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ONTOGENETIC CHANGES IN VISUAL SENSITIVITY OF THE PARASITIC SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS*

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Summary

The salmon louse, *Lepeophtheirus salmonis*, is an ectoparasitic copepod of salmonid fishes whose life cycle involves two broadly defined, free-living larval stages, the nauplius and the copepodid. After settling on a host, the copepodid goes through various transformations to become a mobile adult. We recorded swimming responses of free-swimming salmon lice at the naupliar, copepodid and adult stages to the onset (ON) and offset (OFF) of lights of varying spectral irradiance and polarization. Nauplii showed a prominent swim-up OFF response across the spectrum 352–652 nm, but no ON response. Copepodids exhibited a swim-up ON response and a passive (sinking) OFF response across the same spectral range. Adults showed active swim-up responses to both ON and OFF stimuli, although the OFF response was proportionately stronger. The spectral range of the adult ON and OFF responses was the same as that of the copepodids and slightly greater than that of the nauplii, which did not exhibit responses at 652 nm. The absolute sensitivity of copepodids under white light (approx. 10^{-13} photons m^2 s^1) was higher than that of nauplii (approx. 10^{-17} photons^{-1} m^2 s, OFF response) and that of adult female lice (approx. 10^{-14} photons^{-1} m^2 s). This suggests that the naupliar visual system is best suited for detection of shadows (e.g. the host) under a bright light field (daylight hours), while copepodids and adults may be more specialized for host detection at crepuscular periods and during the night, when light levels are low. None of the developmental stages responded to the rotation of the plane of polarized light or exhibited any difference in directed response when polarized light was used in place of diffuse light.

Key words: copepod, *Lepeophtheirus salmonis*, fish parasite, spectral sensitivity, ON/OFF response, aquaculture.

Introduction

The salmon louse (*Lepeophtheirus salmonis*) is an ectoparasitic copepod that infests both wild and farmed salmonid fishes (Brandal et al., 1976; Pike, 1989; Stuart, 1990; Costello, 1993; Tully et al., 1993; Nagasawa et al., 1993). *L. 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 life cycle consists of several larval stages (two naupliar, one copepodid and four chalimus) before going through two pre-adult stages that culminate in male and female host-resident adults (Johnson and Albright, 1991; Schram, 1993). Since these animals are obligate ectoparasites, they must locate and attach to a host in order to complete their life cycle. *L. salmonis* larvae are free-swimming through to the copepodid stage, which is the primary infectious stage.

Previous investigations have suggested that the visual, chemosensory and mechanosensory systems of *L. salmonis* larvae are involved in host finding and predator avoidance (Johannessen, 1975; Wootten et al., 1982; Bron et al., 1993; Boxshall and Defaye, 1993; Heuch, 1996; Bron and Sommerville, 1998). Research on the visual system has included descriptions of eye morphology (Bron and Sommerville, 1998) and behavioural responses to light stimuli (Johannessen, 1975; Wootten et al., 1982; Bron et al., 1993), though the majority of this work has been restricted to the copepodid stage (but see Gravil, 1996, for observations on nauplii). Several aspects of the visual ecology of the species at different stages during the life cycle have yet to be thoroughly investigated.

Here, we report on the absolute sensitivity, spectral range and polarization response of the salmon louse visual system at the naupliar, copepodid and adult developmental stages. Behavioural responses to the onset and offset (shadow response) of light were obtained for illumination backgrounds that varied in total irradiance, wavelength and polarization content. The results are interpreted within the context of louse visual ecology, with special emphasis on host detection mechanisms.

Materials and methods

Animals

Egg strings were obtained from *Lepeophtheirus salmonis*

(Krøyer) females removed from Atlantic salmon (*Salmo salar*) that were kept in sea pens at the Austevoll Aquaculture Research Station, Storebø, Norway. For each series of experiments, 10–15 egg strings were placed in a 5 l plastic container filled with 30 ‰ salinity filtered seawater at 14 °C and maintained under a 14 h:10 h light:dark photoperiod. Eggs hatched within 2–3 days, and the copepodid stage was reached after 3–4 days. Experiments were performed on adult females the same day that they were removed from the salmon, and on nauplii and copepodids as these stages became available.

Imaging and illumination systems

Silhouette (shadow) video photography (SVP) (Arnold and Nutall-Smith, 1974; Edgerton, 1977; Browman et al., 1989) was used to record the behavioural responses of salmon lice. This method is superior to standard cinegraphic or video imaging techniques because it allows filming of events in a large depth of field (approximately 15 cm) with a relatively large field of view (limited only by the size of the collimating lenses, here 14.5 cm), magnification is independent of distance from the cameras, the resolution is very good (objects as small as 0.2 mm can be resolved) and image quality is unaffected by the ambient light level. In addition, the system does not require intense light sources so that free-swimming lice can be observed under relatively natural conditions.

