Light intensity, salinity, and host velocity influence presettlement intensity and distribution on hosts by copepodids of sea lice, *Lepeophtheirus salmonis*

R.L. Genna, W. Mordue, A.W. Pike, and A.J. Mordue (Luntz)

Abstract: Intensity and distribution of presettlement by the copepodid of the sea louse, *Lepeophtheirus salmonis*, on smolts of its host Atlantic salmon, *Salmo salar*, were quantified for 27 infection regimes under controlled flume conditions. Each infection regime represented a level of interaction between three levels (low, medium, high) of the physical factors of light (10, 300, 800 lx), salinity (20‰, 27‰, 35‰), and host velocity (0.2, 7.0, 15.0 cm·s^{-1}). Light, salinity, and host velocity independently and interactively determined the distribution and number of presettled copepodids on hosts. Host surface area also influenced the number of attached preestablished copepodids. The distribution of presettled copepodids on the host body surface closely corresponded to that of settled copepodids and chalimi reported from other studies, with the greatest levels observed on the fins, in particular the dorsal, caudal, and pectoral fins. Copepodid presettlement occurred on the gills under all conditions. Differential presettlement, not selective mortality, probably produces the distribution pattern of settled stages seen in other studies.

Résumé : Nous avons quantifié l'intensité et la répartition de la pré-fixation des copépodites du pou de mer, *Lepeophtheirus salmonis,* sur les saumoneaux de l'hôte, le saumon atlantique, *Salmo salar*, sous 27 régimes infectieux dans des conditions contrôlées de canalisation artificielle. Chaque régime infectieux représente un degré d'interaction entre trois niveaux (faible, moyen, élevé) de facteurs physiques, soit la lumière (10, 300 et 800 lux), la salinité (20, 27, 35 ‰) et la vitesse de déplacement de l'hôte (0,2, 7,0, 15,0 cm·s^{-1}). La lumière, la salinité et la vitesse de l'hôte déterminent de façon indépendante et de façon interactive la répartition et la densité du nombre de copépodites en pré-fixation sur les hôtes. La surface externe de l'hôte affecte aussi le nombre de copépodites attachés en pré-fixation. La répartition des copépodites en pré-fixation sur la surface corporelle de l'hôte correspond à celles des copépodites et des larves chalimus signalées dans d'autres études, avec les plus grands nombres sur les nageoires, en particulier la dorsale, la caudale et les pectorales. La pré-fixation des copépodites se fait sur les branchies dans toutes les conditions. C'est probablement la pré-fixation différentielle et non la mortalité sélective qui explique les patrons de répartition des stades fixés observés dans les autres études.

[Traduit par la Rédaction]

Introduction

The sea louse, *Lepeophtheirus salmonis*, is a specific ectoparasite of several species of salmonids in the Northern Hemisphere and is a significant pest of farmed salmon, with infections costing the Scottish salmon industry alone £20–£30 million each year in production losses (Pike and Wadsworth 2000). Control strategies should incorporate an understanding of the infection dynamics of the planktonic copepodid, which completes transmission by locating and infecting a new host. *Lepeophtheirus salmonis* copepodids are envisaged to occur in spatially and temporally discrete patches of varying densities in inshore waters in and around marine sea lochs and estuaries through which young hosts forage and swim during seaward migrations (Costelloe et al. 1998*b*; McKibben and Hay 2004; Penston et al. 2004). Such patches can be simulated in a flume with static hosts exposed to a bloom of moving copepodids.

Experimental infections with *L. salmonis* copepodids have been conducted independently at different temperatures and salinities under laboratory conditions in unlit (Bron et al. 1991; Grimnes and Jakobsen 1996) or lit static water (Dawson et al. 1997; Finstad et al. 2000; Tucker et al. 2000) or in unlit flowing water (Bowers et al. 2000). None of these studies has investigated the interactive effects of varying levels of these factors on host attachment and they have identified only the intensity and distribution of successfully settled *L. salmonis* copepodids on a host, ignoring the critical phase of attachment before permanent settlement. Furthermore, artificial infection is unlikely to produce realistic patterns of initial attachment, as lower infection intensities and different distribution patterns of attached copepodids are observed on wild hosts (Dawson 1998; MacKenzie et al. 1998; Finstad et al. 2000) or caged hosts (Boxaspen 1997).

