

Behavioural responses of infective-stage copepodids of the salmon louse (*Lepeophtheirus salmonis*, Copepoda:Caligidae) to host-related sensory cues

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Abstract

The salmon louse (*Lepeophtheirus salmonis* [Krøyer]) is an ectoparasitic copepod that causes disease in farmed Atlantic salmon (*Salmo salar*) and may play a role in the decline of some wild salmonid populations. Controlling lice infestations is a major cost for the salmon industry; this has stimulated the pursuit of alternative approaches to controlling them. One such approach involves determining, and then disrupting, the sensory cues used by the parasite to find its host. In this context, we examined the behavioural responses of lice copepodids to light flicker—simulating light reflecting from the sides of the salmon host and/or the shadows cast by fish passing overhead—and water-soluble chemicals released from the skin of the salmon. From these observations, we estimate that visual cues such as those presented here would operate at relatively long range (metres to tens of metres). A diffuse host-related olfactory cue stimulated swimming, however, it remains unclear whether olfactory cues provide directional information. The observations presented herein could be used to disrupt the link between the parasite and host fish, using a large number of traps deployed at a distance from a salmon farm, for example, thereby reducing sea lice infestation pressure.

KEYWORDS

fish parasite, habituation, host finding, integrated pest management, sensory ecology, trap

1 | INTRODUCTION

The salmon louse (*Lepeophtheirus salmonis*; hereafter referred to as salmon lice) is an ectoparasitic copepod that infests both wild and farmed salmonid fishes (mainly of the generi *Salmo*, *Salvelinus* and *Oncorhynchus*) (Costello 2006; Pike & Wadsworth, 1999). Salmon lice are a major disease problem in farming of Atlantic salmon (*Salmo salar*), costing the industry millions of USD annually in direct losses to keep parasite loads below prescribed levels (Igboeli, Burka, & Fast, 2014; Liu & vanhauwaer Bjelland, 2014; Torrissen et al., 2013). They may also play a role in the decline of some wild salmonid populations (Costello 2009; ICES 2016; Krkosek, Lewis, Morton, Frazer, & Volpe, 2006; Krkosek, Lewis, & Volpe, 2005; Krkosek et al., 2012; Thorstad et al., 2015; Torrissen et al., 2013; Vollset et al., 2016).

These parasites reside on the fish and feed on their mucus, tissue and blood, reducing feed conversion efficiency and causing sores and immunosuppression (Torrissen et al., 2013).

Application of chemotherapeutants has been the predominant approach to control sea lice, typically administered using bath treatments or by addition to feed (Burrige, Weis, Cabello, Pizarro, & Bostick, 2010; Roth, Richards, & Sommerville, 1993). However, over-use of these pharmaceuticals has resulted in the development of resistant strains of salmon lice (Aaen, Helgesen, Bakke, Kaur, & Horsberg, 2015; Besnier et al., 2014). This, and the detrimental effects of chemotherapeutants on the environment and on the fish themselves (e.g., Dounia, Andrea, Lefort, & Van Geest, 2016; Langford, Øxnevad, Schøyen, & Thomas, 2014; Mayor et al., 2008; Van Geest, Burrige, & Kidd, 2014), has stimulated the pursuit of non-pharmaceutical

approaches to controlling sea lice, including the use of cleaner fish, sea lice traps, bivalve filtering (in an integrated multitrophic aquaculture context), selective breeding for sea lice-resistant strains of Atlantic salmon, the use of immuno-stimulatory feeds and various technological solutions (e.g., Browman, Boxaspen, & Kuhn, 2004; Flamarique, Gulbrandsen, Galbraith, & Stucchi, 2009; Gharbi et al., 2015; Skiftesvik, Bjelland, Durif, Johansen, & Browman, 2013; Treasurer, 2002). A growing body of work on the sensory ecology of the free-living life-history stages of the salmon louse has added to our understanding of the link between the parasite and its host, including the possibility of disrupting that link (Fields, Weissburg, & Browman, 2007; Flamarique, Browman, Belanger, & Boxaspen, 2000; Genna, Mordue, Pike, & Mordue, 2005; Heuch, Doall, & Yen, 2007; Ingvarsdottir, Birkett, Duce, Genna et al. 2002). It is in this latter context that the work reported here is set.

Lepeophtheirus salmonis hatch as nauplius I larvae from egg strings carried by adult females (which are attached to the host) and immediately commence a free-swimming planktonic lifestyle. The species' life cycle consists of two naupliar, one copepodid and two chalimus stages before passing through two pre-adult stages which culminate in male and female host resident adults (Hamre et al., 2013). The time span from hatching to infective copepodid is strongly temperature dependent and takes ~4 days at 10°C and ~23 days at 2°C (Boxaspen & Næss, 2000; Samsing et al., 2016). As these animals are obligate ectoparasites, the sole purpose of the free-living larval forms is to locate and attach to a suitable host. The sensory ecology of the salmon louse reflects this need.

