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Where art thou louse? A snapshot of attachment location preferences in salmon lice on Atlantic salmon hosts in sea cages

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Abstract

Problematic sea lice infestations on farmed Atlantic salmon (*Salmo salar*) have motivated extensive research and development into new methods to prevent, monitor and control sea lice. Most of these technologies require detailed information on the behaviour, spatial distribution and demography of lice on host fish. This study investigated how salmon lice (*Lepeophtheirus salmonis*) infestation density varies across the host's surface under sea cage farming conditions. Lice abundance, demography and attachment location were tracked over time, with repeated sampling of 300 individually tagged salmon across three replicate experimental sea cages. The data reveal clear differences in attachment locations according to sex and stage, but with an overall preference for the dorsal surface among mobile stages—dorsal head for adult females and dorsal-posterior section for males and pre-adults. Total lice abundance was highly variable between repeated measures of individual fish, consistent with frequent host-switching or mortality. Total lice numbers also declined between sampling dates, likely due to handling, with lost mobile lice being almost exclusively adult males. As the distribution of sea lice on hosts is likely determined by numerous factors, future image-based automated detection systems should be validated in settings that reflect the complex host–parasite interactions that occur in open farming systems.

KEYWORDS

ectoparasite, host–parasite interaction, life history, salmon aquaculture, sea lice

1 | INTRODUCTION

The ectoparasitic salmon louse (*Lepeophtheirus salmonis*) remains one of the most significant obstacles to sustainable Atlantic salmon (*Salmo salar*) aquaculture, particularly in Norway (Igboeli, Burka, & Fast, 2013; Olaussen, 2018; Torrissen et al., 2013). The industry faces pressure from governing bodies to prevent and control infestations, which has stimulated a surge in innovative solutions (Brakstad, Hagspiel, Lavrutich, & Matanovic, 2019). This has been complemented by increased research focus on biological or ecological aspects of the host–parasite interaction to develop technologies that leverage aspects of the biology of hosts and parasites (Bui, Oppedal, Sievers, & Dempster, 2019).

Spatial distribution and developmental demography of salmon lice on the host are of interest for studies of lice biology and

behavioural studies, and some emerging technologies will also benefit from this knowledge (e.g. image-based automated lice detection). However, the salmon louse has mobile pre-adult and adult stages (Johnson & Albright, 1991b) that often move around on, and between, hosts (Johnson & Albright, 1991a; Jónsdóttir, Bron, Wootten, & Turnbull, 1992; Todd et al., 2000), complicating any understanding of their prevalence and distribution. Numerous previous studies have investigated the attachment location preference of lice (Bjørn & Finstad, 1998; Todd et al., 2000), movement among hosts due to density-dependent competition or mate-searching behaviour (Todd, Stevenson, Reinardy, & Ritchie, 2005; Todd et al., 2000) and influence of abiotic factors on attachment (Genna, Mordue, Pike, & Mordue, 2005; Samsing, Solstorm, Oppedal, Solstorm, & Dempster, 2015). However, these studies were largely conducted in tank environments and would not fully reflect the environmental drivers and connectivity to other potential host groups present in the sea cage production setting (but see Ritchie, 1997).

There is an increasing focus on more efficient and accurate methods to enumerate lice on salmon in sea cages without the need to physically capture and count individuals. The drive for a solution is so great that multiple competing companies, in parallel, are developing camera systems for in situ automated counts (Grøntvedt, 2019). Although these innovations require specific technological advancements, there is also a need for biological data to validate any system's accuracy. Specifically, developers and users of the system must understand size range of stages and how these change with temperature (i.e. Hamre, Bui, Oppedal, Skern-Mauritzen, & Dalvin, 2019), patterns of their distribution on the host (Todd et al., 2000) and expected number of lice on the unimaged areas of the host.

This study aimed to describe the distribution of lice stages and attachment locations on a cohort of salmon hosts held in sea cages, with individuals sampled twice, 15 days apart. These fish were not deloused for ~4 months, which allowed a "natural" development and between-host distribution of lice, undisturbed by treatment efforts.

2 | METHODS

A cohort of ~7,500 salmon was held in a single sea cage (12 m × 12 m × 15 m) at the Austevoll Research Station (Institute of Marine Research, Norway). After sea transfer four months prior to trial start, salmon were held under standard husbandry protocols where they acquired lice infestations naturally (Figure 1). Five weeks prior to trial start, nine groups of 400 salmon were transferred to smaller cages (5 m × 5 m × 6 m) for a separate experiment where fish were not manipulated, but held with low quantities (7.5% stocking density) of cleaner fish (ballan wrasse, *Labrus bergylta*) for 3 weeks. After this period, subsets of these fish were transferred to new cages (5 m × 5 m × 6 m) and held for 2 weeks without cleaner fish present; this fish group formed the stock population for the current study. One day prior to trial start, three cages were stocked with 500 salmon weighing 271 ± 16.3 g (±*SE*).