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R.L. Genna,[1] W. Mordue, A.W. Pike, and A.J. Mordue (Luntz). School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, Scotland, UK.

[1]Corresponding author (e-mail: r.l.genna@btinternet.com).

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Lepeophtheirus salmonis copepodids are known to respond to light (Aarseth and Schram 1999; Novales Flamarique et al. 2000), salinity changes (Heuch 1995), water movements (Heuch and Karlsen 1997), pressure (Bron et al. 1993), and chemicals (R.L. Genna, W. Mordue, A.W. Pike, and A.J. Mordue (Luntz), unpublished data; R.J.E. Bailey, M.A. Birkett, A. Ingvarsdottir, A.J. Mordue (Luntz), W. Mordue, B. O'Shea, J.A. Pickett, and L.J. Wadhams, School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, Scotland AB24 2TZ, UK, unpublished data). Chemical cues dominate specificity during settlement of marine invertebrate larvae (see Pawlik 1992; Rodriguez et al. 1993). In *L. salmonis*, both adults and copepodids exhibit search patterns (R.L. Genna, W. Mordue, A.W. Pike, and A.J. Mordue (Luntz), unpublished; Devine et al. 2000) and directional responses (Ingvarsdottir et al. 2002; R.J.E. Bailey, M.A. Birkett, A. Ingvarsdottir, A.J. Mordue (Luntz), W. Mordue, B. O'Shea, J.A. Pickett, and L.J. Wadhams, unpublished data) to host odors of salmon. In the copepodid, such behavior may serve to bring individuals within the vicinity of potential hosts and switch on settlement behavior by which copepodids will initially attach to a host. Although previously not investigated, the initial phase of host attachment, henceforth termed presettlement, precedes the three phases of attachment described as occurring during host settlement (Bron et al. 1991).

Once activated by chemical cues (R.L. Genna, W. Mordue, A.W. Pike, and A.J. Mordue (Luntz), unpublished data), copepodid host-searching behavior will improve the likelihood of encounter and presettlement on a host. The intensity and distribution of presettled copepodids on a host are hypothesized to be influenced predominantly by interactive physical factors, as identified in flume studies for the settlement of marine larvae of annelids (Butman et al. 1988; Cha et al. 1991; Snelgrove et al. 1999), bivalve mollusks (Gregoire et al. 1996; Tamburri et al. 1996; Snelgrove et al. 1999), decapod crustaceans (Boudreau et al. 1990; Blackmon and Eggleston 2001), and copepod crustaceans (Fleeger et al. 1995) under realistic environmental conditions.

Hence, the aim of this study was to investigate the interactive effects of varying light intensity, salinity, and host swimming velocity on the intensity and distribution of presettlement of *L. salmonis* copepodids to host fish under moving water conditions.

Materials and methods

Copepodid culture

Copepodids were cultured from eggs removed from gravid female *L. salmonis* at a salmon farm at Loch Melfort (GB grid reference NM822116) on the west coast of Scotland after being transferred on ice to the Zoology Building, University of Aberdeen (Scotland). Copepodids were cultured under conditions of ambient light (16 h light – 8 h dark), temperature (10 °C), and salinity (35‰) in fresh, aerated seawater. All copepodids used in experiments originated from the same batch of egg strings collected on 28 April 2001. Copepodids were 24–48 h old when used and, to aid recognition, were stained with neutral red after the protocol of Anstensrud (1989) prior to use.

Flume setup and maintenance

An elliptical gray Perspex flume (inside diameter 200 mm, height 250 mm, length 4200 mm, volume 100 L) was used with a clear Perspex section in the raceway for observation of fish. For host infection within the flume, fish were confined within a clear Perspex infection tube (bore 100 mm, diameter 110 mm, length 300 mm) fitted between steel grids slotted into the raceway, which allowed smolts to swim. Laminar flow through the tube was achieved by use of a collimator placed upstream, while a filter (pore size 100 μm) was placed directly downstream of the infection tube to collect unattached copepodids.

