling should be minimised (e.g. avoid direct sunlight, allowing bin to warm up).

⇒ Fish are left in benzocaine until they cease to move, but never more than 5 min.
⇒ Anaesthetised fish are removed from the holding container one at a time, laid on the plastic sheet, their standard length noted, and mobile lice removed by hand.
⇒ After lice sampling, and assessment of lice damage and general fish condition, the fish is carefully returned to the cage to recover.
⇒ In strong currents or rough sea conditions the fish can be allowed to recover from the anaesthetic while in a holding net.

Lice sampling

⇒ All lice are removed from the fish, counted and categorised as chalimus (sessile larvae), mobile (pre-adult and adult), and egg bearing stages. Chalimus stages are underestimated by this (visual) sampling and must be reported separately from mobile stages.
⇒ If lice are required for population monitoring or research purposes, all lice from a cage are fixed and stored in 70% ethanol in a specimen tube.
⇒ Between cages, the anaesthetic is poured through the sieve into the second holding bin. Any additional lice are counted and added to the specimen tube for that cage.

LICE DAMAGE ASSESSMENT

●	Category 1	No apparent lice damage
●	Category 2	Light skin damage observed as grey patches (where mucus is missing)
●	Category 3	Light bleeding in areas where lice were congregated
●	Category 4	stripping of the skin to expose muscle or bone.
●	Category R	signs of skin recovery after lice damage (indicative of past infection).

RECORDING

A standard recording form is essential to ensure no information is omitted. The form should include sampling date, personnel, sea temperature, cage number, fish number (1-15), number of mobile and sessile lice on each fish, and a comment on the health status, recent lice treatments, and history of the fish sampled.

The described method of fish capture was followed by most farms, and all farms in the west of Ireland and in Shetland during the project. Some alternative methods of fish capture were practised at individual farms:

- Place a 1 m diameter, 2 m deep, floating net into the cage several hours before sampling. Fish will accidentally jump into the net. This method was used for smolts at one site where the farmer considered it minimised stress to the fish. It is time consuming and may obtain a biased sample.
- The cage net can be pulled in to congregate the fish so they can easily be removed with a handnet. This method was used at one site for large (> 2 Kg) salmon.
- A large triangular (1 m each side) net is sunk and raised through the cage, thereby capturing the fish. This method was used at one site

---

Critical comments on lice sampling protocols

Mark J. Costello

Three lice sampling protocols have now been published in Caligus (Treasurer 1998, Kvenseth and Andersen 2000, Costello 2000), and another developed as part of the government lice monitoring programme in Ireland (Jackson *et al*. 1997). There are similarities in each but also differences. Indeed, within each protocol there is scope for some variation in methodology.

Fish capture

There is no information on the effects of different methods of capturing fish on the lice count. If fish capture methods significantly effect results then the accuracy of past and current data may be questionable. Some methods used food to attract fish to a dip net, another recommend against it and advise a vertical sweep of the net to avoid selecting for fish at the surface.

Number of fish sampled

The recommended sample of fish in the protocols varies from 15 (Costello 2000) to 20 (Kvenseth and Andersen 2000) to 30 (Jackson *et al*. 1997) per cage. Only the first mentions any prior statistical analysis and this found > 15 fish to be a minimum to get a 20% precise estimate of mobile lice. This study found over 25 fish would be required to get an estimate of egg bearing lice with a 20% error (Costello, unpublished data). Thus if the aim is to sample egg bearing lice then only the latter protocol (Jackson *et al*. 1997) is likely to achieve this.

Number of cages

The number of cages sampled ranged from all (Costello 2000) to as many as possible and more if few fish sampled per cage (Kvenseth and Andersen 2000), to a ‘standard’ and ‘randomly’ selected cage (Jackson *et al*. 1997). The first study needed to get an accurate estimate of lice from each cage. However, sampling a farm aims to census the lice on a block of cages. It may then be preferable to sample fewer fish in more cages as recommended by Kvenseth and Andersen (2000). However, the accuracy of each of these options should be statistically tested. This may be possible using existing data.

Frequency of counting

On this issue there is more consensus, with monthly sampling in winter (< 10ºC) and fortnightly sampling in summer (> 12ºC).

Data management

While all methods indicate the scope of the data collected this could be more explicit. A standard recording form is required from which the data is entered into a database (or spreadsheet). The details of the information must be retained including the count for each fish (not an average per cage or farm), who conducted the sampling, fish health, lice treatments, environmental conditions, cage numbers, etc.

Lice life-stages censused

All protocols recognise that it is not possible to accurately count chalimus stages by eye in the field. It may be possible to categorise chalimus abundance to speed up counting (e.g. < 5, < 25 etc.). The protocols agree that mobile and egg-bearing lice can be accurately distinguished and counted and egg-bearing *Caligus* from *Lepeophtheirus*. However the Norwegian regulatory monitoring requires all mature female lice to be separately recorded whether with eggs or not (Eithun 2000).

Conclusion

The need for standard methods for sampling sealice on salmon is greater than ever, because of the need to optimise control methods and regulate lice levels on farms. However, the effects of factors influencing the accuracy of lice counts such as fish capture methods, number of fish sampled per cage, number of cages sampled per farm site, have not been sufficiently researched to define the best monitoring methods.