Our SVP observation and motion analysis system consists of two orthogonally oriented optical rails, with the observation aquarium placed at their intersection (Fig. 1). The imaging optics on each rail consist of a far-red-light emitting diode (LED) placed at the focal point of a 14.5 cm diameter biconvex collimating lens whose output passes through the aquarium. The output of the LED was below that measurable using a 100 mm diameter submersible integrating sphere (OL-IS-470-WP) attached via a quartz fiber optic cable to a scanning spectroradiometer (OL 754-O-PMT, Optronic Laboratories Inc.). The use of a collimated beam prevents perspective distortion; clear, sharp shadows of any organism (even a small virtually transparent one) in the beam's path are projected. Shadow images are collected by a lens (Tamron 70–210 mm zoom) attached to a 0.5 inch CCD sensor video camera (Panasonic WV-BL730) and recorded using an S-VHS videotape recorder (Panasonic AG-6730). The optical components on each rail are aligned using helium–neon lasers, which also allow the vertical viewing heights and orthogonal orientation of the two rails to be established precisely. The synchronously recorded orthogonal views allow exact determination of the three-dimensional positions of particles that appear in both fields of view simultaneously.

The illumination system consisted of a light-intensity-controlled 1 kW Xenon arc lamp (Oriel Instruments) connected to an ultraviolet (UV, 280–400 nm)-visible liquid light guide. The light guide was coupled to the arc lamp housing using an Oriel 77800 lensing assembly. The projecting end of the light guide (placed directly above the observation aquarium) was fitted with a lens assembly (Oriel 77800) and a filter holder. The filter holder always contained a KG-3 type quartz substrate heat filter as well as various other optical filters used to modify the spectral and polarization characteristics of the downwelling light as appropriate for any given trial (see below). The light field emanating from this lens assembly–filter holder combination formed a 60 ° aperture cone that projected a uniform circle (30 cm in diameter) into the aquarium. This optical configuration minimized the non-illuminated volume of water and, hence, the chances of edge effects during the experiments. It also provided a large field of view (to mimic natural conditions as closely as possible) while maintaining a uniform polarization field near the centre of the aquarium where measurements took place.

For the absolute sensitivity trials (under a white light field), the filter holder contained a piece of wax paper (used as a diffuser, to eliminate any possible polarization of the light) and a combination of quartz neutral density filters. For the spectral

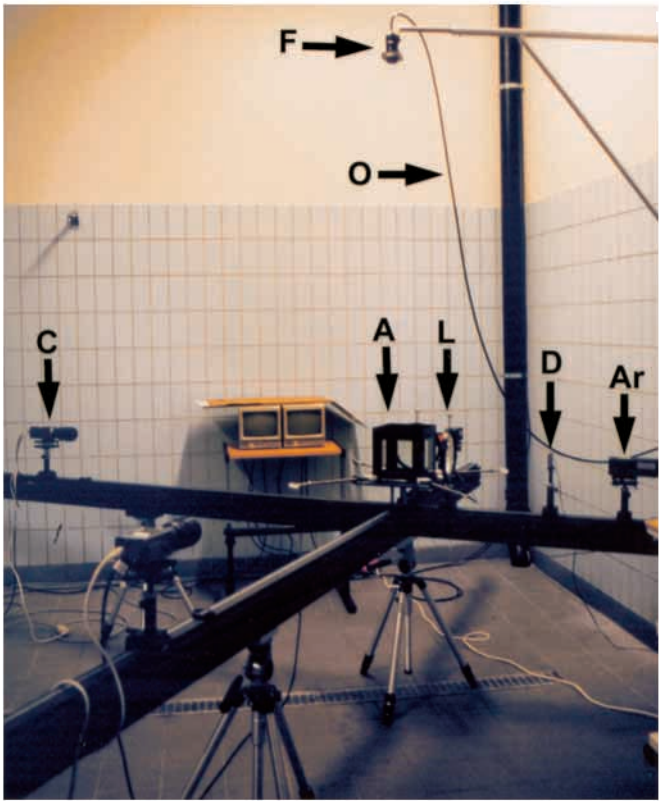

Fig. 1. Silhouette video photography system (SVP) used to make behavioural observations of *L. salmonis*. Light from a 1 kW Xenon arc lamp (not shown) is focussed onto the aperture of a UV-visible liquid light guide (O) and passes through various optical components in a filter holder (F) before reaching the test aquarium (A) in which the animals are freely swimming in 12 l of sea water. The aquarium (30 cm^2) is located at the intersection of two 3 m long optical rails. Each rail supports a far-red-light emitting diode (D) placed at the focal point of a 14.5 cm diameter biconvex collimating lens (L), and a video camera (C) to image the shadows projected by the collimated beam that passes through the aquarium. Also shown are the lasers (Ar) that are used to align the optical components on the rails prior to a given set of experiments. The red spot at the far end of one of the rails is the light emitted by the diode.

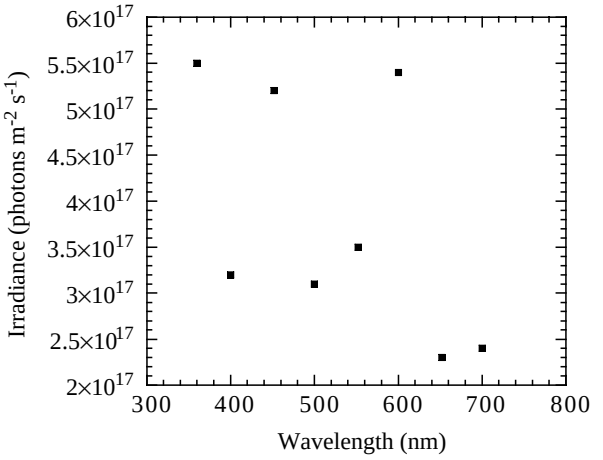

Fig. 2. Total irradiance for the narrow-band wavelength illuminations delivered during the spectral response experiments.

