

Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada

Alexandra Morton, Richard Routledge, Corey Peet, and Aleria Ladwig

Abstract: This study compared sea lice (*Lepeophtheirus salmonis*) infestation rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in five nearshore areas of the British Columbia coast selected on the basis of proximity to salmon farms. A 10-week study in the Broughton Archipelago found sea lice were 8.8 times more abundant on wild fish near farms holding adult salmon and 5.0 times more abundant on wild fish near farms holding smolts than in areas distant from salmon farms. We found that 90% of juvenile pink and chum salmon sampled near salmon farms in the Broughton Archipelago were infected with more than 1.6 lice·(g host mass)⁻¹, a proposed lethal limit when the lice reach mobile stages. Sea lice abundance was near zero in all areas without salmon farms. Salinity and temperature differences could not account for the higher infestation rates near the fish farms. The most immature life stages dominated the lice population throughout the study, suggesting the source of lice was a stationary, local salmonid population. No such wild population could be identified. The evidence from this control–impact study points to a relationship between salmon farms and sea lice on adjacent, wild, juvenile salmon.

Résumé : Notre étude compare les taux d'infestation des poux de mer (*Lepeophtheirus salmonis*) chez le saumon rose (*Oncorhynchus gorbuscha*) et le saumon kéta (*Oncorhynchus keta*) dans cinq régions côtières de la Colombie-Britannique, choisies à cause de la proximité d'élevages de saumons. Une recherche de 10 semaines dans l'archipel de Broughton révèle que les poux de mer sont 8,8 fois plus abondants chez les poissons sauvages à proximité d'élevages contenant des saumons adultes et 5,0 fois plus abondants aux environs d'élevages contenant des saumoneaux que dans les zones situées loin des élevages de saumons. Quatre-vingt-dix pour cent des jeunes saumons roses et kéta échantillonnés près des élevages de saumon dans l'archipel de Broughton portent des infestations supérieures à 1,6 poux·(g de masse de l'hôte)⁻¹, une limite que nous considérons létale lorsque les poux atteignent les stades mobiles. Les densités de poux de mer sont presque nulles dans toutes les régions sans élevage de saumons. Les différences de salinité et de température n'expliquent pas les taux plus élevés d'infestation à proximité des élevages. Ce sont les stades les plus immatures qui dominent dans la population au cours de l'étude, ce qui laisse croire que la source des poux est une population locale et stationnaire de salmonidés. Nous n'avons trouvé aucune population sauvage qui possède de telles caractéristiques. Les données de notre étude de type témoin–impact indiquent qu'il existe une relation entre les élevages de saumon et les poux de mer qui parasitent les jeunes saumons sauvages des régions adjacentes.

[Traduit par la Rédaction]

Introduction

The sea louse *Lepeophtheirus salmonis* is a common salmonid-specific caligid ectoparasite in the Northern Hemisphere. Salmon examined on the high seas are commonly infected with low numbers of adult *L. salmonis* (Nagasawa et

al. 1993). Pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon 20–79 cm (fork length) were the most heavily infected; 91.8% prevalence and 5.83 lice·fish⁻¹ mean intensity for pink salmon and 15.9% prevalence and 2.28 lice·fish⁻¹ mean intensity for chum salmon (Nagasawa 1987; Nagasawa et al. 1993). The scarcity of im-

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A. Morton¹ and C. Peet.² Raincoast Research, Simoom Sound, BC V0P 1S0, Canada.

R. Routledge. Department of Statistics and Actuarial Science, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.

A. Ladwig. Community Advisor for Central Coast, Department of Fisheries and Oceans Canada, P.O. Box 10, Port Hardy, BC V0N 2P0, Canada.

¹Corresponding author (e-mail: wildorca@island.net).

²Present address: University of Victoria, Department of Biology, P.O. Box 3020, Station CSC, Victoria, BC V8W 3N5, Canada.

mature lice on salmon at sea contrasts with the predominantly juvenile lice populations found exclusively on hosts in nearshore, salmon-farmed areas of Ireland and Norway (Tully et al. 1993; Bjørn and Finstad 2002) and is considered evidence of infrequent infection events at sea (Pike and Wadsworth 1999).

Reported epizootics of *L. salmonis* were rare, although noted (White 1940), until acute sea lice infestations were reported on farmed salmon in Norway (Håstein and Bergsjø 1976) and Scotland (Wootten et al. 1982; Stuart 1990). Following these reports, high infestation rates were recorded in wild salmonids near salmon farms in Ireland and Norway (Tully et al. 1993; Birkeland 1996). Today, sea lice epizootics have been reported in most areas where there are large numbers of salmon farms (Anonymous 1997). It is generally accepted that sea lice infestations on farmed salmonids are caused by transfer from wild stocks, because farm salmon enter the marine environment lice free. However, vigorous debate is underway over whether the farms' stationary, high-density host populations in the nearshore marine environment act as a sea lice reservoir capable of reversing the transmission to juvenile wild stocks out-migrating through the zone of influence of farm-origin lice larvae.

