

Overview

Integrated Pest Management of Sea Lice in Salmon Aquaculture



Courtesy of the WATERSHED WATCH Salmon Society



Health Santé Canada Canada



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This publication is the result of a collaborative effort between the Salmon Health Consortium and the Pest Management Regulatory Agency. The content is not regulatory in nature. It reflects the opinions of a diverse group of stakeholders and is intended to support the excellence and the sustainable development of the Canadian salmon industry.

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Foreword

This document was produced by a National Working Group on Integrated Management of Sea Lice, organized by the Pest Management Regulatory Agency (PMRA) and Salmon Health Consortium under a PMRA Integrated Pest Management Partnership Project.

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1. Introduction

Sea lice are parasitic copepods belonging to the family Caligidae. They are natural parasites of wild salmon and other marine fish, but can cause serious disease in farm-reared salmonids. Serious outbreaks of sea lice were first reported in Norway during the mid-seventies following the development of systems for the intensive rearing of salmon in marine net pens. Since then, sea lice infections have been reported in most regions around the world where salmon are farmed.

In Scotland, sea lice are considered the most economically important disease and in 1996 were estimated to cost the industry the equivalent of \$70 million from direct mortality losses, treatment costs, lost growth, and labour to deal with the problem. In Canada, serious disease caused by sea lice has occurred in stocks of farmed salmonids on both coasts, but most notably within the New Brunswick salmon farming industry. In New Brunswick, sea lice infestations of farmed salmon have recently become a major economic and environmental problem for the industry, and in 1995, economic losses due to sea lice were estimated at \$20 million. In addition to direct damage to salmon, sea lice have been implicated as vectors of viral and bacterial diseases of fish.

While the ability to deal with outbreaks is often the most pressing concern of those involved in salmon production, there is acknowledgement that the use of long-term integrated strategies for managing sea lice is important to the sustainability of the industry and the environment in which it operates. This document is produced by a working group comprising salmon producer representatives, therapeutant manufacturers, researchers, federal and provincial government officials, and others in order to address that need. The document provides an overview of sea lice biology and management tools, and recommendations for integrated management of sea lice and for further work.

The information in this report is based on a review of recent scientific literature and experience of the participants. While individual literature citations are not provided, a reference list of useful sources is included.



2. Biology of Sea Lice

Species

In Canada and the northern United States (U.S.), five species of sea lice have been reported to infect pen-reared salmonids, including *Lepeophtheirus salmonis* (Atlantic and Pacific Coast), *L. cuneifer* and *Caligus clemensi* (Pacific Coast), *C. elongatus* and *C. curtus* (Atlantic Coast). Of these species, *Lepeophtheirus salmonis* (commonly referred to as the *salmon louse*) is responsible for the majority of serious disease outbreaks. Being able to distinguish the two genera (*Lepeophtheirus* and *Caligus*) is critical to making informed, effective management decisions. Farmers should become aware of key diagnostic features (Figure 1). Differences between the various species are very subtle and usually of more academic than practical interest. If necessary, expert advice should be sought for definitive identification of species.

Hosts and reservoirs

Lepeophtheirus salmonis is limited in its host range to salmonids, except for very rare cases. Caligus spp. are more cosmopolitan in distribution and have broad host ranges that include both salmonids and non-salmonids. Many of these salmonid and non-salmonid hosts are common in the vicinity of marine grow-out sites and may serve as reservoirs for sea lice infections of cultured salmonids. In most instances wild infections are not severe, but there are documented cases of epizootic outbreaks under certain environmental conditions, such as low river run-off and high temperatures in confined inlets where migrating salmon may congregate before travelling upstream. Due to their preference for salmonids, *L. salmonis* infections tend to be more chronic and persistent in nature. In contrast, *Caligus* spp. infections tend to be more acute and transient.

Life cycle

Sea lice commonly have ten developmental stages (Figure 2). These include two free-living nauplius stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two pre-adult stages, and one adult stage. In some species, notably *Caligus*, the pre-adult stages may not occur. Between each of these stages, sea lice undergo a process called moulting. During the moult, sea lice shed their old external skeleton (exoskeleton), exposing a new exoskeleton that has developed beneath. Once sea lice reach the adult stage, moulting ceases.











Figure 2: The life cycle of the salmon louse



The two naupliar stages are free-swimming in the plankton and serve as dispersal stages. The naupliar stages are non-feeding and not infectious. The copepodid stage is also free-swimming and non-feeding until a suitable host is encountered, and attachment takes place through the use of specially adapted clawed appendages. Moulting to the chalimus stage is characterized by the development of an attachment organ known as the frontal filament, which anchors the growing louse to hard structures such as scales, cartilage and fin rays on the host (Figure 3). Pre-adult and adult lice shed the frontal filament and freely roam over the surface of the host. The attached copepodids, chalimus, pre-adult and adult stages of sea lice feed on mucus, skin and blood. Once settled on the host, lice generally remain. Pre-adult and adult lice, however, may move from host to host. Such movement occurs in Lepeoptheirus spp, but is more prevalent in Caligus spp. As a result, infection by Caligus spp. pre-adults and adults often occurs when large numbers of infected non-salmonid hosts become resident in the vicinity of net pens.

Egg production and development rate

Sea lice carry their eggs as long strings that trail from the posterior edge of the genital segment (Figure 3). The number of eggs produced by sea lice is highly variable, depending on the species of sea lice as well as environmental (seasonal) and host factors. In general, *Caligus* spp. produce fewer eggs per female (<200) than L. salmonis females, which can produce from 100 to over 1200 eggs.

Water temperature is thought to be the primary controlling factor in the number of eggs produced. Photoperiod is also believed to have an effect on egg size and number. Host species, age, state of maturation, health and other physiological factors can also affect the number of eggs carried by sea lice. Low salinity (<15 ppt) and low water temperature (<3°C) markedly reduce egg hatching success. Egg production is a continuous process and once hatching has occurred, new egg sacs can be extruded within one day.