Of the 500 salmon per cage, 100 were tagged for individual identification. Before transfer into the cage, fish were anaesthetized with tricaine methanesulfonate (Finquel, 0.1 g/L). Within 4 min of complete sedation, fish were tagged with a unique coloured anchor T-bar tag (5 cm), photographed, measured for length, and assessed for lice attachment locations. Each fish had a diagrammatic representation of the exact location of lice on the body (Figure 2), marked with the stage of each louse (for detailed biological description of *L. salmonis*, see Costello, 2006, Hull, Pike, Mordue Luntz, & Rae, 1998, Johnson & Albright, 1991b). Precopulating pairs of adult males and pre-adult II females were common (Ritchie, Mordue, Pike, & Rae, 1996) and labelled when observed. Only chalimus II and mobile lice stages were recorded (pre-adults not sex-distinguished), as chalimus II could moult into the mobile stage within this time frame (Hamre et al., 2019). After processing, fish were placed in a recovery tank and monitored for full recovery, after which they were transferred to the cage. Any lice that dislodged in the recovery tank were discarded as they could not be allocated to their original host. This time point, when louse distributions were first recorded, is termed Sample 1.

The groups were left in the cages undisturbed for 15 (cage 1) or 16 days (cage 2 and 3). After this period, tagged fish were hand-netted and anaesthetized in individual buckets so that any lice that were displaced could be assigned to their original host. Individuals were identified and photographed, and lice attachment locations were recorded in the same format. This time point, the second assessment of louse distribution, is termed Sample 2.

For the duration of their time at sea, and throughout this experimental period, the salmon were not deloused, as the study had approval for lice abundances exceeding the standard regulative threshold (Mattilsynet reference 2018/185649). An environmental profile was taken daily at a reference location on the sea facilities using a profiling CTD probe (Model SD204, SAIV AS, Norway).

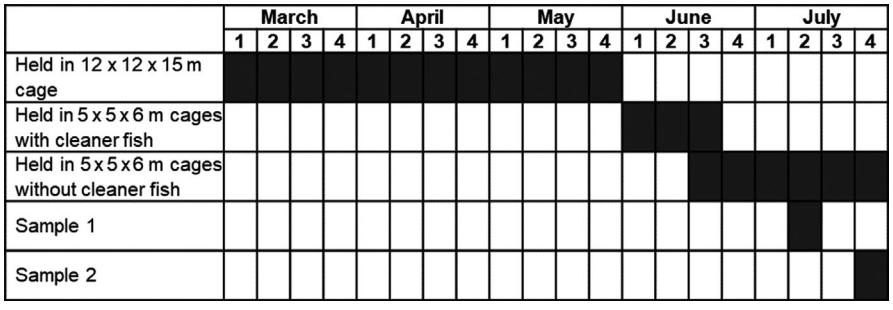

FIGURE 1 Timeline outlining the handling of subjects prior to and during the present study, across months and weeks

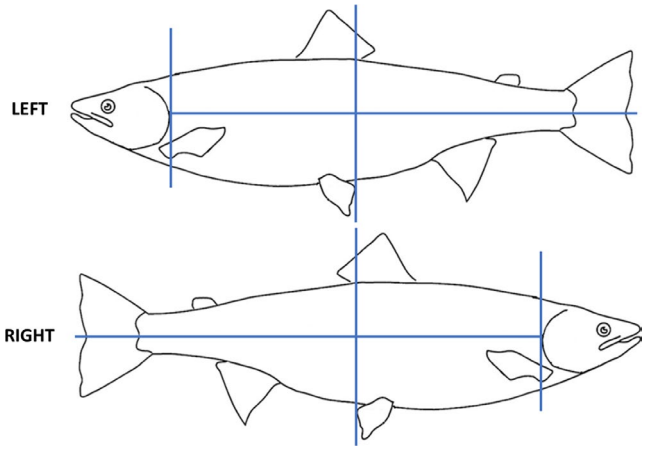

FIGURE 2 Diagrams used for marking attachment locations of lice. Each louse was denoted with their stage and sex (if adults), and precopulating pairs were noted. Individuals attached at the dorsal or ventral midline were considered a separate location quadrant [Colour figure can be viewed at wileyonlinelibrary.com]

Sea temperature between 0 and 5m was on average 14.3°C (range: 11–16.8°C) and salinity on average 31.2 ppt (range: 29.7–32.5 ppt) during the study period. Between 5 m and 10 m, temperature was on average 3.3°C cooler than the top layer (range: 8.4–14.4°C) and salinity 32.2 ppt (31.2–32.9 ppt).