Lepeophtheirus salmonis has a direct life cycle of five discrete phases and 10 stages (Johnson and Albright 1991a) that allows the approximate age of individual sea lice to be determined. This is useful in interpreting infective events as discrete or continuous and local or distant. The ability of sea lice to find a host is limited by time and therefore distance. The eggs hatch from gravid females into free-swimming nauplii (Kabata 1972). The interval between hatching (the naupliar stages) and infective capability (the copepodid stage) is approximately 4 days at 10 °C and 2 days at 15 °C (Johnson and Albright 1991b). The free-swimming, infective copepodid stage, during which host attachment must occur or the parasite dies, lasts for 2–8 days at 5–15 °C (Wootten et al. 1982; Johnson and Albright 1991b). Larval lice, which exhibit phototaxis (Johannessen 1978), disperse via oceanic surface currents (Anonymous 1995) and decline in numbers as distance increases from salmon farms (Costelloe et al. 1996).

Once attached to a host, *L. salmonis* remains anchored in one location as it moults through four chalimus stages. Upon entering the next, pre-adult phase, sea lice become mobile (Johnson and Albright 1991a), and this transition marks the onset of host pathogenicity (Grimnes and Jakobsen 1996). Studies on European salmonids (Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), and sea trout (*Salmo trutta*)) suggest that the lethal infection level of post-smolts is between 0.75 and 1.6 mobile lice·(g host mass)⁻¹, mean mass 60–90 g (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997).

A recent series of events on the central coast of British Columbia (B.C.), Canada, bear similarity to European collapses of wild stocks infected with sea lice near salmon farms and served as the impetus for this study. There are 28 Atlantic salmon farm tenures in the Broughton Archipelago. In 2000, there was an exceptionally high spawning escapement of pink salmon into the rivers of the Broughton Archipelago (PFRCC (Pacific Fisheries Resource Conservation Council) 2002), in addition to a commercial catch of

2×10^6 of this stock (Glen Neidrauer, Department of Fisheries and Oceans Patrolman, Simoom Sound, BC V0P 1S0, Canada, personal communication). In June 2001, Morton and Williams (2004) examined the progeny of this stock to record the first known incidence of *L. salmonis* infestation of juvenile pink salmon. Seventy-five percent (mean host mass 2.26 g) were infected with 1.6 lice·(g host mass)⁻¹ or more. When this generation returned to spawn, a 98% spawning escapement collapse, specific only to the rivers in the Broughton Archipelago, was reported (PFRCC 2002). The PFRCC (2002) suggested "causal factors should be sought in the nearshore environment" because no causative freshwater factors could be identified, and the spatial range of this collapse was so localized. While density-dependent effects in the nearshore environment may have contributed to the population decline, the magnitude of reduction between progeny and parental line is well outside the natural high survival variability recorded for this species over the past 50 years (PFRCC 2002).

Pink and chum salmon biology is significantly distinctive among salmonids because they do not rear in fresh water, and this may be a critical factor when attempting to quantify the magnitude of impact by sea lice. Pink salmon enter the marine environment at 3.5 cm (Heard 1991) and chum salmon enter at 4.0 cm (Bax 1983). These are four to five times smaller than the Atlantic salmonids (Scott and Crossman 1973). Because sea lice impact is host size dependent (i.e., the smaller the fish, the fewer lice required to induce negative effects) (Bjørn and Finstad 1997), it is possible that pink and chum salmon may be more sensitive to sea lice infection than the larger juvenile salmonid species found in Europe. Pink salmon have a fixed 2-year cycle (Heard 1991), while chum salmon in B.C. return at 3, 4, and 5 years old (Salo 1991).