The available studies on the sensory biology of *L. salmonis* suggest that the visual, chemosensory and mechanosensory systems are all involved in host finding (Aarseth & Schram, 1999; Bailey et al., 2006; Bron & Sommerville, 1998; Bron, Sommerville, & Rae, 1993; Browman et al., 2004; Devine et al., 2000; Fields et al., 2007; Flamarique et al., 2000; Genna et al., 2005; Heuch & Karlsen, 1997; Ingvarsdottir, Birkett, Duce, Genna et al., 2002; Ingvarsdóttir, Birkett, Duce, Mordue et al., 2002; Luntz, 2003; Meyer-Rochow, Au, & Keskinen, 2001; Wootten, Smith, & Needham, 1982). The sensory modalities and behaviours involved in host detection and recognition by *L. salmonis* consist of a spatio-temporal hierarchy within which one or more senses operate simultaneously. Visual cues, such as fluctuating light intensity caused by fish swimming overhead, would likely operate at relatively long range (tens of metres) and are not likely species specific (Flamarique et al., 2000), whereas mechanosensory (Heuch et al., 2007) and olfactory (Fields et al., 2007) cues may operate at a shorter range (metres and less) and are likely species specific.

Previous work has investigated the visual and olfactory cues associated with host location in the salmon louse (e.g., Browman et al., 2004; Fields et al., 2007; Flamarique et al., 2000; Genna et al., 2005; Ingvarsdottir, Birkett, Duce, Genna et al., 2002). This research demonstrates the possible utility of the sensory ecology approach for understanding the ecology and epidemiology of salmon lice infestations. In the work reported here, we examined the threshold levels necessary to elicit behavioural responses to fluctuations in light

intensity, simulating light reflecting from the silvered sides of a salmon host (e.g., Browman et al., 2004) and/or the fluctuations caused by a school of salmon passing overhead. We also conducted experiments to determine whether this response habituates. Additionally, we assessed how fish odour and light flicker frequency interact to elicit a stronger swimming response from lice than would either alone. From the results of these experiments, we estimate the distances over which these cues could elicit host-finding behaviour in the natural environment.

2 | METHODS

2.1 | *Lepeophtheirus salmonis* culture

Gravid adult female salmon lice (*L. salmonis*) were collected from live salmon maintained in sea cages or from salmon at a local slaughter house. Adult lice were transported to the laboratory where egg strings were separated from the female using a scalpel. Detached egg strings were placed in a hatching container (50 cm in diameter fitted with a 100 µm sieve on the bottom) suspended in a running seawater bath at 8°C–10°C and under a 12 hr light: 12 hr dark photoperiod. Sieves were checked daily for the presence of hatched nauplii. Newly hatched nauplii were removed from the hatching container and transferred into a separate vessel (a 100 µm sieve, 25 cm in diameter) at the same temperature. Unhatched egg strings were transferred back to the sieve, which was resuspended in the water bath after being cleaned of residue. Nauplii were collected for 11 consecutive days. All experiments were conducted on the infectious copepodid life-history stage which was reached at 9 days post-hatch (dph) for animals raised at 8°C and 6 dph for animals raised at 10°C. The life stage of the animals was confirmed under a dissecting microscope (Olympus SZ-61) before use in the experiments.

2.2 | Measurement of threshold and habituation responses to light

Approximately 200 infectious-stage lice were introduced into a 20 × 20 × 20 cm glass observation tank in which they were exposed to a sequence of ON:OFF light stimuli produced by a 1,000 W Xenon arc lamp (Oriol Instruments). The ON:OFF frequency used was 5:5 s. This sequence was repeated for 30 s with a 60 s pause after each ON:OFF sequence. A new group of animals was added to determine whether the animals habituate to the signal over time. In these experiments, we repeated this entire pattern (30 s sequence–60 s pause) for 1.5 hr. The intensity of the stimulus was adjusted by placing neutral density filters (ND) in the light path. The ON stimulus varied in intensity from “bright” light (ND=0) to “medium light (ND=2) to “dim” light (ND=3). In all treatments, ND=4 was the OFF stimulus (light intensity below the threshold for a response, as determined in preliminary experiments). The same light level was also used to create the background light intensity during controls and pause periods. Three replicates were performed for each of the three light treatments, ND=0, 2, 3. The control trials consisted of

switching from an ND=4 to an ND=4. The absolute light levels produced in these experiments (Figure 1a) were chosen to represent light levels found at different depth in the water column. The spectral irradiances presented in Figure 1a were measured using an OL-754-O-PMT (Gooch and Housego, Orlando, Florida, USA) spectroradiometer.

Lice swimming behaviour was observed using silhouette video photography (SVP, detailed in Flamarique et al., 2000). Video recordings were analysed (using the bespoke MANTRAK software, JASCO Scientific, Halifax, Nova Scotia, Canada) to determine lice swim speeds before the stimulus began (control), immediately after the ON response (time 1) and after 90 min of repeated stimulation (time 2). For each replicate, 20 lice were tracked during the control period, at time 1 and at time 2. These experiments were conducted to determine whether the difference in light intensity produced during the ON:OFF cycles resulted in a change in lice swimming behaviour and/or speed and whether the animals habituate to repeated monotonous stimulation.

A two-way ANOVA was used to compare the swimming speeds of the lice with light level and time as independent variables. All pairwise multiple comparisons were conducted using the Holm–Sidak method and a significance level of $p = <.05$.