2.1 | Data handling and statistical analyses

The abundance of lice stages and their attachment location preference on the host were examined through a series of directed comparisons, either across both time points (mean abundance, sex ratio and attachment location) or between times (repeatability of lice abundance within a host).

To assess repeatability of lice infestation density of a given fish between Samples 1 and 2, lice counts at Sample 2 were regressed against lice counts at Sample 1 using the *lm()* function in R. Fish were treated as replicates. A cage ID factor (3 levels) was also tested, but did not explain a significant amount of variation (p = .3) and was therefore omitted from the final model. Sex ratios were tested for deviation from 50:50 using a Wilcoxon signed-rank test (wilcox.test function in R). The null hypothesis was 50%, with a 2-sided test.

Dorsal–ventral and left–right preferences in attachment locations were tested using a chi-square test of proportions (H_0 = equal numbers). To test whether dorsal–ventral and left–right preference varied according to lice stage, we fitted binomial generalized linear mixed models (GLMMs) to binary response data describing attachment location (2 models: dorsal versus. ventral; left versus. right side). Lice were treated as replicates. Both models were fitted using the *glmmTMB* package for R (Brooks et al., 2017), and included fixed terms for lice stage (5 levels: *L. salmonis* stages chalimus II, pre-adult, adult male, adult female, or *Caligus elongatus*), sample (two levels), cage ID (three levels), and a random intercept term for fish ID. Type II analysis of deviance tables was computed using the *ANOVA()* function in the *car* package for R (Fox & Weisberg, 2011). Lice found on the head were omitted from the dorsal–ventral model (remaining: 2,431 lice on 330 fish), while lice found on the dorsal or ventral midline were omitted from the left–right model (remaining: 2,627 lice on 331 fish).

3 | RESULTS

3.1 | Abundance

Overall, fish had a mean infestation density of 5.9 lice per fish at Sample 1 and 5.0 lice per fish at Sample 2. Abundance of male lice halved between Samples 1 and 2, from 2.0 to 0.9 lice per fish. Abundance of adult female lice (including those with or without egg strings) was similar between Samples 1 and 2 (1.1 lice per fish at both samples), suggesting that any adult females lost between samples were replaced by maturation of pre-adult females. Of all of the lice that were recorded at both samples, 17% of pre-adults, 9% of adult males and 5% of adult females were dislocated from the host and found in the sedation vessel.

3.2 | Sex ratios

Of the 319 fish that had adult lice attached at Sample 1, 70% had adult females present. 34% of adult lice were female (significant deviation from 50:50 sex ratio: Wilcoxon V = 6,187, p < .0001). At Sample 2, only 236 fish had adult lice attached. 75% of fish had adult female lice present, with females making up 52% of the adult lice population (no significant deviation from 50:50 sex ratio: V = 9,375, p = .6). Across Samples 1 and 2, 42% of adult lice were female.

3.3 | Repeatability of lice abundance within fish

A total of 265 fish were assessed at both the sample points; of these, the difference in total lice abundance between the two sample points varied between a loss of 11 lice and a gain of 26. Within fish, infestation density at Sample 1 predicted infestation density at Sample 2 significantly (t_{331} = 2.3, p = .02) but weakly (adjusted R^2 = .01; Figure 3). In other words, the number of lice on a fish at Sample 1 was not a reliable indicator of the likely number of lice on the same fish at Sample 2. Almost equal proportions of the group gained lice (47%) or lost lice (40%), with 75% of the group recording a difference between −4 and 4 lice. Differences in lice loads between Samples 1 and 2 approximately fitted a normal distribution (Figure 4), with most fish experiencing only a small change in lice load, while some fish experienced a very large change (i.e. gain or loss of >10 lice between Samples 1 and 2).

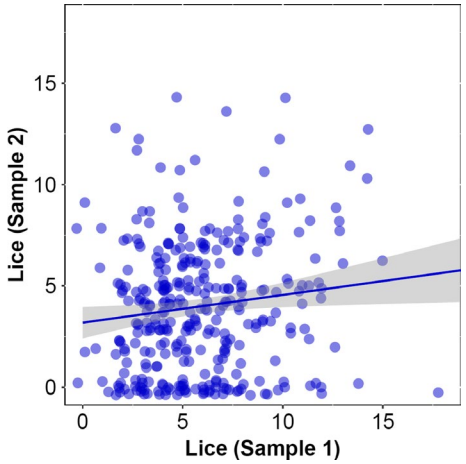

FIGURE 3 Repeatability of lice counts on individual fish between pre- and post-trial samples. New infestations are ignored (i.e. sessile lice stages) in the post-trial sample. Outlier fish with >18 lice at either sample (n = 25) are omitted from the plot, but were included in the statistical analysis. Points are "jittered" to improve visibility of overlapping values [Colour figure can be viewed at wileyonlinelibrary.com]