response trials, the filter holder contained the diffuser, a combination of neutral density filters, and a quartz narrow-band filter to restrict the spectral output. The narrow-band filters had maximum transmission at: 360, 400, 452, 500, 552, 602, 652 and 701 nm (±10 nm FWHM). All these optics are standard stock filters obtained from Melles Griot except the 360 nm filter, which is a multiple-component filter [to eliminate the long-wave light leak common to all single element UV-A (320-400 nm) filters] manufactured by Omega Optical Inc. (Brattleboro). To ensure that the behavioural responses observed were based upon spectral quality and not photon flux, an attempt was made to match (at least to within the same order of magnitude) the irradiances used for each of the test wavebands in the spectral response trials (Fig. 2). For the polarization response experiments, the filter holder contained the diffuser, neutral density filters and a UV-grade linear polarizer (HNP'B, Polaroid) as the final element. This produced a 100 % linearly polarized light field. When the filter holder contained the same elements but with the diffuser as the final element, the light field was diffuse but all other characteristics of the light delivered were identical (this served as a control to the polarized light trial). Polarization response trials were carried out under white light backgrounds of two different intensities (Fig. 3A).

Absolute and spectral sensitivity experiments

For each experiment, 500–700 *L. salmonis* specimens were placed in a 30 cm×30 cm×30 cm glass aquarium filled with 12 l of seawater (the same as that in which they had been reared).

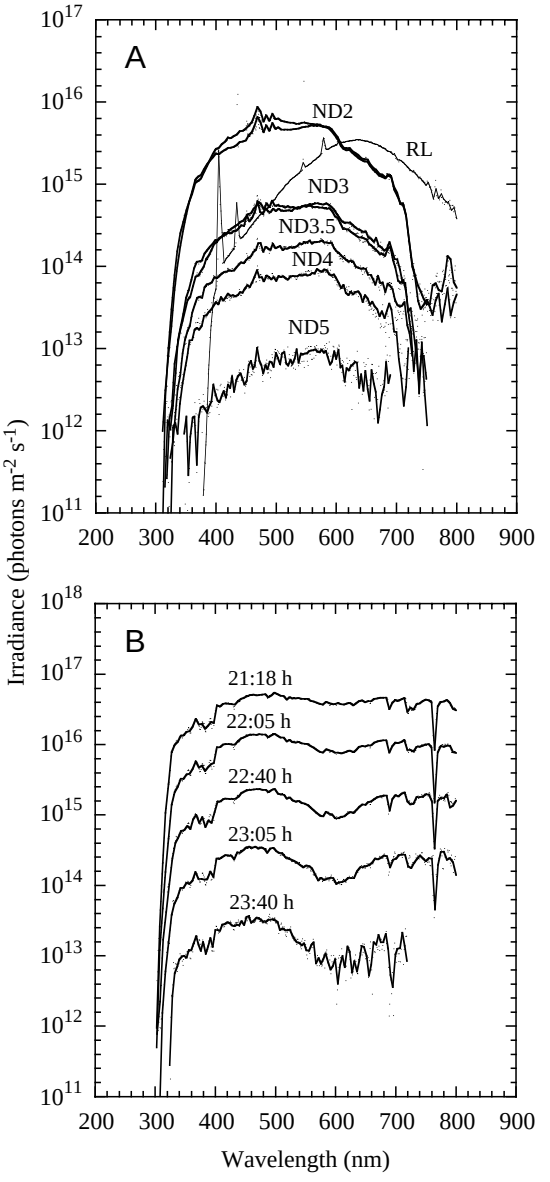

Fig. 3. (A) Spectral irradiance of light delivered in the absolute (white light) sensitivity experiments on nauplii and in the polarization experiments. Irradiances $<1.62\times10^{15}$ photons m^{-2} s^{-1}, also used during experiments with copepodids and adult lice, were below the sensitivity of the scanning spectroradiometer used to make these measurements. The total irradiances for those illumination conditions were computed mathematically from the number of neutral density (ND) filters applied. The numbers associated with each curve correspond to the number of ND filters applied to the output of the Xenon arc lamp. The total irradiance (in photons m^{-2} s^{-1}) for each condition was: ND2= 1.15×10^{18}; ND3=1.22×10^{17}; ND3.5=4.24×10^{16}; ND4=1.83×10^{16} and ND5=1.62×10^{15}. The illumination conditions used for the polarization experiments partially overlap the ND2 and ND3 curves; their total irradiances are: 1.32×10^{18} and 1.21×10^{17} photons m^{-2} s^{-1}, respectively. The curve labelled RL is that for the fluorescent room lights, under which the test animals acclimated for 30 min prior to an experiment. The total irradiance for the RL=6.80×10^{17} photons m^{-2} s^{-1}. (B) Spectral irradiance measured at a depth of 30 cm in the nearshore waters next to the Austevoll Aquaculture Research Station, Storebø, Norway. The numbers on these curves correspond to the time of day when the scans were taken, under clear skies on 28 May 1999. The total irradiance (in photons m^{-2} s^{-1}) corresponding to each of these curves is: 21:18 h=1.79×10^{19}; 22:05 h=4.34×10^{18}; 22:40 h=7.02×10^{17}; 23:05 h=9.86×10^{16}; 23:40 h=6.82×10^{15}. Note the correspondence between the range of spectral irradiance delivered in the experiments and that measured in the surface waters from which the *L. salmonis* specimens were collected.

