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Responses and preferences of salmon louse (*Lepeophtheirus salmonis* Krøyer 1836) copepodids to underwater artificial light sources

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ABSTRACT

The ectoparasitic salmon louse (*Lepeophtheirus salmonis,* Krøyer 1836) remains a major disease problem and cost driver in commercial Atlantic salmon (*Salmo salar* L) farming and is also implicated in the decline of wild salmon stocks. The parasite feeds on mucus and blood causing skin damage on its host (i.e. leads to reduced welfare and disease resistance). Underwater lights (UL) are being used regularly in open-cage salmon aquaculture to delay maturation and increase feeding rates during the dark season. The aim of this study has therefore been to supply basic experimental data on the responses of the infectious copepodid stage of L. *salmonis* to discrete underwater light sources with different light qualities and intensities. The collective movement of a copepodid population in response to light sources was tested in a laboratory-based machine vision system using automated image processing. Copepodids always moved towards the light source even at low light intensities (1.5×10^{-3} µmol m^{-2} s^{-1}) within a broad spectrum of visible light as well as near-UV. It is therefore plausible that subsea light sources frequently used in salmon farming under certain conditions can attract salmon lice copepodids and increase infection pressure. Moreover, the findings of our study support that light traps may be used to catch planktonic salmon lice. The actual effect of underwater light sources on the local distribution of salmon lice should be tested by controlled plankton sampling or monitoring in the vicinity of light sources compared to the surrounding water.

1. Introduction

The ectoparasitic salmon louse (*Lepeophtheirus salmonis,* Krøyer 1836) remains a major disease problem and cost driver in commercial Atlantic salmon (*Salmo salar* L) farming and is also implicated in the decline of wild salmon stocks (Abolofia et al., 2017; Torrissen et al., 2013). The parasites feed on mucus and blood causing skin damage (i.e. skin erosion and hemorrhaging), which leads to reduced welfare and disease resistance for its host (Johnson et al., 1996). Several methods have been used for delousing salmon throughout the past decades including medicinal treatments (i.e. bath- and oral treatments), biological treatments (i.e. cleaner fish), mechanical treatments (i.e. lice flushers, freshwater treatments and thermic treatments) (Jevne and Reitan, 2019) and laser treatments (i.e. Stingray, Stingray Marine Solutions, Norway). Other methods currently in use or under evaluation include shielding technologies which involves physical separation of the infectious copepodid stage, and the salmon based on the assumption that salmon louse copepodids occur mainly in the upper few meters of the water column. Such technologies include lice skirts (Stien et al., 2018), snorkel sea cage technology (Wright et al., 2017), closed sea cages where water is pumped from (lice free) deeper waters (Strand et al., 2013) and submerged or semi-submerged sea cages (Dempster et al., 2009; Glaropoulos et al., 2019). The use of underwater feeding (Dempster et al., 2009) and underwater lights (Juell and Fosseidengen, 2004) aimed at guiding the salmon deeper in the sea cages below the surface waters that contain the highest concentration of planktonic sea lice (Heuch et al.,1995) has also been tested. Additionally, a wide variety of traps have been designed and tested for efficacy to catch the planktonic stages of sea lice before entering the fish cages (Pahl et al., 1999; Flamarique et al., 2009; Selander et al., 2017). Suggested traps are based on creating reinforced stimuli of natural responses such as odor (Bailey et al., 2006), mechanical cues (Heuch et al., 2006) and physical cues such as light and light flickering (Fields et al., 2007; Fields et al., 2018).

Underwater lighting (UL) is being used regularly in open-cage salmon aquaculture to compensate for highly variable diel and seasonal natural photoperiods, and thus, increase growth and delay maturation (Juell and Fosseidengen, 2004; Juell et al., 2003; Oppedal et al., 2001). Since salmon lice copepodids have been shown to display positive phototaxis (Bron et al., 1993; Fields et al., 2018) as well as a distinct

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diel vertical migration pattern (Heuch et al., 1995), it is not unlikely that such underwater light sources under certain conditions (i.e. hydrodynamic, bathymetric or daylight conditions) may increase infection pressure by attracting the parasite to the production sites. This is most likely to happen during night-time when the light sources appear as bright point sources against a dark background and when current velocities are low during high and low tide. It was, however, reported by Frenzl et al. (2014) that the use of submerged lighting significantly reduced infection rates when compared to surface lighting. This was mainly attributed to changes in the behavior of the salmon which migrated to deeper waters due to the submerged light sources.

L. salmonis copepodids ultimately must locate and attach to a host to complete their life cycle. Consequently, they have evolved highly efficient host finding features involving utilization of both chemical and physical cues (Fields et al., 2007; Fields et al., 2018; Mordue and Birkett, 2009). Among the structural features are surprisingly complex eyes with lenses and screening pigments that appear to be capable of highly directional vision (Bron and Sommerville, 1998; Bron et al., 1993). Flamarique et al. (2000) reported a light intensity threshold at 1.24×10^{13} quanta $m^{-2}\,s^{-1}$ (2×10^{-5} μmol $m^{-2}\,s^{-1}$) for behavioral responses in copepodids, which is below the threshold for all the other developmental stages. The copepodids adaptation to low light intensities is also indicated by the presence of a reflecting tapetum, which is typical for night active arthropods (Meyer-Rochow, 2001). The tapetum of salmon louse copepodids consists of stacks of about 20 alternating layers, presumably of different optical densities, arranged as a quarter of a wavelength interference filter (Bron and Sommerville, 1998). The average thickness of the individual layers, according to the illustrations of Bron and Sommerville (1998), is about 105 nm, which theoretically corresponds to a maximum reflectance of blue light (420 nm).