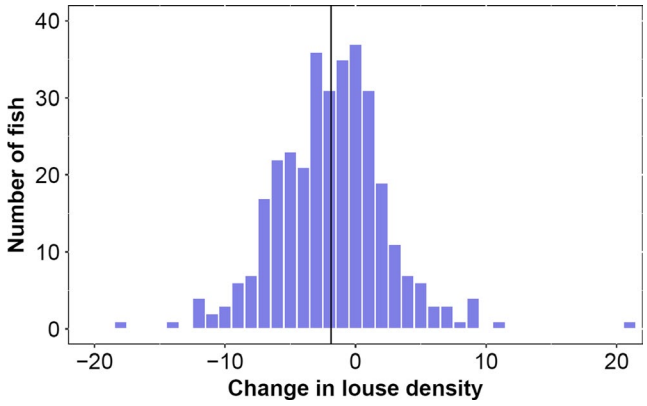

FIGURE 4 Histogram of changes in lice abundance (per fish) between Sample 1 and Sample 2. Vertical line indicates mean change [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Attachment location

Lice abundance varied considerably across quadrants, with lice being most abundant on the left side of the head and the posterior dorsal midline (Figure 5). Adult females, with or without egg strings, showed an especially strong preference for the head (53% were on the head compared to 26% of pre-adults and adult males: Table 1). Among lice that were not attached to the host's head, the dorsal surface was preferred despite a similar available surface area on the ventral half (Table 1). This pattern held for Samples 1 and 2, but was significantly influenced by louse stage (Table 2): 57% of pre-adults and adult males were found in the dorsal half of the body compared with only 27% of adult female lice, corresponding to a stronger preference for the head among adult females (Table 1). There was a slight overall preference for the left side of the fish (41%) compared to the right (36%) or midline (24%) (Table 3). Left–right preference did not depend on louse stage and did not vary significantly between Samples 1 and 2 (Table 2).

4 | DISCUSSION

4.1 | Repeatability of lice abundance

Total louse abundance on a given host was not similar between the two sample points, indicating a large effect of either host transfer, intrinsic mortality, handling, new infections or recruitment from nearby cages or farms. The repeatability of lice abundance, as recorded in this study, reflects the synergistic effect of multiple influences on infection levels and suggests that at least in a cage environment with multiple samplings, there is little reliability in using observed abundances in predicting levels for the near future. Although sources for change in lice abundance within hosts could not have been accurately determined here, we explore potential mechanisms that have been described in existing literature on *L. salmonis* population dynamics.

With the relatively warm sea temperature recorded during the observation period, new infections of salmon lice could contribute to the population quantified at Sample 2. At the average temperature across the 15 days (14°C), new infections within 2–4 days of Sample 1 are likely to represent the pre-adult counts at Sample 2, and all pre-adults would have moulted into adults during that time (Hamre et al., 2019). This shifting demographic creates difficulty in predicting future abundances, as the mortality or transfer rate of mobile lice is unknown. However, with assumed replenishment of the lice population with new infections, the change in lice abundance is likely an underestimation of the true loss of lice within a host.

Host transfer is a possible mechanism for the large variation in lice number between sample points. Across all salmon, there were a total of 377 adult females at the start of the study period and 380 at the end, indicating little change in total adult female abundance or replenishment through development of pre-adult females. However, for adult males, there was a substantial reduction from 654 to 299 over the experimental period, on the same host salmon. This is consistent with high levels of movement between hosts described by Connors, Lagasse, and Dill (2011), whereby 45% of adult males exhibited host transfer in a tank environment. This suggests that males are more likely to seek other hosts to improve their exposure rate to new, reproductively active females once they have interacted with females on the current host (Todd et al., 2005). Stephenson, (2012) recorded approximately 50%–70% of adult males and females transferred hosts within 4 days, both in sea cages and in tanks. Not only are mobile lice reasonably flexible in their movement between hosts in a spatial unit such as a sea cage, but Ritchie, (1997) also reported immigration of lice from neighbouring cages. The open cage environment of the study site used here included high densities of hosts with some infection level in neighbouring cages, which could provide a source for immigration; on the other hand, free-swimming males would have had a lower