2.3 | Vertical tower experiments

A tower tank ($10 \times 10 \times 165$ cm; L \times W \times H; Figure 2a) constructed of clear acrylic was used to determine the ON:OFF stimulus frequency that resulted in the largest number of lice copepodids moving vertically in the water column. Five ON:OFF frequencies were tested (Figure 2 b-e) between 08:00-18:00. Replicate experiments were conducted randomly throughout the day. Once the ON:OFF frequency that elicited the strongest response was determined, the interactive effects of fish odour and fluctuating light intensity on the number of animals attracted to the top of the tower were investigated. At the start of the experiments, 300 infective-stage lice copepodids were transferred into a 300 ml chamber at the base of the vertical column. The chamber was designed so that animals could be retained in isolation from the water column and released,

via a trap door, at the start of ON:OFF stimulus presentation. The movements of the copepodids through the water column was tracked using three video cameras (Sanyo VCB-3524P, outfitted with Nikon, Nikkor AF 50 mm f1.8 lenses) positioned at the bottom, middle and top of the observation tower (30; 63; 96 cm, respectively, from the bottom; Figure 2a). The cameras were optically configured for silhouette imaging, which produces sharp images of all of the lice copepodids in each cameras field of view such that they can be easily identified and counted by object identification software (see below). Silhouette images were produced using a far-red LED that was collimated using 20 cm-diameter biconvex Melles Griot lenses (see Browman, St-Pierre, Skiftesvik, & Racca, 2003 for a complete description of the imaging system). Every second, an individual image was digitally captured from each of the three cameras and the lice that were visible in each view were counted using custom-designed image analysis software (JASCO Scientific). Each camera observed a 15 cm field of view such that there was regional monitoring of the populations as they moved vertically within the tower (Figure 2). Lice copepodids were stimulated to swim upward through the water column by passing a shadow above the tank at various frequencies. The downward-directed light was generated using a 150-W halogen lamp (Dolan Jenner Fiber Lite 180, USA) outfitted with a 1 cm fibre-optic guide. The fibre guide was positioned 25 cm above the surface of the water. The light was focused and projected into the tank. The duration of the shadow was controlled by passing the light from the fibre-optic cable through a series of holes (3 cm diameter) on a large (35 cm) rotating wheel (1 RPM). The rotation of the wheel remained constant throughout all trials. The frequency of the OFF signal (Figure 2 b-e) was altered by changing the number of openings that allowed light to pass through as the wheel rotated. The frequency of the ON signal was changed by dividing the hole size in half to decrease the amount of time the signal remained ON. The spectral irradiance was measured using an Optronic Laboratories OL-754-O-PMT spectroradiometer (Figure 1b). By tracking the number of lice copepodids that passed in front of each the cameras, a time series of vertical movement of the population in the entire tower was obtained. To compare the different light frequency treatments, the number of lice that reached the upper camera was counted from the

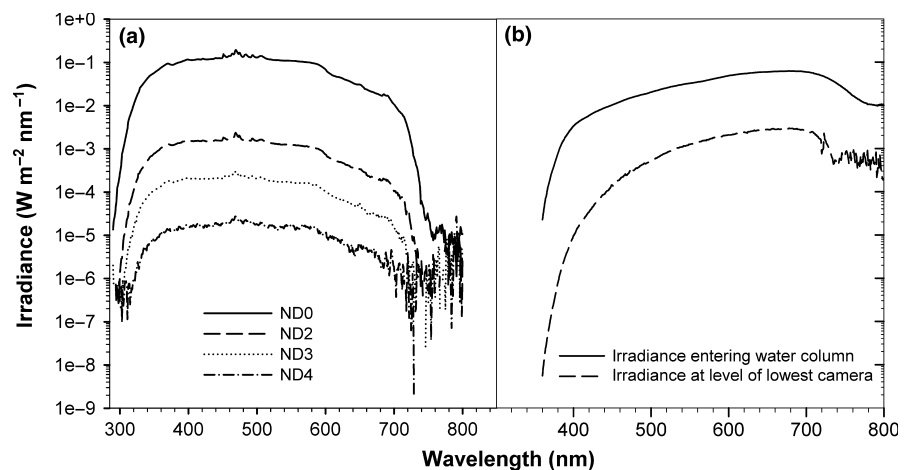


FIGURE 1 (a) Spectral irradiance of light delivered in the visual threshold and habituation response experiments. The numbers associated with each curve are the ND filtering applied. (b). Spectral irradiance of light delivered in the tower tank experiment from a 150-W halogen lamp at the surface of the water (top of the tank) and at the containment chamber (bottom of the tank)

