# Disruption of host-seeking behaviour by the salmon louse, Lepeophtheirus salmonis, using botanically derived repellents

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## Abstract

The potential for developing botanically derived natural products as novel feed-through repellents for disrupting settlement of the salmon louse, Lepeophtheirus salmonis (Caligidae) upon farmed Atlantic salmon, Salmo salar, was investigated using an established laboratory vertical Y-tube beha-vioural bioassay for assessing copepodid behaviour. Responses to artificial sea water conditioned with the odour of salmon, or to the known salmon-derived kairomone component, a-isophorone, in admixture with selected botanical materials previ-ously known to interfere with invertebrate arthro-pod host location were recorded. Materials included oils extracted from garlic, Allium sativum (Amaryllidaceae), rosemary, Rosmarinus officinalis (Lamiaceae), lavender, Lavandula angustifolia (Lamiaceae), and bog myrtle, Myrica gale (Myri-caceae), and individual components (diallyl sul-phide and diallyl disulphide from garlic; allyl, propyl, butyl, 4-pentenyl and 2-phenylethyl isoth-iocyanate from plants in the Brassica genus). Removal of attraction to salmon-conditioned water (SCW) or a-isophorone was observed when listed materials were presented at extremely low parts per trillion (ppt), that is picograms per litre or 10$^{12}$ level. Significant masking of attraction to SCW was observed at a level of 10 ppt for diallyl disulphide and diallyl sulphide, and allyl isothiocyanate and butyl isothiocyanate. The potential of very low con-centrations of masking compounds to disrupt Le. salmonis copepodid settlement on a host fish has been demonstrated in vitro.

Keywords: botanical extracts, isothiocyanates, Lepeophtheirus salmonis, repellency, Y-tube bioassay.

## Introduction

The salmon louse, Lepeophtheirus salmonis Krøyer (Copepoda, Caligidae), is a common marine ectoparasite of both wild and farmed salmonid fish (Kabata 1979). It is a significant pest in aqua-culture, causing substantial annual economic losses in the salmon, Salmo salar (Salmonidae), farming industry (Westcott, Hammell & Burka 2004). The estimated cost of sea lice to the global salmon aquaculture industry is €0.1–€0.2 kg$^{1}$ salmon produced, with total losses of €300 million per annum (Costello 2009). To date, sea louse infec-tions have been controlled using good husbandry techniques, area management agreements and chemotheraputants, but sea lice still remain one of the biggest pest problems to the industry. The threat of resistance to, and the possible environ-mental impact of, chemotheraputants, together with the need for good integrated systems, has led to the search for alternative pest management strategies to control infections (Bravo, Sevatdal & Horsberg 2008; Mordue (Luntz) & Birkett 2009).

Semiochemicals are naturally occurring beha-viour-modifying chemicals that are used by a

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range of arthropods in chemical communication to locate a host, mate or oviposition site (Mordue (Luntz) 2003);. The use of semiochemicals for pest control in agriculture is well established, for example as part of a push-pull strategy where pests are pushed away from crops and pulled towards traps or targets, where they are removed from the environment (Cook, Khan & Pickett 2007; Hassanali et al. 2008). In aquatic environments, many free-living copepods and the parasitic Le. salmonis use chemical cues to identify and seek out mates (Katona 1973; Griffiths & Frost 1976; Ritchie et al. 1996; Ingvarsdottir et al. 2002a). It has also been demonstrated that both Le. salmonis adults and copepodids use semiochemicals in location of the host Atlantic salmon, Salmo salar (Devine et al. 2000; Ingvarsdottir et al. 2002b; Bailey et al. 2006; Mordue (Luntz) & Birkett 2009);. a-Isophorone and 6-methyl-5-hepten-2-one have been identified, from water in which salmon have been swimming (host-conditioned water), as kair-omones that induce upstream rheotaxis swimming behaviours towards the host odours. Furthermore, 4-methylquinazoline and 2-aminoacetophenone, released by the non-host turbot, Psetta maxima (Scophthalmidae), remove attraction of Le. salmonis copepodids to host-conditioned sea water (Bailey et al. 2006). Together, these findings suggest the presence of a sophisticated sensory and behavioural system in Le. salmonis that is responsive to external chemical stimuli. The main olfactory receptors of Le. salmonis are found on the first antennae (antennules). Electrophysiological recordings from the antennules have shown that receptors respond to sea water conditioned with host and non-host odours, and to food-related chemical cues (Ingvarsdottir et al. 2002b; Fields, Weissburg & Browman 2007). Ablation of the distal tip of the chemosensory antennules signifi-cantly reduces pair formation and mating as well as host-finding success in Le. salmonis adult males (Hull et al. 1998), thus revealing the importance of the antennule sensory receptors in host and mate location.

