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(54) **Title**: METHODS AND FEED COMPOSITIONS FOR MASKING OF FISH SEMIOCHEMICALS

(57) **Abstract**: A method for masking the odor of isophorone in water is described Also described is a method for reducing the attraction between an parasite and a fish. The invention also relates to fish feed compositions, and the use of a compound or extract for the prevention and/or treatment of a parasite infection in fish.

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METHODS AND FEED COMPOSITIONS FOR MASKING OF FISH SEMIOCHEMICALS

FIELD OF THE INVENTION

The present invention relates to a method for masking the odor of fish semiochemicals in water. The invention also relates to a method for reducing the attraction between an parasite and a fish. The invention also relates to fish feed compositions, and the use of a compound or extract for the prevention and/or treatment of a parasite infection in fish.

BACKGROUND TO THE INVENTION

Sea lice (*Lepeophtheirus salmonis, Caligus* sp.) are the major pathogen currently affecting the global salmon farming industry and have a significant impact on many areas of production. Economic impact on the aquaculture industry are high due to high annual losses. There is also continued concern over the impact of salmon farming on wild salmon populations with increased density of sea lice adjacent to these production sites. Control measures have been reliant upon the use of a number of chemotherapeutants since the 1970's. Reduced efficacy has now been reported for all compounds, with the exception of the insect growth regulators (IGR) diflubenzuron and teflubenzuron. Further methods are therefore required to effectively control sea lice, in conjunction with sea lice medicines.

Host–specific parasites

The *Lepeophtheirus* genus of sea lice is a host-specific parasite. *L. salmonis* will only complete its life cycle on salmonid species, although mobile stages may occasionally be observed as opportunists on additional fish types. Other *Lepeophtheirus* sp. will target a narrow range of other fish species.

Immune suppression of the host

The *Lepeophtheirus* genus of sea lice has evolved a range of mechanisms to suppress the immune response of their particular hosts. To overcome a potentially fatal inflammatory reaction the sea lice release a series of secretary / excretory products (SEP) into the host tissue, via salivary glands. Prostaglandins (PGE_2), alkaline phosphatase and a range of trypsin-like proteases have been identified as sea lice SEPs. It is thought that several additional unidentified factors such as phosphatase, apyrase and macrophage inhibition factor are also present.

Effect of immune suppressants

L. salmonis has a significant immunosuppressive effect on a range of responses in Atlantic salmon including reduced respiratory burst, lower macrophage activity, increased apoptosis, necrosis, decreased numbers of mucosal cells and down-regulation of immune genes such as interleukin IL-1ß and MHC-1. Suppression occurs at localised attachment sites, although a more generalised effect may occur with higher levels of sea lice infection. Once they have suppressed the immune system of the host, the lice are able to extend a frontal filament for a secure attachment. This is intimately associated with the host tissues and able to survive any subsequent immune response from that species.

A fatal risk of attaching to the wrong host

Lepeophtheirus sp. are not able to suppress the immune system of non-host species. If lice try and settle on to a resistance fish species the immune response will kill it. Thus correct identification of the host is essential for attachment and survival of *Lepeophtheirus* sp.

Correct host identification

Sea lice have advanced olfactory and contact chemoreceptors that are capable of accurate identification of specific host molecules. Semiochemicals (behaviour-modifying chemicals) are used by a range of arthropods in chemical communication systems to locate a host, mate or oviposition site. Similarly, many copepods use chemical cues to identify and seek out mates.

Caligus species

Lice within the *Caligus* genus have an extensive range of potential hosts; *C elongatus* is known to infect over 80 host species world wide. *Caligus* have been found to posses a greater range and quantity of serine and non-serine proteases than *L. salmonis* and this may assist in defeating a greater range of immune responses from many different species. In addition *Caligus* deploy a different attachment mechanism that is not as intimately associated with host tissue. *Caligus* remove the epidermal tissue from the scales and then the frontal filament attaches directly to the cleared scales via a basal plate. The frontal filament is much longer than that deployed by *L. salmonis* and this allows the louse to remain at some distance from the host immune system. Despite these generalists adaption's some *Caligus* species still demonstrate a high degree of host specificity. This may develop in populations in areas where a particular host population is abundant such as *Caligus rogercresseyi* which are now the dominant sea lice species on salmon farms in Chile.

Through behavioral trials, tested the hypothesis that the inter-and intraspecific relationships of salmon louse, *C. rogercresseyi* are mediated by semiochemical compounds has been tested. It has been shown that the host species studied, Coho salmon, Atlantic salmon, and Rainbow trout, emit chemical signals that attract sea lice.

The object of the present invention is to provide a feed composition and a new method for prevention and control of sea lice attraction to, and infections in fish, preferable Salmonidae that is easily applicable, effective in long-term use and are considered as environmentally friendly and less toxic than many known chemotherapeutants. In particular, an object of the present invention is to provide a feed composition and a method for masking the semiochemical compounds in order to reduce the attraction of a sea lice for salmonidae.

SUMMARY OF THE INVENTION

A first aspect of the present invention relates to a method for masking the odor of a fish semiochemical in water, characterized in that an extract or compound is added to said water or is administered to a fish in said water, wherein said extract or compound is selected from;

i) an extract (or oil) of garlic, rosemary, lavender or bog myrtle, or

ii) a compound of formula (I);

$$R^1 - S - S - R^1 \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (II);

$$R^1\text{-}N{=}C{=}S \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

In a preferred embodiment, said fish semiochemical is isophorone. In a preferred embodiment is said fish semiochemical 1-Octen-3-ol. In a preferred embodiment is said fish semiochemical 6-methyl-5-hepten-2-one.

Preferable is said said fish is a Salmonidae.

Preferable, is said water Salmonidae conditioned sea water or said fish in the water is a Salmonidae.

Preferable, said salmonidae is selected from the group consisting of Atlantic salmon, coho salmon, Chinook, rainbow trout, Arctic charr and other farmed salmon species.

Preferable, the method reduces the attraction between a parasite and said fish.

Preferable, said parasite is an ectoparasite, more preferable sea lice (*Lepeophtheirus salmonis, Caligus* sp.).

In a preferred embodiment is said compound a compound of formula (I) above.

Preferable, at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$. Preferable, both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

Preferable, said compound is diallyl disulfide or diallyl sulphide.

In a preferred embodiment is said compound a compound of formula (II) above.

Preferable, R1 is a C_1-C_4 alkyl. Preferable, said compound is butyl isothiocyanate.

Preferable, said compound is propyl isothiocyanate. Preferable, R1 is a C_2-C_3 alkenyl. Preferable, said compound is alkyl isothiocyanate. Preferable, said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl. Preferable, said phenyl alkyl is phenyl ethyl. More preferable, said compound is allyl-, propyl-, butyl-, pentenyl-, phenyethyl-isothiocyanates, and more preferable is said compound 2-phenyl ethyl isothiocyanate.

A second aspect of the present invention relates to a method for reducing the attraction between an parasite and a fish, or for reducing the infestation or infection of an parasite in a fish, or for the treatment of an parasite infection in a fish, characterized in that an extract or compound is added to said water or is administered to a fish in said water, wherein said extract or compound is selected from;

i) an extract (or oil) of rosemary or bog myrtle, or

ii) a compound of formula (I);

$$\mathbf{R^1 - S - S - R^1} \qquad \text{(I)}$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (II);

$$\mathbf{R^1\text{-}N{=}C{=}S} \qquad \text{(II)}$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

Preferable, said fish is a Salmonidae.

Preferable, is said water Salmonidae conditioned sea water or said fish in the water is a Salmonidae.

Preferable, said salmonidae is selected from the group consisting of Atlantic salmon, coho salmon, Chinook, rainbow trout, Arctic charr and other farmed salmon species. Preferable, the method reduces the attraction between a parasite and said fish. Preferable, said parasite is an ectoparasite, more preferable sea lice (*Lepeophtheirus salmonis, Caligus* sp.).