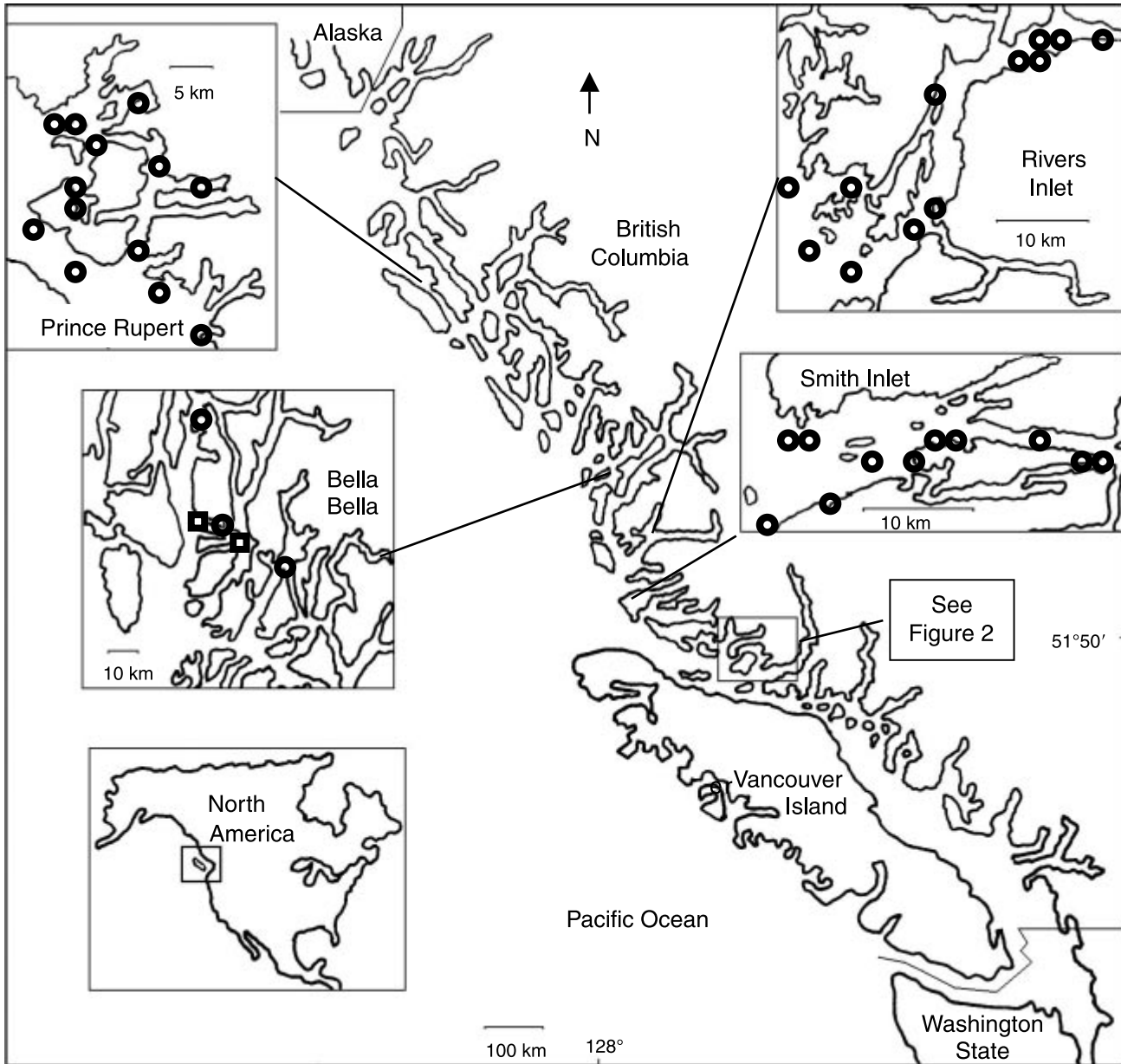
Sea lice infestation intensities show considerable geographic and temporal variability (Boxaspen 1997). To date, no studies have been conducted in B.C. to examine the variability of louse infection intensities across a geographic range.

The object of this study was to compare sea lice infestation rates on wild out-migrating juvenile salmon at locations near to and distant from salmon farms and over time. This was done both within the Broughton Archipelago and less intensively at intervals to the north, well beyond the range of larval lice produced from any salmon farm. We compared the incidence, age, and species of sea lice populations found on juvenile pink and chum salmon among regions of coastal B.C. waters and variation with respect to proximity to salmon farms.

Methods

Juvenile pink and (or) chum salmon were sampled from five areas of the B.C. coast in the spring of 2002 (Fig. 1). In the Broughton Archipelago, up to 20 juvenile pink and (or) chum salmon were collected once a week for 10 weeks from six sites and twice only from an additional site beginning on 16 April for a total of 1072 fish. A dip net (45 cm diameter of 5-mm knotless mesh) on a 2.45-m pole (Bailey et al. 1975) was used for collection. Sites 1, 2, and 3 were control sites: distant from salmon farms but comparable (possessed similar physical and oceanographic conditions) with the ex-

Fig. 1. Map of the coast of British Columbia, Canada, showing all five areas where juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon were sampled and the location of the general area within North America. Collection sites are indicated by a circle, and salmon farms outside the Broughton Archipelago are indicated by a square.



posed categories (smolt and grower) except for proximity to farms. No samples were taken, for example, from brackish water; salinity levels remained above 12‰. Sites 4, 5, and 6 were within 250 m of an active Atlantic salmon farm stocked with growers (i.e., fish in their second year in seawater). Site 7 was within 250 m of a farm stocked with smolts (i.e., salmon in their first year in salt water) and was only sampled twice (Fig. 2).