Aerated, artificial seawater at constant temperature (10 ± 0.1 °C) was recirculated in the flume at variable flow rates by means of an attached recirculating system with pump (model PV71/6; Beresford Pumps, Coventry, UK) and by controlled opening of the connecting valves. Water velocity was monitored and maintained to within 0.1 $cm \cdot s^{-1}$ of the flow regime using a flow meter (model 801; Valeport Ltd., Dartmouth, Devon, UK). Lighting was artificial from overhead fluorescent lights, with the level of lighting controlled by setting the number of lamps illuminating the flume and monitored with a light meter (model Mastersix; Gossen, Nürnberg, Germany). Salinity was maintained to ±0.5‰ of the required level with Peacock's seamix salt using a refractometer (New S-100; Tanaka Sanjiro Co. Ltd., Japan).

Experimental design and infection regimes

Experiments were conducted over the period 30 April – 5 May 2001 in the Zoology aquarium, University of Aberdeen. A multifactorial randomized block design was used, with interaction between the three factors (salinity, light, host velocity), each at three levels (low, medium, high), resulting in 3^3 infection regimes. Ten smolts were infected for each of the 27 infection regimes, with fish selected at random from a stock of 300 smolts (weight, 187.1 ± 3.2 g; fork length, 25.9 ± 0.2 cm). Host swimming velocity was manipulated by maintaining a swimming host within the infection tube and altering current speeds in the flume (low, 0.2 $cm \cdot s^{-1}$; medium, 7.0 $cm \cdot s^{-1}$; high, 15.0 $cm \cdot s^{-1}$) to reflect the range of recorded swimming speeds of Atlantic salmon, *Salmo salar*, smolts (Grottum and Sigholt 1998; Moore et al. 1998; Boucher and Petrell 1999). Salinity (low, 20‰; medium: 27‰; high, 35‰) and light (low, 10 lx; medium, 300 lx; high, 800 lx) were set at values known to affect *L. salmonis* copepodid behavior (Bron et al. 1993; Heuch 1995; Novales Flamarique et al. 2000). To ensure that copepodids were not functioning under conditions of physiological stress, they were exposed to experimental levels of light and salinity only during the infection protocol.

Infection protocol

Prior to each infection, a smolt was introduced into the infection tube by raising and lowering the steel grid and left to acclimate for 5 min. During infection, 1000 copepodids were introduced into the flume upstream of the collimator so that all copepodids were entrained in a laminar flow through the infection tube in a dispersed cloud. Each infection lasted 5 min, to flush unattached copepodids from the infection tube, after which the filter was removed and its contents dis-

carded. The infection tube was lifted from the water, the fish culled, and measurements of fork length (centimetres) and wet weight (grams) taken to allow calculation of surface area (surface area = 14.93 × weight$^{0.5869}$; B. O'Shea, A.J. Mordue (Luntz), R.J. Fryer, and I.R. Bricknell, School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, Scotland AB24 2TZ, UK, unpublished data). The number and location of copepodids presettled on the body surface (including gills) were recorded using a binocular microscope using the areas of the body specified by Bjørn and Finstad (1998): five fin areas (anal, caudal, dorsal (including the adipose), pectoral, pelvic), four body areas (anterior and posterior dorsal, anterior and posterior ventral), the head area, and the gills. Counts were summed for the four body regions (body, fins, gills, head) and per fish.

Statistical analyses

To allow comparison between regimes, we calculated total numbers of presettled copepodids as a proportion of the initial 1000 copepodids released, while the distribution of copepodids on each host body region was calculated as a proportion of the total number presettled per fish. The effects of the three physical factors and fish parameters (length, weight, surface area) on the overall level of presettlement (binomial family, logit link function) and on the distribution patterns of presettled copepodids between body areas (binomial family, logit link function) and body regions (Poisson family, log link function) were analyzed by generalized linear models using the software package RGui© (The Free Software Foundation, Inc., Boston, Massachusetts). Model fitting was conducted by sequential backward elimination of nonsignificant ($p > 0.05$) terms from a full model.