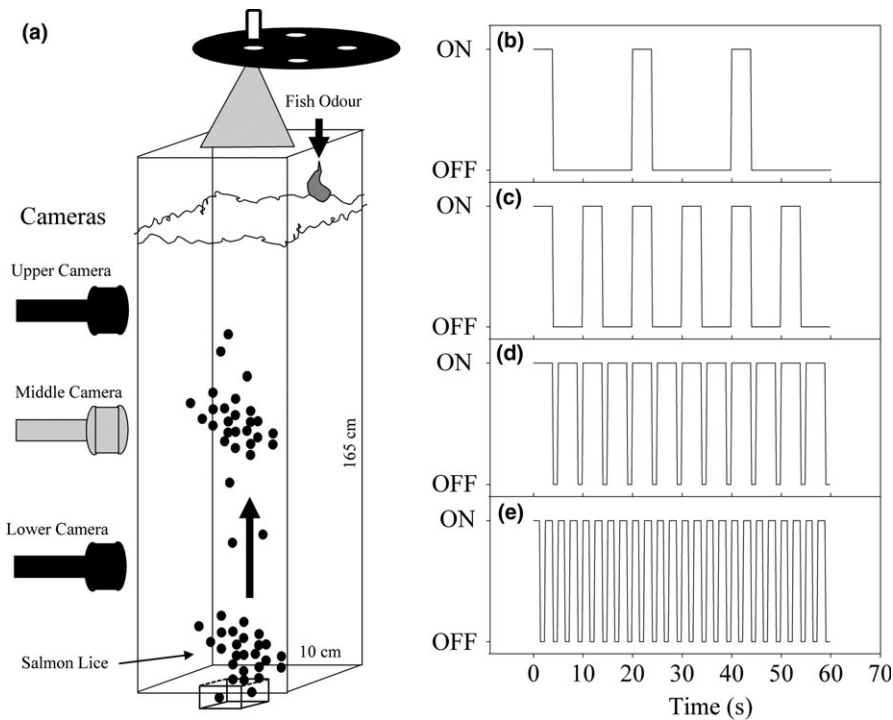


FIGURE 2 (a) Schematic drawing of the 165 cm tower tank showing the containment chamber at the bottom of the tower, the oscillating light at the top of the tower and the three cameras positioned at 30 cm (lower), 65 cm (middle) and 96 cm (upper) above the bottom of the tower. (b-e) Time series of the light stimulus

time of release to 600 s. The change in the number of copepods in the upper camera's field of view (C_t) over time (t) was modelled using a three-parameter sigmoid equation, $C_t = C_o(1 + e^{-t/b})^{-1}$ where C_o is the total number of copepods placed in the chamber and "b" was solved through numerical iterations using SigmaPlot V9.0. The maximum number of animals attracted to the upper camera (C_t) from the three replicate trials was averaged for each treatment and tested using a one-way ANOVA with light frequency as the independent variable. All post hoc comparisons were conducted using the Holm–Sidak procedure. A significance level of 0.05 was applied to in all tests.

2.4 | Responses to combined visual and olfactory signals

The direct and interactive effects of light and salmon-conditioned water (SCW) were tested using a two-way full-factorial design using the optimal light frequency determined above and SCW. Copepodids (300 animals) were introduced into the bottom chamber (which contained filtered sea water) where they remained, in the dark, for 10 min. The water in the tank above the holding chamber was either 0.2 μm filtered sea water or taken from 1,000 L tanks in which five adult salmon had been kept for 1 h (= salmon-conditioned water, SCW). At the onset of the trial, the copepodids were released from the chamber into the tower tank while simultaneously being exposed to darkness or the ON:OFF light signals described above (Figure 2). The vertical movement of the copepodids was tracked as described above to determine how far they travelled in response to the combined chemosensory and visual stimuli. The data were analysed using a two-way ANOVA.

3 | RESULTS

3.1 | Threshold and habituation responses to light

There was a significant effect of light intensity on the swimming speed of salmon lice copepodids (two-way ANOVA; $F_{2,18} = 19.853$; $p < .001$): swimming speed was significantly higher in the ND=0 and the ND=2 treatment (at time 1) relative to the ND=3 treatment (post hoc comparison within Time 1; Table S1C). In the ND=0 treatment, the swimming speed of lice copepodids increased from 18.9 ± 1.5 (SD) mm/s in the controls (ND=4) to 30.2 ± 0.2 (SD) mm/s in the first light flicker sequence ($t = 6.32$; $p < 0.001$; Table S1; Figure 3). Similarly, in ND=2 treatment, the swimming speed increased from 20.4 ± 1.7 (SD) mm/s in the controls (ND=4) to 28.5 ± 2.2 (SD) mm/s in the first light flicker sequence ($t = 4.55$; $p < 0.001$). There was no statistically discernible change in swimming speed when lice copepodids were presented with a one order of magnitude change in light intensity (ND=3 to ND=4) ($t = 0.16$; $p = 0.88$; Table S1; Figure 3).

There was a significant effect of time ($F_{2,18} = 35.780$; $p < 0.001$; Table S1; Figure 3) on the swimming speed of lice copepodids, due to an increase in speed recorded at time 2 in the ND=0 treatment. However, *L. salmonis* copepodids did not decrease swimming speed (i.e., habituate), under any of the light treatments, after 90 min of stimulation by continuous and monotonous ON:OFF stimulation (post hoc analysis). In the ND=0 treatment, the upward swimming speed recorded after 90 min (time 2; 33.8 ± 2.3 [SD] mm/s) was not significantly different from that observed at the beginning of the trial (30.1 ± 0.2 [SD] mm/s). Analogous results were observed for ND=2 (time 2; $29.1 (\pm 0.5)$ [SD] mm/s) and ND=3 (time 2; 20.9 ± 2.0 [SD] mm/s).

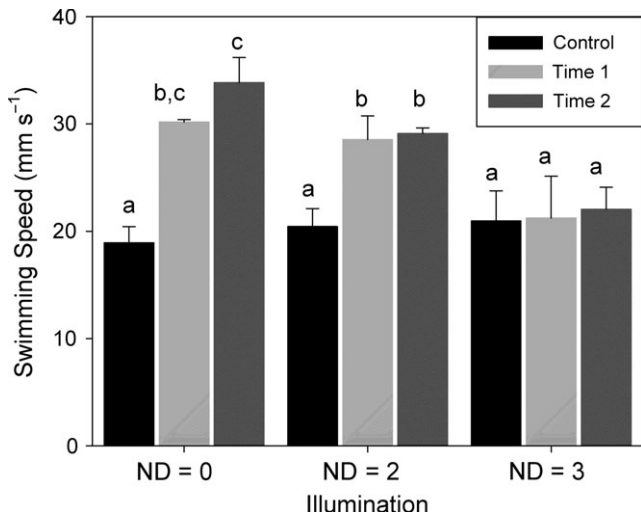


FIGURE 3 Average swim speeds of parasitic stage salmon lice copepodid (\pm SD) in response to ON/OFF visual stimuli of varying ON strength (ND=0,2,3), at the start of the experiment (time 1) and the end (time 2—after 90 min), and in the controls, $n = 3$ for each treatment. In all cases, the OFF intensity was set at ND=4. The ON: OFF cycle was 5:5 sec for 30 s, with a 60 s rest in between ON: OFF sequences; this cycle of ON:OFF:REST was repeated for 1.5 hr. Significant differences are noted by letters above the bars