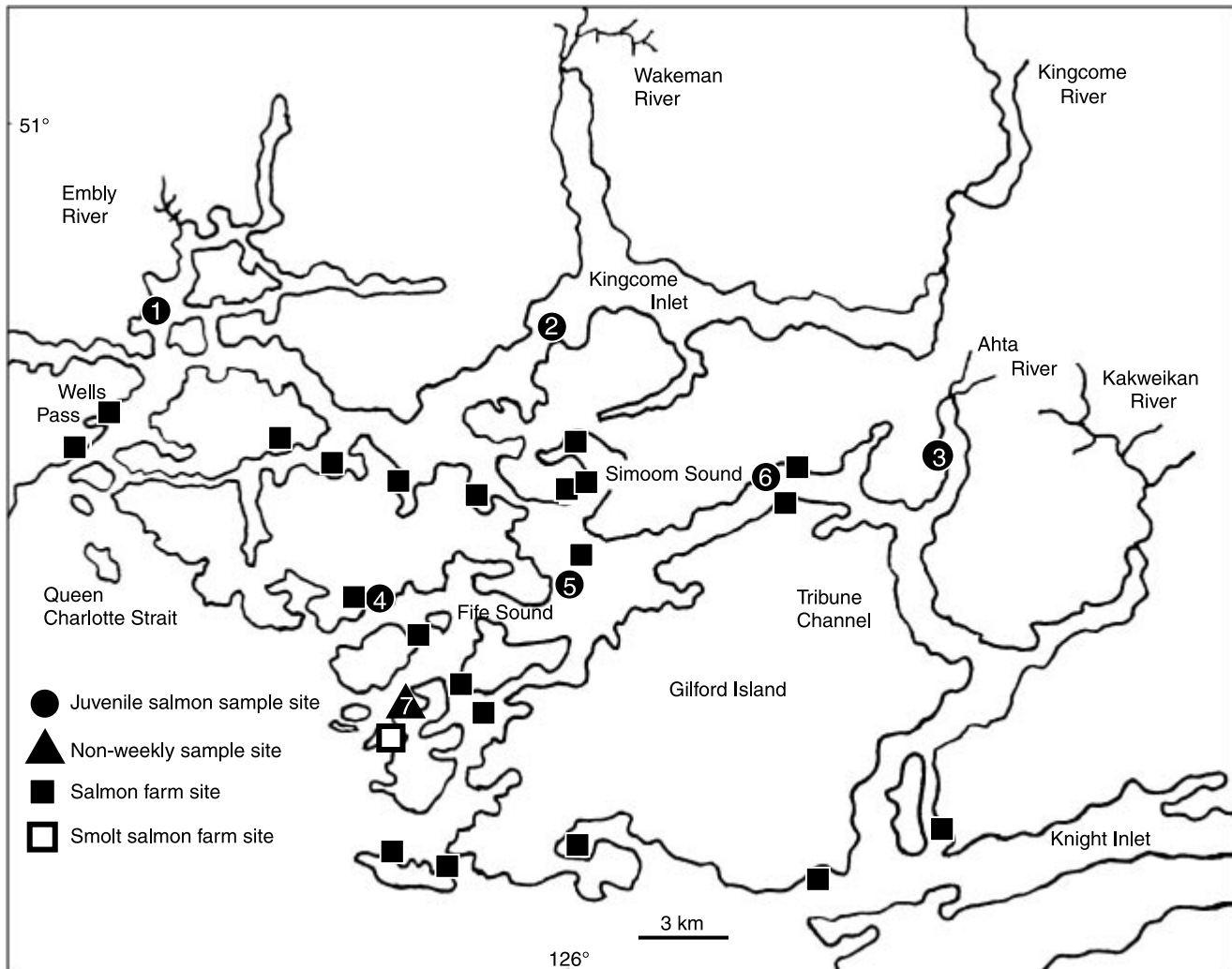
Seventy-five kilometres to the north, 48 juvenile pink salmon were collected on 6 June and 3 July from 11 sites in Smith Inlet. Ninety kilometres north of the Broughton Archipelago, 250 juvenile pink salmon were collected on 10 dates from 13 May through 4 July from 12 sites in Rivers Inlet (Fig. 1). At both of these sites, a 300-m-long purse seine, 30 m deep with 6-mm mesh, was deployed close to shore

from a 15-m seine boat. The net was held in the water and the fish removed individually with a dip net fitted with knotless Marquisette mesh to minimize scale and lice loss.

One hundred eighty kilometres north of the Broughton Archipelago, 154 juvenile pink and chum salmon were collected from three nearshore sites on 27 June through 2 July in the Bella Bella area (Fig. 1) using a dip net (45 cm diameter with 5-mm mesh) on a 2.45-m pole from a shallow draft 4-m boat. As the juvenile salmon out-migration season was nearly completed, sites were selected based on the availability of out-migrating smolts using the following criteria: at least 1 km away from a major freshwater source and close to shore. A maximum of 60 fish were collected from each site.

Four hundred kilometres north of the Broughton Archipelago off Prince Rupert, 566 pink and chum salmon were col-

Fig. 2. Map of the seven sites sampled in the Broughton Archipelago over the 10-week period. Sites 1, 2, and 3 were control sites (distant from salmon farms); sites 4, 5, and 6 were exposed to salmon farms stocked with growers (second year in salt water); and site 7 was exposed to a farm stocked with smolts (first year in salt water) and was only sampled twice during the study.



lected using a 30.5-m-long, 2-m-deep beach seine with 0.3-cm mesh in the bunt and 0.6-cm mesh on the wings and a 13-cm-diameter dip net on a 31-cm pole on 11 dates from 6 May through 2 June from 13 sites (Fig. 1). Over 400 km of shoreline was surveyed and up to 25 juvenile pink and or chum salmon collected at each site where fish were encountered.

At all sites, other than Rivers Inlet and Smith Inlet, fish were placed individually in Whirlpak™ bags immediately after capture, placed on ice, and frozen shortly thereafter. At Rivers Inlet and Smith Inlet, samples were preserved in ethanol in jars. Later, fish were weighed, measured (fork length), and the number and species of sea lice recorded using a $\times 30$ magnification stereoscope.

Sea lice were categorized by species, sex, and life history stage (Johnson and Albright 1991a). *Caligus* and *Lepeophtheirus* chalimus stage lice were distinguished from each other using Piasecki (1996) and Johnson and Albright (1991a).