A variety of light sources intended for use in salmon farming are commercially available. At the same time, several types of traps with light as an attractant have been suggested for catching free-swimming stages of salmon. There is, however, little information on the responses of early stages of salmon lice to submerged discrete light sources. This makes it virtually impossible to evaluate how various light sources used in conjunction with salmon farming will affect infection pressure from salmon lice. The aim of the current study was therefore to supply basic experimental data on the phototactic responses and light preferences of the infectious copepodid stage of L. *salmonis* to discrete underwater light sources. The experiments were designed to simulate the appearance of bright submerged light sources at a distance. Groups of salmon lice copepodids were exposed to submerged low intensity point light sources (LED lights) and their collective movement relative to the light sources were tracked. The experiments included recording both trajectories and velocity of movement towards a single light source (phototaxis) and choices between pairs of equal intensity light sources with different colors or flickering patterns (light preference). These type of data combined with information on ambient water currents are crucial for predicting the potential for spatial efficiency of attracting and retaining salmon lice copepodids by light-traps as well as assessing the potential risk of underwater light increasing local infection pressure from salmon lice.

2. Material and methods

2.1. Animals and husbandry

L. salmonis copepodids were acquired from The Industrial Aquatic Laboratory (ILAB, 5008 Bergen Norway) from a strain (LS-Gulen) that has been cultured since 2008. Copepodids were collected within a day after molting from nauplii and shipped in chilled containers that arrived in our laboratory on the same day. After arrival, the copepodids were counted and distributed in 50 mL vials with 200 individuals each and stored in the laboratory at 10 °C in darkness. Copepodids used in each experiment (n = 200) were transferred to the test arena in dim light and acclimated for at least 15 min prior to the experimental runs. The experiments were performed on the same cohort of copepodids over a period of 9 days with a repeated test regime to test the consistency of the observed responses to different light stimuli (Supplementary material Fig. S3), whereof new batches of dark-adapted copepodids (n = 200) were used for each experimental run.

2.2. Experimental set-up

The experimental system previously described by Kvæstad et al. (2020) consisted of a black polypropylene container (v = 60 L) with an inner circular arena consisting of a 40 cm wide and 10 cm deep tray (volume; 12,5 L) of translucent material (polycarbonate). This arena was filled with seawater (34 ppt, 10 °C) and a 10 mm thick polycarbonate lid was placed on top in direct contact with the water surface to avoid surface distortions. The space between the inner arena and the outer container was continuously circulated with cooled seawater (10 °C) to stabilize the temperature inside the arena. The camera used to record movement within the arena was a Point Grey Grasshopper 3 camera (2448 × 2048, Mono chrome, FLIR, United States) equipped with a wide FOV TECHSPEC lens (4 mm, 84° FOV, Edmund Optics, United States) and a near infrared (NIR) long pass filter (Wratten 2, 800 nm, NIR, long pass filter, Kodak, USA). IR light (85 W) was provided by a near infrared NIR LED-strip (SMD5050-600-IR, 850 nm, IP68, 28.8 W/m, LEDLightsWorld, USA) attached to the inner surface of the 60 L container below the water surface of the circulating cooling water. Two diodes; one RBG diode (total max 200 mW) and one UV diode (max 20 mW) with 5 mm plastic casings were mounted in the wall of the outer container with their tip reaching into the cooling water body to limit differences in light distribution. Identical arrays of diodes were distributed at equal distances from 4 different positions of the circular outer container. At each position, 5 different light qualities (UV, blue, green, red and white) were calibrated using a spectrometer (Flame Spectrometer, Model FLAME-S-UV-VIS-ES, Ocean Optics, Netherlands) to yield the same quantum efficiency at the highest intensity test level when measured in the center of the test arena (Fig. 1A). The intensity of the diodes was adjusted by pulse width modulation (PWM) at 1000 Hz. Three different light intensity levels were used whereof the maximum light intensity was 1.2×10^{-1} μmol $m^{-2}\,s^{-1}$ measured in the center of the arena (Fig. 1B). The corresponding intensity at medium and low intensities were 1.2×10^{-2} and 1.5×10^{-3} μmol $m^{-2}\,s^{-1}$, respectively. Red, green, and blue light was provided by the RBG LEDs. White light was provided by a combination of the three colors at equal intensity whereas UV was provided by separate UV-LEDs placed adjacent to the RBG-LEDs. The peak wavelengths and halfwidth of the discrete light qualities are shown in Table 1. LED spectral distributions are shown in Supplementary material Fig. S1.

The distribution of light within the arena included a sharp gradient in the vicinity close to the active LED sources (Fig. 1B). Due to a slightly higher intensity along the forward axis, compensating for longer distance to the opposite side of the arena, the intensity in front of the inactive LEDs were roughly identical. Fig. 1B is based on light measurements in different positions and show the relative distribution of light when one of the four diodes was activated. The consistency of the responses over time were verified by repeating the test routines at different ages of the copepodids.