The development rate of eggs and the non-feeding planktonic stages (nauplius and copepodid) of sea lice is primarily controlled by water temperature, although other factors such as salinity may have some effect. The development rate of the attached stages (copepodid through adults), in addition to being affected by temperature and other environmental variables, is also affected by host factors such as the species of host and host immunity. For example, development is faster on Atlantic salmon than chinook salmon. Depending on water temperature, development times range from 3 to 13 weeks, and average 7.5 weeks at 10°C for L. salmonis (Table 1). There is little development below about 5°C.







Water Temperature (°C)	C. elongatus	L. salmonis
7	12	13
10	5	7.5
16	3*	5

 Table 1. Estimated egg to gravid female generation time in weeks for two species of sea lice

*Estimate only, no data available.

Behaviour and dispersion of sea lice planktonic stages

Better knowledge of the distribution and behaviour of the planktonic stages of sea lice (nauplius and copepodid) in the wild would assist in site selection and the development of management plans to reduce the level of sea lice infections. Cues such as light, presence of chemicals, water pressure and water flow have been suggested as important in the behaviour of these stages, including host location by the copepodid stage. Unfortunately, knowledge of the importance of these cues and where these stages occur in the water column is extremely limited.

The copepodid stages of sea lice have relatively well-developed eyes, suggesting that vision plays an important role in behaviour and host location. The structure of the eyes of the copepodid suggests that they may have an image-forming capacity allowing for precise location of light and shadow sources when searching for hosts. In the laboratory, both the nauplii and copepodids have been reported to have a strong positive phototaxis to direct light sources. In addition, there is a report that fish reared in deeper pens (20 m) suffer lower rates of infection than fish reared in shallow pens (5 m). It has been speculated that free-swimming larvae are attracted to the surface of the water column, and that salmon become infected during the day when they rise to the surface to feed.

Any factor that decreases water movement through sea cages may result in the retention of higher numbers of nauplii and copepodids within the sea cage. In studies of comparative numbers of larval lice inside and outside net-pens, the highest levels of larvae can consistently be found within the sea cages compared to the relatively few larvae recovered from waters surrounding the sea cages. Reduced water movement through the sea cages due to fouling on nets undoubtedly contributes to retaining larval lice within pens, thereby facilitating infections.

Signs of disease

Disease is caused by the feeding activities of the sea lice as adults and pre-adults, which tend to congregate on the head and the back near the dorsal and adipose fins and the vent. In naturally and experimentally infected fish, copepodids and chalimus larvae can be found on all the body surfaces, but are generally more abundant on the fins. Copepodids are very small (<1 mm in length) and are difficult to see by the untrained eye and can be easily overlooked. Their small size

requires the use of a magnifying glass or dissecting microscope to detect their presence. Thus, pre-adults and adults are usually the first stages observed on fish. They range in size from 3 mm to 10 mm depending on the species and whether they are pre-adults or adults. Their colouration ranges from a translucent yellow or brown to dark brown. Females often trail long paired egg strings that become progressively darker as eggs mature.

Heavily infected salmon may show increased levels of leaping or flashing. Infections with high numbers of chalimus larvae can often lead to fin erosion that in severe cases can result in complete fin loss. As infections progress, lice numbers increase and damage can be seen as a result of the feeding of the larger pre-adult and adult stages. The damage observed on the dorsal surface of fishes usually follows a progressive pattern: grey patches, white patches, red patches.

Red patches represent areas of extensive damage, tissue loss and hemorrhaging. If infections are allowed to progress to this state, the damage reduces the market value of the fish, will often lead to secondary infection, e.g., vibriosis or furunculosis, and can ultimately result in death. It has been suggested that sea lice may act as vectors for some infectious diseases, such as the virus responsible for infectious salmon anemia.

The relationship of the number of sea lice to severity of the disease is dependent on several factors including:

- the size and age of the fish;
- the general state of health of the fish; and
- the species and developmental stages of the sea lice present.

Factors affecting the susceptibility of salmon to sea lice

All species and stages of salmonids reared in sea water are susceptible to sea lice infections. Numerous factors may affect the susceptibility of salmon to infection with sea lice, such as species of salmon, age, maturation state, stress and presence of secondary disease.

Species of salmon are known to differ in susceptibility to infection with *L. salmonis*. Experimental infections with *L. salmonis* of Atlantic, chinook and coho salmon showed that Atlantic salmon were the most susceptible to infections, while coho salmon were the most resistant species. In British Columbia, Atlantic salmon and rainbow trout are generally more heavily infected with sea lice than chinook or coho salmon when raised at the same site. Based on laboratory studies, differences in susceptibility between species are related to innate immunological defence mechanisms. In coho salmon, these responses include well-developed tissue responses (epithelial hyperplasia and inflammatory reactions), while in Atlantic salmon, only minor tissue responses occur. Accordingly, factors that cause stress in fish or depress the immune system will predispose fish to sea lice infections. Sub-optimal environmental conditions and some husbandry procedures can cause stress reactions in fish. High levels of acute stress and chronic stress are known to increase the susceptibility of salmon to many diseases, including sea lice.

3. Management Tools

Therapeutants

Chemicals used

A variety of compounds have been tested and used to treat sea lice on infected salmon. These therapeutants are generally compounds used as drugs or pesticides in terrestrial agricultural applications and have been adapted for use in aquaculture.

Traditionally sea lice therapeutants have been applied topically as *bath treatments*. Compounds used in this manner include the organophosphates azamethiphos, dichlorvos and trichlorfon, which have been used for sea lice control for several years in a variety of countries. Azamethiphos has been available in Canada since 1995, whereas dichlorvos and trichlorfon have never been registered for this use as pesticides in Canada. Hydrogen peroxide has been used in treating sea lice in many countries, including Canada and the United States. It appears to act by forming gas bubbles within lice. Pyrethrum, a naturally occurring pyrethrin, has been used for sea lice control in Norway, Europe and North America, but has been found to have toxic side effects for treated fish. More recently the synthetic pyrethrins (pyrethroids) cypermethrin and deltamethrin have been used for sea lice control in several countries.

Compounds that are currently being developed as potential new oral treatments include teflubenzuron and diflubenzuron. Both compounds inhibit chitin synthesis and, therefore, target control at the larval and pre-adult stages of lice.

The anthelmintic ivermectin has also been reported to be useful in controlling sea lice when administered through the feed. While ivermectin is registered for a variety of agricultural uses, it has not been approved for use in aquaculture. It has, however, found some use in sea lice control in Canada through extra-label prescription. A related compound, emamectin, is being developed for aquaculture use.