In a preferred embodiment is said compound a compound of formula (I) above. Preferable, at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$. Preferable, both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

Preferable, said compound is diallyl disulfide or diallyl sulphide.
In a preferred embodiment is said compound a compound of formula (II) above. Preferable, R1 is a C_1-C_4 alkyl. Preferable, said compound is butyl isothiocyanate. Preferable, said compound is propyl isothiocyanate. Preferable, R1 is a C_2-C_3 alkenyl. Preferable, said compound is alkyl isothiocyanate. Preferable, said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl. Preferable, said phenyl alkyl is phenyl ethyl. More preferable, said compound is allyl-, propyl-, butyl-, pentenyl-, phenyethyl-isothiocyanates, and more preferable is said compound 2-phenyl ethyl isothiocyanate.

A third aspect of the present invention relates to a feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises rosemary or bog myrtle. Preferable, said rosemary or bog myrtle masks the odor of a fish, preferable a Salmonidae. Preferable, said compound or material masks the odor of salmonids in Salmonidae conditioned sea water. Preferable, said compound or material masks the odor of isophorone or 1-Octen-3-ol or 6-methyl-5-hepten-2-one.

A fourth aspect of the present invention relates to a feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises a compound of formula (I);

$$\mathbf{R^1 - S - S - R^1} \qquad \text{(I)}$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl.

Preferable, at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.
Preferable, both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$. Preferable, said compound is diallyl disulfide.

A fifth aspect of the present invention relates to a feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises a compound of formula (II);

$$\mathbf{R^1\text{-}N{=}C{=}S} \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

Preferable. R1 is a C_1-C_4 alkyl.
Preferable, said compound is butyl isothiocyanate.
Preferable, said compound is propyl isothiocyanate.
Preferable, R1 is a C_2-C_3 alkenyl.
Preferable, said compound is alkyl isothiocyanate.
Preferable, said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl.
Preferable, said phenyl alkyl is phenyl ethyl.
Preferable, said compound is 2-phenyl ethyl isothiocyanate.
Preferable, said compound is allyl-, propyl-, butyl-, pentenyl-, phenylethyl-isothiocyanates.

Preferable, according to the feed composition according to the third, fourth and fifth aspect of the present invention, said compound or extract in the feed are in a concentration range of 0.01-0,5, preferably in a concentration of 0.125% by weight of the feed.

A sixth aspect of the present invention relates to the use of a compound or extract for the prevention and/or treatment of a parasite infection in fish, preferable a salmonidae, wherein said extract or compound is selected from;

i) an extract (or oil) of , or

ii) a compound of formula (I);

$$\mathbf{R^1 - S - S - R^1} \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (II);

$$\mathbf{R^1\text{-}N{=}C{=}S} \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

Preferable, the compound or extract is used for the manufacturing of a pharmaceutical or nutraceutical composition, or functional food.

DESCRIPTION OF THE INVENTION

Embodiments of the invention will now be described, by the way of examples with reference to the following figures:

Figure 1 a shows the directional dose response of *L. salmonis* copepodids to seawater control, 100 parts per trillion (ppt) isophorone, 100 ppt isophorone plus 100 ppt garlic oil and 100 ppt garlic oil on its own.

Figure 1b shows the activation dose response of *L. salmonis* copepodids to seawater control, 100 parts per trillion (ppt) isophorone, 100 ppt isophorone plus 100 ppt garlic oil and 100 ppt garlic oil on its own.

Figure 2a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW), SCW plus 50 ppt diallyl disulfide and SCW plus 10 ppt diallyl sulfide.

Figure 2b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW), SCW plus 50 ppt diallyl disulfide and 10 ppt diallyl sulfide.

Figure 3a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 10, 1 and 0.1 ppt diallyl sulfide.

Figure 3b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 10, 1 and 0.1 ppt diallyl sulfide.

Figure 4a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 4-pentenyl, 2-phenylethyl and butyl isothiocyanate at 100 ppt.

Figure 4b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 4-pentenyl, 2-phenylethyl and butyl isothiocyanate at 100 ppt.

Figure 5a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 ppt propyl isothiocyanate.

Figure 5b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 ppt propyl isothiocyanate.

Figure 6a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus butyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 6b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus butyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 7a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus allyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 7b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus allyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 7c shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus allyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 7d shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus allyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt).

Figure 7e shows the aactivation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus allyl isothiocyanate at 1, 10 and 100 parts per trillion (ppt), blocks 1 and 2 combined.

Figure 8a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus bog myrtle, lavender and rosemary at 100 ppt.

Figure 8b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus bog myrtle, lavender and rosemary at 100 ppt.

Figure 9a shows the Directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 and 1000 ppt bog myrtle.

Figure 9b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 and 1000 ppt bog myrtle.

Figure 10a shows the directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 and 1000 ppt lavender.

Figure 10b shows the activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW) and SCW plus 100 and 1000 ppt lavender.

Figure 11. Chemotaxis response of *C. rogercresseyi* Copepodid to stimulus masked with compounds B1(A), B2(B) y B3(C) at different concentration (* $P < 0{,}05$; Chi-square test)

Figure 12. Preference Index of *C. rogercresseyi* copepodids to host signal masked with compounds B1(A), B2 (B) y B3(C) at different concentration.

Figure 13. Fish fed the butyl isothiocyanate (B1) showed a significant reduction of 42% in levels of sea lice compared to controls (Figure 2). There was a trend for a reduction in lice levels with both diallyl sulfide (B2) and diallyl disulfide (B3)

EXPERIMENTAL SECTION

Example 1: *In vitro* assessment of the effect of different compounds on *Lepeophtheirus salmonis*

A number of plant products were tested for their ability to mask salmon odour in order to inhibit the attractant of lice to salmon and to prevent *L.salmonis* settlement on salmon. A Y-tube behavioural arena was developed and used to test the ability of plant extracts/compounds to inhibit copepodid attraction to salmon conditioned water.

Products tested were:

- garlic constituents; garlic oil, diallyl disulphide and diallyl sulphide
- cruciferous isothiocyanates; allyl-, propyl-, butyl-, pentenyl-, phenyethyl-isothiocyanates
- plant extracts; bog myrtle, lavender, rosemary

Material and methods:

Lice collection

Ovigerous female *Lepeophtheirus salmonis* were collected from Atlantic salmon. Material was transported on ice to the laboratory with clean seawater for sorting. Water from the source site was collected and used for subsequent rearing of egg strings. Strings were removed gently from their point of attachment to adult females using ultra-fine forceps and placed in 2 L glass conical flasks. All flasks were aerated to keep the strings in suspension and promote hatching. Egg strings were reared under a 16 h light - 8 h dark regime and at 12°C ambient temperature in water from the source site.

Development of the egg to the copepodid was determined as a function of the mean temperature following Johnson and Albright (1991). Strings were monitored twice daily for hatching of nauplii and subsequent development to the copepodid stage, at which point they were removed for use in behavioural bioassays.

Fish Conditioned Water

Fish conditioned water was collected as described by Devine *et al.* (2000) and Ingvarsdottir *et al.* (2002b). Atlantic salmon, *S. salar* were maintained in aquaria containing artificial seawater (32 ‰). Fish conditioned water was obtained by placing the fish for 24 h into a circulating flume (20 cm x 25 cm x 420 cm) filled with artificial seawater (100 L) circulated at a rate of 30 cm s^{-1}. Aeration was provided by bubbling compressed air into the raceway. Standardisation of fish odour in the water was achieved by using the water at a concentration of 8-10 g live fish L^{-1} 24 h^{-1}. Conditioned water was either used immediately or frozen for later use.