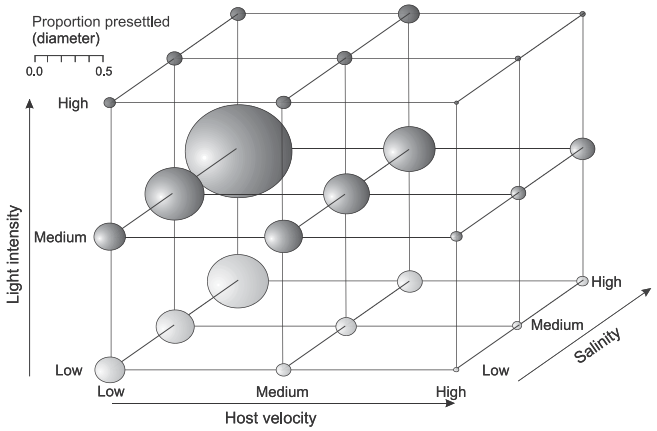
Results

Total presettlement

Light, salinity, and host velocity showed interactive effects in determining the proportion of presettled copepodids, with variability within and between infection regimes (Fig. 1). The generalized linear model showed good fit (null deviance, 37 665.50, 266 df; residual deviance, 1294.50, 239 df; Akaike's Information Criterion (AIC), 3060.90). In this model, the proportion of presettled copepodids was significantly determined by fish surface area (deviance, 1343.50, 1 df; AIC, 3107.90; $p < 0.001$) and the three factors with full interactions (deviance, 2001.90, 8 df; AIC, 3752.20; $p < 0.001$). Host surface area showed a negative relationship ($b = -7.756 \times 10^{-4} \pm 1.110 \times 10^{-4}$) with the total proportion of copepodids that presettled (P), such that $P = a\exp(b \times \text{surface area})$, where a is the number of copepodids that presettled under the interactive effects of light, salinity, and host velocity. For the small range in size of hosts used, variability in the proportion of presettled copepodids owing to host surface area was calculated as less than 0.005 (i.e., <0.5%): hence, the effects of surface area on presettlement were disregarded in this study.

Presettlement was maximal at medium light, low host velocity, and high salinity (0.708‰ ± 0.015‰) and minimal at high light, high host velocity, and low salinity (0.021‰ ± 0.001‰) (Fig. 1). Copepodid presettlement was influenced differently by each physical factor, greater at medium light intensity with sequentially fewer at low and high light intensity, greater at low to medium than high host velocity, and increasing with salinity for all regimes (Fig. 1).

Fig. 1. Mean proportion of *Lepeophtheirus salmonis* copepodids per infection that presettled on *Salmo salar* smolts exposed to one of 27 infection regimes ($n = 10$ per point). Each infection regime represents one combination of low, medium, and high levels of light (10, 300, 800 lx), salinity (20‰, 27‰, 35‰), and host velocity (0.2, 7.0, 15.0 cm·s^{-1}).