Responsibilities for registering or licensing therapeutants in Canada are summarized in Table 2. In Canada, fish farmers should always consult with their attending veterinarian, the PMRA or the Veterinary Drugs Directorate (Health Canada) for information on the current status of compounds licensed for use as sea lice therapeutants.



Table 2. Registration and licensing of sea lice therapeutants in Canada and the U.S.

In Canada, therapeutants for control of sea lice and similar ectoparasites on fish grown in aquaculture and that are administered externally by direct or indirect application are subject to the *Pest Control Products Act*, administered by the PMRA. Products added to water for bath or dip treatments, therefore, are considered pesticides. Products that are administered orally, e.g., in feed, or parenterally, e.g., by injection, are regulated as drugs under the *Food and Drugs Act* administered by the Veterinary Drugs Directorate, Health Canada.

In Canada, drugs that are licensed for use in animals can be prescribed by licensed veterinarians to treat conditions other than those indicated on the label (extra-label use). Extra-label prescriptions have many restrictions and rely on the prescribing veterinarian to justify their necessity, effectiveness and withdrawal periods for food safety. Extra-label prescription is not permitted, however, for drugs mixed in feeds used for food animals in the U.S. or for therapeutants regulated as pesticides in Canada. Pesticides can only be used as directed on the label.

For information on licensed treatments, contact:

Pest Management Regulatory Agency, Health Canada Phone: 1-800-267-3615 (in Canada) or 1-613-736-3799 E-mail: <u>pmra_infoserv@hc-sc.gc.ca</u> Web site: <u>http://www.hc-sc.gc.ca/pmra-arla/</u>

Veterinary Drugs Directorate, Health Canada Phone: (613) 954-5687 E-mail: <u>vetdrugs-medsvet@hc-sc.gc.ca</u> Web site: <u>http://www.hc-sc.gc.ca/vetdrugs-medsvet/e_home.html</u>

In the U.S., all products that are used for control of sea lice and similar ectoparasites on fish grown in aquaculture are considered as drugs, and regulated under the *Federal Food*, *Drug*, *and Cosmetic Act* by the Center for Veterinary Medicine, Food and Drug Administration.

Regulatory evaluations of food safety, applicator safety and effects on fish and non-target animals are reflected in use instructions and restrictions for individual products. Important use instructions may include withdrawal times, applicator protection measures, application methods, and restrictions to protect sensitive non-target species. For safe use of therapeutants it is essential that users follow the label directions and seek veterinarian advice. Provincial departments responsible for the environment and for aquaculture should be contacted prior to treatments to determine any regulatory requirements that must be met.

Application methods

The most common method of application of therapeutants is bath treatment, where the compound is added to the water of the net pen. In order to better contain the therapeutant, enclosures surrounding the cage are used, usually tarpaulins that completely enclose the sides and bottom of a cage. Bath treatments are usually conducted by hauling up the net to reduce its volume and surrounding it with a tarpaulin. Because the fish are concentrated, oxygen must be supplied throughout the treatment. Oxygenation also aids in mixing the therapeutant within the tarpaulin. While administering therapeutants topically is very effective for controlling sea lice, the use of tarpaulins is time consuming, physically demanding, and requires expertise. Quantification of dose rate cannot be exact given the field conditions, which include the influence of currents on tarpaulin shape and visual estimation of water volume. However, with smaller tarpaulin sizes and experience, the range of doses is generally within two times the target dose.

The use of skirts that surround only the sides of a cage is generally reserved for very large pens in which fitting a tarpaulin would be impractical or where currents are too strong. However, dose rate is much less controlled and the efficacy of treatment may be compromised. In addition, in some jurisdictions, or for some treatments, the use of skirts instead of tarpaulins is not permissible. Label instructions for particular therapeutants may specify application methods. These instructions must be respected.

As an alternative to tarpaulins, the use of well boats for sea lice treatment has been successfully used in Norway and Scotland. The use of well boats avoids the difficulties of tarpaulins, allowing more accurate treatment dosing and, depending on the equipment used, shorter treatment-to-treatment intervals. Fish can be moved into the well boat with pumps or by brailing, and large numbers of fish can be treated at one time. As with the use of tarpaulins, the treatment solution is released at the end of each treatment. Stress and trauma can be caused when pumping or brailing fish. As with the tarpaulin treatments, the use of well boats and pumping equipment is expensive and time consuming. When considering such treatments, steps may be needed to prevent transmission of disease from one site to another.

Dipping individual fish has been investigated by researchers as an alternative application method that reduces the amount of treatment chemical released to the environment. While the procedure could be done during size grading, it is very labour intensive, entails more handling of fish than in-pen treatments and is less amenable to treating large numbers of fish. A variation on the *dip* theme is the application of the treatment solution while fish are passing through a transfer device. An Archimedes' screw device is currently under investigation for such an application. A major issue will be in establishing a safe and effective dose level for the short treatment times that would be involved.

With any of these external treatment methods, it must be recognized that fish handling can be stressful to fish, especially for clinically ill fish or during periods of warm water temperature. Preparations for treatment should include plans for monitoring the fish and dissolved oxygen levels. Backup plans for provision of oxygen may be necessary. As with any application of therapeutants in closed systems, fish under treatment should never be left unattended.

The other major route of application of therapeutants is orally through feed. Teflubenzuron, diflubenzuron and ivermectin would be administered through this route. This method offers ease of application and allows all pens on a site to be treated simultaneously, maximizing the effectiveness of treatments within a given area. One significant disadvantage to in-feed treatments is potentially long withdrawal times. For example, the withdrawal periods recommended for the Investigational New Drug trials of teflubenzuron for treatment of sea lice in Atlantic Salmon were 21 days and 42 days at water temperatures at 10°C and 5°C, respectively. Farmers should consult with their attending veterinarian and follow instructions given on prescriptions with respect to withdrawal times.