Lice Behaviour *L. salmonis*

A vertical Y-tube bioassay modified by Bailey *et al.* (2006) from that previously described by Ingvarsdóttir *et al.* (2002a) was used to study *L. salmonis* copepodid activation and directional (taxis) responses to host semiochemical components and potential host-masking compounds. The Y-tube was constructed from glass (1 cm diameter bore) moulded into a 'Y' design between two glass sheets of glass (2 mm thick). The arms were 6.5 cm in length and the main leg was 8 cm long. The main leg of the Y-tube was fitted with a glass stopper and filter to prevent copepodids from entering the outflow tubing running to waste. A syringe pump (SP 200 iz, World Precision Instruments, Florida, USA) held two plastic 60 mL syringes (Terumo Monoject, New Jersey, USA), which were loaded with test odours prior to use. The syringe pump was programmed to deliver a consistent flow rate of 2 mL min^{-1}. Chemical dyes demonstrated a clear demarcation of the flow down each arm and no mixing of water in the main leg of the T-tube.

When single chemical stimuli were tested e.g. salmon conditioned water (SCW), the test water was introduced to one arm whilst artificial seawater (ASW) at 32 ‰ was introduced into the other. When one of the isothiocyanates for example was tested, seawater was introduced into one arm whilst SCW plus the isothiocyanate at the desired concentration were introduced to the other. The introduction of stimuli was alternated between left and right inflow arms during each experiment, with washing in between, to eliminate positional bias. At the beginning of each experiment, the Y-tube was allowed to fill and run with seawater or seawater plus a cue/masking chemical, and a single copepodid was introduced using PTFE tubing and syringe into the tube at a point 1.5 cm above the base of the main leg. The copepodid was allowed a maximum of 3 min to respond. Each trial consisted of 1 copepodid.

Replicate tests were carried out over a period of four days to monitor for age effects of the lice on results.

Behaviour was defined by the degree of movement within the Y-Tube, as described by Ingvarsdóttir *et al.* (2002b). Behaviour was divided into two categories, low and high. Low activity was defined as the movement of the copepodid less than the length of the main leg. High activity was defined as movement of the copepodid more than the length of the main-leg. Movement into either arm was also regarded as high activity. Both activation and directional responses of copepodids were measured. For directional responses, the number of copepodids choosing the stimulus arm rather than the control arm within the allocated 3 min period were compared to the control in which seawater was presented in both arms.

<u>Chemicals</u>

Chemicals used in behavioural bioassays were supplied by the Chemical Ecology Group at Rothamsted Research, Harpenden, Hertfordshire, UK. Solutions of individual chemicals in ethanol (0.001, 0.01, 0.1 and 1 mg/mL) were prepared and diluted to 1 µL/L in artificial seawater (Ingvarsdóttir *et al.*, 2002b) to give a final concentration of 0.1, 1, 10, 100 and 1,000 parts per trillion (ppt) respectively.

<u>Data Analysis</u>

Copepodid responses to ASW (Artificial sea water) and SCW (Salmon conditioned sea water) across all experiment days were compared in the first instance using a chi-square test to determine if there was a day effect on louse behaviour. If this proved to be non-significant, it implies that the data are consistent across days and therefore can be pooled.

For directional responses and experiments on activity, the null hypothesis that all lice in all treatments behaved the same was tested using a 'global' χ^2 contingency table (Zar, 1999). Upon rejection of that hypothesis, data were analysed by *post hoc* targeted pairwise comparisons using a 2 x 2 χ^2 contingency table (Zar, 1999) to identify whether pairs of treatments of interest were significantly different.

Experiments testing whether allyl isothiocyanate can mask the attractiveness of salmon conditioned water were conducted in two blocks. In addition to χ^2 analysis of the original data (block 1), binomial logistic regression was used to test whether copepodid directional and activation responses differed both between experimental treatments (salmon conditioned water presented alone, or with three concentrations of ally isothiocyanate, against an artificial seawater control) and between blocks. Two separate models were constructed, with either copepodid directional response (test or control) or activity (high or low) entered as the dependent variable. In both cases, treatment and block were entered as factors, with a treatment by block interaction included to test if louse responses to each treatment varied between blocks. Significance of terms in both models was investigated through stepwise deletion (changes in deviance assessed through χ^2 tests) and comparisons of responses at each concentration of allyl isothiocyanate with respect to salmon conditioned water (no allyl isothiocyanate) made using Wald statistics.

Results *in vitro* assessment *Lepeophtheirus salmonis*

Garlic Oil

For directional responses i.e. upstream positive rheotaxis, the global χ^2 showed that lice did not behave the same in all treatments ($\chi^2 = 26.42$, df = 3, $P < 0.001$). When compared with the seawater control, significantly more copepodids chose the arm containing the isophorone ($\chi^2 = 6.87$, df = 1, $P < 0.01$), a component of salmon conditioned water. A significant decrease in copepodid responses was detected with isophorone plus garlic oil ($\chi^2 = 8.72$, df = 1, $P < 0.01$) and with garlic oil alone ($\chi^2 = 25.1$, df = 1, $P < 0.001$) when compared against isophorone responses (Figure 1a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2a.

Table 2a

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	36	76
ASW v Isophorone	100	0	100
ASW v Isophorone + Garlic Oil	58	21	79
ASW v Garlic Oil	50	0	50

Under control conditions, when only seawater was present in both arms of the Y tube, 68% of copepodids showed low activity, and 32% were in the high activity category. The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 72.81, df = 3, P < 0.001). When compared with the seawater control, a significant increase in high activity behaviour was observed in the presence of isophorone (χ^2 = 36.57, df = 1, P < 0.001). Significantly more copepodids showed low activity with isophorone plus garlic oil when compared against isophorone responses (χ^2 = 7.25, df = 1, P < 0.01) however. No difference in activity was detected between garlic oil alone and isophorone (χ^2 = 0, df = 1, NS; Figure 1b).

Garlic Oil Compounds: Diallyl Disulfide and Diallyl Sulphide

For directional responses, the global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 14.17, df = 3, P < 0.001). When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 11.82, df = 1, P < 0.001). A significant decrease in copepodid responses was seen with SCW plus 50 ppt diallyl disulfide (χ^2 = 9.43, df = 1, P < 0.01) and SCW plus 10 ppt diallyl sulphide (χ^2 = 16.54, df = 1, P < 0.001) when compared against SCW responses (Figure 2a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2b.

Table 2b

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	36	84	120
ASW v SCW	81	2	83
ASW v SCW + 50 ppt DDS	27	28	55
ASW v SCW + 10 ppt DS	27	28	55

Under control conditions, when only seawater was present in both arms of the Y tube, 62% of copepodids showed low activity, and 38% were in the high activity category. The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 80.89, df = 3, P < 0.001). When compared with the seawater control, a significant increase in high activity was seen in the presence of SCW (χ^2 = 80.54, df = 1, P < 0.001). Significantly more copepodids showed low activity in the presence of SCW plus 50 ppt diallyl disulfide (χ^2 = 43.84, df = 1, P < 0.001) and SCW plus 10 ppt diallyl sulphide (χ^2 = 33.25, df = 1, P < 0.001) when compared against SCW responses (Figure 2b).

Diallyl Sulphide Dose Response

The global χ^2 showed that lice behaved the same in all treatments (χ^2 = 7.25, df = 4, NS) in directional response assays. As a result, further pair wise comparisons were not carried out (Figure 3a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2c.

Table 2c

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	113	153
ASW v SCW	19	1	20
ASW v SCW + 10 ppt DS	15	5	20
ASW v SCW + 1 ppt DS	14	6	20
ASW v SCW + 0.1 ppt DS	17	3	20

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 42.02, df = 4, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW (χ^2 = 19.64, df = 1, P < 0.001). Significantly more copepodid showed low activity with SCW plus 1 ppt diallyl sulfide (χ^2 = 4.44, df = 1, P < 0.05) when compared against SCW responses. No difference in activity was detected between SCW plus 0.1 (χ^2 = 1.03, df = 1, NS) and 10 ppt diallyl sulphide (χ^2 = 2.11, df = 1, NS; Figure 3b) however.