Recently, our investigations into the semiochemical basis of repellency, or avoidance of unsuitable hosts, with respect to the behaviour of haematophagous or carnivorous arthropods, have led us to hypothesize that botanically derived materials, in conveying a strong plant cue, interfere with host location prior to colonization and/or feeding (Pickett et al. 2010). The aim of this study was therefore to test this hypothesis by assessing Le. salmonis behaviour, using an established Y-tube behavioural bioassay (Mordue (Luntz) & Birkett 2009 and refs therein), in response to sea water containing host-derived semiochemicals (salmon-conditioned water (SCW) or a-isophorone), compared with that in response to sea water containing both host-derived semiochemicals and selected botanically derived materials. The materials were selected on the basis of known ability to interfere with arthropod host location. These included garlic, Allium sativum (Amaryllidaceae) (Block 2010), rosemary, Rosmarinus officinalis (Lamiaceae) (Hori 1998), lavender, Lavandula angustifolia (Lamiaceae) (Mauchline et al. 2008), bog myrtle, Myrica gale (Myricaceae) (Blackwell, Stuart & Estambale 2003) and isothiocyanates from members of the family Brassicaceae (Nottingham et al. 1991; Fahey, Zalcmann & Talalay 2001; Vig et al. 2009). From a practical perspective, demonstration of interference with attraction by some or all of these materials would provide underpinning science for their development as feed-through repellents for disrupting Le. salmonis settlement, which could form part of an overall integrated pest management strategy for louse control aimed at minimizing dependence on chemotheraputants.

## Materials and methods

### Sea lice

Ovigerous female Le. salmonis were collected from Atlantic salmon, Salmo salar L., and sea trout, Salmo trutta L., at a wild fish netting station at Montrose on the east coast or from salmon farms on the west coast of Scotland. Material was trans-ported on ice to the laboratory at the University of Aberdeen in bags with clean sea water for sort-ing. Water from the source site was collected and used for subsequent rearing of egg strings (John-son & 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

Atlantic salmon, S. salar, were maintained in aquaria containing artificial sea water (ASW) (32

&). Fish-conditioned water was collected as described by Ingvarsdottir et al. (2002b) by placing the fish for 24 h into a circulating flume (20 cm 9 25 cm 9 420 cm), filled with ASW (100 L) circulated at a rate of 30 cm s $^{1}$. Aeration was provided by bubbling compressed air into the raceway. Standardization of fish odour in the water was achieved by weighing the fish and using the correct number of fish to give a standard strength of odour that was collected over a 24-h period (8–10 g live fish L $^{1}$ 24 h $^{1}$). Conditioned water was either used immediately or frozen for later use.

## Louse behaviour

A vertical Y-tube bioassay modified by Bailey et al. (2006) from that previously described by Ingvarsdottir et al. (2002a) was used to study Le. salmonis copepodid activation, and directional (taxis) responses to host odour components and potential host-masking compounds. The Y-tube was constructed from glass (1 cm diameter bore) moulded into a ‘Y’ design between two sheets of glass (2 mm thick) held vertically. 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) held two plastic 60-mL syringes (Terumo Monoject), which were loaded with the waters to be tested (clean sea water, fish-conditioned water or fish-conditioned water with test compounds) prior to use. The syringe pump was programmed to deliver a constant 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 Y-tube. SCW was tested by having the test water introduced into one arm of the Y-tube whilst ASW at 32 & was introduced into the other. When test compounds were used, ASW was introduced into one arm whilst SCW plus the test compound at the desired concentration was introduced into 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 the waters to be tested, 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. For most experiments, replicates were carried out over a period of 4 days to allow for age effects of the copepodid. Behaviour was defined by the degree of movement within the Y-tube (Ingvarsdottir et al. 2002b) as low and high activities. 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 cope-podid more than the length of the main leg, or as movement into either arm. In the latter case, choice of arm was recorded as a taxis response if the copepodid had moved half way or more up an arm of the Y. In these cases, the test was considered com-plete and was terminated. Very often highly active copepodids could be seen tracking in a zigzag fashion up the main arm of the Y before selecting an arm. Taxis responses were often rapid and could occur within 20–40 s from the start of each assay. Both activation and directional responses of copepodids were measured. All assays were conducted using sea lice collected from fish farms (west coast of Scotland), apart from the allyl isothiocyanate and butyl isothiocyanate (BITC) dose–response studies, which were conducted using lice collected from Montrose (east coast of Scotland).

## Chemicals

Garlic oil, A. sativum, was purchased from Elixarome Ltd; rosemary, R. officinalis, and lavender, La. angustifolia, essential oils were provided by Botanix Ltd, and bog myrtle oil, Myrica gale, was purchased from Amphora Aromatics Ltd. Diallyl sulphide, diallyl disulphide, allyl, propyl, butyl and 2-phenylethyl isothiocyanate were purchased from Sigma-Aldrich. 4-Pentenyl isothiocyanate was synthesized from 4-pentenylamine hydrochloride as described previously (Dawson et al. 1993). Solutions of extracts and individual compounds in absolute ethanol were prepared (0.1, 0.05, 0.01 and 0.001 mg mL $^{1}$) and diluted to 1 lL L $^{1}$ in ASW (Ingvarsdottir et al. 2002b) to give a final concentration of 100, 50, 10 and 1 parts per trillion, respectively, for use in Y-tube behavioural bioassays.