Activity against sea lice developmental stages

Compounds such as the organophosphates and hydrogen peroxide are effective against pre-adult and adult sea lice, but have little, if any, effect on chalimus larvae. Pyrethroids also act on preadult and adult lice, but effects on larval stages vary with the compound and formulation used. In contrast, the insect growth regulators, teflubenzuron and diflubenzuron, inhibit moulting and are, therefore, primarily effective against larval and pre-adult lice, with reduced efficacy against adult lice. Ivermectin has been shown to have a broad spectrum efficacy against all stages of sea lice. Given the wide range of effects that sea lice therapeutants have on different stages of lice, selecting the appropriate available compound for a given treatment situation is critical and this emphasizes the importance of routine lice monitoring on the farm.

Effects on fish

The depression of levels of acetylcholinesterase (an important enzyme of the nervous system) in fish by organophosphates such as azamethiphos can adversely affect fish. Signs of toxicity include irregular movements, seizures, loss of balance and reduced respiration and growth. Adequate oxygenation during treatment can reduce the effects of depressed acetylcholinesterase levels. It is also important to allow sufficient intervals between treatments to allow the enzyme to recover following treatment.

Where hydrogen peroxide is used, toxicity due to irritation of the gill tissues increases with water temperature. While it is safe to fish at low water temperatures ($<10^{\circ}$ C), treatment at temperatures above 14°C is not recommended.

There is little information on the effects of ivermectin on fish, but the low therapeutic margin can result in toxicity problems with improper or prolonged use. Cases have been reported involving darkened colouration, lethargy and mortality in Atlantic salmon that had been overdosed with ivermectin or treated for prolonged periods at low water temperatures ($<10^{\circ}$ C).

Therapeutic ratios, or safety margins (the difference between treatment dose and toxic dose) are relatively small for some sea lice therapeutants, emphasizing the importance of accurately controlling the dose delivered to the fish.

Toxicity to non-target organisms and environmental impact

The potential environmental impact of compounds for sea lice control is a key consideration in the regulatory approval process, especially given the relatively recent development of intensive

salmon farming and increasing public awareness on environmental stewardship. The conditions of use for approved products can include measures to manage potential environmental risks. Those risks are usually estimated using a combination of laboratory toxicology studies on non-target species, field studies of effects on caged organisms, and modelling and measurement of dispersion, persistence, breakdown and inactivation of the compounds in the environment.

Some of the information developed on sea lice treatments has been published in scientific journals. The published information indicates that, not surprisingly, crustacean species are most sensitive to therapeutants in laboratory studies, while molluscs are not usually sensitive. Published measurements of environmental concentrations usually suggest a high degree of dissipation, although these types of parameters will tend to be site specific.

Potential environmental risks may be mitigated by label instructions related to dosage, number of treatments and timing of treatments. Farmers, therefore, can ensure that the risk of potential impacts is minimized by following all label instructions when using sea lice therapeutants, by employing good husbandry practices, and by minimizing feed wastage by optimizing feeding practices. In addition, using integrated pest management (IPM) to manage sea lice infestations can contribute to a reduced need for the use of therapeutants.

Resistance management

Beyond safe use of individual products, a longer term concern is the development of resistance by sea lice to chemotherapeutic agents. Resistance of parasites and arthropods has become an issue of major importance in livestock and crop production, and has been a prime motivation for the development and adoption of modern IPM. While it is not possible to predict whether resistance to therapeutants will be a major problem in sea lice control in salmonid aquaculture, there are already indications of decreasing efficacy of organophosphates against *L. salmonis* in Europe and possibly in eastern Canada.

Some general principles concerning resistance to pesticides may be relevant to sea lice control. Resistance is favoured when frequency of use of a therapeutic agent is high or pests are exposed to sublethal doses. Resistance development is not favoured in systems where resistant genes are continually diluted by high rates of migration from an unexposed susceptible population. It is clear from the terrestrial models that over-reliance on frequent pesticide use will result in a strong selective pressure for resistance. As well, pests may develop multiple- and cross-resistance to pesticides, which may lead to decreased susceptibility to therapeutic agents to which they have never been previously exposed.

One tactic for decreasing resistance pressure is to use, in rotation, therapeutants having different modes of action. IPM control strategies also incorporate other control measures and preventive tactics. Adoption of an integrated management approach that includes the strategic use of multiple control and prevention strategies for sea lice control will also be key to reducing the development of therapeutic resistance.

Fallowing of sites and other fish cultural practices

Fish culture and management practices can be particularly useful in reducing and preventing sea lice infections. In particular, the use of single-year class sites, and the fallowing of farm sites before restocking can eliminate or significantly reduce infestations and the need for chemotherapeutic intervention. Excellent results have been obtained using these practices and they have been identified as key parts of a sea lice management strategy.

The level of sea lice on farmed salmon tends to increase with the time the fish are in the net pens. Smolts introduced into a site with older fish can be exposed to a source of infection and can quickly acquire lice. On the other hand, if a site is stocked with a single generation of fish, acquisition of lice can be avoided or delayed significantly and both damage to fish and the number of treatments can be reduced.

Leaving a site fallow removes the major local source of sea lice for new fish, and breaks the life cycle of the parasite. The length of time that a site should be left fallow depends on the developmental time of the parasite with respect to water temperature during the fallow period. A longer period of time may be necessary to account for the time when an egg-bearing female may remain viable after being separated from a host. The minimum fallowing times recommended by various authors are four to six weeks, and can be as long as twelve weeks or more if adult female lice can produce viable eggs when not attached to a host. It should be noted that fallowing may be less useful for management of *Caligus* than for *Lepeophtheirus*. *Caligus* occurs on a wide range of fish hosts and there is greater opportunity for continual reinfection of farms from wild fish carriers.

Fallowing should cover an area within which substantial numbers of larval sea lice can disperse. Unfortunately, the size of that area cannot be precisely defined. By maximizing the distance between sites, the rate of transmission between farm sites and the need to fallow sites simultaneously can be reduced. In cases where farms belonging to different companies are in proximity, cooperative agreements between companies with respect to single-year class stocking, periods of fallowing and timing of sea lice treatments have proven useful in reducing sea lice infections and damage.

Where possible, avoiding areas with abundant wild hosts of sea lice can be useful. Estuaries at the mouth of salmon rivers may provide particular foci of infection and sources of larvae from wild hosts and should therefore be avoided if possible. Where abundant wild hosts of sea lice are present, the effectiveness of fallowing can be reduced.