Isothiocyanate Compounds

The global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 26.50, df = 4, P < 0.001) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 11.82, df = 1, P < 0.001). A significant decrease in copepodid responses was detected with SCW plus 100 ppt 2-phenylethyl (χ^2 = 13.06, df = 1, P < 0.001) and SCW plus 100 ppt butyl isothiocyanate (χ^2 = 15.14, df = 1, P < 0.001) when compared against SCW responses. No difference in directional responses was detected between SCW plus 100 ppt 4-pentenyl isothiocyanate and SCW responses (χ^2 = 0.7, df = 1, NS; Figure 4a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2d.

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 97.56, df = 4, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW (χ^2 = 80.54, df = 1, P < 0.001). Significantly more copepodids showed low activity with SCW plus 100 ppt 4-pentenyl (χ^2 = 25.97, df = 1, P < 0.001), 100 ppt 2-phenylethyl (χ^2 = 41.40, df = 1, P < 0.001) and 100 ppt butyl isothiocyanate (χ^2 = 75.42, df = 1, P < 0.001) when compared against SCW responses (Figure 4b).

Table 2d

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	36	84	120
ASW v SCW	81	2	83
ASW v SCW + 4-Pentenyl Isothiocyanate	24	26	50
ASW v SCW + 2-Phenylethyl Isothiocyanate	15	35	50
ASW v SCW + Butyl Isothiocyanate	12	40	52

<u>Propyl Isothiocyanate</u>

The global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 39.84, df = 2, P < 0.001) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 7.42, df = 1, P < 0.01). A significant decrease in copepodid responses was detected with SCW plus 100 ppt propyl isothiocyanate ($\chi^2 = 39.58$, df = 1, $P < 0.001$) when compared against SCW responses (Figure 5a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2e.

The global χ^2 showed that lice did not behave the same in all activity treatments ($\chi^2 = 59.78$, df = 2, $P < 0.001$). When compared with the seawater control, a significant increase in high activity was detected with SCW ($\chi^2 = 26.69$, df = 1, $P < 0.001$). No difference in activity was detected between SCW plus 100 ppt propyl isothiocyanate however ($\chi^2 = 0$, df = 1, NS; Figure 5b).

Table 2e

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	113	153
ASW v SCW	56	0	56
ASW v SCW + 100 ppt Propyl Isothiocyanate	95	5	100

Butyl Isothiocyanate Dose Response

The global χ^2 showed that lice did not behave the same in all treatments ($\chi^2 = 23.99$, df = 4, $P < 0.001$) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW ($\chi^2 = 8.01$, df = 1, $P < 0.01$). A significant decrease in copepodid responses was detected with SCW plus 10 ppt ($\chi^2 = 5.84$, df = 1, $P < 0.05$) and 100 ppt butyl isothiocyanate ($\chi^2 = 20.81$, df = 1, $P < 0.001$) when compared against SCW responses. However, no difference in directional responses was detected between SCW plus 1 ppt butyl isothiocyanate and SCW responses ($\chi^2 = 1.84$, df = 1, NS; Figure 6a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2f.

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 91.94, df = 4, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW (χ^2 = 75.04, df = 1, P < 0.001). Significantly more copepodids showed low activity with SCW plus 100 ppt butyl isothiocyanate (χ^2 = 15.43, df = 1, P < 0.001) when compared against SCW responses. No difference in activity was detected between SCW plus 1 and 10 ppt butyl isothiocyanate however (χ^2 = 2.65 and 2.64 respectively, df = 1, NS; Figure 6b).

Table 2f

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	14	51	65
ASW v SCW	89	31	120
ASW v SCW + 1 ppt Butyl Isothiocyanate	40	20	60
ASW v SCW + 10 ppt Butyl Isothiocyanate	35	25	60
ASW v SCW + 100 ppt Butyl Isothiocyanate	34	31	65

Allyl Isothiocyanate Dose Response

The global χ^2 showed that lice behaved the same in all treatments (χ^2 = 4.65, df = 4, NS) in directional response assays. As a result, further pairwise comparisons were not carried out (Figure 7a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2g.

Table 2g

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	12	33	45
ASW v SCW	45	16	61
ASW v SCW + 1 ppt Allyl Isothiocyanate	28	22	50
ASW v SCW + 10 ppt Allyl Isothiocyanate	25	25	50
ASW v SCW + 100 ppt Allyl Isothiocyanate	26	24	50

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 37.24, df = 4, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW (χ^2 = 27.99, df = 1, P < 0.001). No difference in activity was detected between SCW plus 1, 10 or 100 ppt allyl isothiocyanate and SCW responses however (χ^2 = 2.24, 1.54, 3.04 respectively, df = 1, NS; Figure 7b).

Allyl Isothiocyanate Dose Response - Updated Analysis

The effect of treatment on louse directional responses was found to differ between blocks, as demonstrated by a significant treatment by block interaction term (χ^2 = 8.24, df = 3, P < 0.05). While no overall difference in louse behaviour was found between treatments in experiments conducted in block 1 (July 2005; χ^2 = 4.24, df = 3, NS; Figure 7c), an overall effect of treatment was found in block 2 (June-October

2006; χ^2 = 9.11, df = 3, P < 0.05; Figure 7d), with fewer copepodids chose the test arm in the presence of 100 ppt allyl isothiocyanate than SCW presented unmasked (Wald = 4.65, df = 1, P < 0.05; Figure 7c).

There was no significant effect of block by treatment (χ^2 = 5.09, df =3, NS) or block (χ^2 = 0.001, df = 1, NS) on activation responses, allowing data to be pooled across blocks. No subsequent overall difference in activation responses was found across treatments (χ^2 = 1.90, df =3, NS; Figure 7d). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2h.

Table 2h

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment (blocks 1 and 2).

Assay	Directional Responses	No Choice	Total No. Replicates
ASW v SCW	147	68	215
ASW v SCW + 1 ppt Allyl Isothiocyanate	72	66	138
ASW v SCW + 10 ppt Allyl Isothiocyanate	58	44	102
ASW v SCW + 100 ppt Allyl Isothiocyanate	37	29	66

Plant Extracts

The global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 33.38, df = 4, P < 0.001) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 7.89, df = 1, P < 0.01). A significant decrease in copepodid responses was detected with SCW plus 100 ppt lavender (χ^2 = 19.03, df =

1, $P < 0.001$) and 100 ppt rosemary ($\chi^2 = 17.89$, df = 1, $P < 0.001$) when compared against SCW responses. However, no difference in directional responses was detected between SCW plus 100 ppt bog myrtle and SCW responses ($\chi^2 = 0.01$, df = 1, NS; Figure 8a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2i.

Table 2i

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	113	153
ASW v SCW	136	15	151
ASW v SCW + 100 ppt Bog Myrtle	70	30	100
ASW v SCW + 100 ppt Lavender	97	4	101
ASW v SCW + 100 ppt Rosemary	48	52	100

The global χ^2 showed that lice did not behave the same in all activity treatments ($\chi^2 = 144.34$, df = 4, $P < 0.001$). When compared with the seawater control, a significant increase in high activity was detected with SCW ($\chi^2 = 91.70$, df = 1, $P < 0.001$). Significantly more copepodids showed low activity with SCW plus 100 ppt bog myrtle ($\chi^2 = 12.23$, df = 1, $P < 0.001$) and SCW plus 100 ppt rosemary ($\chi^2 = 43.24$, df = 1, $P < 0.001$) when compared against SCW responses. No difference in activity was detected between SCW plus 100 ppt lavender however ($\chi^2 = 2.03$, df = 1, NS; Figure 8b).