## Data analysis

Copepodid responses to ASW and SCW across all experimental days were compared, in the first instance, using a chi-square test to determine whether there was a day effect on louse behaviour. If non-significant, data were pooled. For experiments studying directional and activity responses, the null hypothesis was that all lice in all treatments behaved the same. This hypothesis was tested using a global chi-square contingency table (Zar 1999). Upon rejection of that hypothesis, data were analysed by post hoc targeted pairwise comparisons using a 2 9 2 chi-square contingency table (Zar 1999) to identify whether pairs of treatments of interest were significantly different.

## Results

### Garlic oil

For directional responses, that is upstream positive rheotaxis, global chi-square analysis showed that lice did not behave the same in all treatments (Table 1; $v^2 = 26.42$, df = 3, P < 0.001). When compared with a seawater control, significantly more copepodids chose the arm containing 100 ppt a-isophorone ($v^2 = 6.87$, df = 1, P < 0.01), a component of SCW. Significantly fewer copepodids chose the arm containing 100 ppt garlic oil when compared to the response to 100 ppt a-isophorone alone ($v^2 = 8.72$, df = 1, P < 0.01) compared with 100 ppt a-isophorone alone. When garlic oil was added to 100 ppt a-iso-phorone at 100 ppt, a significant decrease in cope-podid attraction was observed when compared to the response to 100 ppt a-isophorone alone ($v^2 = 25.1$, df = 1, P < 0.001) (Table 1). For activity responses, global chi-square analysis showed that lice did not show the same activation levels in all treatments (Table 2; $v^2 = 72.81$, df = 3, P < 0.001). When compared with the seawater control, a significant increase in high activity behaviour of copepodids was observed in the presence of 100 ppt a-isophorone alone ($v^2 = 36.57$, df = 1, P < 0.001). No difference in activity was detected between 100 ppt garlic oil and 100 ppt a-isophorone ($v^2 = 0$, df = 1, NS). When 100 ppt a-isophorone was combined with 100 ppt garlic oil, significantly more copepodids showed low activity when compared to responses to 100 ppt a-isophorone alone ($v^2 = 7.25$, df = 1, P < 0.01).

### Diallyl disulphide and diallyl sulphide

For directional responses, global chi-square analysis showed that lice did not behave the same in all

Table 2 Activation response of Lepeophtheirus salmonis copepodids to seawater control, a-isophorone, salmon-conditioned water (SCW), garlic oil Allium sativum, diallyl disulphide (DDS) and diallyl sulphide (DS) in a Y-tube olfactometer assay. Data are expressed as the number of copepodids with low or high activity (see Materials and Methods for definition), and were analysed using global chi-square analysis, comparing responses to a-isophorone or SCW

| Experiment | Low activity | High activity | N |
|---|---|---|---|
| Seawater control *** | 24 | 52 | 76 |
| a-Isophorone (100 ppt) | 0 | 100 | 100 |
| Garlic oil (100 ppt)[NS] | 0 | 100 | 100 |
| Isophorone (100 ppt) + garlic oil (100 ppt) ** | 7 | 93 | 100 |
| Seawater control *** | 74 | 46 | 120 |
| SCW | 0 | 83 | 83 |
| SCW + DDS (50 ppt) *** | 24 | 31 | 55 |
| SCW + DS (10 ppt)*** | 19 | 36 | 55 |

N, number of replicates; NS, no significant difference; ppt, parts per trillion.
**P < 0.01, ***P < 0.001.

Table 1 Direction response of Lepeophtheirus salmonis copepodids to seawater control, a-isophorone, salmon-conditioned water (SCW), garlic oil Allium sativum, diallyl disulphide (DDS) and diallyl sulphide (DS) in a Y-tube olfactometer assay. Data are expressed as the percentage (%) of copepodids choosing a specific (control or test) arm and were analysed using global chi-square analysis, comparing responses to a-isophorone or SCW

| Experiment | Seawater control | Test | Non-choosers | N |
|---|---|---|---|---|
| Seawater control ** | 21 | 19 | 36 | 76 |
| a-Isophorone (100 ppt) | 29 | 71 | 0 | 100 |
| Garlic oil (100 ppt)** | 40 | 39 | 0 | 79 |
| Isophorone (100 ppt) + garlic oil (100 ppt) *** | 51 | 49 | 21 | 121 |
| Seawater control *** | 19 | 17 | 84 | 120 |
| SCW | 17 | 64 | 2 | 83 |
| SCW + DDS (50 ppt) ** | 42 | 58 | 28 | 128 |
| SCW + DS (10 ppt) *** | 63 | 37 | 28 | 128 |