For each test session, 200 *L. salmonis* copepodids were introduced to the test arena and subjected to pre-programmed routines of light combinations emitted from the diodes pointing into the test arena. During this test routine, the light stimuli in terms of light intensity, color or position was changed every 10 min. The position of individual copepodids in the horizontal plane was recorded with high resolution images at a rate of two frames per second over a period of 120 min for each routine (12 different light combinations), corresponding to a total

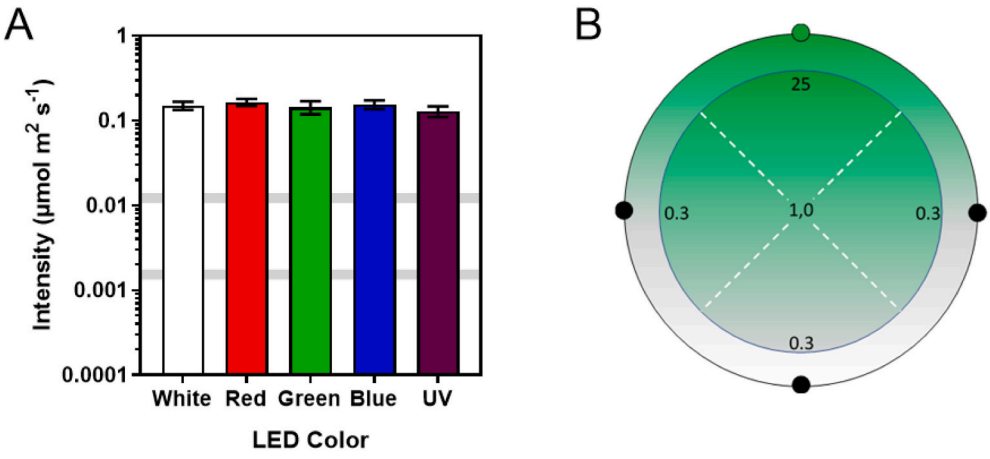

Fig. 1. Intensity and distribution of LED light A; Maximum intensity used for the various light qualities (average of 4 LEDs ± SD) used to assess light responses in *L. salmonis* copepodids, measured in the center of the circular test arena. Horizontal grey lines indicate the light intensity of medium and low intensity treatments. B; Distribution of light within the test arena (inner circle). Numbers inside the circle indicate intensity in front of each LED position relative to the center position (1.0) with one active diode (on top). The diameter of the test arena was 40 cm diameter and the positions of the inactive LEDs in the wall of the outer container (50 cm diameter) are marked with dots (green "on" and black "off"). Dotted white lines indicate subdivisions into quadrants used for statistical calculations of the copepodid distribution.

Table 1
Characteristics of LED's used to assess light responses in L. *salmonis* copepodids.

Color	UV	Blue	Green	Red
Peak wavelength (nm)	385	460	515	645
Dominant wavelength (nm)	–	465	525	630
Halfwidth (nm)	35	25	30	28

of 14,400 high resolution images (72 Gigabytes) per experimental run. Each new combination of light stimuli was presented with the population positioned in front of the preceding preferred stimulus. This assured that the majority of the copepodids were at the same approximate distance from the new stimulus that was presented either diagonally on the same axis, or along the transverse axis of the arena, 45 degrees on either side of the position of the preceding stimulus (Fig. 2).

2.3. Responses to different light intensities

Responses to different light intensities and intensity preferences were tested for white light and near-UV (UVA). Changes in light stimuli were arranged either as a random shift in position of a single stimulus, or as a choice between combinations of two different light intensities. Examples are shown in Fig. 2 where the average position of the active copepodid population within the arena is plotted every 2.5 s on a time scale (0–600 s), which is indicated by a change in color from red (start) to blue (end).

2.4. Responses to colors of equal light intensity (quantum flux)

Color preferences were tested by the choice situation were pairs of color combinations were presented in the arena alternating between the two transverse axes (horizontal and vertical, cf. Fig. 2). All experiments with choices between different colors were conducted at the lowest intensity (1.5×10^{-3} µmol m^{-2} s^{-1}).

2.5. Responses to flickering light

The copepods were presented to choices between flickering light and constant light during 10-min test periods. The L:D cycles were presented in random order and the tested cycles are shown in Table 2. All experiments with flickering light were conducted with white light at the low intensity (1.5×10^{-3} µmol m^{-2} s^{-1}).

2.6. Data treatment

The collected images were processed with regards to positioning, movement trajectories, horizontal bulk movement and response times in relation to the tested light regimes as described in detail by Kvæstad et al. (2020). In short, frames acquired by the camera during the