Bog Myrtle Dose Response

The global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 19.35, df = 3, P < 0.001) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 7.89, df = 1, P < 0.01). A significant decrease in copepodid responses was detected with SCW plus 1,000 ppt bog myrtle (χ^2 = 15.88, df = 1, P < 0.001) when compared against SCW responses. No difference in directional responses was detected between SCW plus 100 ppt bog myrtle and SCW responses however (χ^2 = 0.01, df = 1, NS; Figure 9a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2j.

Table 2j

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	113	153
ASW v SCW	136	15	151
ASW v SCW + 100 ppt Bog Myrtle	70	30	100
ASW v SCW + 1,000 ppt Bog Myrtle	48	7	55

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 122.56, df = 3, P < 0.001). When compared with the seawater control, a significant increase in high activity was seen with SCW (χ^2 = 91.70, df = 1, P < 0.001). Significantly more copepodids showed low activity with SCW plus 100 ppt bog myrtle (χ^2 = 12.23, df = 1, P < 0.001) when compared against SCW responses. No difference in activity was detected between SCW plus 1,000 ppt bog myrtle however (χ^2 = 0.81, df = 1, NS; Figure 9b).

Lavender Dose Response

The global χ^2 showed that lice did not behave the same in all treatments (χ^2 = 19.46, df = 3, P < 0.001) in directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing the salmon conditioned water, SCW (χ^2 = 7.89, df = 1, P < 0.01). A significant decrease in copepodid responses was detected when SCW plus 100 ppt (χ^2 = 19.03, df = 1, P < 0.001) and 1,000 ppt lavender (χ^2 = 7.02, df = 1, P < 0.01) were compared against SCW responses (Figure 10a). The number of *L. salmonis* copepodids making directional responses, not choosing and the total number of replicates for each treatment are presented in Table 2k.

Table 2k

Number of *L. salmonis* copepodids making directional responses, non-choosers and the total number of replicates for each treatment.

Assay	Directional Responses	No Choice	Total No. Replicates
ASW Control	40	113	153
ASW v SCW	136	15	151
ASW v SCW + 100 ppt Lavender	97	4	101
ASW v SCW + 1,000 ppt Lavender	34	2	36

The global χ^2 showed that lice did not behave the same in all activity treatments (χ^2 = 160.36, df = 3, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW (χ^2 = 91.70, df = 1, P < 0.001). No difference in activity was detected between SCW plus 100 ppt (χ^2 = 2.03, df = 1, NS) and 1,000 ppt lavender (χ^2 = 0.81, df = 1, NS; Figure 10b) however.

Discussion:

In this study, it has been shown that copepodid larvae of the salmon louse, *L. salmonis*, show significant directional responses to isophorone, a component of salmon conditioned water. Isophorone has been identified as a behaviourally active component of salmon-conditioned water (Bailey *et al.*, 2006) and was therefore used as a host cue to elicit a response in preliminary experiments. The inclusion of garlic oil with isophorone, removed the attraction to isophorone. On its own, garlic oil appears to repel lice to the artificial seawater. It is suggested here that garlic oil may act as a lice repellent and/or mask host odour cues, i.e. as a semiochemical masking and attraction reducing material.

We have also found that the addition of 50 and 10 parts per trillion diallyl sulphide removed the attraction to salmon conditioned water. Diallyl sulphide at 10 parts per trillion however appeared to be the more effective masking compound.

Further, we have shown that 2-phenylethyl, butyl and propyl isothiocyanate at the 100 parts per trillion concentration, removed the attraction of copepodids to salmon conditioned water..4-pentenyl isothiocyanate did not mask copepodid responses to salmon conditioned water however. Dose response experiments with butyl isothiocyanate showed 100 parts per trillion to be the most effective concentration for switching off responses to salmon conditioned water. Allyl isothiocyanate dose response assays suggest a possible effect at 100 parts per trillion.

For the plant extracts, both rosemary and lavender at 100 parts per trillion were effective at masking the salmon conditioned water. Bog myrtle dose response assays showed significant masking to occur at the 1,000 ppt concentration however.

A high number of non-choosers were seen in all seawater controls and is due to a lack of cues to stimulate a behavioural response from the lice.

In general, the seawater controls showed predominantly low activity behaviour in copepodids. This switched to high activity in the presence of a positive cue i.e. either isophorone or salmon conditioned water. Low activity re-appeared in the profile when test compounds were introduced, suggesting that the chemicals masked the effect of the isophorone or salmon conditioned water in copepodids. The extent of masking was variable between compounds and is thought to be related to the original field source of *L. salmonis*.

Conclusions from example 1:

The use of plant derived masking compounds has been shown to significantly disrupt *L. salmonis* copepodid attraction to host (salmon) conditioned water *in vitro*. By masking the profile of the key host recognition molecules it was surprisingly possible to significantly reduce the host response of both *L. salmonis* and C. *rogercresseyi*. In the shown series of Y-tube assessments, sea lice showed a significant activity towards host odours from control Atlantic salmon. Inclusion of a series of masking compounds of vegetable origin effectively reduced this response in both species. Diallyl sulphide, diallyl disulphide, butyl isothiocyanate, allyl isothiocyanate, propyl Isothiocyanate, rosemary oil, lavender oil and bog myrtle were identified as candidate compounds for masking salmon host compounds.

The following compounds and concentrations were especially promising: Diallyl sulphide (10parts per trillion), diallyl disulphide (100parts per trillion), butyl isothiocyanate (100 parts per trillion), propyl isothiocyanate (100 parts per trillion) rosemary oil (100 parts per trillion), lavender oil (100 parts per trillion) and bog myrtle (1,000 parts per trillion).

Example 2

Evaluation of the effect of masking compounds on chemicals cues released by Atlantic salmon

Material and methods

Fish conditioned water

Atlantic salmon, *S. salar* were hatchery-reared stock produced at the west coast of Puerto Montt (Chile). For the preparation of Salmon Conditioned Water (SCW), one fish-host (100–200 g) was placed in a flume during 24 h with artificial seawater (100 L) (Aquarium salt; SERA, Heinsberg/Germany) with a salinity of 32‰ at 12 °C. The flow rate in the flume was 30 cm s−1 (Ingvarsdottir et al., 2002b). The water kept in the flume was used for bioassays, or frozen for use in chemical analysis.

Lice

Ovigerous C. rogercresseyi females were collected from freshly harvested Atlantic salmon, on commercial fish farms on the west coast of Puerto Montt (Chile). Egg strings were removed gently from their point of attachment to adult females using ultra-fine forceps and were placed in a 500 mL glass culture flask with artificial seawater and held in suspension by an air supply through the stem at 12 °C keep them in absolute darkness until the copepodid stage was reached.

Semiochemical Masking Compounds

Butyl isothiocyanate (B1), Diallyl sulphide (B2) and Diallyl disulfide (B3) were selected as test compounds. Each of the compounds were prepared in three solution with ethanol 1.0; 0.01 and 0.001 mg mL^{-1}, then where diluted to 1 µL L-1 in Salmon Conditioned Water (SCW).

Preference Bioassays

A vertical Y-tube bioassay modified from that previously described was used to study *C. rogercresseyi* copepodid activation and directional (taxis) responses to host odours. The Y tube was made from perspex. The arms were 5 cm in length and the main leg was 6 cm long.

Water flowed through into each arm from reservoirs positioned immediately above the Y tube at a rate of 2 mL·min–1. In control assays, artificial seawater was introduced into both arms of the Y tube. When salmon-conditioned water (SCW) plus masking compounds were tested, the test water was introduced into one arm while seawater was introduced into the other.