N, number of replicates; ppt, parts per trillion.
**P < 0.01, ***P < 0.001.

treatments ($v^2$ = 14.17, df = 3, P < 0.001). When compared with the seawater control, significantly more copepodids chose the arm containing SCW ($v^2$ = 11.82, df = 1, P < 0.001) (Table 1). A significant decrease in copepodid responses was seen with SCW plus 50 ppt diallyl disulphide ($v^2$ = 9.43, df = 1, P < 0.01) and SCW plus 10 ppt diallyl sulphide ($v^2$ = 16.54, df = 1, P < 0.001) when compared with responses to SCW (Table 1). For activity responses, global chi-square analysis showed that lice did not behave the same in all activity treatments (Table 2; $v^2$ = 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 ($v^2$ = 80.54, df = 1, P < 0.001). Significantly more copepodids showed low activity in the pres-ence of SCW plus 50 ppt diallyl disulphide ($v^2$ = 43.84, df = 1, P < 0.001) and SCW plus 10 ppt diallyl sulphide ($v^2$ = 33.25, df = 1, P < 0.001) when compared with responses to SCW.

## Isothiocyanates

Global chi-square analysis showed that lice did not behave the same in all treatments ($v^2$ = 26.50, df = 4, P < 0.001) in directional response assays (Fig. 1a). When compared with the seawater control, significantly more copepo-dids chose the arm containing SCW ($v^2$ = 11.82, df = 1, P < 0.001). A significant decrease in copepodid responses was detected with SCW plus 100 ppt 2-phenylethyl isothiocyanate ($v^2$ = 13.06, df = 1, P < 0.001) and SCW plus 100 ppt BITC ($v^2$ = 15.14, df = 1, P < 0.001) when compared with the response to SCW. No difference in direc-tional response was detected between responses to SCW, and SCW plus 100 ppt 4-pentenyl isothiocyanate ($v^2$ = 0.7, df = 1, NS; Fig. 1a). Global chi-square analysis showed that lice did not show the same activity in all treatments (Fig. 1b) ($v^2$ = 97.56, df = 4, P < 0.001). When compared with the seawater control, a significant increase in high activity was detected with SCW ($v^2$ = 80.54, df = 1, P < 0.001). Significantly more copepodids showed low activity with SCW combined with either 100 ppt 4-pentenyl isothiocyanate ($v^2$ = 25.97, df = 1, P < 0.001), 100 ppt 2-phenylethyl isothiocyanate ($v^2$ = = 41.40, df = 1, P < 0.001) or 100 ppt BITC ($v^2$ = 75.42, df = 1, P < 0.001) when compared to

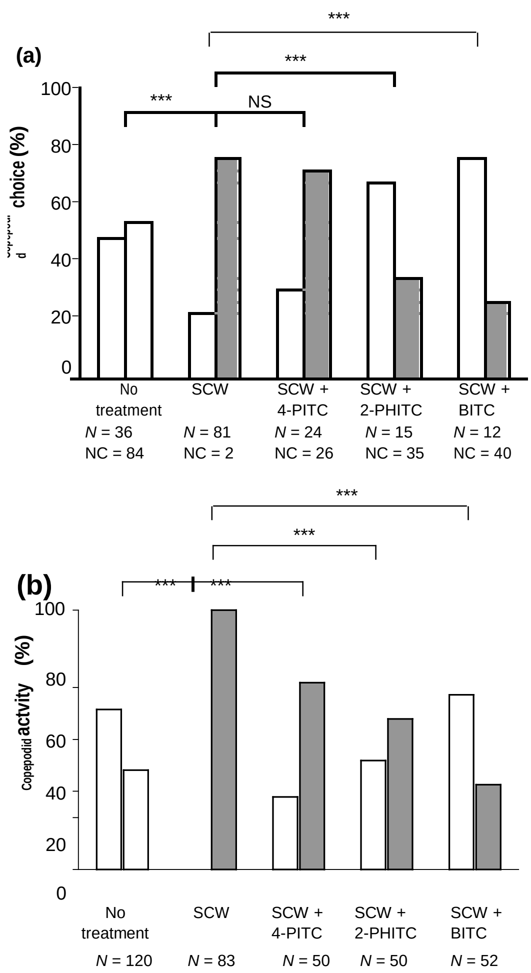



Figure 1 Response of Lepeophtheirus salmonis copepodids in Y-tube behaviour assays to different treatments: salmon-conditioned water (SCW), SCW plus 100 ppt 4-pentenyl isothiocyanate (4PITC), SCW plus 100 ppt 2-phenylethyl isothiocyanate (2-PHITC) and SCW plus 100 ppt butyl isothiocyanate. N = number of replicates. NC = non-choosers. (a) Directional responses; data are expressed as the percentage of copepodids choosing a specific arm of the Y-tube. White bar = artificial sea water (ASW), grey bar = ASW plus treat-ment. Data were analysed using global chi-square analysis for individuals making a choice between ASW and treatments. ***P < 0.001. NS = no significant difference. (b) Activation responses to treatments; data are expressed as the percentage of copepodids with low or high activity (see Materials and Meth-ods for definition). White bar = low activity, grey bar = high activity. Data were analysed using global chi-square analysis for individuals showing differences in activity compared with ASW. ***P < 0.001.