The presence of adequate currents, which is usually a criterion for site selection for several reasons, can help ensure the infectious copepodid larvae are not retained on site. Good husbandry through use of clean nets can also reduce retention of larvae in nets.

Cleaner fish

Several species of wrasse (family Labridae) have been shown to act as *cleaner fish* by grazing sea lice from infected salmon. These fish are capable of removing mobile lice, especially females with egg sacs, and some chalimus larvae. In trials conducted in Europe, stocking with wrasse has been

demonstrated to reduce sea lice populations, thereby reducing the need for chemical treatments. In the early 1990s, use of wrasse as a biological control of sea lice was increasingly used in Norway, Scotland and Ireland. Although most reports of successful use of wrasse in commercial operation are anecdotal, there have been successful controlled trials on operating farms. However, the use of wrasse in Scotland in recent years has declined rapidly because they do not survive in net pens during cold winter months. In Norway, there is an abundant supply of wrasse, and government support for their use, so use of wrasse is continuing.

There is little experience in the use of cleaner fish in North America. There are no native wrasse species on the Pacific coast of Canada. There are two native wrasse species on the Atlantic coast of Canada, but only one, the cunner, has been reported as having cleaning behaviour. One trial with this species was not successful at controlling sea lice. While further work may be necessary before coming to a definite conclusion on the use of wrasse or other candidate species in North America, e.g., with different ratios of cleaner fish to salmon, the use of cleaner fish does not appear to be promising for use in sea lice management in North America.

Mechanical devices

The apparent movement of infective sea lice larvae towards light has led to trials with light traps. These devices are intended to attract copepodid larvae in the vicinity of net pens. The larvae can then be collected and destroyed. There is little published evidence, however, about the effectiveness of light traps. Some success has occurred in trials in tanks, but similar success in net pens has not been reported.

Pumping fish has been reported to remove lice from salmon. Reductions of 20–60% have been reported anecdotally, but there is little quantitative information. This technique may be most effective for small fish, as mechanical damage increases in larger fish. On the other hand, larger fish may work harder against the water flow and dislodge more lice. Heavily infected fish may be less tolerant of the stress of pumping. In some instances, a post-pumping increase in lice number has been observed due to the stress of the procedure. Such strategies should, therefore, be approached with caution.

Nevertheless, some farmers have used this method in response to heavy infections and, if used judiciously, it can be effective when other treatment options are limited. When infected fish are pumped, the outflow must be collected and filtered in order to remove the lice and possible sources of infection (unhatched eggs) in the vicinity of a farm.

Vaccines

Prevention of sea lice infection through site selection and site management is an important part of integrated management of sea lice. Another approach to prevention would be the use of an effective vaccine. There is much interest in the development of a vaccine and some progress has been reported; however, the work has many challenges, including a generally poor understanding of the immunological relation between lice and fish. Moreover, lice feed on mucus and tissue

and, by the time blood feeding begins, substantial damage has already been done. Furthermore, infected salmon do not appear to acquire an immunity after they are first infected.

Current work on vaccines, especially vaccines that are targeting antigens in the gut of lice, is showing some promise. None is currently available for use but vaccines remain an important potential tool for the future.



4. Recommended IPM Program

Background

The following are the general elements of an integrated management strategy for sea lice using currently available tools. The goal is to provide an overall management strategy that uses all available measures to suppress pests effectively, economically, and in an environmentally sound manner.

The types of action that can be taken to satisfy these criteria are indicated; however, details of an implemented management plan will need to be adapted to local situations and to the development of new tools. There will, therefore, be variation in the specific measures that are taken to prevent and reduce lice numbers. Using the greatest number of management tools possible will provide better control with the least chance of relying solely on chemical treatments to deal with outbreaks. In turn, this approach will maximize the effectiveness of therapeutants and extend the useful life span of compounds by avoiding resistance.

Some elements of the strategy may be easier to implement in new operations. Existing operations may need to adapt the strategy and emphasize some techniques over others. For example, the ability to separate sites will vary: with less separation, there would need to be greater emphasis on other measures such as coordination in management and treatment.

The key steps in IPM involve prevention, monitoring, identification, thresholds for action, and control. Proper management of sea lice will require ongoing preventive activity, with defined and practical steps.

There are also some cross-cutting themes in the management strategy, including training (monitoring, prevention, application, availability of expertise) and availability of sites (fallowing, single-year classes, site selection for healthy fish).

Management for prevention

Prevention is fundamental to IPM. A number of measures can be taken that should reduce the likelihood of sea lice becoming a problem. Not all of these measures can be readily implemented in all locations, and some may be more applicable to new than to existing operations. A number of the preventive measures are dependent on the number of suitable sites for net pens and, in some cases, may require access to additional sites.

Nevertheless, whatever preventive measures can be adopted will both reduce losses due to sea lice and contribute to the success of treatments and other control measures. The use of therapeutants should supplement preventive measures, not replace them, as one part of an overall management plan.

Location of sites is important with respect to sources of infection and water quality. In addition to enhancing production, use of good quality sites leads to healthier fish that may be less susceptible to infection with sea lice. Characteristics such as water depth, tidal range, currents, bottom type, temperature and salinity are generally considered as part of site selection and will have an influence on the quality of the site and general health of the fish. In particular, adequate water flow can help prevent build-up of sea lice larvae in a site and contribute to dispersion of therapeutants following a treatment.

Siting of farms at a distance from potential sources of infection is an important preventive practice. At least initially, infections are probably derived from wild hosts. Siting farms away from locations where salmon are known to concentrate, e.g., salmon-bearing rivers and estuarine holding areas, can be useful for prevention of infection with *L. salmonis*. Similar measures can be taken to avoid hosts of *Caligus* spp. Since those lice infect a larger number of wild host species, it is more difficult to avoid contact.

Another source of infection can be adjacent farms that are infested with the parasite. An appropriate spacing between farms may help reduce the transmission of the parasite. One study in Europe found a 90% reduction in larvae at a distance of one kilometre from a farm. However, the optimum spacing has not been defined and will depend on local conditions. Local decisions on the spacing of farms will depend on an understanding of currents, tidal flow, and other factors that may enhance or restrict larval dispersal.