The outermost 10 cm of the aquarium walls were covered with black plastic (matt-surface) contact paper. This restricted the field of view to the central 20 cm^3 volume of water and ensured that the behaviour observed was not influenced by surface or edge effects; only lice freely swimming in the water column were imaged and their displacements analyzed.

A typical absolute or spectral sensitivity experiment consisted of transferring the animals to the observation aquarium and allowing them 30 min to acclimate (under standard white fluorescent tubes, the spectral output of which is presented as RL in Fig. 3A), before beginning testing. Initial testing consisted of observations made under the following sequence of illuminations (using the Xenon arc lamp): (1) complete darkness for 10 min; (2) photopic conditions for 10 min; (3) complete darkness for 10 min. The onset of light at the transition from (1) to (2) in this sequence allowed the responses of the louse to a sudden (and then maintained) increase in light intensity (the ON response) to be observed. The offset of light at the transition from (2) to (3) in this sequence allowed the responses of the louse to a sudden decrement in light intensity (the OFF response) to be observed. Following these initial observations under white light, the intensity and/or spectral composition of the illumination was altered by changing the filter combinations (as above, and see Fig. 3A), and steps (2) and (3) were repeated (that is, an alternation between 10 min of light and 10 min of dark). The spectral sensitivity experiments were designed to assess the overall response of lice to different wavelengths as the lights were turned on or off. The light intensities used were low, corresponding to those found at crepuscular periods (Fig. 3B). Such stimuli are particularly relevant to lice biology because they may approach natural stimulations such as those produced by transient (ON) reflected flashes generated when fish turn against a low light intensity (crepuscular) background, or the quick (OFF) shadow signal generated by a fish swimming overhead and blocking the downwelling light. In addition, ON/OFF responses could provide the sensory basis for communication using weak photonic emissions, as suggested for *Daphnia magna* (Galle et al., 1991).

In the absolute sensitivity experiments to a white light field, responses were recorded over both a decreasing and an increasing series of background intensities. For the polarization experiments, responses were recorded under diffuse light and under five rotations of the polarizer (0 °, 45 °, 90 °, 135 ° and 180 ° with respect to the direction of one of the optical rails of the SVP system). We carried out five replicate trials per experiment type (i.e. intensity, spectral or polarization experiments). The study was performed on at least two populations of lice at any given life stage.

Behavioural observations

Videotaped observations from the trials were analyzed frame-by-frame to identify individual louse responses. In experiments on nauplii and copepodids, ON and OFF responses were quantified using the average swimming velocity of ten randomly selected individuals per trial. The velocity of a given louse was calculated as the vertical component of the displacement vector on the screen during a 1–3 s period immediately following the transition from lights on to off (or vice versa). For adults, ON and OFF responses were quantified as the number of adults, from a test group of 20, that swam upward during the first 2 s following the onset or offset of illumination. This measurement may underestimate the response of adults as only those that appeared simultaneously in both orthogonal views were counted. Average velocities of active swimming and passive sinking were also calculated for eight adults, although this response was not included in further analyses because of the often erratic swimming patterns exhibited (which rendered vertical velocities inappropriate as a measure of response strength).

Statistical analyses

One-way analyses of variance (ANOVA, SPSS v. 6.1.3 for Windows 95) were performed to test for differences in response amplitude between illumination conditions. A Student–Newman–Keuls (S–N–K) test was also applied, when necessary, to identify statistically similar groups in the ANOVA (α=0.05).

Results

Absolute and spectral sensitivities

Under dark adaptation, naupliar movement alternated between short active upward-directed swimming (‘hops’) and slow sinking. The net result was that animals maintained their approximate position in the water column (net vertical velocity approximately 0 mm s^{-1}; Fig. 4A,B). When the Xenon lamp was turned on (full spectrum white light, Fig. 3A), there was no statistically discernible change in swimming velocity (ON response) for any of the intensities tested (analysis of pooled dark and ON responses: $F_{9,40}$=0.349, P=0.952; Fig. 4A). In contrast, when the illumination was turned off, there was a noticeable change in upward swimming (the OFF response), which was positively correlated with light intensity (Fig. 4A). Following the strong initial OFF response, nauplii slowly returned to their pre-response level of swimming activity. There was no OFF response at light intensities equal to or below 4.24×10^{16} photons m^{-2} s^{-1} (Fig. 4A): at this intensity, the OFF response was statistically equal to the ON response and to the swimming velocity in the dark ($F_{1,8}$=1.84, P=0.211). Sensitivity is defined as the reciprocal of the light intensity that induces a given response; thus, 2.36×10^{-17} photons^{-1} m^2 s would correspond to the absolute sensitivity of the OFF response, within the resolution of our experiments.