3.2 | Response to changes in flicker frequency

There was a significant treatment effect of light flicker frequency on the number of animals attracted to the top of the 1 metre tank (ANOVA $F_{4,14} = 167.433$; $p < 0.001$). The control trials (darkness) attracted less than 5% of the animals to the upper camera 600 s after being released (Figure 4). All of the flicker frequency treatments attracted significantly greater numbers of animals to the top of the tower than the dark treatment ($p < 0.001$ in all pairwise comparisons, Table S2). The treatment with the shortest ON:OFF cycle (1.8:0.9 s) attracted the lowest percentage of animals (Table S2A); only 24% of the population was present in the upper camera's field of view at the end of the 600 s observation period. A doubling of the duration of the ON time to 3.5 s, while maintaining a constant OFF time of 0.9 s, resulted in a significant increase in the percentage of animals reaching the surface (37% of the population) (Table S2). Similarly, an increase in the duration of the OFF cycle to 5.5 s increased the percentage of the population present in the upper camera's field of view to ~80%. A further increase of the OFF time to 16.5 s did not result in a further significant increase in the percentage of the population that reached the upper camera (Table S2).

3.3 | Responses to combined visual and olfactory signals

There was a significant increase in the per cent of the population that was present in the upper camera's field of view in the light treatment vs. the dark treatment (2-way ANOVA; $F_{1,18} = 33.83$, $p < 0.001$; Table S3; Figure 5). There was no significant direct effect

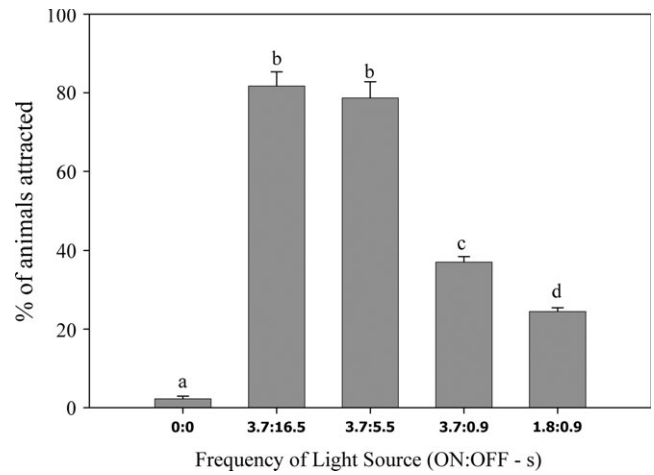


FIGURE 4 The per cent of salmon lice attracted to the field of view of the upper camera in the tower tank under each ON:OFF treatment

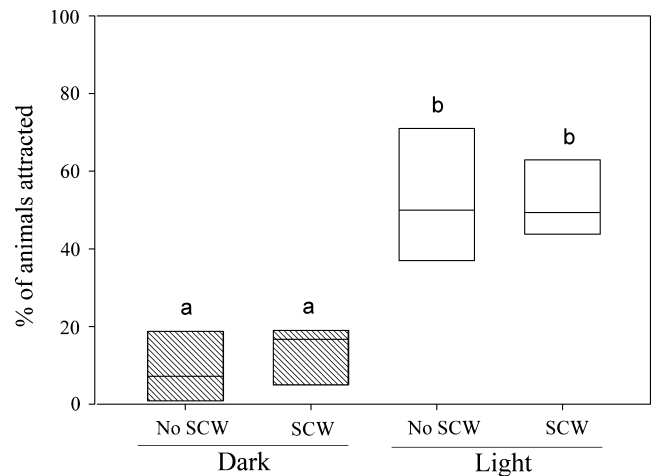


FIGURE 5 Box plots showing the results of the two-way full-factorial ANOVA testing the effect of light (ON:OFF-3.7:5.5 s) and salmon-conditioned water (SCW) in attracting parasitic stage salmon lice copepodids through a 1-metre water column. The upper and lower edges of the boxes are the 25th percentile and 75th percentile, respectively. The dark line in the box is the median value. Dark treatments are shown with hatched bars and light treatments in white. Treatments with salmon-conditioned water are labelled SCW

of SCW (two-way ANOVA; $F_{1,18} = 0.01$, $p > .93$), nor an interactive effect of light and SCW, on the percentage of the population of lice copepodids that reached the upper camera's field of view (two-way ANOVA; $F_{1,18} = 0.03$, $p > 0.83$). Although the addition of SCW did not produce a significant increase in the percentage of the population that reached the upper camera (compared with dark controls; Figure 6a), the addition of SCW increased the number of animals that reached the lower camera (Figure 6b). In the dark treatment (no SCW), the population of animals did not reach the lower camera (30 cm) during the 600 s after being released from the holding chamber. In contrast, the addition of SCW (no light; Figure 6b) produced a surge in the movement of the population. Within 60 s of