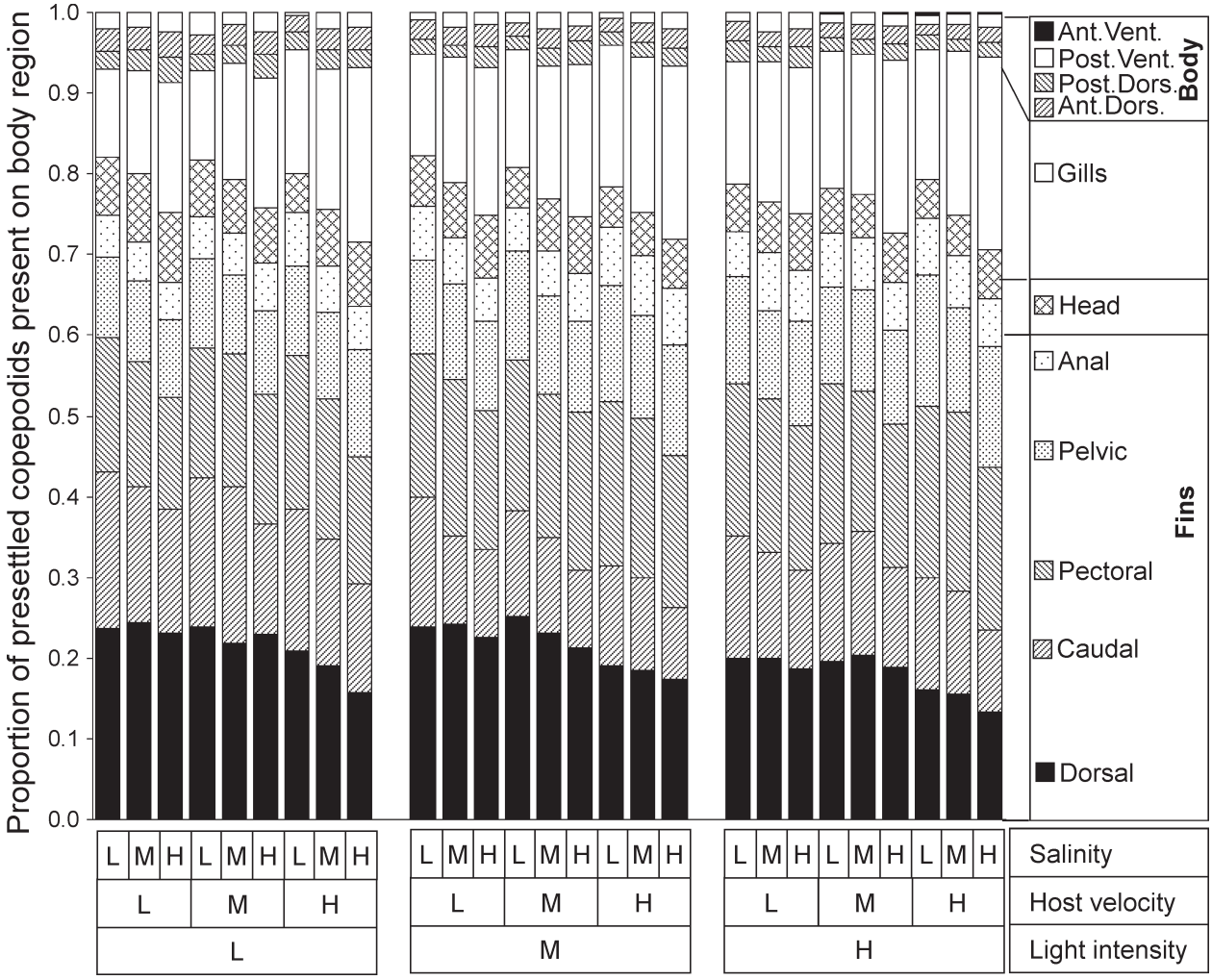


Presettled copepodid distribution

Presettled copepodids showed a differential distribution over the body surface of smolts under all regimes, both for individual areas (null deviance, 64 688.00, 2936 df; residual deviance, 11 160.10, 2902 df; AIC, 21817.10) and for grouped regions (null deviance, 71 245.80, 1067 df; residual deviance, 3539.80, 1035 df; AIC, 8608.90) of the host surface (Fig. 2). Differential presettlement occurred with greatest intensity on the fins and then the gills and head, with few on the body (Fig. 2). Specifically, the dorsal, caudal, and pectoral fins had greater intensities, with lower intensities on the pelvic and anal fins and the lowest intensity on the body (Fig. 2).

Physical factors determined the distribution of presettled copepodids on all body areas, but fish parameters did not ($p > 0.05$). Independent factors determined presettlement to all except two body areas (caudal and pectoral fins), where the interaction between factors was responsible. Light, host velocity, and salinity influenced presettlement on the gills ($p < 0.001$), the head ($p < 0.001$), and the body ($p < 0.001$), with only host velocity and salinity influencing presettlement on the fins ($p < 0.001$). Both light and host velocity determined presettlement on the anterior dorsal ($p < 0.05$) and posterior ventral ($p < 0.05$) body areas, with presettlement on the posterior dorsal body area determined by light ($p < 0.05$). Presettlement on the dorsal fin was independently influenced by all three factors ($p < 0.001$), while presettlement on the pelvic fins was independently influenced by salinity and light ($p < 0.001$). Presettlement on the pectoral and caudal fins was determined independently by light ($p < 0.001$) and interactively by host velocity and salinity ($p < 0.05$).

Fig. 2. Proportional distribution of presettled *Lepeophtheirus salmonis* copepodids between areas of the host (*Salmo salar* smolts) body surface for each of the 27 infection regimes. Each regime is the average of 10 fish (n = 10) infected at one combination of low (L), medium (M), and high (H) levels of light (10, 300, 800 lx), salinity (20‰, 27‰, 35‰), and host velocity (0.2, 7.0, 15.0 cm·s^{-1}).



The distribution of presettled copepodids changed between body regions with light (deviance, 3553.80, 2 df; AIC, 8619.00; $p < 0.001$) and host velocity (deviance, 3688.10, 2 df; AIC, 8753.20; $p < 0.001$) and between body areas with all three factors, with host velocity inducing the greatest effects (light: deviance, 11 169.00, 2 df, $p < 0.05$; salinity: deviance, 11 184.00, 2 df, $p < 0.001$; host velocity: deviance, 11 245.60, 2 df, $p < 0.001$).