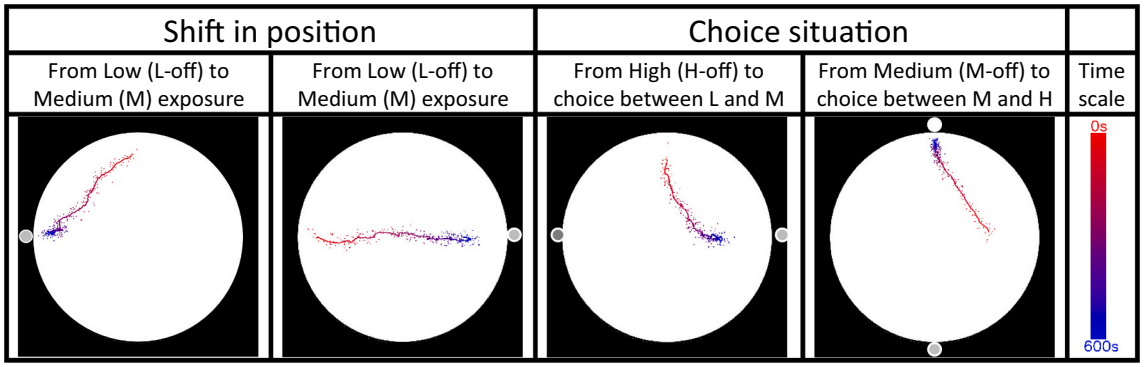

Fig. 2. Basic test routines showing average trajectories of population movement for L. *salmonis* copepodids in response to discrete light sources. Two approaches were used, one was a shift in position of a single light source and the other included a choice between two light sources with specific light quality and intensity pre-sets. The panels show the average trajectory of the copepodid population (solid line) during a course of 10 min after a change in the configuration of white light stimuli. Average position of the population in 2.5 s intervals is shown as dots. Time is represented by a color gradient from red at the beginning and blue at the end of the 10-min exposure period. Light intensity is indicated by dots with different shades of grey outside the representation of the test area. The starting point of the copepods were in front of the previous stimulus represented here by the "off" stimuli.

Table 2
Tested light flickering cycles compared to constant light with equal intensity.

Test no.	1	2	3	4	5	6	7	8	9	10
Frequency (Hz)	20	10	1	0.25	0.167	0.125	0.125	0.1	0.083	0.0625
Light (s)	0.025	0.05	0.5	2	2	2	4	4	4	4
Darkness (s)	0.025	0.05	0.5	2	4	6	4	6	8	12

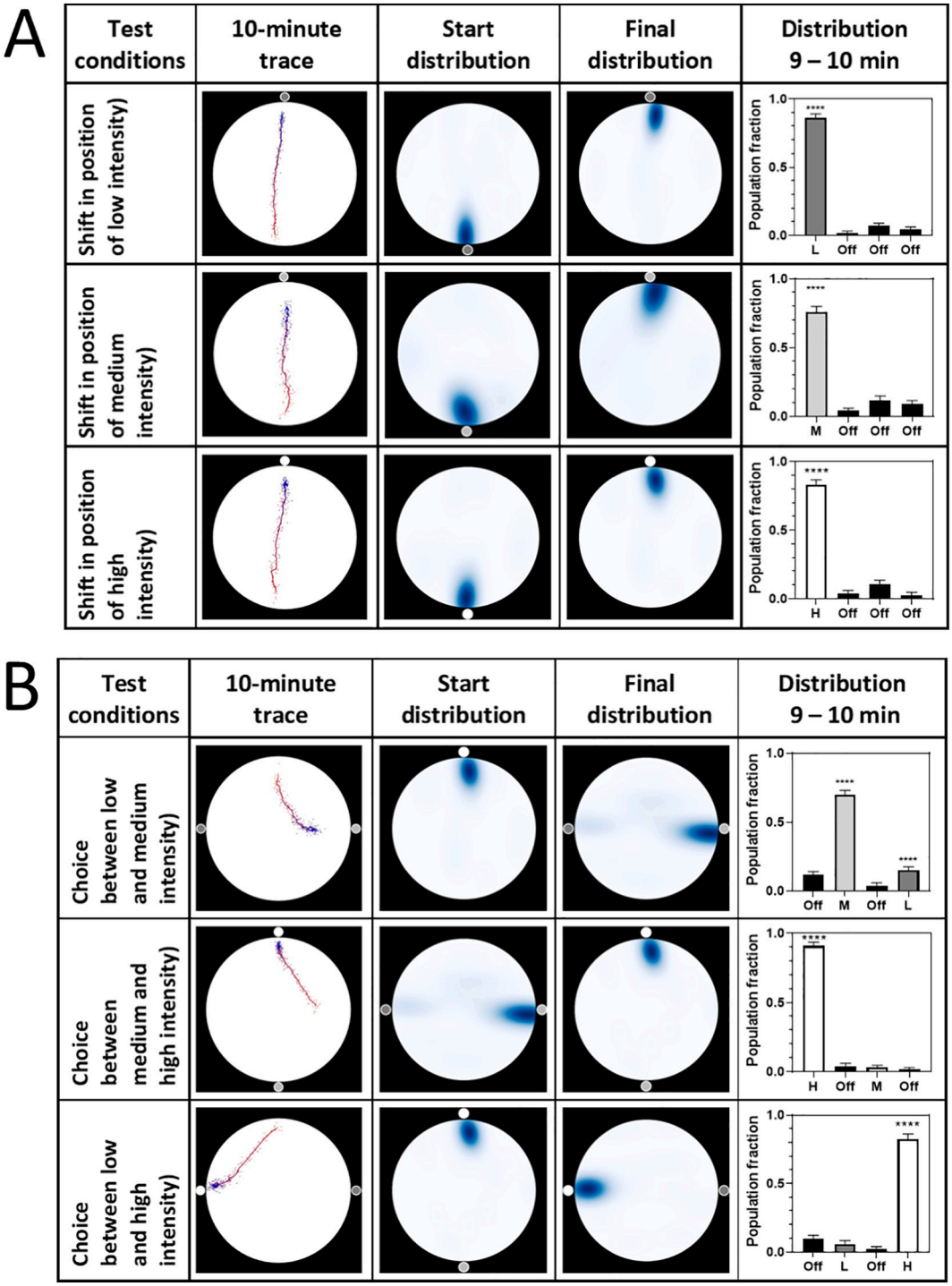

Fig. 3. Responses of L. *salmonis* copepodids to three different intensities of white light. A: Shift in position of white light with similar intensity. B: Choice between different intensities. Intensities and position of active light sources are indicated by dots with different shades of grey illustrating low (L), medium (M) and high (H) light intensity, respectively. Left panels show average trajectory for population movement with time indicated by a gradual change in color from red (start) to blue (end). Middle panels are heat-plots of the initial and final distribution, respectively. Right panels show relative distribution of copepodids between quadrants (see Fig. 1B) during the last minute of each stimulus period (9–10 min., SD indicated). Asterixis denote significant differences from quadrants without light (**** = p <0,0001).