Appropriate husbandry practices are a key aspect of prevention. Some of the practices are dependent on access to a number of sites. In an already established operation where new sites are not available these practices may, therefore, be difficult to fully implement. Nevertheless, they can be incorporated into new operations and may constitute one consideration in expansion of available sites.

Year-class separation is probably the most effective husbandry technique, whereby smolts are not introduced to a site with older fish. Consequently, smolts will not be immediately infected from older fish at the site, thereby slowing down the acquisition of sea lice. This practice requires an operator to have at least two sites to allow for continuous production. Where year-class separation is not feasible, it may be useful to treat salmon already on a site in order to reduce lice numbers as much as possible before introducing smolts.

Fallowing of sites is another important practice that can reduce or eliminate self-sustaining lice populations. After harvest, a site is left without fish for a certain period, thus removing the source of reinfection and breaking the life cycle of the sea louse. The fallow period will depend on location and temperature but, in general, a period of at least four to six weeks should be used. Fallowing should be coordinated between farms within an area. Sites within a short distance should be treated similarly, i.e., put on the same fallowing rotation. Fallowing is another of the preventive practices that requires access to an adequate number of sites.

Proper management of fish densities can help reduce the infection, and attention is needed to determine critical densities at which sea lice and other diseases may become unmanageable.

Clean nets help maintain greater water circulation, which can prevent the build-up of larval stages within a pen, as well as contribute to overall fish health.

Susceptibility to sea lice may not be the major consideration in fish species selection, but an awareness of this factor is needed. In laboratory studies, coho salmon were less susceptible to infection by *L. salmonis* than Atlantic salmon, with chinook salmon showing intermediate susceptibility. These results are similar to observations in wild and cultured salmon.

Monitoring pest populations and pest damage

Decisions about when to conduct a treatment should be based on a program of monitoring lice numbers established with the attending veterinarian. Treatment triggers should be established, and treatment conducted when those triggers are reached, rather than waiting for damage to become apparent. The treatment triggers will need to be chosen in consultation with the farm veterinarian and based on local conditions. The setting of treatment triggers, counting of lice and initiation of treatment require accurate record-keeping, exchange of information and coordination between farms.

The monitoring program should address:

- how many fish and how many pens to sample;
- ► how to catch fish for a representative sub-sample, and how to handle fish to minimize stress;
- ► how often to sample;
- which stages to count; and
- damage assessment.

Sampling frequency should be in the range of once a month to once a week. In general, lice should be counted more frequently when water is warm (>12°C) and in the spring when temperatures and lice populations are increasing.

The number of fish and pens sampled should be large enough to provide a representative sample but also small enough that sampling can be completed in the chosen sampling interval. One cage should be chosen to include in every sample, in order to provide a consistent reference point. Additional pens can be chosen at random each time. The reference cage can be a cage that tends to be more heavily infected, e.g., an end cage.

A method is needed, e.g., sampling net or other enclosure, to take a sub-sample of the fish in a pen. Moribund or obviously weak fish are usually not representative and should be excluded. Feed is sometimes used to attract fish for a sample. However, this sampling method probably biases the lice counts toward a gross underestimate of the true lice load. Sampling methods should be determined in consultation with the farm veterinarian.

Fish should be put into an anaesthetic bath, a few at a time, then examined closely and lice counted immediately after removal from anaesthetic. This will minimize stress and enhance detection of the lice. Any lice falling off into the bath must be counted, and the numbers attributed across the sample.

Operators will encounter sea lice species of the genera *Lepeophtheirus* or *Caligus*. Differences in the biology of these species will affect management options, and it is important to be able to distinguish them. The main differences between *Lepeophtheirus* spp. and *Caligus* spp. that have an impact on management are the potential for damage and the host range.

Lepeophtheirus salmonis is capable of causing severe damage and becoming a long-term chronic problem. This species has a restricted host range, occurring almost exclusively on salmonids, and can become established on populations of net-cage salmon. If present, they must be controlled. Because of the greater potential for damage, the trigger levels that indicate treatment is necessary would need to be lower than for *Caligus*.

In contrast, *C. elongatus* may cause short-term acute damage to salmon, but tend not to establish endemic populations on caged salmon. Because this sea louse species has a broad host range, there are multiple sources of infection and the presence of *C. elongatus* is largely dependent on movements of wild fish. If large populations of wild fish are in the vicinity, a *Caligus* infection may need to be controlled in order to prevent a chronic problem. However, one-time infections may not need to be controlled.

Adult and pre-adult *Caligus* species possess a pair of lunules, small semicircular translucent structures on the anterior margin, which distinguishes them from *Lepeophtheirus* spp (Figure 1). *Caligus* adults are generally smaller (up to 5 mm) than *Lepeophtheirus salmonis* adults (up to 10 mm).

The following publications provide keys or descriptions for distinguishing species of adult sea lice:

- Johnson, S. C. and M.L. Kent. 1992. Sea Lice (Caligid Copepod Parasites), *in* M. L. Kent, Diseases of Seawater Netpen-reared Salmonid Fishes in the Pacific Northwest. Canadian Special Publication of Fisheries and Aquatic Sciences 116: 76 (1992). Reprinted in The Biology of Sea Lice and a Key for Species Identification. Aquaculture Update No. 59. Fisheries and Oceans Canada, Pacific Biological Station. p. 7 (1992)
- Johnson, S. C. and L. Margolis. 1992. Sea Lice *in* J. C. Thoesen (ed.). Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, 4th ed., Version 1, Fish Health Section, American Fisheries Society. Chapter XV: 1–10.

The need for, and choice and timing of, treatment will also depend on the number of lice and population structure. It is, therefore, important to distinguish the basic life cycle stages (attached chalimus larvae, mobile pre-adults and adults) and to identify gravid females (Figs. 2, 4). Gravid

females are distinguished by the presence of long thin sacs containing eggs, which trail behind the genital segment. Adult males and pre-adults are frequently counted together. Adults and preadults, however, will need to be distinguished if moult inhibitors are being used for treatment.