Faster host velocity increased presettlement on the fins, particularly the dorsal and pectoral fins, but decreased presettlement on the gills, head, and body, reflecting the changes induced in individual body areas (Table 1). Greater salinity increased presettlement on the fins overall, with both increased (dorsal, caudal) and decreased (pelvic, pectoral) levels noted. Greater salinity also increased presettlement on the body and head but decreased presettlement on the gill tissues (Table 1). Increasing light intensity decreased gill presettlement but increased presettlement to the head and the body region, particularly on the anterior and posterior dorsal part of the body, and either increased (dorsal fin) or decreased (pectoral and pelvic fins) presettlement on individual fins but induced no overall effect on the fins as a group (Table 1).

Discussion

Light, salinity, and host swimming velocity interactively determined copepodid presettlement in this study, with almost all infection regimes showing different intensities. Presettlement showed a negative relationship with fish surface area, although this did not account for significant variability in the level of presettlement over the range of sizes of hosts used in this study. In a comparable study of *L. salmonis* settlement, no effect of surface area was found (B. O'Shea, A.J. Mordue (Luntz), R.J. Fryer, and I.R. Bricknell, School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, Scotland AB24 2TZ, UK, unpublished data). The decrease in presettlement with increasing host surface area in this study may reflect the greater increase in the body than the fins to the total host surface area, reinforced by the preference shown for presettlement on the fins.

Table 1. Outcomes of generalized linear models of physical factors affecting the distribution of *Lepeophtheirus salmonis* copepodid presettlement on *Salmo salar* smolts.

	Physical factor														
	Model fit, residuals			Light intensity				Host velocity				Salinity				
Region/area	AIC	Dev	df	AIC	Dev	*p*	Effect	AIC	Dev	*p*	Effect	AIC	Dev	*p*	Effect	Interactions
All fins	1440.0	86.0	262	—	—	ns	nc	1605.0	255.1	***	↑	1451.0	101.0	***	↑	NA
Dorsal fin	1413.4	121.4	260	1484.6	196.7	***	↑	1426.6	138.6	***	↑	1525.7	237.7	***	↑	NA
Caudal fin	1358.1	142.9	256	1474.0	262.8	***	Med ↓ High ↑	—	—	ns	↑	—	—	ns	↑	Yes[a]
Pectoral fins	1385.9	108.4	256	1413.6	140.2	***	↓	—	—	ns	nc	—	—	ns	↓	Yes[b]
Pelvic fins	1313.8	139.0	262	1333.6	162.8	***	↓	—	—	ns	nc	1334.3	163.5	***	↓	NA
Anal fin	—	—	—	—	—	ns	nc	—	—	ns	nc	—	—	ns	nc	NA
Gills	1351.9	100.5	260	1384.7	137.2	***	↓	1441.1	193.6	***	↓	1421.2	173.7	***	↓	NA
Head	1108.0	80.6	260	1120.8	97.4	***	↑	1123.0	99.6	***	↓	1123.4	99.9	***	↑	NA
Body	1111.2	103.1	260	1128.3	122.1	***	↑	1133.3	127.1	***	↓	1115.4	109.2	*	↑	NA
Anterior dorsal	837.6	91.8	262	841.4	99.5	*	↑	860.9	119.1	***	↓	—	—	ns	nc	NA
Posterior dorsal	858.2	109.4	264	863.2	118.4	*	↑	—	—	ns	nc	—	—	ns	nc	NA
Anterior ventral	—	—	—	—	—	ns	nc	—	—	ns	nc	—	—	ns	nc	NA
Posterior ventral	827.4	174.2	262	829.9	180.8	*	nc	834.1	184.9	**	↓	—	—	ns	nc	NA

Note: Independent factors showed 2 df with 4 df for interactions. Significance codes: not significant (ns), $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Changes in copepodid presettlement on each body region are shown in response to increasing levels of each significant factor (↑, increase; ↓, decrease; nc, no change). AIC, Akaike's Information Criterion; Dev, deviance; *p*, probability score of likelihood ratios; NA, not applicable.

[a]Host velocity and salinity (AIC = 1365.3, Dev = 158.3, $p < 0.05$), complex effects.