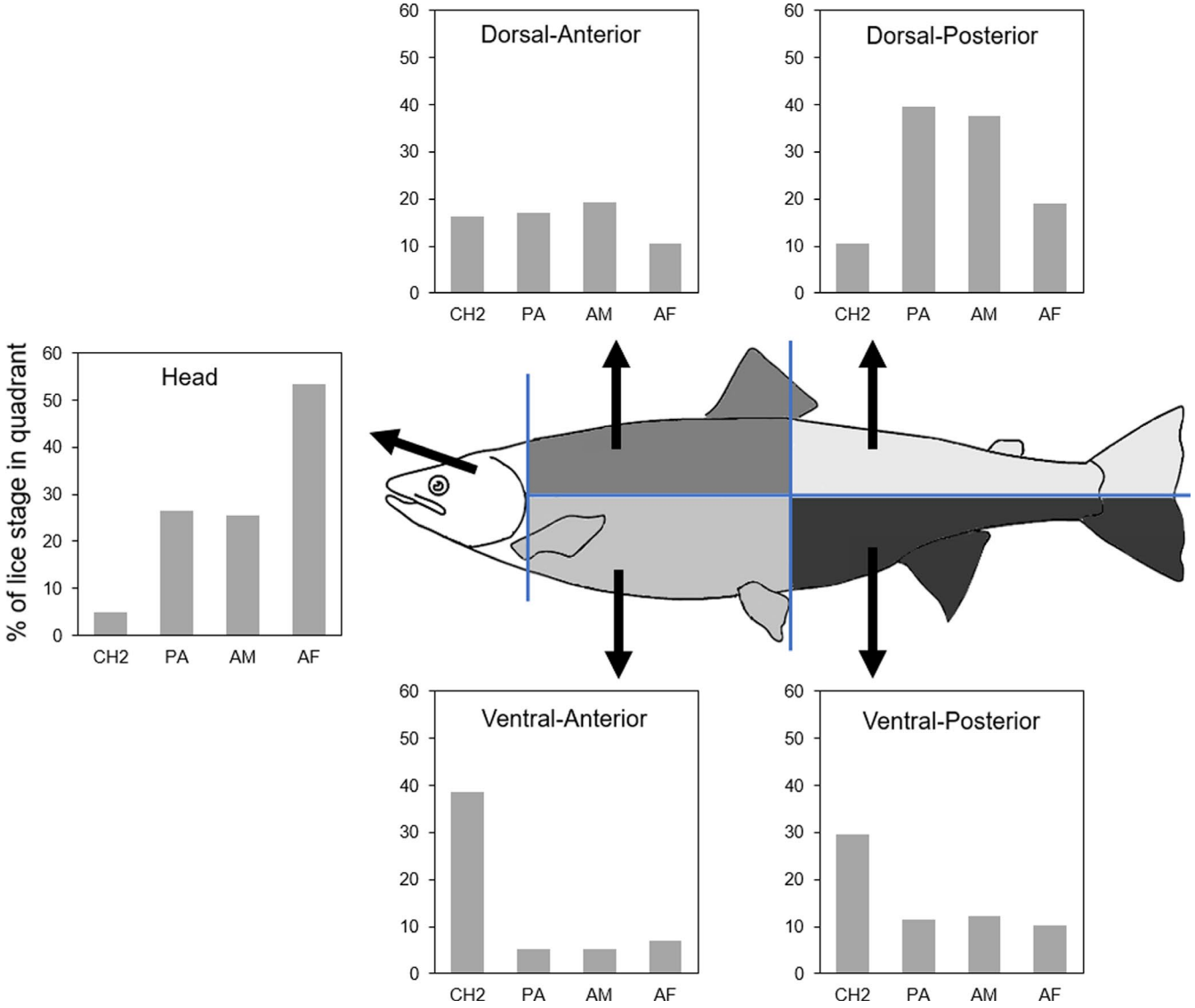

FIGURE 5 Distribution of lice stages on the body of host salmon. Bars represent the percentage of lice of a given stage that occurred in the corresponding quadrant (percentages for each stage across all five quadrants sum to 100%). Stages shown are chalimus II (CH2), combined pre-adults 1 and 2 of both sexes (PA), adult males (AM) and adult females (AF). Data are pooled across Samples 1 and 2 and are irrespective of side (left, right, midline). Quadrants were defined as head (delineated at the opercula), dorsal-anterior section (including the dorsal fin), dorsal-posterior section (including the adipose fin and dorsal part of the caudal fin), ventral-anterior section (including the pectoral and pelvic fins) and ventral-posterior section (including the anal fin and ventral part of the caudal fin) [Colour figure can be viewed at wileyonlinelibrary.com]

probability of encountering and attaching to a new host before being exported from the cage due to natural current velocities at the site. Commercial sites with multiple net pens, holding tens of thousands of hosts, would have a substantially different host-transfer dynamic within the site and between other nearby farms.