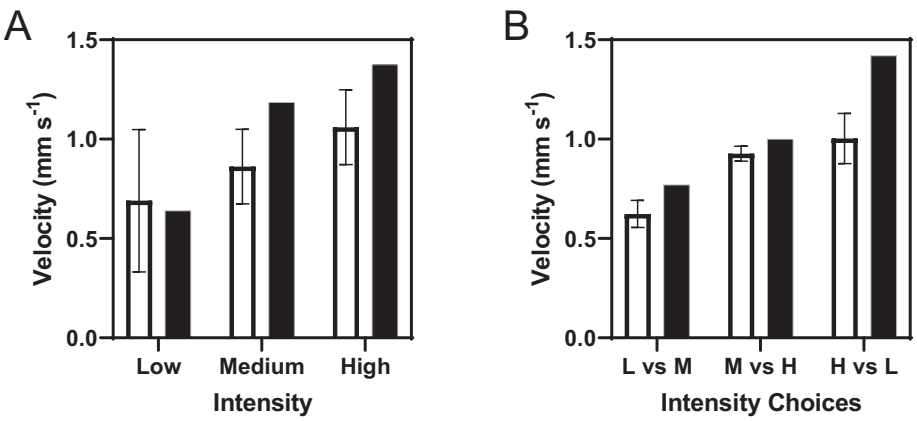

Fig. 4. Comparison of average response velocities for L. *salmonis* copepodids to equal intensities of white (white bars) and UVA light (black bars) recorded during the first 2 min after onset of the light stimuli. A; Shift in position of a single light source. B; Choice between two light sources with different intensity. Six tests were conducted with white light (SD indicated) and two tests were conducted with UVA. An approximately tenfold increase in intensity was applied for each step from low ($L = 1.5 \times 10^{-3}$ µmol m^{-2} s^{-1}) medium ($M = 1.2 \times 10^{-2}$ µmol m^{-2} s^{-1}) and high ($H = 1.2 \times 10^{-1}$ µmol m^{-2} s^{-1}) light intensities and the velocity represents the velocity towards the preferred light source.

experiments were postprocessed using an algorithm written in Python 3.6 using OpenCV 3.4. Each frame was calibrated using the OpenCV (v3.4) implementation of Zhang's "A flexible new technique for camera calibration" (Zhang, 2000) before being processed. The data processing algorithm was designed to detect moving particles from one frame to another, removing particles that have been stationary for more than 5 s, extracting only moving particles for further processing. Thus, copepodids were only detected when they were moving. The calculations of spatial distributions and velocity of movement assumed that the median position of the lice population was in the middle of the water column which introduced an error of ± 6.2% due to perspective distortion for individuals moving along the surface and bottom of the 10 cm water column, respectively (Kvæstad et al., 2020).

For each 10-minute stimulus period, time-traces of average position of the copepodids where plotted in intervals of 2.5 s along with a heatplot of the final distribution. Additional representations with time traces of the average distance to each diode position, velocity of movement, and the number of active copepodids, are shown in Supplementary material. For statistical purposes, the test arena was divided in quadrants centered around the four light sources (Fig. 1B). In tests of light preferences between equal intensity stimuli, the change in the number of copepodids in the two quadrants associated with the stimuli was compared before and after the stimulus. In tests with different light intensities and flickering versus constant light, the number of observations were compared for all quadrants at the end of the stimulus period. Data were tested for normality by Shapiro Wilk normality which failed for sectors with few copepodid observations. Comparisons between quadrants were therefor performed using Kruskal-Wallis tests (non-parametric). Statistical analysis and bar graphs were generated using GraphPad Prism version 6.00 for Windows, (GraphPad Software, La Jolla California USA, www.graphpad.com).

3. Results

3.1. Consistency of the measurements over time

To test the variation of responses throughout the course of the experimental runs, as well as with the ageing of the copepodid cohort tested, one of the experimental routines was repeated three times over the course 9 days (2–10 days post molt). Response patterns did not appear to change with age. Additionally, the responses were independent of the number of actively swimming copepodids recorded by the camera system (Supplementary material Fig. S3). However, the average number of active copepodids at any point in time decreased somewhat after repeated sessions (several hours) indicating reduced swimming activity of the copepodids.

An initial test with comparison of light and dark adapted copepodids exposed to a wider range of intensities than that used in the current tests indicated that light adapted individuals initially responded slower to defined light intensities than individuals collected from darkness and kept in dim light as was the case in all other experiments. After 30–40 min exposure to reduced light intensities within the range used in other tests, the initially light adapted copepodids responded similar to those initially kept in darkness (Supplementary material Fig. S4).