The measures of lice infestation rates are defined as follows. Prevalence, usually expressed as a percentage, is the

number of individual hosts infected with lice. Intensity is the number of lice on each infected host. Abundance is the total number of lice divided by the total number of hosts (infected and uninfected) (Margolis et al. 1982).

The null hypothesis of primary concern was that the average abundance of sea lice did not depend on proximity of a sampling site to a fish farm or on its geographic area. Choosing the appropriate statistical method to test that hypothesis was a complex task. The analysis also had to account for (and assess) possible differences over time and potential confounding effects that are unavoidably present in any control–impact study. Here, salinity and temperature were of primary concern. Potential differences between host species (pink vs. chum salmon) also needed to be included in the analysis. Furthermore, with count data, the variance usually increases with the mean. Hence, the analysis had to be able to handle multiple factors, some categorical (e.g., geographic area) and others continuous (e.g., salinity and temperature), and to handle heteroscedasticity. In addition, so that inferences on the impacts of other factors would not be limited to the particular sites sampled, these had to be

viewed as random. This also implied that any site-week interactions be treated as random.

Where appropriate, we chose the now standard method of loglinear modeling for handling count data with mixed (fixed and random) effects (McCulloch and Searle 2001), analyzed using the SAS macro, GLIMMIX. The response variable was the number of lice per fish. For *L. salmonis*, we were able to simplify the analysis by focusing on the Broughton Archipelago, the only area with significant louse abundance. We first fit a full model with all potentially relevant factors as listed above. Then for subsequent analyses, we dropped those factors that were not significant at the conventional level $p = 0.05$. The tests were conducted by examining the ability of each variable in turn to explain further variation in the data with each of the other variables in the model. The p value for salinity, for example, assessed the existence of any remaining relationship between salinity and lice abundance after the influence of all other factors was accounted for. The reasoning is analogous to that used in multiple regression modeling (Kleinbaum et al. 1998). We also calculated denominator degrees of freedom with a Satterthwaite approximation (using the SAS option, `ddfm = satterth`). The resulting degrees of freedom were not always whole numbers.

We checked for autocorrelation between successive weeks at a single site (positive autocorrelation being of particular concern in that it could lead to inflated significance). Since the lag-1 autocorrelation estimate was not even positive ($r = -0.21$) and not statistically significant ($t = -1.77$, $p = 0.084$), autocorrelation was not included in any of the subsequent statistical analyses.

Multiple comparisons were made with conservative adjustments for the number of comparisons based on Bonferroni's inequality (Miller 1981). The experiment-wise significance level for these tests was set to 5%. These analyses were then repeated for *L. salmonis* adults and then again for juveniles. For the adults, however, there were very low abundances (lice per fish) at the start of the sampling period. Although this is a valuable observation, it created a situation where the nonzero counts were too sparse for us to trust the theoretical approximations underlying the GLIMMIX algorithms and output. We therefore chose to analyze these counts with the more traditional (e.g., Steel and Torrie 1980) yet conceptually similar expedient of transforming the counts (y) to $\log_e(y + 0.5)$ and then applying a linear model. Because sites were still to be viewed as random, we used the SAS procedure MIXED for this analysis.

The *Caligus* counts were, by contrast, not restricted so intensively to the Broughton Archipelago, nor were they anywhere as abundant. Hence, we were again faced with sparse nonzero counts. To promote the accuracy of the theoretical approximations, we made two alterations to the modeling strategy. We performed the more traditional analysis described above, with a further a priori exclusion of all but the essential terms in the model for testing for differences in mean abundances among exposure categories and geographic areas. The exclusion of these other terms, while necessary to promote the validity of the theoretical approximations, will have potentially inflated the amount of unexplained variation. This in turn will have made it more difficult to detect the influence of the remaining factors in the model. Hence,

the resulting tests of significance should be viewed as conservative.

As there were no funds dedicated solely to this study, we opportunistically shared juvenile salmon sampled by other research projects outside the Broughton Archipelago. While this allowed for a quick response to the question "How do lice loads on juvenile Pacific salmonids compare coastwide?", it meant we did not control collection methods. Each method used in this study has advantages and each introduces a form of bias.

Dip-netting subjects fish to the minimum duration in a net and therefore may be least likely to remove lice or scales. Dip nets successfully capture juvenile pink salmon because at this life stage, these fish typically remain in shallow water (Healey 1980) where they occupy only the top few centimetres of the water column (Parker 1965). As chum salmon were equally abundant in our samples, they appeared to behave similarly, at least while schooled with pink salmon.

Dip-netting could select for weaker fish; however, any such sampling bias would have been consistent over all sites throughout the 10 weeks of the Broughton study, the Bella Bella samples, and most of the Prince Rupert samples.

The seine net used in Smith and Rivers inlets could remove scales and lice if hauled aboard, but the net was held in the water against the boat and the fish removed individually with a knotless mesh dip net. The seine also introduces unknown potential for selectivity that differs from the dip net. Once the fish were trapped in the bunt, there was no escape, but before that time escape was possible, particularly on sets where tidal turbulence, gusty winds, etc., caused difficulties, and some fish almost surely did escape. However, one would anticipate that the vigorous fish would be most likely to escape and that the more sluggish, lice-infested fish would be more likely to be caught. Hence, sampling bias appears not to be a reasonable explanation for the near-zero lice infestation rates in these samples.