References:

Costello, M. J. 2000. A lice sampling protocol developed as part of a research project on cleaner-fish technology. *Caligus* No. 6, 22-23.

Eithun, I. 2000. Measures to control sea lice in Norwegian fish farms. *Caligus* No. 6, 4-5.

Kvenseth, P.G. & Andersen 2000. A standard protocol for lice sampling on salmon farms. *Caligus* No. 6, 21-22.

Jackson, D., Deady, S., Leahy, Y. & Hassett, D. 1997. Variations in parasitic caligid infestations on farmed salmonids and implications for their management. *ICES Journal of Marine Science* 54, 1104-1112.

Treasurer, J. & Grant, A. 1998. The Marine Harvest McConnell surveillance system for sealice infesting farmed Atlantic salmon. *Caligus* No.4, 5-6.

Development of data based models for effective treatment and the environmentally safe use of veterinary methods in the control of sea lice infestation of farmed salmon

(A LINK Aquaculture Project - ENV12 - funded by MAFF, Marine Harvest and SSGA)

This multidisciplinary project was initiated in response to the LINK Aquaculture call for the development of disease control methods that minimise the use of veterinary medicines as well as the environmental impact of aquaculture operations. Its scope was informed by a Working Group report to the Co-ordinator of Fisheries Research and Development (June 1998) which recommended that target areas for research should include, the development of mathematical models for sea lice population dynamics, standardisation of data collection methods & the establishment of a central database

Project Objectives

- Audit of Marine Harvest (and other accessible) databases
- Identification of risk factors
- Creation of appropriate mathematical models
- Validation of models
- Proposals for designed experiments

Approaches and Methods

The project will explore a range of research questions and adopt methods which are deemed to be appropriate to address the key issues in the light of on-going interaction between the academic and industrial partners. It seems likely these will include:

- ***Database Auditing and Analysis*** - what data exist?; what data are necessary?; which standards apply?; how can data be converted to knowledge? Methods: data cleaning/aggregation; meta-data description; data models; KDD; data fusion
- ***Statistical Analysis*** - what are the correlates of risk?; how might models be parameterised? Methods: multifactorial regression; GLM, log linear and logistic approaches; time series analysis; data mining and neural networks
- ***Mathematical Modelling*** - what are the effects of control on sea lice populations?; how might the emergence of resistance be modelled?; which key factors must be included? Methods: compartmental and site-specific modelling; other population modelling approaches
- ***Designed Experiments*** - what and how should new data be generated? Methods: statistical process control; process specification/design; Taguchi methods
- ***Decision Support*** - how can any findings be delivered at policy and farm level? Methods: user requirements analysis; knowledge representation and delivery; systems integration of models, knowledge bases and hypermedia

If successful these models and their appropriate delivery have the potential to support management decisions and aid the conservation of useful veterinary product.

Participants:

- University of Strathclyde and Glasgow University
- Marine Harvest McConnell
- Scottish Salmon Growers Association

The project commenced on the 1st October 1999 and will run for a duration of three years.

Contact Details: Crawford Revie, Department of Statistics and Modelling Science, University of Strathclyde, Livingstone Tower, Richmond Street, Glasgow G1 1XH. Tel:0141-548 3805; Fax:0141-552 2079; Email: crawford@dis.strath.ac.u

Acknowledgement and project information

This newsletter is part funded under the European Union FAIR (aquaculture) research programme as part of a three year project (1997-1999) entitled 'Biology and management in the control of lice on fish farms' (contract number CT96-1615). The project was a 'Concerted Action' and so did not involve new research but rather facilitated the communication of information related to the control of lice on fish farms. This is the last issue of Caligus to be published under this project.

Achievements of the project:

provided a World Wide Web site	*http://www.ecoserve.ie/projects/sealice* which has received over 2500 visits
established an e-mail network	over 180 people are subscribed to <Caligus@listserv.heanet.ie>
published newsletters	6 issues of *Caligus* newsletter sent to an average of 250 persons per issue
established register of persons interested in lice control	over 300 persons on current mailing list from 27 countries, of whom 33 % are from industry
produced a computerised list of scientific literature relevant to sealice control	bibliography of over 640 publications compiled in database, published in *Caligus*, and on web site
hosted research-industry workshops and scientific conferences	In Trondheim 1997, Amsterdam 1998, and Dublin 1999
Published two special issues of international scientific journals	'Contributions to Zoology' and 'Aquaculture Research'

The project was co-ordinated by Dr Mark J. Costello (EcoServe, Dublin) in partnership with Dr Geoffrey A. Boxshall (Natural History Museum, London), Mr Kjell Maroni (KPMG, Lauvnes), Dr Andrew Barbour (and Mr Per Gunnar Kvenseth) (Norsk Hydro a/s), and Dr Carmel Mothersill (Dublin Institute of Technology). Enquires about the project or newsletter should be sent to: mcostello@ecoserve.ie; Dr M. J. Costello, Ecological Consultancy Services Ltd, 17 Rathfarnham Rd, Terenure, Dublin 6W, Ireland.

This newsletter is printed on recycled paper