The strong OFF response and lack of an ON response observed under white light was also observed for restricted wavelength illumination (Fig. 4B). The spectral range of the OFF response (expressed in terms of response swimming velocity) extended across the spectrum from the UV-A through the red (Fig. 4B), but there were no statistically discernible differences in response between wavelengths ($F_{1,8}$=1.84, P=0.211). No responses were found to 652 nm light; the OFF

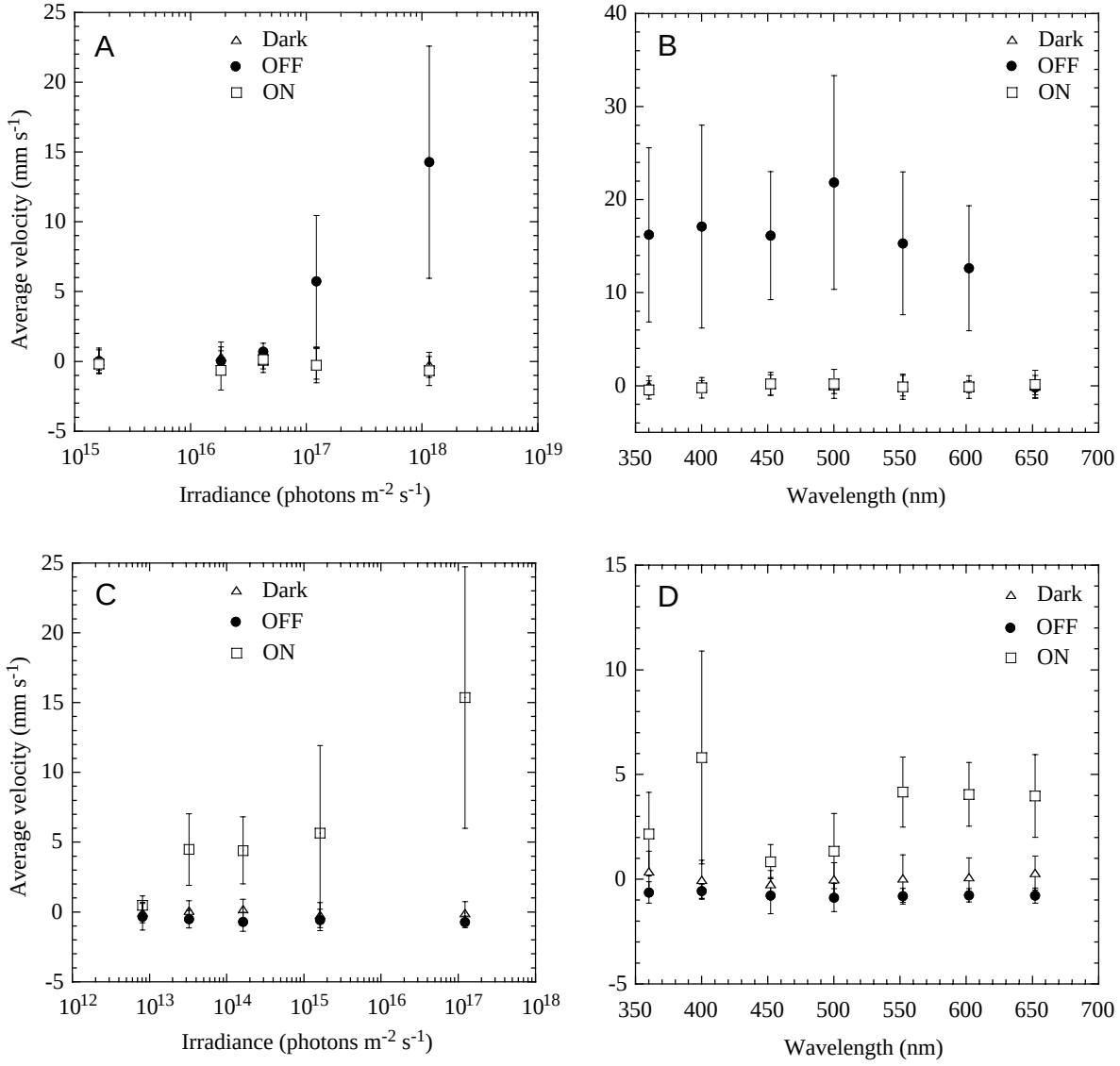

Fig. 4. Average response velocity of *L. salmonis* as a function of total irradiance and wavelength. (A,B) Nauplii. (C,D) Copepodids. Note that in A and B, the data points for the dark response are hidden behind those for the ON response and that for C and D some of the data points for the dark response are hidden behind those for the OFF response. Values are means ± S.E.M. (N=10 individuals per trial, 5 trials).

response at this wavelength was statistically discernible from those at the other wavelengths (S–N–K, $F_{6,28}$=5.52, P=0.0007).

The ON and OFF responses of copepodids were different from those of nauplii. Copepodids exhibited a strong response to the onset of light (the ON response) that was positively correlated with light intensity (Fig. 4C,D). Following the strong initial ON response, copepodids progressively drifted downward as the animals light-adapted. When the illumination was turned off, copepodids stopped swimming and sank (Fig. 4C,D). The ON response to white light was no longer statistically discernible from the dark response at an intensity of 8.07×10^{12} photons m^{-2} s^{-1} ($F_{1,8}$=1.05, P=0.334; Fig. 4C). Thus, the absolute sensitivity of the ON response (under white light) for copepodids is 1.24×10^{-13} photons^{-1} m^2 s.