The reduction of lice numbers between sample points could be partially due to natural mortality; natural loss of mobile lice stages is not uncommon, even in tank environments which are relatively contained. In communal and single fish tanks, between 36% and 51% of mobile lice can be lost over 24 days (Hamre & Nilsen, 2011). Interestingly, farmed strains of salmon lost 27%–32% of attached lice over 34 days in tanks; however, this was not the case with wild and landlocked Atlantic salmon in the same tanks (Bui, Dalvin, et al., 2018), suggesting a genetic effect on lice developmental success or movement between hosts. However, experience in unspecific loss of lice over development and between replicate tanks has been reported as quite high and very variable (Bjørn & Finstad, 1998; Hamre, Glover, & Nilsen, 2009), but when infested fish are housed in an very controlled manner, variation and loss of lice can be almost eliminated (S. Dalvin, unpublished data). Natural loss of lice could be more prevalent at sites with unfavourable environmental conditions for the lice, such as consistent brackish layers (increasing exposure to low-salinity water; Sievers, Oppedal, Ditria, & Wright, 2019).

The reduced lice abundance in Sample 2 could be driven by fish handling during capture and counting, rather than natural attrition.

TABLE 1 Dorsal–ventral distribution of lice stages for pre- and post-trial samples. $p < .05$ indicates significant deviation from 50:50 dorsal:ventral distribution

Stage	Sample	Head	%	Dorsal body	%	Ventral body	%	Total	*p*
All stages	1	553	28	970	50	426	22	1,949	<.0001
	2	456	31	642	43	393	26	1,491	
	Combined	1,009	29	1612	47	819	24	3,440	
Chalimus II	1	3	3	41	37	66	60	110	
	2	18	6	74	23	226	71	318	
	Combined	21	5	115	27	292	68	428	
Pre-adult	1	209	27	424	54	153	19	786	<.0001
	2	140	26	322	61	69	13	531	
	Combined	349	26	746	57	222	17	1,317	
Adult male	1	160	24	375	57	119	18	654	<.0001
	2	77	28	153	56	42	15	272	
	Combined	237	26	528	57	161	17	926	
Adult female	1	177	47	126	33	74	20	377	.002
	2	218	60	92	25	52	14	362	
	Combined	395	53	218	29	126	17	739	

Notably, some individual salmon lost >10 mobile lice over the 15-day trial—this is unlikely to be due to natural attrition (Figure 4). The choice of capture method when collecting subsamples of fish to assess infestation status has a large impact on observed lice abundance (Holst, Nilsen, Hodneland, & Nylund, 1993; Nagasawa, 1985; Tully, Gargan, Poole, & Whelan, 1999). In this study, fish were gently crowded in the cage and hand-netted, which is likely to result in increased rubbing and abrasion against the net before transfer into the sedation vessel. Previous work indicates that immobilizing fish using sedatives reduces lice loss during handling compared to mechanical euthanasia (Copley, O'Donohoe, McGrath, & Jackson, 2005; Jackson & Minchin, 1993). Depending on the sampling conditions and procedure, up to 68% of mobile lice can be displaced from the host during handling and therefore were found in the sedation or anaesthesia bucket (Table 4; Berntsen et al., 2018). This occurs with a variety of sedative compounds (personal observation S. Bui). In fact, Copley et al. (2005) reported that 71% of pre-adult male lice were found in the sedation vessel on one sampling occasion, with lice of all mobile stages also found displaced from the host. In comparison, the present study found only 5%–17% of mobile lice where dislodged, likely due to the use of individual sedation buckets where lice cannot experience mechanical disturbance from other salmon hosts.

4.2 | Sex ratios

Females were underrepresented among adult lice at Sample 1 (37% of adult lice). However, the decline in total abundance at Sample 2 was almost entirely due to loss of male lice, bringing the female prevalence up to 56% of adult lice. This follows previous reports of higher ratios of male to female lice on farmed hosts (Bron, Sommerville, Wootten, & Rae, 1993; Todd et al., 2000), whereas females have been found predominant (up to 72%) on wild hosts (Copley et al., 2005; Jacobsen & Gaard, 1997; Todd et al., 2000). The question of why adult male lice are more prevalent on farmed salmon has received little attention since those early reports; previous hypotheses attributed this observation to the faster developmental rate of male lice (Hamre et al., 2019), sex-biased impact of delousing therapeutants (specifically, to dichlorvos; Jaworski & Holm, 1992) or greater mobility of male versus female lice between hosts (e.g. Stephenson, 2012).