responses to SCW alone. For propyl isothiocyanate (Fig. 2a), global chi-square analysis showed that lice did not behave the same in all treatments ($v^2$ = 39.84, df = 2, P < 0.001) in

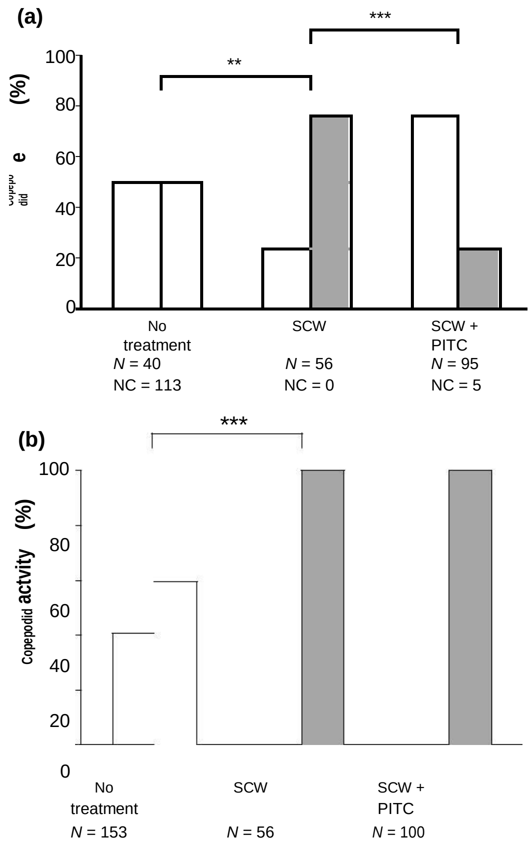



Figure 2 Response of Lepeophtheirus salmonis copepodids in Y-tube behaviour assays to different treatments: salmon-conditioned water (SCW) and SCW plus 100 ppt propyl isothio-cyanate (PITC). N = number of replicates. NC = non-choosers. (a) Directional responses; data are expressed as the percentage of copepodids choosing a specific arm of the Y-tube. White bar = artificial sea water (ASW), grey bar = ASW plus treatment. Data were analysed using global chi-square analysis for individuals making a choice between ASW and treatments. **P < 0.01, ***P < 0.001. (b) Activation responses to treat-ments; data are expressed as the percentage of copepodids with low or high activity (see Materials and Methods for definition). White bar = low activity, grey bar = high activity. Data were analysed using global chi-square analysis for individuals showing differences in activity compared with ASW. ***P < 0.001.

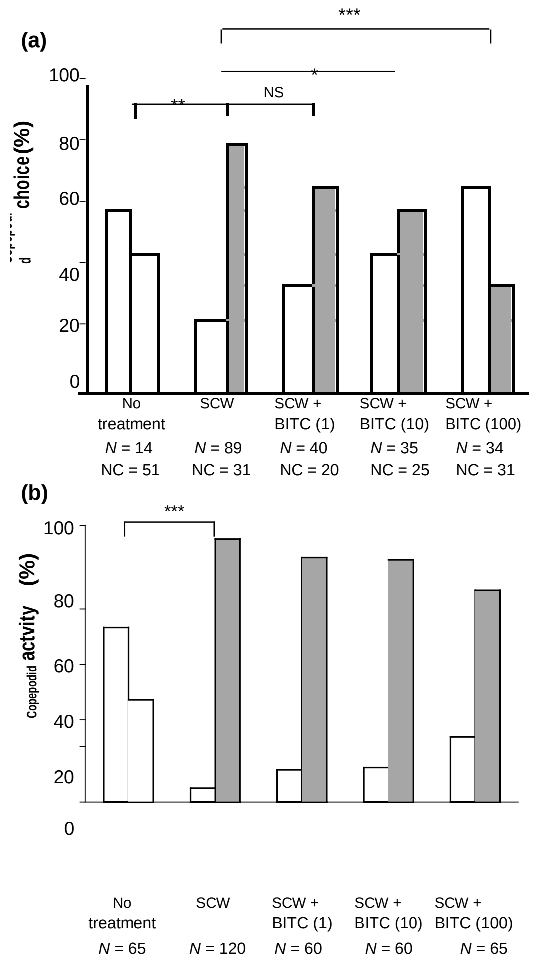
directional response assays. When compared with the seawater control, significantly more copepodids chose the arm containing SCW ($v^2$ = 7.42, df = 1, P < 0.01). A significant decrease in copepodid attraction was detected with SCW combined with 100 ppt propyl isothiocyanate ($v^2$ = 39.58, df = 1, P < 0.001) when compared to responses to SCW alone. Global chi-square analysis showed that lice did not behave the same in all activity treatments ($v^2$ = 59.78, df = 2, P < 0.001) (Fig. 2b). When compared with the seawater control, a significant increase in high activity was detected with SCW ($v^2$ = 26.69, df = 1, P < 0.001). However, no difference in activity was detected between SCW and SCW combined with 100 ppt propyl isothiocyanate ($v^2$ = 0, df = 1, NS; Fig. 2b).