At the beginning of each experiment, the Y tube was allowed to fill, and a single copepodid was introduced by polytetrafluoroethylene tubing (1 mm internal diameter (i.d.)) and syringe into the tube at a point 1 cm above the base of the main arm.

The copepodid was allowed a maximum of 5 min to respond. Behaviour was defined by the degree of movement within the Y tube, as described previously. Behaviour was divided into two categories, low and high. Low activity was defined as the movement of the copepodid less than the length of the main leg. High activity was defined as movement of the copepodid more than the length of the main leg. Preference was observed when the copepodid with high activity choose either arm. Both activation and directional responses of copepodids were measured. Each trial consisted of one copepodid, and each copepodid was never used more than once. There were 30–100 trials conducted for each experiment.

Results

Table 1

Response of copepodids Caligus rogercresseyi to different concentration of masking compounds in a vertical Y-tube bioassays.

	Activity (%)				
	High	Low	X^2	P	N
Control	67.50	32.50	4.9	0.027	40
SCW	62.64	37.36	5.8	0.016	100
B1. Butyl isotiocianate					
SCW + (0,001 mg/mL)	68.63	31.37	7.1	0.008	50
SCW + (0,01 mg/mL)	62.86	37.14	4.2	0.031	70
SCW + (1,00 mg/mL)	69.00	31.00	4.7	0.029	70
B2. Dialyl sulfide					
SCW + (0,001 mg/mL)	86.67	13.33	24.2	0.000	45
SCW + (0,01 mg/mL)	85.00	15.00	19.6	0.000	40
SCW + (1,00 mg/mL)	93.33	6.67	22.5	0.000	30
B3. Dialyl disulfide					
SCW + (0,001 mg/mL)	80.65	19.35	24.2	0.000	31
SCW + (0,01 mg/mL)	67.50	32.50	4.9	0.027	40
SCW + (1,00 mg/mL)	93.33	6.67	22.5	0.000	30

$P > 0,05$ (test X^2).

The level of preference was affected when the masking compounds were added. B1 at 0.01 and 1 mg / mL tends to change the preference shown by the sea lice at a lower concentration and control (Fig. 11A).

B2, at all the concentrations, showed a masking effect on the chemical cues released by Atlantic salmon, although no significant differences (Fig. 11B). B3 at 0.001 mg / mL significantly ($P > 0.05$) changed the preference of the copepodids (Fig. 11C).

An index of preference (IP) were calculated. IP = # visits at stimulus zones / # visits in the control zone. Which indicates, if IP = 1, there is no avoidance neither preference, if IP > 1, it indicates that there is preference for the stimulus, and if IP <1, it indicates an avoidance for the stimulus or a preference for control.

This study found that the IP calculated for B1 showed that the highest concentrations (0.01 and 1.0 mg mL-1) reduced the preference for the stimulus of Atlantic salmon. In the case of masked B2 and B3, the IP showed that both compounds were effective in their action of masking chemical cues (Fig. 12).

Example 3

Effects of B's in feed on disruption of copepodids settlement of *Caligus rogercresseyi*

The aim of this experiment was to validate the effect of three masking compounds in feeds on the disruption of copepodid settlement and *in vivo* challenge assays.

Materials and Method

Semiochemical Masking Compounds

Isobutyl thiocyanate (B1), Diallyl sulfide (B2) and Diallyl disulfide (B3) were selected as test compounds.

Tank Trails

Fish

Atlantic salmon, *Salmo salar* (N = 168; 500g avg), hatchery-reared stock produced and maintained in Chile prior to the experiment, were in Chile, smolted gradually through a freshwater to seawater gradient and held in a circular tank (12 m^3). Fish were pit-tagged at the end of smoltification.

Sealice

100 ovigerous females *C. rogercresseyi* (5000 copepodids) for each tank were collected from freshly harvested Atlantic salmon, *S. salar* were placed in 2000 mL glass culture flasks with clean seawater, and held in suspension by an air supply through the stem at 12°C in absolute darkness. Egg strings were removed gently from their point of attachment using ultra-fine forceps and were placed in a 2000 mL glass culture flask with clean seawater and held in suspension with air supply at 12°C keep them in absolute darkness until the copepodid stage was reached. The emerged copepodid were used for infestation during the trail.

Tank distribution.

14 fish, individually weight and tagged, were distributed in 12 fibreglass tanks (350L) with a flow through seawater (32 ‰) system at 13-14°C. Three tanks (replicas) were used for each masking compound dose and control diet.

Masking compound feed formulation.

A dose of masking compounds (B1, B2 and B3) (0.125%) were tested against sea lice settlement compare with a commercial diet used as a Control. Feeding periods were held for 21 days, before sea lice infestation (Table 1). Post-Infestation feeding was held for 8 days.

Table 1

Setting up experiments.

Feeding Tanks	Formulation	Number of Fish	Fish weight (g)	Feeding days)
1, 2, 3	FormB1 (0.125%)	14	500	21
4, 5, 6	FormB2 (0.125%)	14	500	21
7, 8, 9	FormB3 (0.125%)	14	500	21
10, 11, 12	Control	14	500	21

Sea lice Counting

Fish were culled and removed for the sampling. Sea lice were counted individually on each fish at 8 days post challenge.

Results

Fish fed the butyl isothiocyanate (B1) showed a significant reduction of 42% in levels of sea lice compared to controls (Figure 13). There was a trend for a reduction in lice levels with both diallyl sulfide (B2) and diallyl disulfide (B3)

It will be appreciated that the features of the invention described in the foregoing can be modified without departing from the scope of the invention.

Definitions of terms:

The term "semiochemical" (semeon means a signal in Greek) is a generic term used for a chemical substance or mixture that carries a message. These chemicals acts as messengers for members of the same species or in some cases other species. It is usually used in the field of chemical ecology to encompass pheromones, allomones, kairomones, attractants and repellents. Please note especially that the term in respect of this application is not restricted to messengers between the same species, and that the term specifically is used to denote messengers between different species, such as between a Salmonidae and a parasite. The term is intended to include the chemical compounds which are specific for the attraction of parasites to Salmonidae, and especially to the attraction of sea lice to Salmonidae.

Claims

1. A method for masking the odor of a fish semiochemical in water, characterized in that an extract or compound is added to said water or is administered to a fish in said water, wherein said extract or compound is selected from;

i) an extract of garlic, rosemary, lavender or bog myrtle, or

ii) a compound of formula (I);

$$R^1 - S - S - R^1 \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (II);

$$R^1\text{-}N{=}C{=}S \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

2. A method according to claim 1, wherein said fish semiochemical is isophorone.

3. A method according to claim 1, wherein said fish semiochemical is 1-Octen-3-ol or 6-methyl-5-hepten-2-one.

4. A method according to claim 1, wherein said fish is a Salmonidae.

5. A method according to claim 1, wherein said water is Salmonidae conditioned sea water or said fish in the water is a Salmonidae.

6. A method according to claim 4 or 5, wherein said salmonidae is selected from the group consisting of Atlantic salmon, coho salmon, Chinook, rainbow trout, Arctic charr and other farmed salmon species.

7. A method according to any of the preceding claims, wherein the method reduces the attraction between a parasite and said fish.

8. A method according to claim 7, wherein said parasite is an ectoparasite.

9. A method according to claim 8, wherein said ectoparasite is sea lice (*Lepeophtheirus salmonis, Caligus* sp.).

10. A method according to claim 1, wherein said compound is a compound of formula (I).

11. A method according to claim 10, wherein at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

12. A method according to claim 10, wherein both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

13 A method according to claim 10, wherein said compound is diallyl disulfide or diallyl sulphide.

14. A method according to claim 1, wherein said compound is a compound of formula (II).

15. A method according to claim 14, wherein R1 is a C_1-C_4 alkyl.

16. A method according to claim 14, wherein said compound is butyl isothiocyanate.

17. A method according to claim 14, wherein said compound is propyl isothiocyanate.

18. A method according to claim 14, wherein R1 is a C_2-C_3 alkenyl.

19. A method according to claim 14, wherein said compound is alkyl isothiocyanate.

20. A method according to claim 14, wherein said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl.

21. A method according to claim 20, wherein said phenyl alkyl is phenyl ethyl.

22. A method according to claim 14, wherein said compound is allyl-, propyl-, butyl-, pentenyl-, phenyethyl-isothiocyanates.