Results

We collected a total of 2090 fish and all were examined for lice. The mean fork lengths and masses were smallest in Bella Bella, nearly identical between Prince Rupert and the Broughton Archipelago, and largest in Rivers and Smith inlets (Table 1). The average mass of the pink and chum salmon we captured in the first 3 weeks in the Broughton Archipelago was 0.33 g. Mean salinity values were recorded (Table 1). Average temperature for the Broughton Archipelago was 10.4 °C.

Lepeophtheirus salmonis was rare in our samples outside the Broughton Archipelago: three lice total on 1018 juvenile pink and chum salmon (Fig. 3, Table 2). By contrast, 4338 *L. salmonis* individuals were found on 1138 juvenile pink and chum salmon sampled from the Broughton Archipelago (an additional 364 lice were *Caligus*). Over the 10-week study period, the overall abundance of *L. salmonis* increased to a peak in week 9 (Fig. 4).

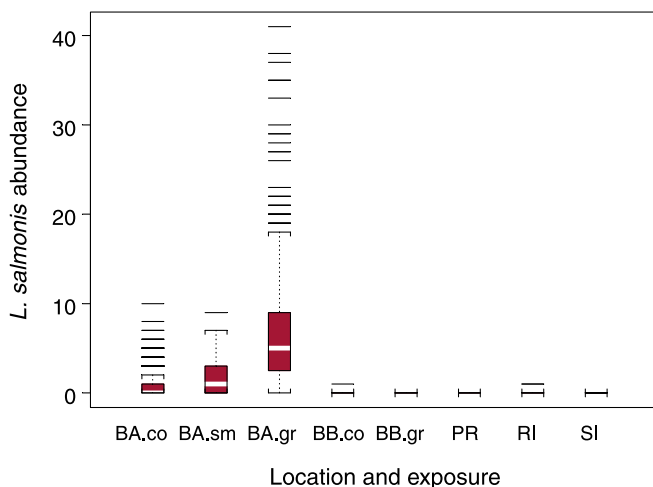
The abundance of *L. salmonis* was much higher on fish sampled at grower sites than on those at control sites, with an intermediate abundance at the smolt site (Fig. 5, Table 2). Overall (grower, smolt, and controls sites), we found 90% of pink and chum salmon to be infected at or above what could

Table 1. Mean fork length (cm) and mass (g) of juvenile salmon and salinity (‰) per location and exposure.

Location	Fork length (SE)*	Mass (SE)*	Exposure	Salinity
Prince Rupert	4.44 (0.05)	1.02 (0.05)	—	33.6
Bella Bella	3.76 (0.06)	0.63 (0.05)	Grower	21.0
			Control	11.9
Rivers Inlet	7.78 (0.09)	3.97 (0.12)	—	1.3–14.0
Smith Inlet	7.59 (0.20)	3.82 (0.32)	—	23.0
Broughton Archipelago	4.45 (0.04)	1.07 (0.04)	Grower	28.6
			Control	23.2

*Standard error is given in parentheses.

Fig. 3. Presentation of abundance of sea lice (*Lepeophtheirus salmonis*) on juvenile salmon sampled in all five areas for exposure categories. In each instance, the darkened box represents the middle half of the values (between the lower and upper quartiles). The lighter line inside this box is at the median. The whiskers extend beyond each quartile by 1.5 times the interquartile range (the height of the box). Isolated bars beyond these whiskers designate individual values beyond these limits (outliers). BA.co, Broughton Archipelago control (distant from salmon farm); BA.sm, Broughton Archipelago smolt site; BA.gr, Broughton Archipelago grower sites; BB.co, Bella Bella control (distant from salmon farms); BB.gr, Bella Bella grower sites; PR, Prince Rupert (no salmon farms in region); RI, Rivers Inlet (no salmon farm in region); SI, Smith Inlet (no salmon farm in region).



become the lethal limit when and if the lice reach a mobile stage ($1.6 \text{ mobile lice} \cdot (\text{g host mass})^{-1}$), as described by Bjørn and Finstad (1997) for sea trout. The mean number of sea lice on salmon near salmon farms was 6.99, while the mean mass of the pink and chum salmon was 1.07 g. As outlined below, infestation was not significantly different between pink salmon and chum salmon ($p = 0.89$) (Fig. 6, Table 3).

The *L. salmonis* counts were fit with a generalized linear mixed model to test for the influence of the following factors: salinity, temperature, host species, week, and exposure category. Also, to allow for different time dependence for different exposure categories, we included the interaction of the last two factors. In addition, location (nested within ex-

posure category) was added as a random factor to allow inferences to extend beyond these particular locations. We used this analysis to test the null hypotheses that each of these factors had no influence on abundance (results are summarized in Table 4). Of the fixed factors in this model, only exposure category ($p = 0.027$), week ($p < 0.0001$), and their interaction ($p = 0.013$) were significant. A more parsimonious model excluding the nonsignificant factors (temperature, salinity, and host species) was then fit. This model generated the same main conclusions: (i) differences in lice levels between exposure categories could not be attributed to chance ($p = 0.001$), (ii) lice levels also changed significantly over time ($p < 0.0001$), and (iii) this time behaviour depended on the exposure category ($p = 0.009$). Furthermore, the coefficients in this model can be used to estimate that mean lice infestation rates, after accounting for effects of other uncontrolled but influential factors, were about eight times higher in those sites impacted by grower farms vs. the control sites.