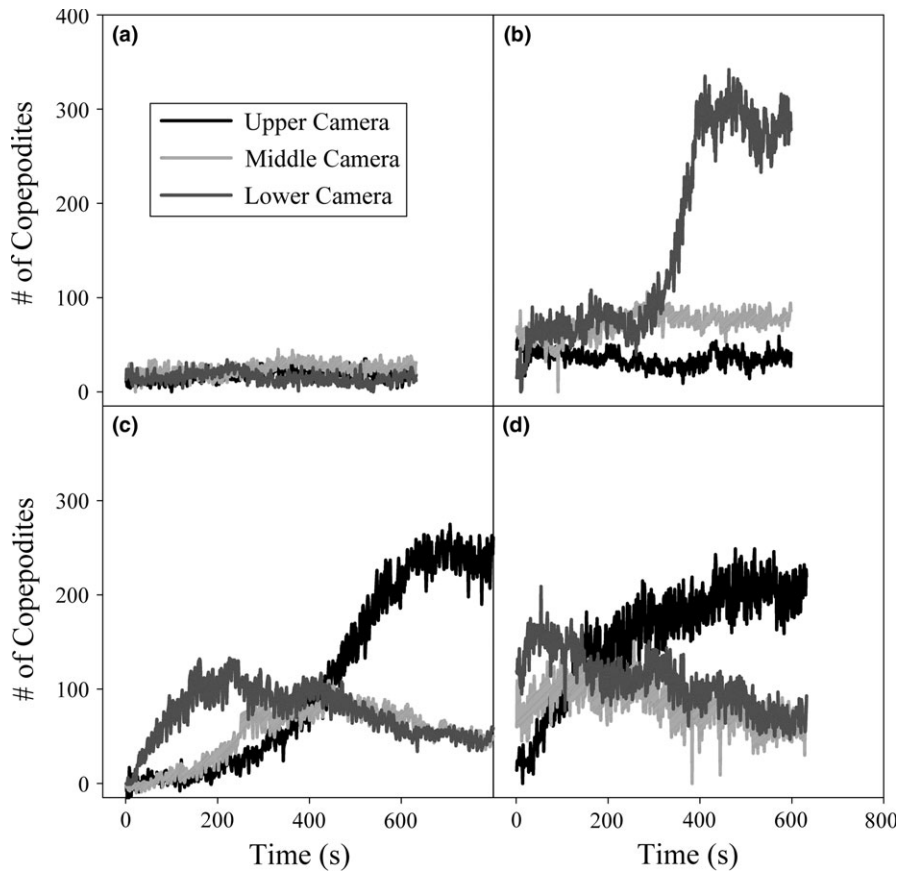


FIGURE 6 Vertical movement of 300 *L. salmonis* copepodids in response to an oscillating light source and salmon-conditioned water (SCW). Oscillation frequency was set at three flashes per minute (ON:OFF of ~3.7:16.5 s). Animals in the field of view of each camera (Figure 2) were counted every second as they travelled vertically in a 1-metre water column. Panels (a-d) show the movement of the population over time when exposed to (a) no light and no SCW, (b) SCW and no light, (c) light with no SCW, (d) light and SCW

being released from the bottom holding chamber, 25% of the copepodids appeared in the lower camera's field of view. After 350 s (350-400 s), up to 100% (73%-100%) of the population had reached the lowest camera. Although the copepodids experienced a pronounced initial increase in their vertical displacement, this SCW-driven upward swimming behaviour was short-lived. During the 600 s of observations, only ~25% (19%-31%) of the total population reached the middle camera and on average less than 20% reached the upper camera. Similarly, the addition of SCW to the light-stimulated trials (ON:OFF-3.5:5.5 s) did not result in a significant increase in the total number of copepodids reaching the upper camera's field of view (Figure 5). In both treatments, ~80% of the population reached the upper camera within 600 s of being released. Although there was no significant increase in the per cent of the animals reaching the upper camera (two-way ANOVA; $F=0.007_{1,18}$; $p = 0.93$; Table S3), the addition of SCW increased the speed at which the population centre animals moved vertically in the tower (compare Figure 6c, d). The addition of SCW increased the speed at which copepodids arrived at the upper camera by ~22%, with the population mode arriving in the surface at 415 s in the SCW trials and 600 s in the light-only trials.

4 | DISCUSSION

The objective of these experiments was to investigate the roles of light and chemoreception in host detection by the parasitic copepod,

L. salmonis. Previous field-oriented results (Hevrøy, Boxaspen, Oppeidal, Taranger, & Holm, 2003), and experimental work on the swimming responses of lice to changes in light intensity (Flamarique et al., 2000), highlight the importance of light in host finding by *L. salmonis*. Other work identifies the importance of chemical (Fields et al., 2007) and mechanical signals (Heuch et al., 2007) in detecting potential hosts. In this study, we used behavioural observations to characterize the specific aspects of light signals—alone and in combination with SCW—that maximize the directional swimming of infectious-stage copepodids towards a potential host.