3.2. The effect of different intensities

For the experiments using white light with shifts in light positions, the copepodid population always moved towards the light source (Fig. 3A). In all instances where the copepodids were exposed to a single light source, the final number of copepodids in quadrants associated with the light source was significantly higher than in all other quadrants ($p < 0.0001$). When subjected to a choice between light sources having different intensities, the movement was always directed towards the brightest light source (Fig. 3B) causing a significantly higher number of copepodids to migrate into the quadrant associated with the highest intensity. The maximum average population velocity towards the preferred light sources ranged from 0.5 to 2 mm/s. The maximum velocity was in most cases reached at 75–150 s after the shift in position of the stimuli. By comparing the velocity towards white light with different intensities, we found a positive log-linear correlation between velocity and intensity. This was the case for both changes in light positions and movement towards the highest intensity in a choice situation (Fig. 4).

When tested with near-UV alone, the copepodids showed positive phototaxis at all three intensity levels. The average population velocity towards the UV light was in fact as fast or faster than the responses to comparative intensities of white light (Fig. 4). A comparison between response patterns towards similar intensities of white and UVA light is shown in Supplementary material (Fig. S5).

3.3. Color preferences

When the copepods were subjected to choices between light sources of equal intensities (1×10^{-4} µmol m^{-2} s^{-1}), we found a distinct preference for white and blue light. Moreover, by comparing all light quality data to each other the ranking of responses in terms of most positive phototactic responses were: blue ≈ white > green > UV ≈ red (Fig. 3). UV and red light was never the preferred cue when the copepodids were subjected to blue and green light at equal quantum fluxes.

3.4. Light flickering versus constant light

When presented to a choice between constant and flickering light at low frequencies of the same light intensity, the copepodids clearly preferred the constant light source (Fig. 6). It appeared that the attraction to the flickering light was reduced in proportion to the reduction in average light intensity.

4. Discussion

The current work relates to the potential risk of attracting planktonic salmon louse infective copepodids by using underwater light sources. The results are relevant in a salmon-farming context as artificial lights are commonly used to prevent maturation. Furthermore, information on positive phototaxis can be used to optimize different conceptual light-traps proposed for catching early developmental stages of the salmon louse. The stimuli were designed to simulate the appearance of bright light sources perceived from a distance and the measured responses are assumed to represent phototactic responses to various characteristics of submerged artificial light sources used in conjunction with salmon farms. The applied light intensities were deliberately kept low to mimic a distant light source and to reduce the potential impact of uncontrolled transitions between light and dark adaptation. We assume that the copepodids were photomechanically dark adapted during the testing (Supplementary material Fig. S4). The experiments were designed to record the collective movement of the tested population rather than observing individual swimming patterns. The results are therefore based on changes of the average position the copepodid population rather than individual behavior in response to defined light stimuli. In darkness, the copepodids showed a random distribution within the circular 40 cm diameter test arena (Supplementary material Fig. S2). However, when exposed to low intensity illumination by discrete LED lights the copepodid population always moved towards the light sources.

The aim of our experimental system was to push the limits for water volume and extension of the measurement area while being able to track movement of many L. *salmonis* copepodids simultaneously without blind zones. Technical limitations restricted the design to recording the movement in the horizontal plane (Kvæstad et al., 2020). Consequently, without the possibility of detecting vertical movement, the perspective distortion of the lens had to be considered. For simplicity it was assumed that all copepods were situated in the middle of the water column, which is in the same plane as the light sources. The resulting horizontal positional error not accounted for in the peripheral margin of the arena was maximum ± 6.2% for individuals situated at the bottom and the surface, respectively (Kvæstad et al., 2020). This will affect the calculation of average velocity if the copepodids are unevenly distributed vertically in the water column. Without information of vertical movement, the recorded velocities are not representative of the average individual swimming velocity, but only represent the average horizontal movement of the active population relative to the stimuli.

The swimming pattern of salmon louse copepodids is described as repeated bursts of swimming intervened with periods of passive sinking (Bron, 1993; Gravil, 1996), which is probably an adaption for saving energy (Haury and Weihs, 1976). The data processing algorithm used in the current work was designed to detect moving particles from one frame to another, temporarily removing particles that have been stationary for more than 5 s. Thus, a single individual resting on the bottom of the arena for more than 5 s will not be recorded as active, whereas the same individual will reappear as active when it starts moving again. The recorded maximum number of active copepodids at any point in time rarely exceeded 50% of the total copepodid population. Despite variation in the number of active individuals recorded over time, the response pattern and velocity of the moving population was, however, surprisingly constant (Supplementary material Fig. S3). It is unclear if variation in the number of active individuals was due to inactivity in parts of the population, longer pauses between bursts of activity, or both.

There are observations that the infection efficiency of L. *salmonis* copepodids declines with age (Brooker et al., 2018).(Repeated measurements showed that both the pattern of population movement and response velocities of the phototactic response were maintained during the 9-day experimental period with the same cohort of copepodids (Supplementary material Fig. S3). There were, however, indications of a reduction in the number of active individuals during extended experimental sessions. The activity did not appear to change within the nine-day experimental period with copepodids 2–10 days after hatch for nauplii. This may however not be representative for free-swimming copepodids since we stored the copepodids in darkness prior to the testing and this may have affected their activity. Due to technical limitations, the copepdids were not subjected to controlled diurnal light regimes and it cannot be excluded that endogenous rhythms affected the results. The consistency of the observed responses, however, indicate that this effect was limited (Supplementary material Fig. S3).