When the counts of juvenile *L. salmonis* were analyzed separately, the same effects remained significant. However, in the parsimonious model for adult *L. salmonis*, the only significant factor was time. Thus, the time fluctuations in Fig. 7 are not attributable to chance fluctuations, and exposure status has a significant impact on juvenile lice abundance but not on adult lice abundance. Note also the sustained presence of the copepodid stage and a roughly exponential growth of adult lice up to week 9 of this study in the Broughton Archipelago (Fig. 7).

A sample of *Caligus* from this study was identified as *Caligus clemensi* (P.A. Heuch, National Veterinary Institute, P.O. Box 8156, Dep., N-0033 Oslo, Norway, personal communication). These lice were more dispersed, with particularly substantial numbers found in the sites exposed to salmon farms near Bella Bella (Fig. 8). They were also nowhere as abundant as *L. salmonis* were in the Broughton Archipelago. These smaller numbers and the geographic dispersion ruled out not only the generalized linear modeling approach, but also a more geographically focused and complex analysis of the abundance patterns. We therefore included in the statistical model terms only for (i) fixed effects for area and exposure status and (ii) random effects for sites. The exclusion of other terms will likely have led to the overestimation of the degree of chance variation in the random site effects, and hence the production of conservative (larger) p values. We tested the null hypotheses that area and expo-

Table 2. Mean abundance of lice per fish for all areas and exposure categories moving from the most southerly site to the most northerly.

Area	No. of fish sampled	Total	<i>Lepeophtheirus salmonis</i>			<i>Caligus</i> spp.			
			Mean abundance	SD	SE of mean	Total	Mean abundance	SD	SE of mean
Broughton									
Control	537	437	0.814	1.356	0.059	21	0.039	0.329	0.014
Smolt	37	79	2.135	2.371	0.390	79	2.135	3.591	0.590
Grower	564	3822	6.777	6.361	0.268	264	0.468	2.308	0.097
Smith Inlet	48	0	0.000	0.000	0.000	0	0.000	0.000	0.000
Rivers Inlet	250	0	0.000	0.000	0.000	1	0.004	0.063	0.004
Bella Bella									
Control	118	1	0.008	0.092	0.008	1	0.008	0.092	0.008
Grower	36	0	0.000	0.000	0.000	28	0.778	1.072	0.179
Prince Rupert	566	2	0.004	0.059	0.002	4	0.007	0.084	0.004

Note: SD, standard deviation; SE, standard error.

Fig. 4. Weekly abundance of sea lice (*Lepeophtheirus salmonis*) on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the Broughton Archipelago over 10 weeks. See Fig. 3 for interpretation of box plots.

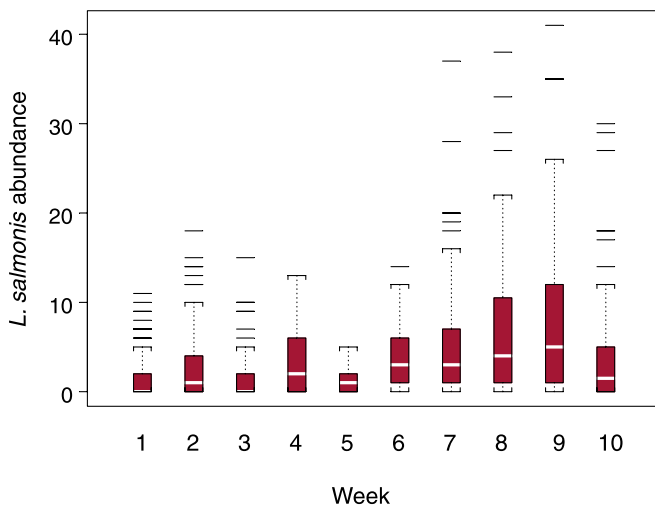
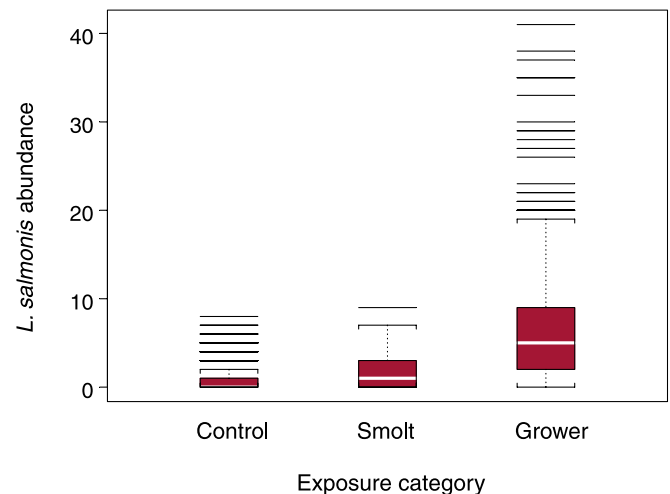


Fig. 5. Abundance of sea lice (*Lepeophtheirus salmonis*) on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the Broughton Archipelago for each of the three exposure categories: control, smolt, and grower. See Fig. 3 for interpretation of box plots.



sure status had no influence on *Caligus* abundance and then performed pairwise comparisons to search for specific differences.

