Sensory biology and behaviour of the free-living stages of salmon lice, *Lepeophtheirus salmonis* (Copepoda, Caligidae)

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This proposal is drafted by
Anne Berit Skiftesvik, Karin Boxaspen and Howard Browman
Institute of Marine Research
Austevoll Aquaculture Research Station

INTRODUCTION

Salmon lice are recognised as the major disease problem in farming of Atlantic salmon (*Salmo salar* L.) and the possibility of salmon lice playing a role in the decline of wild anadromous species has also been questioned. Most research since the late 70's on sea- and salmon lice have been centred around chemical delousing in commercial farming (see reviews of Roth et al., 1993, Costello, 1993) rather than on basic biology. Some recent attention has been given to the general biology of salmon lice (Boxshall and Defaye, 1993) but the overall contribution has been rudimentary considering the scope of the problem. Many studies have been descriptive, reporting on numbers of salmon lice found on salmon under different conditions, but any permanent solution to this problem will eventually be built on considerably better biological knowledge of the salmon lice itself.

Salmon lice larvae biology

Salmon lice hatch as nauplius I and remain free-swimming through the moults to nauplius II and to the infective copepodid stage. The time span from hatching to infective copepodite is strongly temperature dependent and takes from 4 days at 10°C to 23 days at 2°C (Boxaspen and Næss, 1999). The copepodite is infective from day 1, but the actual number of days it will stay infective is not known. Larvae are positively phototactic (Bron et al., 1993) and display a vertical migration by active movement upwards during the day and a more passive sinking behaviour at night in closed trial units (Heuch et al., 1995). Aggregation of larvae around salinity gradients can also occur (Heuch, 1995).

In Norway, salmon farmers who undertake regular monitoring can in some places (especially in fjord systems) observe pulses of salmon lice settlement onto hosts. These pulses appear to be connected to changes in the physical environment (Wallace 1998). A more even temporal distribution in the settlement pattern is observed in coastal regions (Hevrøy and Mikalsen, 1994, Boxaspen, 1997). These variances are typically explained as being the result of the synchronous hatching of eggs (released from unidentified source populations) combined with tidal and/or coastal currents. Whether the inherent behaviour of the salmon lice itself can account for some of these observations have been postulated but is yet to be documented.

Sensory apparatus

Bron and Sommerville (1998) point out that little work has been done on behaviour and sensory apparatus of salmon lice. The copepodid larvae - although being the principal infective stage - has been particularly neglected. ***Thus, the active swimming of salmon lice free-living stages, and the cues that affect/guide these movements, have not been studied extensively.***

Eye structure

Whilst the eyes of a wide variety of both free-living and parasitic copepods have been described in varying degrees of detail (Boxshall, 1992)., the structure of these receptors in both the larval

and adult stages of caligids had been largely ignored. Bron and Sommerville (1998), however, describe the functional and comparative morphology of the photoreceptors in the salmon lice copepodite. They report that the optic photoreceptor of the copepodite comprises a median nauplius eye consisting of two lensed dorsolateral ocelli and a single unlensed ventral ocellus. Each dorsolateral ocellus has a slightly larger maximum transverse diameter than other species reported and thus, *L. salmonis* has a far higher eye to body length ratio. This suggests that the eye is important for the copepodid or that it plays a major role in the life of later developmental stages. The eye of *L. salmonis* corresponds closely to the «type B» lensed eye with reflective tapetum of Land (1981). The nauplius eye should, therefore be able to detect all of the principal categories of light information available in the marine environment (Bron and Sommerville, 1998). The morphological basis also exists for the eye of *L. salmonis* to act as a polarisation detector as reported for other invertebrates with virtually identical eye structure (Elofsson, 1976, Wehner, 1997). Polarisation cues have been documented in intra- and interspecies identification and in prey retention (Shashar and Cronin, 1996, Shashar et al., 1996 Shashar and Hanlon, 1997, Shashar et al., 1998) and, in the case of salmon lice, this could indicate a possible host finding mechanism since the silvery sides of fish to some extent polarise light (Land, 1991). Thus, we will test the reaction of the salmon lice larvae to various qualities and quantities of light and in particular towards polarised light.

Antennules

The antennules are the primary chemo- and mechano-receptors in copepods (Boxshall and Huys, 1998). Antennulary sensor elements characterised by their thin cuticle and the large number of small dendrites are considered to be chemoreceptors (Gretsky et al., 1993). Bron et al. (1993) also found a pair of large chemosensors in the frontal region of the head of the salmon lice copepodite. Chemotaxis has been proposed as a mechanism for both long-range location and the close-range recognition of a host (Kabata, 1981). The postulate is however not documented. Thus, we will test the salmon lice larvae response to various exudates from salmon, such as mucus and blood. The system described in this proposal will enable direct observations of chemotaxis in salmon lice larvae.