The response pattern of copepodids under different wavelength illumination was similar to that under white light: a strong ON response and a downward drift OFF response (Fig. 4D). The spectral range of the ON response in copepodids (expressed in terms of response swimming velocity) was broad, extending across the spectrum from the UV-A through the red (Fig. 4D). There was no response to 701 nm light (not shown). With the exception of 701 nm, there were no statistically discernible differences between responses at any of the test wavelengths for either the ON or OFF responses (S–N–K, $F_{6,28}$=1.21, P=0.332).

Adult female lice exhibited strong OFF and ON responses that were both positively correlated with light intensity (Fig. 5A). The ON response was consistently smaller than the OFF (Fig. 5). Both ON and OFF responses were characterized by abrupt bursts of swimming that were sometimes interrupted by passive sinking. The average swimming velocity of adult female lice was 3.6±1.0 cm s^{-1} and the average sinking velocity

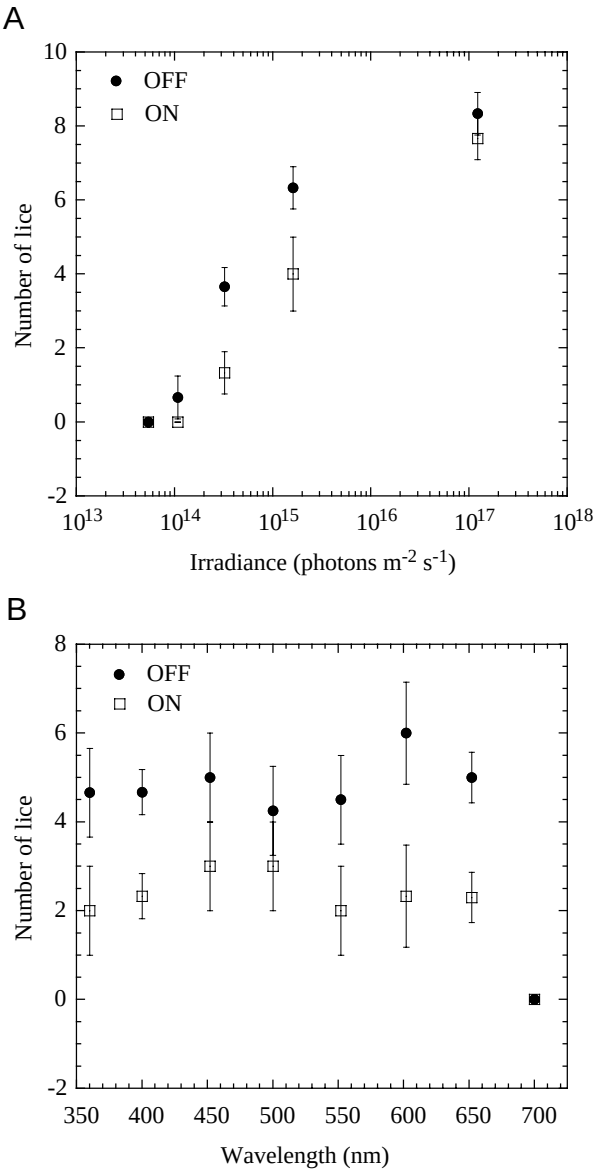

Fig. 5. Numbers of adult *L. salmonis* (from a total of 20) that swam upward as a function of (A) irradiance and (B) wavelength.

was 3.8±0.4 cm s^{-1} (*N*=8). Adult lice had an absolute sensitivity response to white light which was intermediate between that of nauplii and copepodids; no response was detected at a light intensity of 5.4×10^{13} photons m^{-2} s^{-1} (Fig. 5A). Thus, the absolute sensitivity of the ON and OFF responses (under white light) for adult female lice was 1.85×10^{-14} photons^{-1} m^2 s.

The ON and OFF response patterns of adult female lice under white light were similar under different wavelength illumination showing both ON and OFF responses, with the ON response consistently smaller than the OFF (Fig. 5B). The spectral range of the ON and OFF responses was broad, extending across the spectrum from the UV-A through the red (Fig. 5B). The OFF response was similar at all wavelengths, but was highest at 602 nm (S–N–K, $F_{6,28}$=2.97, *P*=0.022). There was no statistically discernible difference between the ON responses at any of the test wavelengths (S–N–K, $F_{6,28}$=1.74, *P*=0.216). No response was found for 701 nm illumination.

Responses to linearly polarized light

There was no statistically discernible response in swimming velocity, orientation, or numbers of responding adults when lice were exposed to linearly polarized light *versus* diffuse light, nor was there any response to the rotation of the plane of polarization.

Discussion

Absolute sensitivity of ON and OFF responses

There were pronounced life-stage-related changes in the absolute sensitivity of *L. salmonis* to light. Absolute sensitivity to white light increased from the naupliar (approximately 10^{-17} photons^{-1} m^2 s) to the copepodid stage (approximately 10^{-13} photons^{-1} m^2 s), and remained high in the adult (approximately 10^{-14} photons^{-1} m^2 s). The corresponding response-threshold irradiances for copepodids and adult lice (see Figs 4, 5) are one to several orders of magnitude below the total irradiance available during crepuscular periods (Fig. 3B). Such sensitivities are comparable to those of their salmonid hosts (approximately 10^{-13} photons^{-1} m^2 s; Novales Flamarique and Hawryshyn, 1997). Thus, both host and parasite appear able to use visual cues to guide their behaviour during crepuscular periods, and perhaps even at night under moonlight. The absolute sensitivity of nauplii is significantly lower than that for the older life stages; although their visual system can still respond to OFF stimuli during crepuscular periods, it may be better suited for daylight activity.