The maximum swimming velocities of salmon lice copepodids have been reported up to about 3 cm s^{-1}, and over longer periods of time they can maintain a velocity of approximately 0.5 cm s^{-1} (Fields et al., 2018). If copepodids are attracted to a stationary light source, the trajectories of free-swimming lice depend on the detection distance, the average swimming velocity and the ambient current. The results obtained in this paper suggest that in dark conditions, the copepodids will start moving towards a bright light source irrespective of color at a distance. The average velocities of movement of the whole population of 0.5–2 mm /s is well below that observed in individual copepodids by other authors (Fields et al., 2018). This may to some extent be caused by the low light intensities used but is also impacted by the one-dimensional recording excluding horizontal movement. The velocity of movement increased at higher intensities in line with previous reports using a variety of light conditions (Bron et al., 1993; Fields et al., 2018; Flamarique et al., 2000). Results from using bright laser beams (unpublished data) indicate that copepodids display the same phototaxis even at very high intensities, suggesting that the velocity of movement increases both with increased intensity and also reduced distance to the light source.

The intensity threshold for instant behavioral responses of copepodids to light on and off is reported to be about 10^{13} photons m^{-2} s^{-1} (Flamarique et al., 2000) corresponding to 1.7×10^{-5} μmol m^{-2} s^{-1}. This was considerably below the corresponding intensity threshold found for the nauplii by four orders of magnitude as well as the adult stage by one order of magnitude. This, along with the structural design of the dorsal eye of the copepodids displaying a discrete lens combined with lateral screening pigments (Bron and Sommerville, 1998), indicate that directional visual clues play an important role in their life. The lowest intensity tested during our experiments was about 1.5×10^{-3} μmol m^{-2} s^{-1}, which for example is two orders of magnitude above the range for photomechanical dark adaptation of 10^{-5}–10^{-8} μmol m^{-2} s^{-1} ($10^{-9,5}$ to $10^{-5.5}$ W/cm^2 – white light) in the superposition eye of the hyperbenthic shrimp *Eualus gaimardii* (Nordtug and Krekling, 1989). However, we have no indication that the sensitivity threshold for phototactic responses of the copepodids was approached in our experiments and this should be further investigated.

The results show that the copepodids display positive phototaxis to all the tested light qualities, whereof the most efficient light qualities to attract the copepodis were white and blue light (Fig. 5). The color resolution used in our experiments was insufficient to determine the spectral profile. In contrast to Gravil (1996) who reported sensitivity maxima at 500 and 560 (cyan and green) we observed that blue was preferred over green. In general, the spectral response in behavioral tests support that the copepodids detect light over a wide range of what we define as visible light (400–700 nm) (Bron et al., 1993; Flamarique et al., 2000; Gravil, 1996). UV and red light were never preferred when presented together with similar intensities of white, blue, or green light. However, when UV was presented alone the copepodids displayed positive phototaxis like that seen for white light. Interestingly, the maximum velocity towards the UV light was as high or higher than towards the same intensity of white light (Fig. 4). There are several examples of copepods showing a negative phototaxis when exposed to high “daylight” intensities of UV and this is probably a response to avoid harmful effects of UV-radiation. It is unclear if these responses are triggered by