### Butyl isothiocyanate dose response

Global chi-square analysis showed that lice did not behave the same in all treatments ($v^2$ = 23.99, df = 4, P < 0.001) in directional response assays (Fig. 3a). When compared with the seawater control, significantly more copepodids chose the arm containing SCW ($v^2$ = 8.01, df = 1, P < 0.01). A significant decrease in copepodid attraction was detected with SCW combined with either 10 ppt ($v^2$ = 5.84, df = 1, P < 0.05) or 100 ppt BITC ($v^2$ = 20.81, df = 1, P < 0.001) when compared with responses to SCW alone. However, no difference in directional responses was detected between SCW combined with 1 ppt BITC and responses to SCW alone ($v^2$ = 1.84, df = 1, NS; Fig. 3a). Global chi-square analysis showed that lice did not behave the same in all activity treatments ($v^2$ = 91.94, df = 4, P < 0.001) (Fig. 3b). When compared with the seawater control, a significant increase in high activity was detected with SCW ($v^2$ = 75.04, df = 1, P < 0.001). Significantly more copepodids showed low activity with SCW plus 100 ppt BITC ($v^2$ = 15.43, df = 1, P < 0.001) when compared with the response to SCW. However, no difference in activity was detected between SCW and SCW plus 1 and 10 ppt BITC ($v^2$ = 2.65 and 2.64, respectively, df = 1, NS; Fig. 3b).

### Allyl isothiocyanate dose response

Global chi-square analysis showed that lice behaved the same in all treatments ($v^2$ = 4.65, df = 4, NS) in directional response assays (Fig. 4a). As a result, further pairwise comparisons were not carried out. Global chi-square analysis showed that lice did not behave the same in all activity treatments ($v^2$ = 37.24, df = 4, P < 0.001) (Fig. 4b). When compared with the seawater control, a significant increase in high activity was detected with SCW ($v^2$ = 27.99, df = 1, P < 0.001). However, no difference in activity was detected between responses to SCW



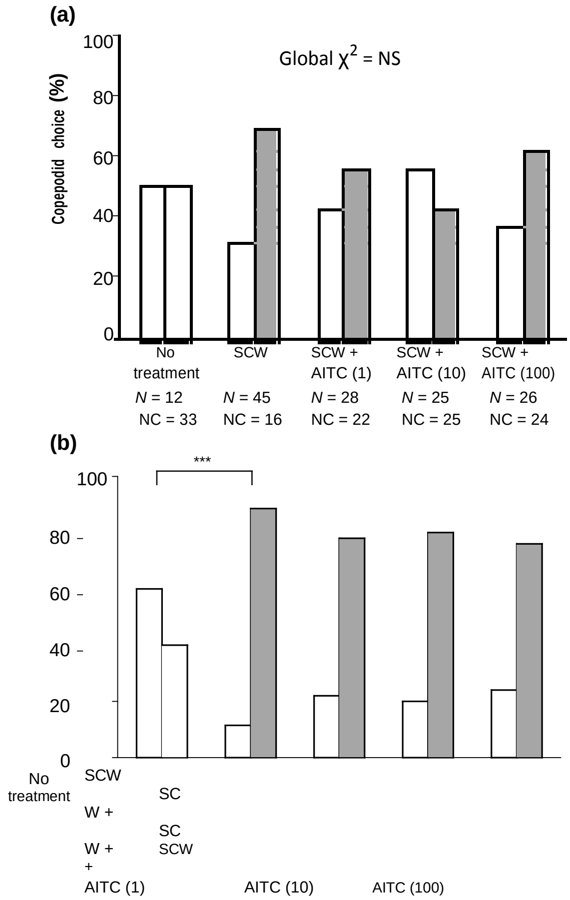




Figure 3 Response of Lepeophtheirus salmonis copepodids in Y-tube behaviour assays to different treatments: salmon-conditioned water (SCW) and SCW plus 1, 10 and 100 ppt butyl isothiocyanate. N = number of replicates. NC = non-choosers. (a) Directional responses; data are expressed as the percentage of copepodids choosing a specific arm of the Y-tube. White bar = artificial sea water (ASW), grey bar = ASW plus treatment. Data were analysed using global chi-square analysis for individuals making a choice between ASW and treatments. *P < 0.05, *P < 0.01, ***P < 0.001. NS = no significant difference. (b) Activation responses to treatments; data are expressed as the percentage of copepodids with low or high activity (see Materials and Methods for definition). White bar = low activity, grey bar = high activity. Data were analysed using global chi-square analysis for individuals showing differences in activity compared with ASW. ***P < 0.001.

plus 1, 10 or 100 ppt allyl isothiocyanate and responses to SCW alone ($v^2$ = 2.24, 1.54, 3.04, respectively, df = 1, NS).