4.1 | Copepodid swimming speed is not dependent on the intensity of the shadow

L. salmonis strongly increased their swimming speed in response to a decrease in light intensity analogous to that which would be caused by a fish (any species) passing overhead (Flamarique et al., 2000). The observations presented here suggest that this response functions more like an on/off switch with a threshold trigger rather than one which is modulated by changes in the absolute intensity of light. Specifically, changing light intensity from full strength (ND=0) to an intensity four orders of magnitude lower (ND=4) had indistinguishable effects from experiments during which the shadow was generated by decreasing the light levels by two orders of magnitude. Both experiments induced maximum swimming velocities in individual lice. In contrast, a shadow caused by a decrease in light level of only one order of magnitude (ND=3 to ND=4) elicited no difference from

background swimming speeds despite the fact that both intensities were well above the sensitivity threshold of visual responses in lice copepodids (Flamarique et al., 2000). Therefore, if the light stimulus is sufficient to elicit increased swimming speeds, the copepodids swim at maximum speed. Contextualizing this: if the shadows cast by a grouping of salmon (or other species—a shadow would not provide a species-specific cue) swimming overhead can be perceived by lice copepodids, they will continuously swim towards the stimulus at high velocity.

4.2 | Estimating the distances over which visual stimuli operate in the natural environment

The effectiveness of a light stimulus in inducing a response is dependent on its attenuation in the water column and the sensitivity of the observer's photoreceptive organ. Epipelagic calanoid copepodids can perceive light intensities as low as 2.8×10^{11} photons/m²/s across a broad range of wavelengths (453–620 nm) (Stearns & Forward, 1984; but even greater sensitivities are reported for mesopelagic copepodids, see Cohen & Frank, 2013). Based on the difference in light intensity between the ON:OFF flicker found in this study (Figures 1a and 3), the depth at which there would be sufficient light intensity (ON) to create a detectable shadow can be calculated. This depth would depend on the attenuation coefficient of light for the specific water column of interest. For example, assuming an attenuation coefficients (K_d) of 0.14 and 0.12 m⁻¹ reported from stations 3 (60°18'N, 05°38'E) and 5 (60°13'N, 05°38'E), respectively, in the Samnanger fiord (western Norway), (Kjeldstad et al., 2003), the intensity of light needed to generate a stimulus with sufficient difference in the ON:OFF flicker shadow would occur above a depth of 31–37 m. Animals below these depths would not respond to the change in light intensity generated by the shadow cast by fish swimming overhead. Although the threshold depths at which lice might react to shadows of fish passing overhead will vary greatly with water clarity, these calculations provide a first approximation of the distances over which lice copepodids can use visual cues to detect potential hosts in their natural environment. Once stimulated, the copepodids can swim (hop) at speeds of up to ~30 mm/s (as reported in the habituation trials); however, they also sink between hops. Assuming an average swim speed of 5.5 mm/s (based on the fastest swimmers in the combined SCW/light trials), it would take ~3 min for a lice copepodid to travel 1 m vertically. Based on our results, if the copepodids are continually stimulated, they can sustain this for well over an hour, at least, meaning that they could move towards a population of fish swimming overhead at a rate of 20 m/hr.

4.3 | Copepodid response is dependent on the frequency at which the light stimulus is delivered

The optimal frequency of any stimulus reflects a balance between overstimulation, where the nervous system cannot distinguish between the incoming signals (flicker fusion frequency), and understimulation where stimulus frequency does not maximally motivate

the organism. The frequencies tested in this study were well above previously reported critical flicker fusion frequencies (7.2–12 Hz, Cohen & Frank, 2013). When unstimulated, infectious-stage lice copepodids hop at speeds of 18.9 ± 1.5 mm/s (Figure 3) with long periods of inactivity (sinking) between hops. As a result, over time, populations of copepodids become aggregated in the lower water column. When stimulated with a flickering light source, *L. salmonis* copepodids swim towards the source of the stimulus through a rapid series of propulsive thrusts punctuated by short periods of sinking. Maximizing the distance covered is achieved by either increased swimming speed, decreased sinking time or a combination of both. The results described above (threshold and habituation responses to visual signals) demonstrate that if the copepodids react to the changes in light intensity, they do so with an all-or-nothing response. The copepodids either swim at sustained speeds of ~30 mm/s or at background swimming speeds of ~20 mm/s. Therefore, for a population of copepodids to move vertically through the water column, the signal must arrive at a frequency that minimizes the time spent sinking. Our results suggest that the frequency of ON/OFF visual stimuli that results in a maximal vertical displacement of *L. salmonis* copepodids is between 3.5; 5.5 and 3.5:16.5 s (Figure 3; Table S2). Frequencies higher than this appear to either overstimulate the animals or decrease the time spent swimming. Although it is unclear how long the shadow can remain while still providing a stimulatory effect, it is clear that extended durations of darkness (shadow) will not produce maximum upward swimming responses (Figure 6—dark treatment). In a natural context, this would be equivalent to a group of salmon (or other object—they would not be able to tell what species it is at this point) having passed by overhead—the copepodids would then stop their rapid upward swimming, waiting for the next group of fish to pass by.

4.4 | The effect of SCW

Chemical cues can provide information on the presence and identity of a host. This is supported by previous behavioural observations showing that copepodid (Bailey et al., 2006) and adult (Ingvarsdóttir, Birkett, Duce, Genna et al., 2002) *L. salmonis* are attracted to SCW and showed directional choice in response to extracts in y-tube choice experiments (Bailey et al., 2006), and neurophysiological measurements that show chemosensors of adult *L. salmonis* respond to low-molecular-weight hydrophilic chemicals present in salmon flesh (Fields et al., 2007). Our observations support these earlier studies: diffuse chemical signals, devoid of spatial and temporal structure, transiently stimulated swimming activity. However, without a spatial or temporal gradient in the chemical signal, chemoreceptors habituate (Fields et al., 2007), explaining why the copepodids returned to background swimming speeds after minute-long exposure to chemical signals.