AIM OF THE STUDY:

Study the responses of larval salmon lice to light of various spectral qualities, intensities, and the degree of polarisation, olfactory and to salinity cues and integrate the findings with possible mechanisms for host localisation in the copepodite. Attempt to develop a salmon lice trap.

Main objectives:

1. Evaluate the vision of salmon lice larvae related to UVA plane polarised light (Laboratory)
2. Test responses to olfactory and salinity cues (Laboratory)
3. Verify the results from 1 and 2 in field trials (Net pens)
4. Develop and test a trap for salmon lice

MATERIAL AND METHODS

Salmon lice larvae

Ovigorous female salmon lice will be collected from Atlantic salmon kept in net-pens. The egg strings will be removed if necessary by cutting them from the female with a scalpel, and transferred to the hatching systems. Viability of egg strings will be determined following the method of Ritchie et al. (1993). Briefly, only opaque egg strings with symmetrically stacked eggs can be regarded as viable. This can be determined using dissecting microscope.

Hatching systems

Egg strings will be placed in cylindrical hatching containers (40 cm Ø, 15 l) with a sieve bottom placed in up welling sea water. The sea water is taken from a depth of 50 m and sand filtered before temperature adjustments. The rearing conditions will thus be stable with respect to salinity and temperature (Boxaspen and Næss, 1998).

On-growing systems
Each group, usually all hatched within 24 hours, will be placed in individual cylindrical containers (20 cm Ø, 5 l) with a sieve bottom. All the age groups will then be kept in a flow through water bath.

3D imaging system
Three-dimensional silhouette (shadow) video photography will be used to record the behaviour of salmon lice larvae. This method, which produces a sharp shadow (dark on a white background) image of objects in the field of view, has several advantages over standard cinegraphic or video techniques. First, it can be used to make detailed observations of small transparent organisms such as small fish and their prey (Browman et al. 1989; Browman & O'Brien 1992a,b; Browman et al. 1994). Second, events can be filmed in a large depth of field (approximately 15 cm) with a relatively large field of view (18 cm); free-swimming larvae can be viewed under laboratory conditions. Third, magnification is independent of distance from the camera, and the resolution of the system is extremely high: objects as small as 0.2 mm in diameter can be resolved. The salmon lice copepodite is about 0.7 mm long and 0.2 mm wide. This is clearly visible using this system. Our existing system is configured for three-dimensional imaging using two orthogonally-oriented side views.

Light system
We will use a 1000 W Xenon arc lamp (Oriel Corporation), outfitted with optical filters, to deliver light of various spectral qualities, and intensity and degree of polarisation to the observation aquarium. The spectral irradiance (at 1 nm intervals) of the light delivered will be measured using an OL754-O-PMT scanning spectroradiometer (Optronic Laboratories, Orlando, Florida) outfitted with a WP470 submersible integrating sphere.

Path analysis
Images will be analysed, frame-by-frame, using TRAKFISH, ANAPATHS, and MEASURE, unique software designed by H.I. Browman and Racca Scientific Consulting. TRAKFISH automates the acquisition of movement data. The software interfaces with two video tape machines (VTRs), ordering them to move the videotape forward a specified temporal interval at each iteration of the analysis. The images on each frame - from both orthogonal views - are grabbed, using a Matrox Meteor board, and the positions of all larvae are identified. Spacial coordinates from the two views are combined into one three-dimensional position for each larva at each temporal interval. In this manner, movement paths for all of the larvae being tracked are generated. To avoid edge effects, only images from the central 15 cm of the 30 cm observation tank are analysed. Path lengths of 50 - 150 cm are routine using this system and, as a result, the path analysis, and the interpretations based upon them, are extremely reliable.

ANAPATHS software is used to evaluate the movement paths generated by TRAKFISH. The spatial co-ordinates from each path are input to ANAPATHS, and the following indices of the larva's movements are calculated for each: percent time actively swimming; the number and duration of stationary periods; move distances, times and velocities; turn angles; the fractal dimension of the swim path (yielding an index of path complexity, in three-dimensions). All of

these data are output as histograms, so that the inherent variability in these data is retained throughout the analysis.

Our existing software will be modified so that the motion vectors of all lice larvae in the field of view can be analysed (VECTOR CORRELATION ANALYSIS)as a population. This involves using a statistical test to evaluate whether the lice swim paths are all correlated with respect to their movement vectors. Essentially, this will allow us to determine whether the lice larvae are all swimming in the same direction with respect to visual (e.g. the e-vector of the polarised light field) or chemical (e.g. fish skin exudate) cues.

EXPERIMENTAL DESIGN

Objectives 1 and 2 (Laboratory)

Silhouette video images will be made of larvae free-swimming in a 30 x 30 x 30 cm aquarium filled with 10 l of water. Larvae of known age will be collected from the hatching system described.

1: Evaluate the vision of salmon lice larvae related to plane polarised light (320-400 nm) with or without a UVA component.