An alternative mechanism for biased sex ratios could be differential effects of host handling on males versus females (see below for further discussion of handling effects). Females are larger than males at all mobile stages (Johnson & Albright, 1991b) and therefore may be more robust to mechanical disturbances. If so, male lice would be at higher risk of detachment during fish handling. Two studies conducted at commercial sites (14–39 sample dates with similar sampling procedures for each) reported that 41%–76% of adult lice were found in the sedation vessel (Table 4). Adult males were 17% (Site 1) and 22% (Site 2) more likely to be found in the sedation vessel than females (Bui, Stien, Nilsson, Trengereid, & Oppedal, 2020; Geitung et al., 2019); in the present study, adult males were 91% more likely. The likelihood of lice being detached during handling is likely to be influenced by the sedation or euthanasia protocol—the fish sampled at the above commercial sites were quickly killed, probably reducing mechanical stress on attached lice relative to the present study, where fish were sedated individually in smaller buckets.

4.3 | Preference of lice attachment locations on the host

The mobile nature of pre-adult and adult lice stages results in a distribution around the host body that varies according to environmental

TABLE 2 Summary of fitted GLMMs. Tests are Wald's chi-square (type II) performed using the car package for R

Model 1: Effect of louse stage on dorsal–ventral preference (binary response)			
BodyDV ~ Stage +Sample + CageID + (1|FishID)			
	χ^2	*df*	*p*
Stage	271	4	<.0001***
Sample	3.0	1	.08·
CageN	4.8	1	.03*
Stage:CageN	19.7	4	.0006***
Split by CageN:			
BodyDV ~ Stage +Sample + (1|FishID)			
	χ^2	*df*	*p*
Cage 1			
Stage	143	4	<.0001***
Sample	0.3	1	.58
Cage 2			
Stage	70	4	<.0001***
Sample	3.9	1	.049*
Cage 3			
Stage	75	4	<.0001***
Sample	2.1	1	.15
Model 2: Effect of louse stage on left–right preference (binary response)			
Side ~ Stage +Sample + CageN + (1|FishID)			
	χ^2	*df*	*p*
Stage	0.80	4	.94
Sample	1.0	1	.31
CageN	3.0	1	.09·

TABLE 3 Left–right distribution of attached lice stages for pre- and post-trial samples. $p < .05$ indicates significant deviation from 50:50 left:right distribution

Stage	Sample	Left	%	Midline	%	Right	%	Total	*p*
All stages	1	757	39	504	26	688	35	1,949	.0006
	2	645	43	309	21	537	36	1,491	
	Combined	1,402	41	813	24	1,225	36	3,440	
Pre-adult	1	297	38	216	27	273	35	786	.14
	2	205	39	144	27	182	34	531	
	Combined	502	38	360	27	455	35	1,317	
Adult male	1	245	37	192	29	217	33	654	.08
	2	100	37	90	33	82	30	272	
	Combined	345	37	282	30	299	32	926	
Adult female	1	151	40	84	22	142	38	377	.08
	2	165	46	66	18	131	36	362	
	Combined	316	43	150	20	273	37	739	

TABLE 4 Total lice found across all sample points in the study, and the proportion of those lice recorded that were found dislodged from the host into the sedation vessel. Mobile stages are represented (PA = pre-adult, A = adult) with sexes (*M* = male, *F* = female) differentiated. Note that the present study did not distinguish between pre-adults 1 and 2, or between sexes; all pre-adults are pooled

	PA1	PA2 M	PA2 F	AM	AF	Mobile lice pooled[c]	Ratio AM:AF[d]
Site 1[a]							
Total lice found	1,229	681	409	823	852	3,994	1.17
% found in sedation vessel	58	78	73	76	66	68	
Site 2[b]							
Total lice found	1,760	985	978	1761	1,049	6,553	1.22
% found in sedation vessel	32	39	48	50	41	42	
This study							
Total lice found	639			299	380	1,318	1.90
% found in sedation vessel	17			9	5	12	

[a]Site 1 was located in the Rogaland District of south-western Norway and contained 6 cages that were sampled 39 times over 12 months (2016–2017). At every sample, each cage had 20 fish collected and transferred into a single sedation vessel (one per cage), before lice assessment.

[b]Site 2 was located in the Rogaland District of south-western Norway and contained 12 cages that were sampled 14 times over 12 months (2016–2017). At every sample, each cage had 20 fish collected and transferred into a single sedation vessel (one per cage), before lice assessment.

[c]The total number recorded of pre-adults 1 and 2, and adult stages of both sexes.

[d]Ratio of the proportion of adult male and adult female lice found in the sedation vessel

Note that the present study did not distinguish between pre-adults 1 and 2, or between sexes; all pre-adults are pooled (grey shaded values).

factors and the presence of conspecifics. This study is among the first to describe this behaviour in a sea cage rather than tank setting and therefore reflects responses to complex environmental conditions experienced in the field.