[b]Host velocity and salinity (AIC = 1387.5, Dev = 118.0, $p < 0.05$). Increase with salinity at low host velocity; increase with salinity at medium host veocity, mixed effects with host velocity at high salinity.

Although this study investigated copepodid presettlement, infection intensities compare well with the range of successful settlers recorded in artificial infections in low water flows with low (Bron et al. 1991; Grimnes and Jakobsen 1996) to high (Dawson et al. 1997; Finstad et al. 2000; Tucker et al. 2000) light conditions or low light and flowing water (Bowers et al. 2000) but are generally greater than those seen on wild hosts (Dawson 1998; MacKenzie et al. 1998; Finstad et al. 2000), which may reflect the high infection intensity used or indicate that more copepodids presettle than successfully settle on a host.

The environmental factors induced differential presettlement to areas of the host body, with the fins, particularly the dorsal, and the gills preferred. *Lepeophtheirus salmonis* settlement on wild hosts occurs predominantly on the fins, particularly the dorsal fin, with gill attachment seldom reported (Dawson 1998; MacKenzie et al. 1998; Finstad et al. 2000). Such infections occur at high salinity (>25‰) and under low light levels and during fast host swimming (Moore et al. 1998; Petersen and DeAngelis 2000). Comparable infection regimes in this study produced similar distribution but greater levels on the gills and pectoral fins and lower levels on the dorsal fin and body. These differences may be due to the swimming speeds being at the lower end of locomotory speeds for Atlantic salmon smolts in the wild (Grottum and Sigholt 1998; Moore et al. 1998; Boucher and Petrell 1999) or that the distribution of settled chalimi on wild hosts represents serial independent infections under different regimes. Hosts infected artificially in static water show chalimi predominantly settled on the gills and fins, particularly the dorsal fin, in lit tanks (Grimnes and Jakobsen 1996; Dawson et al. 1997; Bjørn and Finstad 1998) or the dorsal, ventral, and caudal fins and the gills in unlit tanks (Bron et al. 1991; Johnson and Albright 1991; Tucker et al. 2000). As in this study, low light intensity resulted in greater copepodid settlement on the ventral surfaces of paired fins and the entire caudal fin, the ventral flanks, opercula, and the gill filaments (Bron et al. 1991; Johnson and Albright 1991; Dawson et al. 1997).

The sites of copepodid presettlement will be determined by the copepodid's ability to attach to and remain on the host skin, with the suitability of a given area dependent on the nature of the epidermis and the degree of exposure to water currents (Bron et al. 1993). It may be a feature of the epidermis on the fins that allows more secure attachment than other areas, as differential settlement on the fins has been noted in other parasitic copepods, with local current speed, position maintenance in the host boundary layer, and movement towards areas of water movement proposed as factors during copepodid settlement in other parasitic copepods (Kabata and Cousens 1977; Boxshall 1976; Anstensrud and Schram 1988).

Detection of a swimming host by *L. salmonis* copepodids will predominantly involve the use of mechanical stimuli, namely the anterolateral flow field about a host, a low-frequency hydrodynamic dipole field derived from water being pushed ahead and along the sides of an advancing fish (Kalmijn 1989). Heuch and Karlsen (1997) showed that *L. salmonis* copepodids respond to uniform linear accelerations, similar to those found in front of a swimming fish, and suggested that copepodids react to such near-field accelerations produced within centimetres of a swimming fish with high-speed burst swimming and subsequent attachment to the host. The burst swimming response is likely to aid host attachment by increasing the chances of encountering a host or entering and remaining in the fish boundary layer, that region of reduced current flow and negligible current shear that exists around the host body. Copepodids may utilize the host boundary layer to facilitate presettlement on the host surface, and therefore, boundary layer dynamics will influence the distribution of presettled copepodids. This study supports this belief, with greater presettlement at slow swimming speeds when the boundary layer is thicker but declining at faster swimming speeds that thin the boundary layer. Faster host swimming may also change the flow field around a host, altering the distribution of copepodid presettlement. Increased, turbulent water flow would induce eddies behind fin regions and the fin rays, lying perpendicular to the current, and may provide shelter and hence make settlement in their lee easier (Bron et al. 1991). A thinner boundary layer would also reduce presettlement to areas without such eddies, namely the flanks and head, as in this study.