The observation of an OFF but no ON response in nauplii, ON and OFF responses that induce opposite vertical displacement in copepodids, and both ON and OFF positive responses (albeit with a stronger OFF) in adults, has significant implications for louse visual ecology at the various life stages.

Positive phototaxis in lice copepodids has been hypothesized to increase the probability of encountering a host by bringing the animals closer to the water surface (Wootten et al., 1982; Bron et al., 1993) where salmonid fishes are often found (Groot, 1972; Westerberg, 1982; Holm et al., 1982; Quinn et al., 1989; Ruggerone et al., 1990). Heuch et al. (1995) observed diel vertical migration of copepodids in sea enclosures and proposed that these movements increased the probability of their encountering a host. This conclusion is based on the diel pattern of vertical movements in salmonids (i.e. ascension to the surface to feed at dusk and descent at dawn), which appears to be opposite to that of lice copepodids (these sink from the surface at dusk and swim upward at dawn; note their negative vertical velocity in Fig. 4D) (Groot, 1972; Scarsbrook et al., 1978; Healey, 1980; Huse and Holm, 1993; Heuch et al., 1995; Heuch, 1996).

In contrast to the copepodids, both nauplii and adult female lice exhibit strong shadow (OFF) responses, a feature that

probably plays a role in the localization of potential hosts (see Bron and Sommerville, 1998). In fishes and most other aquatic organisms, the shadow response is believed to be a mechanism for improving target contrast under bright light conditions (e.g. Beaudet et al., 1993). Thus, our results suggest that the naupliar visual system is designed to detect shadows of potential hosts as they swim overhead. The active swimming exhibited by copepodids in response to ON stimuli suggests that their visual system may be tuned to respond to flashes of light from the sides of potential fish hosts (once they are already in reasonably close proximity). The ON response may also be used to detect optimal sites for settling once on the host. Presumably, these sites would exhibit optical properties (e.g. higher reflection, specific colours or polarization patterns) that the ON response would be able to discriminate. The higher absolute sensitivity of the copepodid visual system may allow discrimination between potential settlement sites characterized by small differences in reflected light intensity.

The passive sinking exhibited by lice copepodids in response to a shadow (OFF) stimulus is difficult to interpret in the context of host-finding behaviour. It is possible that this slow passive sinking reduces the probability of encounters with predators, as has been suggested for other crustacean larvae (e.g. Forward, 1988). This passive OFF response is contrary to the strong shadow responses reported for other parasitic copepods (Kabata and Cousens, 1977; Poulin et al., 1990).

The reappearance of a strong OFF response in adults, in combination with a strong ON response, suggests that their visual system is configured for a broader range of responses than those of the earlier life stages.

Spectral response

The spectral range of the OFF response in lice nauplii and adults was similarly broad, extending from the UV-A portion of the spectrum to the red. However, nauplii showed no OFF response at 652 nm, while adults exhibited both ON and OFF responses at 652 nm, but neither at 701 nm. To our knowledge, these are the first such observations for lice at these life stages. The observation that lice nauplii do not respond to wavelengths around and above 652 nm may be related to the fact that they occur deeper in the water column than adults (since far red light is rapidly attenuated in the water column).

The spectral range of copepodid responses was similar to that of adults, and a little broader than the OFF response in nauplii. Unlike nauplii, copepodids responded to 652 nm light, which may be related to their presence at shallower depths than nauplii. Reflected light from the sides of fishes can vary in intensity and spectral content (Denton and Nicol, 1966), and it is possible that life-stage-dependent spectral characteristics of ON responses in lice may improve their detection of surface contrast on fishes.

Our spectral response results for copepodids differ somewhat from previous studies. In accordance with our results, Bron et al. (1993) also reported broad spectral sensitivity in the range 400–700 nm, while Gravil (1996) found sensitivity peaks at 500 and 561 nm. It is possible that our method of measuring spectral response (swimming behaviour) was insufficiently sensitive to resolve such peaks. Experiments with two light sources using chromatic adaptation would help to resolve the number of photoreceptor mechanisms that underlie the broad responses observed in this and previous studies.

