An improved method for experimental infection of salmon (Salmo salar L.) with salmon lice, Lepeophtheirus salmonis (Krøyer)

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Abstract

The aim of this study was to develop a simplified infection method for salmon lice (Lepeophtheirus salmonis). A comparative study was performed between two infection methods; 1) copepodids (infective stage) are added to the tank containing salmon and 2) salmon are anaesthetized and dipped in a solution containing copepodids. The infection success (% infection intensity = infection intensity / infective dose x 100) was investigated on different body parts (gills, body and fins) of the salmon hosts at the copepodid stage (1 – 2 days post infection, dpi), chalimus stage (14 dpi) and the preadult stage (25 dpi). Infection with method 1 resulted in an average infection success of 33.2 % (range: 9.4 – 57.1 %) for copepodids, 28.1 % (21.7 – 38.7 %) for chalimus larvae and 21.3 % (16.5 – 27.0 %) for pre-adults. Using method 2, the infection success was higher: 77.9 % (68.5 – 85.2 %) for copepodids, 59.5 % (47.6 – 75.2 %) for chalimus larvae and 50.9 % (30.8 – 69.5 %) for pre-adults. Method 2 gives a higher infection success compared to method 1. Method 2 is suitable for infecting a small number of fish when the number of infective copepodids is limited.

Introduction

The salmon louse (Lepeophtheirus salmonis K.) is a marine ectoparasitic copepod (Copepoda: Caligidae) of salmonid fish. It is a common parasite of wild salmonid populations in Northern Europe, USA and Canada. The life cycle consists of three planktonic (nauplius I – II and copepodid), four immobile (chalimus) and three mobile (pre-adult I – II and adult) stages (Schram 1993). The copepodid is also the infective stage. The parasite is the most severe parasitic pathogen in marine farming of salmon (Salmo salar L.) (Wootten et al. 1982, Costello 1993) causing damage to the skin (Jones et al. 1990; Jónsdóttir et al. 1992) and osmoregulatory problems (Grimnes and Jakobsen, 1996). Infection methods incorporating cultivated salmon lice have been described by several authors (Johnson and Albright 1991, Heuch et al. 1995, Grimnes and Jakobsen 1996). Typically, these describe addition of a copepodid suspension to a tank containing the fish to be infected. Water flow is normally turned off for several hours to facilitate infection of the fish. When egg strings from certain populations of lice are available in limited numbers, there is a need for methods providing as many pre-adults from the first cultivated generation of salmon lice as possible. The aim of this study was to develop such a simplified infection method.

Materials and Methods

Experimental parameters are given in table 1. Adult female salmon lice were collected from farmed Atlantic salmon. Egg strings were re-
moved and placed in a chemical separation funnel containing one litre of seawater. Air, supplied via an aquarium pump, was introduced via the base of the system and the airflow regulated, allowing the egg strings to circulate gently in the water column. After hatching, the egg strings and the nauplii larvae were separated after stopping aeration. The nauplii larvae gathered at the top and the remaining egg strings sank to the bottom of the water column. The egg strings and the larvae were then separated. The nauplii larvae were placed in a 15-litre aquarium with a slow water exchange (approx. 6 l per hour) and a filter (mesh size: 85 µm) on the outlet water to prevent larvae from leaving. This procedure was repeated 1 – 2 x each day until all egg strings were hatched. Infection of salmon was performed 7 days after 50 % hatching of the egg strings. Copepodids were concentrated by filtering the water in the aquarium through the outlet filter, to 2 l in studies 1, 2 and 3 and to 1 l in studies 4, 5 and 6.

The number of live copepodids was calculated by examination of 25 ml of the concentrated solution in a graded glass cylinder. Live copepodids were counted 3 times for each study. Percent infection intensity (infection intensity / infective dose x 100) was used as a measurement of infection success (infection intensity = number of lice per fish).

Method 1: An estimated number of copepodids were placed together with the salmon in a tank containing 200 l seawater. The water flow was stopped and oxygen concentration kept at a minimum of 7 mg/l by aeration. After 4 h of infection, normal water flow was restored.

Method 2: The fish were anaesthetized using benzocaine (10 ml 5 % benzocaine in 10 l seawater) until operculum movement stopped. The fish were rinsed with clean seawater, then dipped for 30 sec in the concentrated copepodid solution. Two fish were infected simultaneously, each held by the head. After infection the fish were placed in a recovery tank containing seawater. New fish were infected as long as live (moving) copepodids could be observed in the infection container.