Copepodid and chalimus larvae are small (1–3 mm) and are hard to detect even by experienced observers. They can sometimes be felt as small bumps with the tips of fingers. The use of a flashlight can also help to highlight them, for example, in crevices behind fins. While it may not be feasible in some situations to accurately count chalimus, their presence must at least be noted with some type of index, e.g., none, few, many. Especially at warm temperatures, weekly counting of chalimus may be necessary to predict the need to control mobile lice stages.

Descriptions of larvae are available in the following:

- ► Johnson, S. C. and L. J. Albright. 1991. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). Can. J. Zool. 69: 929–950.
- Piasecki, W. 1996. The developmental stages of *Caligus elongatus* von Nordmann, 1832 (Copepoda: Caligidae). Can. J. Zool. 74: 1459–1478.
- Schram, T. A. 1993. Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *In* Boxshall, G. A. and D. DeFaye, [eds.] Pathogens of wild and farmed fish: Sea lice. Ellis Horwood, New York. pp. 30–47.



Figure 4: Female and male sea lice



The presence of lesions should be noted, perhaps with a simple severity index. Damage from lice may be seen as characteristic white, grey or red lesions on the head, back or perianal region. Before damage occurs, small congregations of brown-coloured lice can often be seen. Minor lice damage, eg., grey backs, is more visible when the fish are in the water, and general observations of such damage can be used as an early indicator of the need for treatments.

Every farm needs to have staff trained in identifying the stages and signs of damage due to sea lice. Regular consultations with the farm veterinarian are important to the collecting of information useful for making decisions regarding lice control options. It can be useful for a farm to develop a collection of representative samples of the various species, stages and sexes of sea lice to use as a reference for identifying lice on fish. Key personnel should have a specific duty to regularly examine fish, count lice and keep records. It is recommended that trained and experienced staff conduct the counting on a farm.

Information that should be recorded for each fish includes net cage sampled, genus, number of lice, stage of lice, and an index of lesions. Other information should include date of sampling, date of last treatment, water temperature, fish weight and name of the person conducting the sampling.

The monitoring program will provide the basis for making decisions on when to treat. In establishing the monitoring program, therefore, it is also necessary to establish the treatment triggers, i.e., the populations of lice that will indicate a treatment is necessary. The triggers will depend on local situations, the types of treatments available, and advice from the farm veterinarian. In addition to total counts, the relative proportion of chalimus and mobile stages (pre-adults and adults) and whether the proportion is changing are also useful in determining the need for treatment. High numbers of larval stages may indicate that a site has become self-propagating and requires urgent treatment or other management strategies. Examples of monitoring programs are given in Appendix I.

While the treatment triggers must be low enough to protect the salmon, it must be recognized that too low a trigger can lead to unnecessary therapeutant use, which can be difficult, costly and environmentally imprudent.

Reducing pest populations to acceptable levels

The treatment strategy should be chosen to provide effective treatment while minimizing the potential for negative impact on the environment and human health. In general, IPM uses a combination of biological, mechanical, and chemical controls. Whatever method is chosen, treatments in an area should be coordinated, and the closer the sites, the greater the need for coordination.

Currently, treatments of infestations of sea lice involve the almost exclusive use of therapeutants. Within a farm site, the treatment of all pens within as short a period as possible is preferable to the treatment of a few pens only. Untreated pens can be sources of reinfection of treated pens. In addition, sea lice in those pens may be exposed to lower levels of therapeutants, risking the

development of resistance. Strategies in Scotland and Ireland are placing considerable emphasis on the synchronous treatment in a management area at the time when lice populations are at their most vulnerable, i.e., in the spring, as this effectively reduces the whole population and leads to much reduced infection levels during the summer.

Pumping of fish can be used to remove a proportion of mobile lice. Because of the length of time involved and the stress it puts on fish, this may not be a suitable approach for an outbreak situation. Attached stages are not removed, and there is a possibility that the fish may be more susceptible to reinfection because of the stress from pumping. Nevertheless, pumping as a control measure can be useful if used as part of routine management practices, e.g., at the time of grading or splitting fish, or as a short-term control near harvest time. When pumps are used, they should be fitted with screens to retain dislodged sea lice.

Conducting an emergency harvest can also be considered, although the feasibility and desirability are obviously dependent on opportunities for marketing the fish.

A veterinarian with expertise in fish health management should be consulted to develop monitoring and control programs and to assist in decisions on therapeutant options and frequency. Regular and periodic review of monitoring records with the veterinarian is advisable to facilitate treatment decisions.

The choice of a therapeutant will be based primarily on products registered or licensed for treating sea lice. In most cases, labels specify that products are available only through veterinary prescription. In addition, in-feed product may be available in Canada for extra-label use through prescription by a veterinarian with expertise in fish health management. Extra-label use of such products is not permitted in the U.S., or for products regulated as pesticides in Canada, i.e., external treatments.

Product labels and use instructions need to be understood and carefully followed. Specific treatment procedures such as application method, withdrawal times, and worker and environmental safety are reviewed as part of the registration and licensing of products, and are reflected on product labels and use instructions. Veterinarians and local provincial or state agencies need to be consulted to be sure regulatory requirements concerning environmental issues are known and followed.

Considerations when choosing a treatment include the method of application (bath or in-feed treatment), the cost per treatment, cost of total treatments over the treatment season, efficacy against different stages, and withdrawal times for consumption. Repeated usage of the same therapeutic compound over an extended period may lead to resistance in the lice. Where feasible, consideration should be given to using alternative compounds during a course of treatments, especially if a compound is not fully effective against all stages. Alternating the use of therapeutants can also reduce resistance development.

Planning is needed because both trained staff and appropriate material must be on hand to conduct treatments. For bath treatments, equipment and established procedures are needed for

lifting nets, deploying a tarpaulin, quantifying the dose and adding the treatment chemical. There should be provisions for monitoring the fish for signs of distress during the treatment, monitoring levels of dissolved oxygen, and providing oxygenation if necessary. Any required worker protection equipment and procedures must be in place. For in-feed treatments, some planning is required to ensure proper product milling and feed delivery to the site. The correct dosage of prescribed therapeutant needs to be added to feed of an appropriate size for the fish, and those feeding the fish must obtain instructions on handling and feeding out the medicated product.