23. A method according to claim 14, wherein said compound is 2-phenyl ethyl isothiocyanate.

24. A method for reducing the attraction between a parasite and a fish, or for reducing the infestation or infection of a parasite in a fish, or for the treatment of a parasite infection in a fish, characterized in that an extract or compound is added to said water or is administered to a fish in said water, wherein said extract or compound is selected from;

i) an extract of rosemary or bog myrtle, or

ii) a compound of formula (I);

$$\mathbf{R^1 - S - S - R^1} \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (II);

$$\mathbf{R^1\text{-}N{=}C{=}S} \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

25. A method according to claim 24, wherein said fish is a Salmonidae.

26. A method according to claim 24, wherein said water is Salmonidae conditioned sea water or said fish in the water is a Salmonidae.

27. A method according to claim 25, wherein said salmonidae is selected from the group consisting of Atlantic salmon, coho salmon, Chinook, rainbow trout, Arctic charr and other farmed salmon species.

28. A method according to claim 25, wherein said salmonidae is Atlantic salmon.

29. A method according to claim 25, wherein said salmonidae is rainbow trout.

30. A method according to any of the preceding claims, wherein the method reduces the attraction between a parasite and said fish.

31. A method according to claim 30, wherein said parasite is an ectoparasite.

32. A method according to claim 31, wherein said ectoparasite is sea lice (*Lepeophtheirus salmonis, Caligus* sp.).

33. A method according to claim 24, wherein said compound is a compound of formula (I).

34. A method according to claim 33, wherein at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

35. A method according to claim 33, wherein both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

36 A method according to claim 33, wherein said compound is diallyl disulfide or diallyl sulphide.

37. A method according to claim 24, wherein said compound is a compound of formula (II).

38. A method according to claim 37, wherein R1 is a C_1-C_4 alkyl.

39. A method according to claim 37, wherein said compound is butyl isothiocyanate.

40. A method according to claim 37, wherein said compound is propyl isothiocyanate.

41. A method according to claim 37, wherein R1 is a C_2-C_3 alkenyl.

42. A method according to claim 37, wherein said compound is alkyl isothiocyanate.

43. A method according to claim 37, wherein said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl.

44. A method according to claim 43, wherein said phenyl alkyl is phenyl ethyl.

44. A method according to claim 37, wherein said compound is allyl-, propyl-, butyl-, pentenyl-, phenyethyl-isothiocyanates

45. A method according to claim 37, wherein said compound is 2-phenyl ethyl isothiocyanate.

46. A feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises rosemary or bog myrtle.

47. A feed composition according to claim 46, wherein said rosemary or bog myrtle masks the odor of a fish, preferable a Salmonidae.

48. A feed composition according to claim 46, wherein said compound or material masks the odor of salmonids in Salmonidae conditioned sea water.

49. A feed composition according to claim 46, wherein said compound or material masks the odor of isophorone or 1-Octen-3-ol or 6-methyl-5-hepten-2-one.

50. A feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises a compound of formula (I);

$$\mathbf{R^1 - S - S - R^1} \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl.

51. A feed composition according to claim 50, wherein at least one R^1 is $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

52. A feed composition according to claim 50, wherein both R^1 groups are identical, and are either $-CH_2-CH=CH_2$ or $-CH=CH-CH_3$.

53. A feed composition according to claim 50, wherein said compound is diallyl disulfide.

54. A feed composition comprising conventional feed ingredients such as lipids, proteins, vitamins, carbohydrates and minerals, characterized in that the feed comprises a compound of formula (II);

$$\mathbf{R^1\text{-}N{=}C{=}S} \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

55. A feed composition according to claim 54, wherein R1 is a C_1-C_4 alkyl.

56. A feed composition according to claim 54, wherein said compound is butyl isothiocyanate.

57. A feed composition according to claim 54, wherein said compound is propyl isothiocyanate.

58. A feed composition according to claim 54, wherein R1 is a C_2-C_3 alkenyl.

59. A feed composition according to claim 54, wherein said compound is alkyl isothiocyanate.

60. A feed composition according to claim 54, wherein said phenyl alkyl is phenyl methyl, phenyl ethyl or phenyl propyl.

61. A feed composition according to claim 60, wherein said phenyl alkyl is phenyl ethyl.

62. A feed composition according to claim 161, wherein said compound is 2-phenyl ethyl isothiocyanate.

63. A feed composistion according to claim 54, wherein said compound is allyl-, propyl-, butyl-, pentenyl-, phenylethyl-isothiocyanates.

64. A feed composition according to one of the claims 46, 50 or 54, said compound or extract in the feed are in a concentration range of 0.01-0,5, preferably in a concentration of 0.125% by weight of the feed.

65. Use of a compound or extract for the prevention and/or treatment of a parasite infection in fish, preferable a salmonidae, wherein said extract or compound is selected from;

i) an extract (or oil) of , or

ii) a compound of formula (**I**);

$$R^1 - S - S - R^1 \qquad (I)$$

wherein each R^1 independently of each other is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3 alkynyl, or

iii) a compound of formula (**II**);

$$R^1\text{-}N{=}C{=}S \qquad (II)$$

wherein R^1 is C_1-C_4 alkyl or C_2-C_3 alkenyl or C_2-C_3-alkynyl or phenyl alkyl.