## Plant extracts

Global chi-square analysis showed that lice did not behave the same in all treatments

*N* = 45 *N* = 61 *N* = 50 *N* = 50 *N* = 50

Figure 4 Response of Lepeophtheirus salmonis copepodids in Y-tube behaviour assays to different treatments: salmon-condi-tioned water (SCW) and SCW plus 1, 10 and 100 ppt allyl isothiocyanate (AITC). N = number of replicates. NC = non-choosers. (a) Directional responses; data are expressed as the percentage of copepodids choosing a specific arm of the Y-tube. White bar = artificial sea water (ASW), grey bar = ASW plus treatment. Data were analysed using global chi-square analysis for individuals making a choice between ASW and treatments. NS = no significant difference. (b) Activation responses to treatments; data are expressed as the per-centage of copepodids with low or high activity (see Materials and Methods for definition). White bar = low activity, grey bar = high activity. Data were analysed using global chi-square analysis for individuals showing differences in activity com-pared with ASW. ***P < 0.001.

($v^2$ = 33.38, df = 4, P < 0.001) in directional response assays (Fig. 5a). When compared with the seawater control, significantly more copepo-dids chose the arm containing SCW ($v^2$ = 7.89, df = 1, P < 0.01). A significant decrease in cope-podid attraction was detected with SCW plus 100 ppt lavender ($v^2$ = 19.03, df = 1, P < 0.001) and 100 ppt rosemary ($v^2$ = 17.89, df = 1, P < 0.001) when compared with

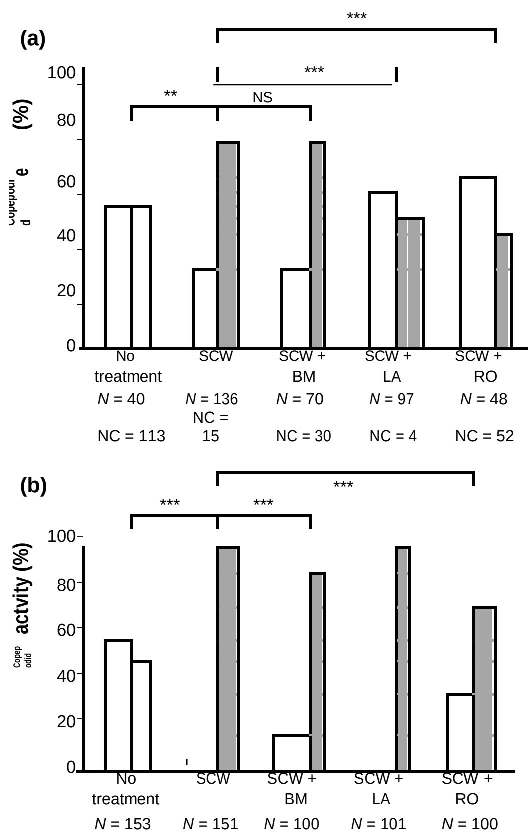



Figure 5 Response of Lepeophtheirus salmonis copepodids in Y-tube behaviour assays to different treatments: salmon-condi-tioned water (SCW) and SCW plus 100 ppt bog myrtle (BM), 100 ppt lavender (LA) and 100 ppt rosemary (RO). N = num-ber of replicates. NC = non-choosers. (a) Directional responses; data are expressed as the percentage of copepodids choosing a specific arm of the Y-tube. White bar = artificial sea water (ASW), grey bar = ASW plus treatment. Data were analysed using global chi-square analysis for individuals making a choice between ASW and treatments. **P < 0.01, ***P < 0.001. NS = no significant difference. (b) Activation responses to treatments; data are expressed as the percentage of copepodids with low or high activity (see Materials and Meth-ods for definition). White bar = low activity, grey bar = high activity. Data were analysed using global chi-square analysis for individuals showing differences in activity compared with ASW. ***P < 0.001.

responses to SCW alone. However, no difference in directional responses was detected between responses to SCW plus 100 ppt bog myrtle and responses to SCW alone ($v^2 = 0.01$, df = 1, NS; Fig. 5a). Global chi-square analysis showed that lice did not behave the same in all activity treat-ments ($v^2 = 144.34$, df = 4, $P < 0.001$) (Fig. 5b). When compared with the seawater con-trol, a significant increase in high activity was detected with SCW ($v^2 = 91.70$, df = 1, $P < 0.001$). Significantly more copepodids showed low activity with SCW plus 100 ppt bog myrtle ($v^2 = 12.23$, df = 1, $P < 0.001$) and SCW plus 100 ppt rosemary ($v^2 = 43.24$, df = 1, $P < 0.001$) when compared to responses to SCW alone. However, no difference in activity was detected between SCW plus 100 ppt lavender and responses to SCW alone ($v^2 = 2.03$, df = 1, NS).