Follow-up monitoring is almost always required, and should be planned, because treatments rarely remove all stages, eggs will hatch even if the female dies, and larvae already in the adjacent sea will reinfect.



5. Future Needs and Issues

Development and implementation of an integrated management approach to sea lice is feasible with currently available tools. Nevertheless, some areas have been identified that require further development and could contribute to more sustainable management of sea lice.

Viability and development of additional management options

The development of safe and effective vaccines would drastically change and improve the ability to manage sea lice. Research in this area should be encouraged.

Increased options in available therapeutants

Given the numerous factors that affect the choice of therapeutant, including efficacy against different stages, withdrawal time, and application method, a greater choice of therapeutants would provide greater opportunity to develop suitable treatment regimes. In addition, rotation of the treatments used is a key strategy for avoiding the development of resistance. Activity on a number of fronts can contribute to the availability of therapeutants: identification of candidate products by users, manufacturers and researchers; development of suitable registration data by manufacturers and user support groups; and provision of clear registration guidelines and performance standards by regulatory agencies. A particular need is research to develop suitable laboratory models of infection for testing purposes.

• Advances in application methods

Application of therapeutants presents a number of challenges, including the technical difficulty of deploying tarpaulins, especially as cage size increases: quantifying sizes of pens for accurate dosing; ensuring adequate dosing of all fish; and controlling dispersion of therapeutants if necessary to mitigate potential risks. While closed treatment systems would provide a number of benefits, a practical system remains to be developed, and further development in this area would be useful.

• Effective documentation of sea lice infestations and management

A coordinated approach to prevention and control of sea lice is part of an IPM approach. There is a need for effective record keeping at the farm and regional levels about sea lice infestations, and the preventive and control strategies employed. Accurate and coordinated record-keeping is essential to making control decisions, but is also needed so that the effectiveness of the strategies can be properly assessed and adjusted if necessary. It may also justify the need for, and development of, new treatments. Sample size, sampling methods, and identification of factors that contribute to lice infestation levels need to be investigated further to improve the efficiency of monitoring and control programs.

Looking beyond salmon and sea lice, the prevention and management of potential disease and parasite problems need to be considered early in the process of future species development for aquaculture. The need for, and means of, effectively applying therapeutants can have an impact on culture methods being developed. There needs to be a recognition of increasing public scrutiny of therapeutant release into open water. Early consideration and planning should reduce the need to make costly adjustments later on.



6. Additional Sources of Information

There are numerous scientific papers on various aspects of sea lice biology and management that provided the basis for information in this document. The following publications provide an overview of information available. The list also includes a few recent publications on specific topics addressed in this document.

Many aspects of sea lice biology and management are reviewed in the volume edited by Boxshall and DeFaye (1993), and in a recent review by Pike and Wadsworth (2000).

Excellent reviews on the control of sea lice with therapeutants and other methods are given in Roth *et al.* (1993) and Costello (1993).

A complete bibliography of sea lice information is being collected as part of the European Union Concerted Action Programme on sea lice. The bibliography was published in 1997 in Volume 3 of "Caligus", a newsletter funded under the EU FAIR Programme. Updated versions are available on the World Wide Web at: <u>http://www.ecoserve.ie/projects/sealice/index.html</u>.

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Appendix I: Examples of monitoring and treatment triggers

- ► The first week of December, conduct counts on 20 fish per cage, and treat every cage that is above five pre-adult lice per fish, or 0.25 gravid female lice per fish. Treat until target levels reached. The second week of May, conduct counts on 20 fish per cage, and treat by the end of the month every cage on a site that is above five pre-adult lice per fish, or 0.25 gravid female lice per fish. (New Brunswick strategy, fall 1995 and spring 1996.)
- Starting June 1, conduct bi-weekly counts on five fish from five pens a year/class/system/site. Treatment threshold will be established based on summary of all counts, taking into account number of lice, condition of fish, water and climatic conditions, and season. A threshold of five pre-adult or one gravid female *L. salmonis* per fish is recommended as a guide. Fall clean-up treatments should be done when water temperature falls below 8°C. A spring clean-up treatment should occur when water temperature is still below 8°C, if fall treatment could not be done or if spring lice counts are above the threshold. (Maine strategy, 1997.)
- Use a sampling net to separate a sub-sample of fish in the net pen. Collect fish using a dip net to get a vertical cross-section sample. Count lice on 10-20 fish from every second pen on the farm. Choose one pen that is sampled each time, and three to four others, selecting those with the highest apparent lice numbers. Conduct counts once a month, more frequently when water temperature is high and in the spring. Lice should be counted in four categories: adult female *Caligus*; adult female *Lepeophtheirus*, pre-adults and adult males of both species; and chalimus of both species. (Draft standard protocol for lice sampling, presented by P. Kvenseth and P. Andersen at workshop on Lice Control on Fish Farms, Trondheim, Norway, November 1997.)
- Samples are taken 14 times a year (twice a month in March to May; once a month the rest of the year, except once during the December to January period). Counts are conducted on 30 fish from a standard cage, i.e., the same one every time, and 30 from a second cage, randomly selected each time. All mobile lice of each species are recorded, then removed and preserved in alcohol. Chalimus are not individually counted, but a notation is made of many (≥5), few (<5), or none. (Monitoring protocol in Ireland; presented by D. Jackson at workshop on Lice Control on Fish Farms, Trondheim, Norway, November 1997.)</p>
- Counts of lice are undertaken at least weekly in summer, when water temperatures are over 10°C. Counts may be less frequent in winter. Five fish are selected from each net pen to be sampled, and a total of 25 to 40 fish are sampled at one site. Depending on the layout of the site and the manner in which pens are grouped, two or more pens in a group are selected. End pens and those with visibly higher lice numbers are selected for sampling. It is desirable to have some specific pens included in each sampling for continuity. Fish are removed with a hand net after attracting them to the surface with feed. *L. salmonis* are categorized as gravid female, non-gravid adult female, adult male or preadult male and female. For *Caligus*, total counts of mobile stages are recorded. Total

counts of chalimus of either species are recorded, and multiplied because counts of chalimus consistently under-estimate the population. (Monitoring strategy used by Marine Harvest McConnell; described by J. Treasurer and A. Grant in Fish Farmer, November/December 1997.)