There will be four treatment groups: two for which the illumination will be diffuse (NOPOL), one with and the other without UVA, and two for which the illumination will be 100% polarised (POL) one with and one without UVA. Five replicate experiments will be run for each treatment, with 1000 larvae per experiment. This will allow us to see whether the salmon lice will react to polarised light and if UVA is necessary for this response.

2: Test responses to olfactory and salinity cues

A directional pattern of any fluid (olfactory or salinity) is introduced into the aquarium by a micro pump injector and micro pipettes. This allows us to have a constant flow or pulse of exact quantities of cue material. By mixing the cues with water of slightly different salinity the plume exiting the micro pipette will be visible as a dark line on the video imaging system. It will thus be feasible to visualise and quantify changes in orientation by the larvae in response to various cues.

Olfactory cues

Olfactory cues to be tested will be 1) sea water from a tank with saithe, 2) sea water from a tank with salmon, 3) mucus from non-salmonids (saithe), 4) mucus from salmonids (salmon, sea trout), 5) blood and(or) urine from salmon. Five replicate experiments will be run for each treatment, with 1000 larvae per experiment.

Salinity cues

Water of different salinity can be introduced in the same manner as the olfactory cues. This will allow us to establish to what extent the larvae will respond to a directional variation in salinity.

3: Verify the results from 1 and 2 in field trials (Net pens)

The results from the laboratory experiments will be tested in 5 x 5 x 5 m cages with salmon. Will for instance reduced polarised light reduce or enhance the settlement of salmon lice onto the salmon? We will use diffusing material to reduce the polarisation in two of the cages while two will have no diffusing material (and thus high polarisation).

4: Develop and test a trap for salmon lice

The results from objectives 1 to 3 will hopefully enable us to know what will attract the salmon lice larvae. Both quality and quantity of light plus olfactory cues that have proven effective in the laboratory can be tested. The work will start with a larval fish and invertebrate light trap modified from that of Floyd et al. (1984).

RESOURCES AND AVAILABLE EXPERTISE

Infrastructure

The three-dimensional silhouette (shadow) video photography system described in this proposal is already in use at Austevoll Aquaculture Station. Drs Browman and Skiftesvik have built the system after model from a Canadian system earlier run by Dr. Browman. Recently a new light source (described earlier) have been bought able to control both quality (wavelength) and quantity of the light. The analysis of the data is done by unique software designed by H.I. Browman and Racca Scientific Consulting. Salmon lice larvae have already been tested in the system (Boxaspen et al., 1998) so we know the proposed experiments are feasible.

In addition, the station has recently made significant investments in support of the type of research proposed here: (1) Rearing tanks (25 x 50 l units) for conducting controlled and well-replicated experiments on invertebrates and vertebrates: 1.000.000 NOK; (2) UVB-optimised high-resolution scanning spectroradiometer for the accurate measurement of light intensity and spectral quality: 650.000 NOK. (3) 1000 W Xenon arc lamp for the delivery of high-intensity and tightly controlled light environments: 150.000 NOK.

Personnel

Project leader: Anne Berit Skiftesvik, a Senior Scientist with IMR-Austevoll, has been working on behaviour in fish larvae for the past 15 years. As detailed on her Curriculum Vitae (attached), Dr. Skiftesvik has developed new and innovative techniques for observing and assessing behavioural responses in fish larvae. Dr. Skiftesvik will participate in the entire project, but will be responsible mainly for generating the behavioural observations required to test different conditions. Dr. Skiftesvik has many years of experience conducting this type of work. Further, she was involved in setting up the motion tracking system at IMR-Austevoll and is already an experienced user. Finally, Dr. Skiftesvik has already done preliminary work on both ballan wrasse and hake.

Principal Investigator: Dr. Howard Browman holds a 1183 Forsker position with the Institute of Marine Research's Aquaculture Centre. Over the past 16 years, he has worked on several aspects of the physiology, behaviour and ecology of fish early life stages. Dr. Browman has trained and worked in several laboratories in Canada and the United States, and has developed, from direct experience, expertise in the subject-area of the research proposed here. Evidence of Dr. Browman's expertise in the subject area of this proposal is presented in the Appendices, and the Vita included. Dr. Browman's responsibilities will include taking the lead role in planning and implementing the experiments on marine species, in interpreting the results, and in writing them up for publication in scholarly journals.

Principal Investigator: Karin Boxaspen have been working with salmon lice with the Institute of Marine Research's Aquaculture Centre since 1990. In the first years chemical treatment of salmon in cages was the main topic but the need for more basic understanding of the biology of salmon lice arose. Since 1995 she has been project leader of three projects from the Norwegian Research Council (each of three years) looking into preventive and integrated treatment of salmon lice, development of salmon lice at low temperatures and in the last one started the behavioural studies of salmon lice in the project «factors and stimuli influencing dispersal of salmon lice larvae» (see reference list in this proposal).

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