Figure 1a

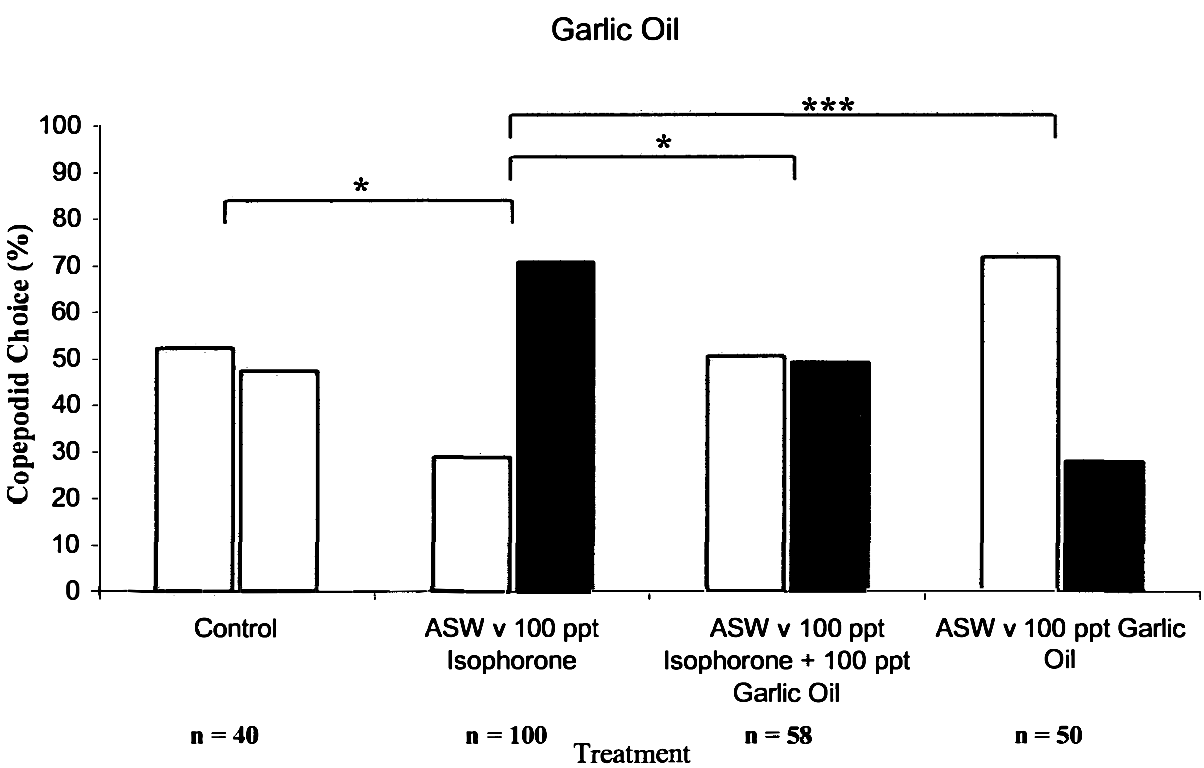

Figure 1a: Directional dose response of *L. salmonis* copepodids to seawater control, 100 parts per trillion (ppt) isophorone, 100 ppt isophorone plus 100 ppt garlic oil and 100 ppt garlic oil on its own.

Key □ Seawater ■ Odour ppt, parts per trillion

Figure 1b

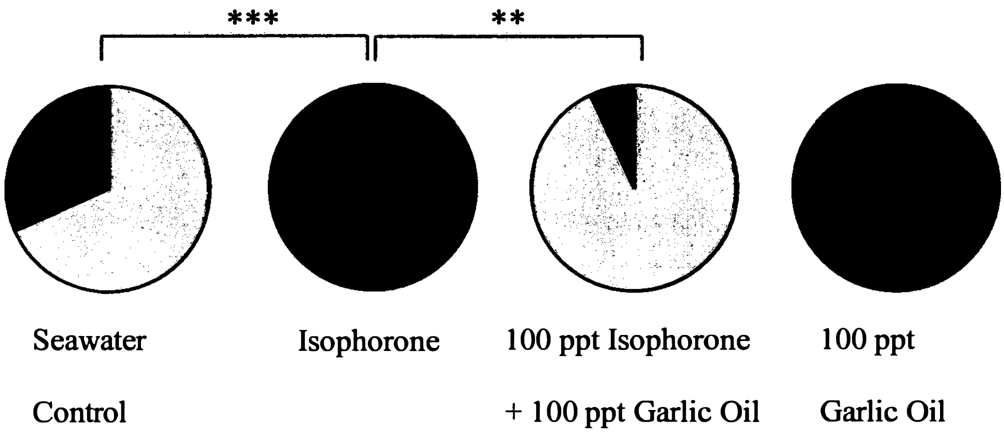

Figure 1b: Activation dose response of *L. salmonis* copepodids to seawater control, 100 parts per trillion (ppt) isophorone, 100 ppt isophorone plus 100 ppt garlic oil and 100 ppt garlic oil on its own.

Key □ Low Activity ■ High Activity ppt, parts per trillion

Figure 2a

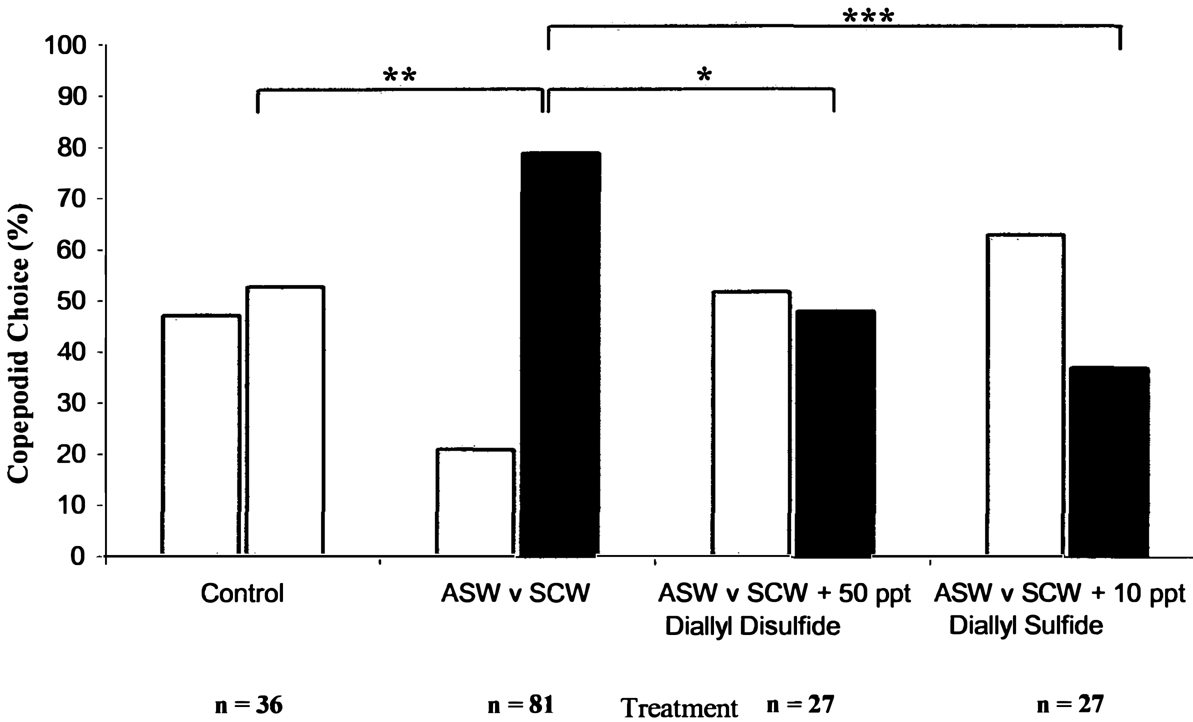

Figure 2a: Directional dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW), SCW plus 50 ppt diallyl disulfide and SCW plus 10 ppt diallyl sulfide.

Key: □ Seawater ■ Odour ppt, parts per trillion

Figure 2b

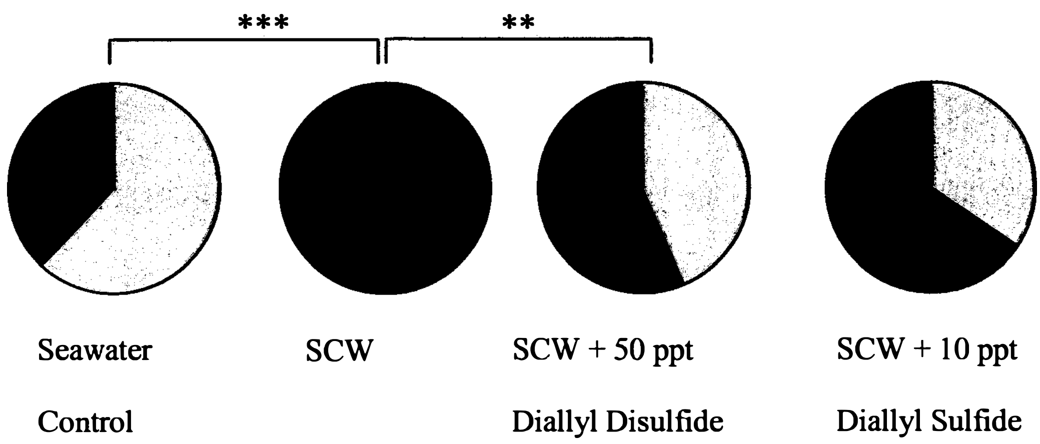

Figure 2b: Activation dose response of *L. salmonis* copepodids to seawater control, salmon conditioned water (SCW), SCW plus 50 ppt diallyl disulfide and 10 ppt diallyl sulfide.

Key □ Low Activity ■ High Activity ppt, parts per trillion