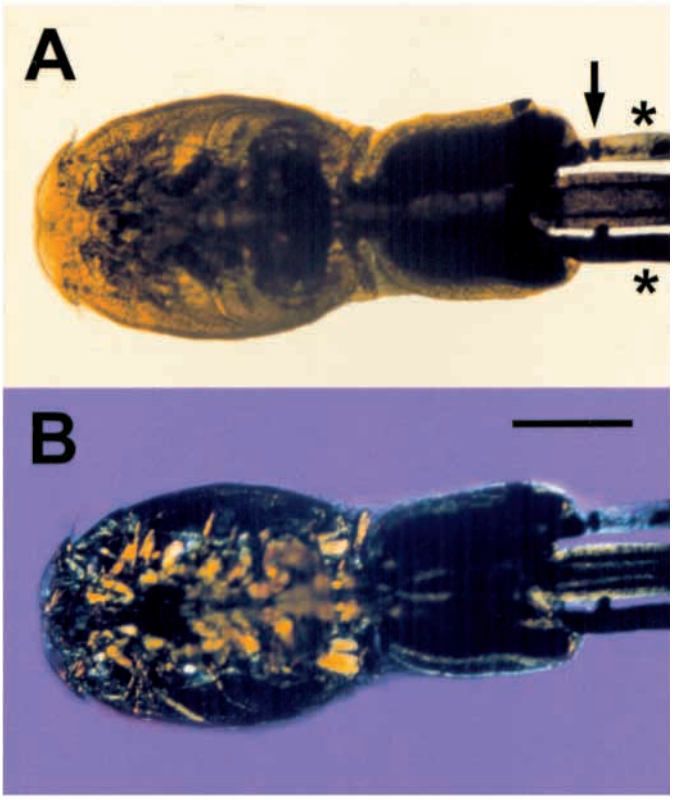

Fig. 6. Adult female *L. salmonis* illuminated under partially polarized (approximately 40 %) light, observed (A) with no analyzer, and (B) with an analyzer oriented perpendicular to the maximum plane of polarization. Imaging was achieved using a Zeiss Universal R microscope without a condensor and with a 1× objective in place. The polarization background was created using a linear polarizer and a 560 nm quarter wave plate (Zeiss). Note that internal features of the louse become more apparent when an analyzer is used, due to the imparted light retardance and the resulting interference as the light rays exit the specimen. Asterisks in A identify two strings of eggs on either side of the female. The upper one has lost most of its eggs while the lower one is still full. The arrow identifies a small stack of 2–3 eggs still contained within the upper string. Scale bar, 1 cm.

The upward swimming that we observed for *L. salmonis* in response to both UV-A and white light backgrounds is contrary to the downward swimming response reported by Aarseth and Schram (1999) under backgrounds that contained UV wavelengths. These authors measured the settling depth of salmon lice copepodids in 1 m tubes when exposed to white light backgrounds that either included or excluded UV wavelengths. Superficially, these results seem contradictory. However, the UV lamp used by Aarseth and Schram (1999) had a maximum output at 313 nm (i.e. in the UV-B), a region of the spectrum known to be deleterious to many invertebrates (e.g. Damkaer et al., 1980; Storz and Paul, 1998). In contrast,

our light backgrounds contained little if any UV-B (Fig. 3A). Furthermore, Aarseth and Schram (1999) did not control for differences in the intensity of the light backgrounds that they used, i.e. the UV irradiance was superimposed upon the white light using a second lamp. Hence, the deeper settling depths that they observed under backgrounds that contained UV light are probably responses to higher irradiance; such intensity responses are well documented for invertebrates (e.g. Forward, 1988). In this study, in which intensities delivered under different spectral backgrounds were controlled, lice at all life stages responded positively to narrow-band UV-A light. Our observations are nonetheless insufficient to determine whether this response is based upon the secondary absorption band (β band) of a red or green photopigment, or from the action of a separate UV-A photoreceptor mechanism.

Polarization response

Various copepods, including one parasitic species, exhibit polarotactic movements, i.e. the directionality of their swimming behaviour is directly related to the polarization of incident light (Umminger, 1968a,b). As in most other invertebrates, all of the polarotactic copepod species studied by Umminger possessed at least two retinula cells with perpendicularly oriented microvilli that have optical axes transverse to the direction along which incident light propagates in the retina (e.g. Wehner, 1983; Labhart, 1988). An ultrastructural study of the *L. salmonis* copepodid eye identified two dorsolateral ocelli (part of the median nauplius eye) that possess the required retinula cells for polarization detection (Bron and Sommerville, 1998). Furthermore, the arrangement of tapetal cells at the back of these ocelli (Bron and Sommerville, 1998) would probably improve the efficiency of the polarization detection system, in a manner analogous to that proposed for tapetal crystals in the eye of the northern anchovy (*Engraulis mordax*) (Novales Flamarique and Hawryshyn, 1998). Polarization sensitivity in *L. salmonis*, however, has never been demonstrated.

We were unable to detect any change in the swimming behaviour of lice, at any life stage, in response to linearly polarized light. Umminger (1968b) suggested that negative polarization results obtained for various species of copepods might be due to a natural rhythm of polarotaxis whose activity peaks are often missed by investigators. It is unlikely that our negative results were related to such a rhythm, since we tested the lice at different times of the day (morning, midday and dusk).

The absence of an orientation response to the polarization of light does not mean that lice cannot detect this light cue, nor that they cannot use it for some other purpose. Our results, however, do not support a role of polarization detection in the orientation behaviour of *L. salmonis*. Since the structural basis for polarization sensitivity is present in at least the copepodids of this species, we suggest that polarization detection in *L. salmonis* (if present) may be used in the analysis of reflected light to detect targets of interest, such as a host or features on a host.

Like many crustaceans with exoskeletons based on calcium carbonate, *L. salmonis* exhibits birefringence under partially polarized illumination (Fig. 6). This change in polarization with respect to that of the surrounding water (or, perhaps, that reflecting from the sides of a fish) may increase the contrast of lice to their predators. Such contrast enhancement may be used by fishes such as the cleaner wrasse (e.g. *Ctenolabrus rupestris*, *Crenilabrus melops*) to locate *L. salmonis* or other copepod ectoparasites on the sides of fish. This may be one of the reasons why these fishes can be used so successfully on salmon farms to control lice infestations (e.g. Costello, 1993).

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