Conversely, increased water viscosity at higher salinity would thicken the boundary layer aiding copepodid attachment. This positive relationship between salinity and *L. salmonis* attachment has been reported previously (Tucker et al. 2000). Furthermore, as larval survival and development are affected by low salinity (<25‰), it will affect behavior and so presettlement (Johnson and Albright 1991). Low salinity may cause copepodids to sink or swim downwards and out of suspension, as observed in other meroplanktonic larvae during estuarine vertical migration, to locate deeper and more saline waters (reviewed by Forward 1987). This may explain the change in presettlement from the gills and ventral surfaces at low salinity to the head and dorsal surfaces at higher salinity as a function of increased copepodid activity and with less reliance on passive entrainment as described below.

Maximal copepodid presettlement occurred at low to medium light, with increasing light levels causing a shift in attachment from the ventral surfaces and fins to the head and dorsal and caudal areas and fins, indicative of a behavioral response to light. The structure of the visual system in *L. salmonis* copepodids allows location of a light or shadow source (Bron et al. 1993) and is sensitive to low light levels, such as those available during night time and crepuscular periods (Novales Flamarique et al. 2000), and may account for greater attachment at low to medium light intensity. Increased activity and the resultant higher position in the water column in response to rising light levels may account for a switch in distribution from ventral to dorsal surfaces. Poor presettlement at high light levels may reflect overstimulation of the visual system, with positive phototaxis known to occur in *L. salmonis* copepodids (Bron et al. 1993; Heuch 1995; Aarseth and Schram 1999).

The switch in presettled copepodids from ventral to dorsal surfaces and fins with increasing levels of salinity and light reflects their position in the water column from which they are able to attach to these areas. Changes in salinity and light induce the translocation of copepodids from ventral to dorsal areas of the body, mediated through increased copepodid activity and behavior.

To date, significant settlement on the gills has been observed only in laboratory infections, with the inference that settlement on the gills is a systematic artifact resulting from reduced host swimming speed and passive entrainment across the gills by the respiratory current (Bron et al. 1993; Tucker et al. 2000; Treasurer and Wadsworth 2004). This study partially supports this view, as the highest levels of presettlement on the gills occur under nonstimulatory conditions, namely static water and low light and salinity. However, although presettlement on the gills decreases at faster swimming speeds and higher salinities and light levels, it still persists under the most natural conditions. This suggests that presettlement on the gills is not an experimental artifact, despite infection levels greater than those observed in wild hosts infected at comparable swimming velocities. As such, initial gill attachment may be followed by selective removal–mortality mediated by water flow or host behavior. Other studies support this view, with reported changes in the intensity and distribution of attached copepodids over time after artificial infection (Bjørn and Finstad 1998) and total elimination from the gills 1–2 weeks postinfection (Dawson et al. 1997). Such a mechanism accounts for the respective greater and lesser copepodid presettlement on the gills and the rest of the body in this study in comparison with chalimi in other studies.

Given the influence of physical factors on successful copepodid presettlement, it is probable that copepodids exhibit behaviors to seek out areas with environmental conditions for optimal host attachment; namely medium to high salinity with low to medium light and slow host velocities. These conditions are found around estuarine haloclines at night when young hosts forage at slow speeds close to the water surface (Moore et al. 1998), with aggregations of *L. salmonis* copepodids previously reported in connection with such areas (Heuch 1995; Costelloe et al. 1998*a*, 1998*b*). These findings have implications for the siting of salmon farms in areas with physical conditions that minimize copepodid presettlement. Given that host swimming speed has the greatest influence on presettlement intensity, allowing greater host swimming speeds by siting salmon farms in areas of fast water currents or through lower stocking densities in individual pens may minimize the potential for farmed salmon to become infected. Siting of fish in pens at levels removed from the halocline or removed from the surface may also further reduce the overall intensity of sea lice presettlement on farmed salmonids.

In summary, light, salinity and host swimming velocity interactively affect the intensity and distribution of presettled *L. salmonis* copepodids on the body of a salmon by altering the boundary layer of fish hosts and the behavior of *L. salmonis* copepodids. Given the similarity between copepodid presettlement on all host body areas other than the gills in this study and the settlement of chalimi in other studies, it is proposed that the distribution on a host is predominantly produced by presettlement, with some differential mortality of copepodids presettled on the gills.

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