## Discussion

In this study, copepodid larvae of the salmon louse, Le. salmonis, showed significant directional responses towards a-isophorone, a behaviourally active component of SCW (Bailey et al. 2006). This provided the basis for assessing the impact of garlic, A. sativum, and its individual components, diallyl sulphide and diallyl disulphide, on the response to this host-derived semiochemical. The inclusion of A. sativum at 100 parts per trillion (ppt) with 100 ppt a-isophorone removed attrac-tion to the attractant. These data suggest that A. sativum interferes with the attractiveness of host odour cues, and whilst repellent properties of

A. sativum for other ectoparasites has been reported (Anthony, Fyfe & Smith 2005; Block 2010), to our knowledge, this is the first pub-lished report of repellency of garlic observed for this marine pest. In a second set of experiments, copepodid larvae showed significant directional responses to SCW, as reported by Bailey et al. (2006). Ingvarsdottir et al. (2002a) also found that adult male Le. salmonis responded to and were activated by SCW. The addition of diallyl disulphide and diallyl sulphide to SCW at concen-trations found in A. sativum (50 and 10 ppt, respectively) removed the attraction to SCW, in a similar vein to that observed above with A. sativum and a-isophorone.

Other botanical materials tested in this study were selected on the basis of their ability to inter-fere with host location by invertebrate arthropods that are major pests affecting crop, livestock and human health. Isothiocyanates produced by Brassi-caceous plants, and rosemary, Rosmarinus offici-nalis, were selected on the basis of their ability to repel aphids and earthworms (Nottingham et al. 1991; Hori 1998; Chan & Munro 2001). Laven-der, La. angustifolia, was selected because of its ability to repel pollen beetles (Mauchline et al. 2008), and bog myrtle, Myrica gale, because of its ability to repel mosquitoes (Blackwell et al. 2003). For the isothiocyanates, 2-phenylethyl, butyl and propyl isothiocyanate, at 100 ppt in sea water, removed the attraction of copepodids to SCW. However, neither 4-pentenyl isothiocyanate nor allyl isothiocyanate significantly masked copepodid responses to SCW at the 100 ppt level. Subse-quent dose–response assays were conducted with BITC because of its higher stability, and signifi-cant interference of the response to SCW was still observed at the 10 ppt level. At the lowest dose tested (1 ppt), no significant difference was observed. Of the other materials tested, both R. officinalis and La. angustifolia at 100 ppt were effective at interfering with the response to SCW, whereas M. gale showed no significant interference.

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 predomi-nantly low activity behaviour in copepodids. This switched to high activity in the presence of a posi-tive cue, that is either a-isophorone or SCW. Low activity re-appeared in the profile when test com-pounds were introduced, suggesting that the chemicals masked the effect of the isophorone or SCW in copepodids. High activity was also observed in some cases, with copepodids moving towards the seawater control suggesting a repellent effect of the plant compounds.

Botanically derived materials have long been considered as candidate materials for use in crop, livestock and human protection, typically originat-ing via ethnobotanical principles (Birkett et al. 2008; Ukeh et al. 2009, 2010; Brenes & Roura 2010; Leicach & Chludil 2014). Our investiga-tions into the semiochemical basis of repellency, or avoidance of unsuitable hosts, with respect to the behaviour of haematophagous or carnivorous arthropods have led us to hypothesize that such materials, in conveying a strong plant cue, inter-fere with host location prior to colonization and/ or feeding (Pickett et al. 2010). The results in this study provide evidence that this hypothesis extends to a major aquatic pest that severely impacts upon wild and farmed populations of sal-monid fish. From a practical perspective, demon-stration of interference with attraction by some or all of these materials would provide underpinning science for their development as feed-through repellents for disrupting Le. salmonis settlement, which could form part of an overall integrated pest management strategy for louse control in farmed salmonid populations, aimed at minimiz-ing dependence on chemotheraputants, that is vet-erinary medicines.

In summary, the use of botanically derived materials has been shown to disrupt, significantly, Le. salmonis copepodid attraction to host (sal-mon)-conditioned water in vitro. Further work is underway to investigate their impact upon Le. salmonis populations in vivo, and to explore the potential for their deployment as feed addi-tives, that is as feed-through repellents, which would be the most convenient method of deploy-ment in the farmed environment. Whilst evidence for linking the excretion of dietary components to modification of ectoparasite behaviour is scarce (Rajan et al. 2005), such an approach could be effective at suppressing sea louse attachment. Work is underway to conduct in vivo experiments with active materials identified in this study. Compounds will be included in diets and lice levels assessed post challenge to determine any dis-ruptive effects upon settlement and attachment.

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