The infection success for both methods was
determined by netting individual fish and killing them rapidly by a blow to the head prior to lice counting at the following time points after infection: 1-2 days post infection (dpi) (copepodids), 14 dpi (chalimus III and IV) and 25 dpi (pre-adults). Three separate zones were examined by visual examination: gills, head and body (except gills) and fins. Numbers of salmon lice were recorded for each zone.

Water temperature was 14 – 15 °C during hatching and infection, and 12 °C post infection. Natural seawater from 60 m depth with a salinity ranging from 32 – 34 o/oo was used in all studies. The light cycle was 9 h light, 9 h dark and 3 h dusk and dawn. The salmon (60 – 115 g) were acclimatized to natural seawater at least two weeks before infection and fed twice daily with commercial fish feed. The salmon were starved 48 hours prior to infection.

Results

The results are shown in Table 2. Infection with method 1 resulted in an average infection success of 33.2 % (range: 9.4 – 57.1 %) for copepodids, 28.1 % (21.7 – 38.7 %) for chalimus larvae and 21.3 % (16.5 – 27.0 %) for pre-adults. Using method 2, the infection success was higher: 77.9 % (68.5 – 85.2 %) for copepodids, 59.5 % (47.6 – 75.2 %) for chalimus larvae and 50.9 % (30.8 – 69.5 %) for pre-adults.

Discussion

Using method 1, up to 30 % of the parasites settled on the gill filaments. The period of infection was 4 hours, during which time the fish filtered copepod-containing water through the gills. In contrast, following infection with method 2, almost no copepodids were found on the gills as these fish were anaesthetized and had few gill movements during infection. With the exception of study 1, the numbers of chalimus larvae found were reduced compared to the numbers of copepodids. The fraction attached to gill filaments was reduced more than the other fractions (body and fins). Johnson and Albright (1991) proposed gills to be an important site for initial attachment, while Bron et al. (1991) described that copepodids were searching for a suitable site of attachment and concluded that they preferred the fins for settlement. Pre-adults were found on the head, behind the dorsal and adipose fins and behind the anus.

<table>
<thead>
<tr>
<th>Study</th>
<th>Infective dose (copepod / fish)</th>
<th>Method of infection</th>
<th>Infection success*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Copepodids (1-2 dpi, n=3)</td>
<td>Chalimus (14 dpi, n=3)</td>
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<tr>
<td></td>
<td>Total A</td>
<td>B</td>
<td>C</td>
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<tr>
<td>6</td>
<td>12.0</td>
<td>80.0</td>
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</table>

Table 2. Study number, infective dose, and infection success (% infection intensity = (infection intensity / infective dose) x 100) of different stages of salmon lice L. salmonis) copepodids, chalimus larvae, pre-adults and adults on each body part (A: gills; B: head and body except gills, and C: Fins) following infection. dpi=days post infection, n=number of fish sampled.
Mobile stages of salmon lice prefer areas of low water drag (Jónsdóttir et al. 1992) and areas without scales, such as the head and operculum (Kabata 1981). The fraction on the head and body showed an increasing tendency, confirming that pre-adults migrated towards the head and body.

In general, the infection intensity was reduced between the copepodid and pre-adult stages. This is in accordance with the results of Grimnes and Jakobsen (1996) who found a reduction in infection intensity from early chalimus to pre-adult II of 34%. In study 2, 3, 4, 5 and 6 all fish were killed and pre-adults counted and harvested 25 dpi, while in study 1, 14 fish were sampled 35 dpi when the parasites had developed to adults. The average number of adult salmon lice on each fish was 1.1 (- infection success of 0.6 %), which was a dramatic fall in the population. This has also been registered by others under laboratory conditions (S. Alexandersen, C. Wallace, pers. comm.). It is possible that adult salmon lice were eaten by fish or left the tank with the outlet water when they moved between hosts. To maintain salmon lice in a laboratory for more than one generation, isolation of each fish and low water flow will be necessary.

Deviation in infection intensity was lower at the copepodid and chalimus stages for both methods (8 – 38 % coefficient of variance, C.V.) than the pre-adult stage (42 – 50 % C.V.), supporting the finding that pre-adults move between hosts (Ritchie 1997). Using method 2, the concentration of copepodids in the infection container was reduced as more and more fish were infected. This probably explains the increased variation in the number of copepodids and chalimus larvae on each fish using this method.

In conclusion, method 1 gives lower infection success compared to method 2. Method 1 may be suitable for infecting a large number of fish. Method 2 gives a higher infection success and is suitable for infecting a small number of fish when the number of infective copepodids is limited.

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References