Synergistic effects between different sensory modalities can increase the behavioural sensitivity of an organism well beyond the thresholds found for signals presented alone (Bowen, 1991; Mikheev, Pasternak, & Valtonen, 2004; Uetz, Roberts, & Taylor,

2009). In the tower experiments (Figure 6), there was no difference in the number of animals that reached the upper camera's field of view in the light treatment alone compared with the light treatment with SCW. However, in the SCW treatment, the time that it took for the population to swim towards the surface decreased. The lice that received only the shadow signal (no SCW) took ~ 450 s for 50% of the population to reach the top camera in the 1 m tower (Figure 6b); however, when the chemical and light stimuli were administered together, 50% of copepodids reached the upper water column at nearly twice the speed of animals exposed to the light signal alone (compare Figure 6c, d).

4.5 | Spatio-temporal hierarchy of sensory cues

The sensory modalities and behaviours involved in host detection and recognition by *L. salmonis* consist of a spatio-temporal hierarchy within which one or more senses operate simultaneously. Visual cues, such as decreases in light intensity resulting from shadows cast down into the water column by fish swimming overhead, would operate at long range—metres to tens of metres. Such non-species-specific signals alter the parasite's overall activity level and/or swim pattern, typically motivating it to move towards the source (Flamarique et al., 2000; Mikheev, Pasternak, & Valtonen, 2003; Browman et al., 2004; observations presented here). *L. salmonis* is very sensitive to decreases in light intensity (Flamarique et al., 2000). Increases in light intensity, such as flashes off the side of a fish, may also induce directed swimming behaviour, as is the case for the fish ectoparasite *Argulus foliaceus* (e.g., Browman et al., 2004; Mikheev et al., 2003). For a flashing light to be perceived requires that the flash is not too rapid to surpass the flick or fusion rate of the animals' sensory system nor too slow, resulting in neurological adaptation. Further, the flash must be of sufficient intensity relative to the background to be perceived as being different and noteworthy.

From the swim speeds quantified here, we calculated that copepodids could travel 1 m within 0.5–3 min. Extrapolating the swim speed of 5.5 mm/s (which represents the fastest swimmers in the combined SCW/light trials) over the amount of time the habituation experiments ran (90 min), lice can travel a minimum of 30 m without habituating. Thus, shadows and light flicker of a perceivable frequency could conceivably attract free-swimming lice towards a population of potential host fish over spatial scales up to tens of metres.

A diffuse host-related chemical signal (likely species-specific), in conjunction with a light stimulus, augments the swimming speed of lice copepodids (Figures 5 and 6). This increased swimming speed would improve the chance of a lice copepodid reaching a potential host. Our observations indicate that a diffuse host-related olfactory cue can operate over a distance of ~1 m. This is supported by electrophysiological data showing that *L. salmonis* can detect water-soluble chemical compounds released from one gram of salmon flesh at a dilution of 10^{-4} (Fields et al., 2007). Whether a lice copepodid can detect and follow a chemical gradient was not directly tested in this study; however, previous work suggests that odour trail following may operate on scales of centimetres. It has been suggested

that the chemical trails associated with an individual fish function on spatio-temporal scales of only a few cm (e.g., Ingvarsdóttir, Birkett, Duce, Genna et al. 2002; Ingvarsdóttir, Birkett, Duce, Mordue et al., 2002; Okubo, Armstrong, & Yen, 2001). It is plausible that a chemical cue gradient produced by a high concentration of salmon in an aquaculture setting (i.e., an enormous source of olfactory cues), or from a large group of migratory salmon, would operate over distances of several tens of metres and, in the case of the former, would persist longer than shadows and flashes of light from a passing school. Around salmon farms, where salmon concentrations can exceed 10^6 fish, both olfactory and visual sensory cues would be powerful and omnipresent. For most copepods, hydrodynamic cues are only successful over scales of mm to a few cm (e.g., Doall, Strickler, Fields, & Yen, 2002; Yen & Okubo, 2002). This is also the case for *L. salmonis* copepodids, which responded to a moving plaster cast of a salmon head over distances of 3–4 cm by jumping towards the signal (Heuch & Karlsen, 1997; Heuch et al., 2007). Assuming a 50 cm fish swimming at an average speed of 100 cm/s, a lice copepodid would have 0.5 s to leap on to the passing fish. In this scenario, and assuming a maximum speed of 30 mm/s (as reported in the habituation trials), lice copepodids would have to be within 15 mm of the fish to complete a successful encounter. Slower swimming salmon could be settled on from greater distances, and shoals of fish would also increase the likelihood of successful encounters. Once a parasitic copepod settles on its target fish, chemical and tactile cues linked with the skin and mucous are probably most important in host identification (e.g., Buchmann & Bresciani, 1998; Núñez-Acuña, Marambio, Valenzuela, Wadsworth, & Gallardo-Escárate, 2016).

The information presented herein could be used to disrupt the link between the parasite and host fish, using a large number of traps deployed at a distance from a salmon farm for example, thereby reducing sea lice infestation pressure.

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