Initial settlement location is influenced by host swimming behaviour (Bui, Oppedal, Samsing, & Dempster, 2018b) and velocity (Genna et al., 2005), as well as light conditions (Genna et al., 2005; Hamoutene et al., 2016). Here, chalimus II stages were most abundant in the ventral-anterior quadrant, consistent with tank studies finding that newly attached lice are most abundant on the fins and ventral aspect of Atlantic salmon (Bron, Sommerville, Jones, & Rae, 1991; Bui, Oppedal, et al., 2018; Genna et al., 2005; Samsing et al., 2015) and sea trout (Bjørn & Finstad, 1998).

Mobile stages were most densely aggregated on the head and dorso-posterior sections of the host in this study, indicating that these areas are optimal for fitness under the prevailing conditions. Interestingly, the distributions observed in this study are almost exactly opposite to those reported by Todd et al. (2000). That study also described a significant difference between distribution of adult males and females on wild Atlantic salmon hosts, but with favourable attachment locations for females being the areas adjacent and posterior to the anal fin, and the males predominating the head and anterior dorsal midline. Jackson and Minchin (1993) similarly noted a more posterior-preferred distribution of adult females on wild salmonids, whereas adult males and pre-adults of both sexes were found more uniformly across the body. This contrasts to observations on farmed Atlantic salmon held in sea cages (both in this study and anecdotally), where there was an equal preference for the head compared to the posterior region of the host (Wootten, Smith, & Needham, 1982), albeit dependent on louse stage and sex. Jaworski and Holm (1992) reported that 42%–81% of adult females were found solely in the post-anal body region on salmon held in sea cages, whereas the largest proportion (54%) were found on the head in this study. Females are thought to situate themselves to exploit areas of optimal resources and maximize hydrodynamic streamlining due to their larger body size (although this was suggested for the posterior preference of females on wild salmon; Todd et al., 2000).

Differences between farmed and wild salmon may be vast from a parasitic perspective, and variable distribution preferences may also be influenced by host-specific traits such as mucosal thickness (Pittmann 2013). On a population level, differences over time and between wild and farmed environments may indicate evolution of preferences in response to novel conditions at salmon farms. In Norway, the vast majority of salmonid hosts are contained within farms. Farm-specific conditions include (a) a very high density of hosts, likely reducing the fitness cost of detaching by increasing the odds of finding a new host with new mates, (b) circular, schooling swimming behaviour of the host fish and (c) threat of removal by cleaner fish and lasers. Any of the above may affect the fitness outcomes resulting from specific attachment locations and host-switching behaviour for lice.

Previous studies have not highlighted lateral asymmetry in attachment preferences in lice; however, in this cohort there was a greater abundance of lice found on the left side of the host. This preference was also not specific for any developmental stages, indicating that the benefits of being situated on the left side are likely equal across stage and sex. As Atlantic salmon are essential bilaterally symmetrical, competition or attraction with conspecifics could drive this preference, but we consider it more likely due to site-specific environmental factors, whereby the exposed outer side of a salmon is more affected by hydrodynamic forces or potential scraping against the cage net. It is interesting to note that in this study, fish were not continuously schooling tightly as is often observed in

salmon aquaculture (Oppedal, Dempster, Stien, 2011), but rather that individuals swam in varied directions in a loose shoal, which is often observed when group size is small or just after sea transfer (e.g. Glaropoulos, Stien, Folkedal, Dempster, & Oppedal, 2019). The lateral preference observed here should be explored across a range of hosts and environmental conditions to discover whether this pattern is generalizable and, if so, elucidate the drivers of such a preference. This will be particularly important for in situ lice assessments using camera technologies that will typically image hosts from one side only. Users of these systems will need to predict or track any asymmetrical distribution in order to estimate cage lice abundances with confidence.

5 | CONCLUSIONS

Innovative techniques to prevent, monitor and remove sea lice infestations on farmed salmon can benefit from detailed information on the biology and behaviour of hosts and parasites. Here, we described how the spatial distribution of lice on farmed salmon varies over time and according to the developmental stage and sex of the louse, at a single research site. Further investigation to map these preferences with wider variation in environmental conditions and host characteristics is required to further validate these results, particularly as lice are so mobile across the host's surface and development is temperature-dependent. Confident use of image-based automated lice detection systems will depend on an understanding of how lice are distributed on the host's body (including areas that are not imaged) and how this behaviour changes with season, host size and health status, parasite density and environmental conditions. With the use of cleaner fish and mechanical or optical delousers, there may be behavioural plasticity or adaption in attachment location preferences to persist on farmed hosts. These details are particularly significant if legislation shifts to the use of automated systems in place of the current physical inspections, where validation across these different biotic and abiotic conditions would be essential.

ETHICS STATEMENT

This study adhered to the Norwegian regulations for animal experimentation, as assessed by the Norwegian Food Safety Authority (application ID: 16289).

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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