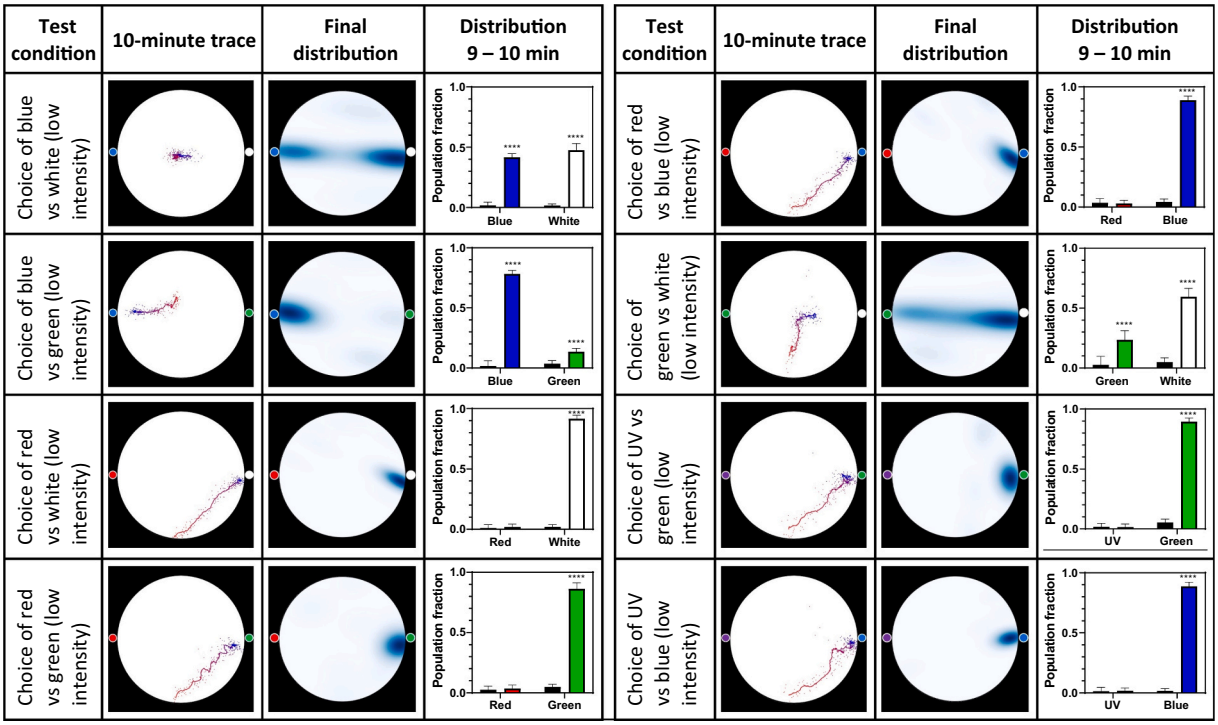

Fig. 5. Choices of L. *salmonis* copepodids exposed point light sources with different colors of equal intensity. Left panels show average 10-min trajectory for population where time is indicated by change in trace color (see Fig. 2). Middle panels are heat-plots of the final distribution. Right panel bars show the fraction of copepodids in the illuminated quadrants during 1 min before start (black) and termination of a test period (colored) with SD indicated. Intensity of stimulus was 1.5×10^{-3} µmol m^{-1} s^{-1} in the center of the arena. Active light sources are indicated with colored dots in trace plots and heat plots. Asterixis denote significant differences between fractions of copepodids before and after the light was turned on (**** = $p < 0{,}0001$).

vision or extraocular photoreceptors such as the dorsoventral ocelli described by (Bron and Sommerville, 1998). Due to the highly directional response to the point sources of low intensity UVA, we assume that lice movement is driven by visual detection. Positive phototaxis to background illumination of both white and UVA light have previously been reported (Flamarique et al., 2000). To the authors knowledge the visual pigments of salmon lice copepodids has not been characterized. We can therefore not conclude if the response is detected by the tail end of pigment with maximum at longer wavelengths or a discrete UV visual pigment. In a study of phototransduction genes of salmon louse four types of photopigment transcripts were identified of which three were middle wavelength sensitive (MWS) opsins and one C-type pteropsin known to be associated with extraocular photoreception (Porter et al., 2017).

Fields et al. (2018) showed that in a column system, flickering light increased vertical swimming speed (light off response). To test if flickering light can be used to enhance the efficiency of light traps, we tested similar light on/off cycles as well as higher frequencies and compared these to a constant light with the same intensity (Fig. 6). In our set-up, the copepodids always preferred the constant light source. Since we detected only horizontal movement, we cannot exclude that the flickering induced increased vertical swimming. However, the stimulus situations are very different in that Fields et al. (2018) used flickering of overhead light to simulate a shoal of fish whereas we used light on/off by small point source pointing in the horizontal direction. Thus, for discrete light sources used for instance in subsea light traps, our results indicate that light flickering will reduce the efficiency in attracting copepodids compared to constant light.

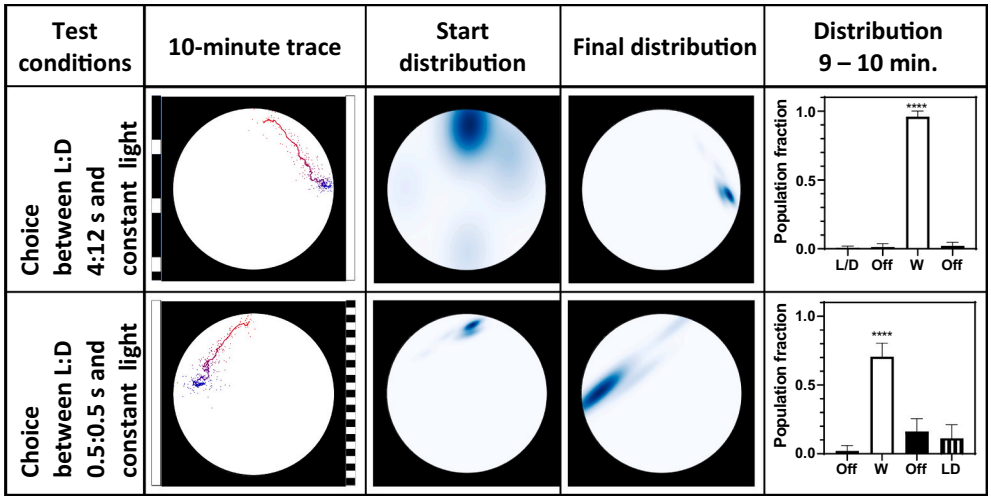

Fig. 6. Examples of responses of L. *salmonis* copepodids when exposed to a choice between constant and flickering light at equal light intensities. Patterns of light on and off are indicated on the sides if the trace plot (left panels). Trace color gradually changes from red at start to blue at the end of the exposure period (se scale in Fig. 2). Middle panels show distribution as heat plots at start and termination of the 10 min test period. Right panels show average distribution between quadrants (see Fig. 1B) during the last minute of the 10-min stimulus period (SD indicated). Asterixis denote significant differences from quadrants without light (**** = p < 0,0001).

5. Conclusions

Based on the current results we find it plausible that subsea light sources frequently used in salmon farming under certain conditions can attract salmon lice copepodids and increase infection pressure. Except for red light, the intensity rather than spectral composition within the visible spectrum of light sources determines how effective the light sources are in attracting copepodids. The impact of adding near-UV to light sources is unclear, but a strong positive phototactic response was observed at low intensities of near-UV alone. To evaluate the potential impact of artificial lighting on the distribution and potential aggregation of planktonic sea lice, further information is needed on the thresholds for phototaxis and responses to background contrast. The actual effect of discrete light sources on the local distribution of salmon lice should be tested by controlled plankton sampling or monitoring in the vicinity of light sources compared to the surrounding water.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.736036.

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