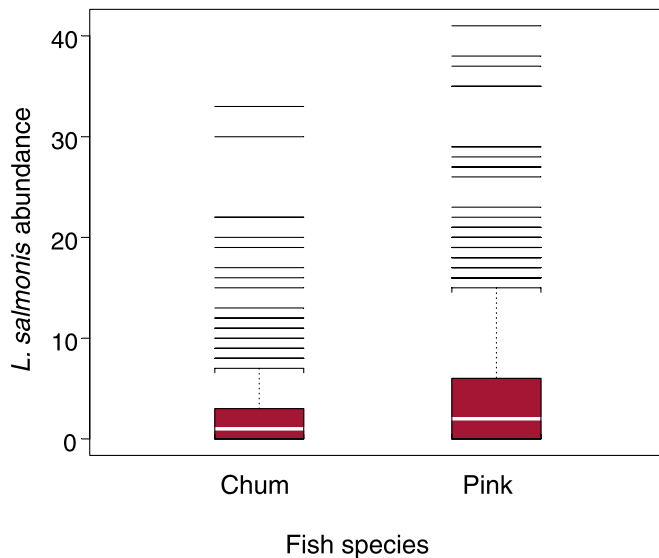
Even with this potential conservatism, there remains strong evidence of differences in *Caligus* abundance among the different exposure categories (Broughton grower, Broughton smolt, Bella Bella grower, and the control sites) ($F = 13.85$, $df = 3$ and 19.4 , $p < 0.0001$). Indeed, nowhere in the control areas did the mean *Caligus* count per fish exceed 0.04, and at all control sites outside the Broughton Archipelago, the mean *Caligus* count was uniformly less than 0.008. In addition, multiple comparisons of the means of each of the three impact categories (Table 2) and the combined mean for the control categories showed that the two categories with the highest observed mean count per fish, Bella Bella grower and Broughton smolt, were significantly higher than all the rest. By contrast, when we performed pairwise comparisons for means of control observations from the five geographic regions, there were no significant differences at

the 5% level, even without any adjustment for multiple comparisons. Hence, chance variation cannot explain the higher *Caligus* incidence at the Bella Bella grower and Broughton grower sites.

Discussion

Our results do not support the hypothesis that the average abundance of sea lice on pink and chum salmon was unrelated to proximity to a salmon farm. In particular, the overall pattern of *L. salmonis* abundance we observed within the Broughton Archipelago counters the hypothesis that *L. salmonis* is naturally more abundant in the Broughton Archipelago than in the other regions sampled. Estimated abundances increased eightfold between control sites and those close to farms at the grower stage. While this study cannot provide a causal link among salmon farms, sea lice, and juvenile wild salmonid infection rates, five findings add

Fig. 6. Comparison of abundance of sea lice (*Lepeophtheirus salmonis*) on pink (*Oncorhynchus gorbuscha*) vs. chum (*Oncorhynchus keta*) salmon. See Fig. 3 for interpretation of box plots.



considerably to the concern that salmon farms are a major source of sea lice in the Broughton Archipelago and have significantly altered the population dynamics of sea lice in the region.

In all areas throughout the study period, there were substantially more lice near salmon farms, whereas in geographic areas where there were no farms, lice numbers were near zero. Second, the sustained dominance of juvenile sea lice life stages indicates that infective events were continuous and local. The fact that the juvenile (but not adult) lice abundances were significantly higher in the near-farm sites reinforces this point.

Third, we found that the highest occurrence of lice occurred not only near salmon farms, but also near those holding year 2 (grower) salmon. Although the formal statistical analysis showed that this may be a result of partial confounding with time effects, the number of sea lice per farm-salmon host has been reported to triple from year 1 to year 2 of a sea farm's production cycle in Scotland (Revie et al. 2002). Hence, if salmon farms were the source of lice on wild salmon, one would expect higher infection rates near grower sites than smolt sites.

Fourth, the anomalously high lice infection rates recorded in 2001 (Morton and Williams 2004) reoccurred in 2002. And finally, 90% of pink and chum salmon in the densely salmon-farmed waters of the Broughton Archipelago were infected at or above the level considered lethal (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997).

Both the 2001 and 2002 sea lice epizootics on wild juvenile salmon near salmon farms in B.C. share key characteristics with sea lice epizootics in Europe. The onset of this phenomenon in Pacific waters strongly suggests that a common variable between the two areas could be the arrival of novel, stationary, salmonid hosts into nearshore marine habitat. Salmon farms do offer sea lice ideal overwintering habitat not previously available. While salmon farms have been in operation in the Broughton Archipelago since 1987, the

Table 3. The proportions of fish sampled in the Broughton Archipelago infected with more and less than 1.6 lice-(g host mass)⁻¹ for both pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon at the control sites (distant from salmon farms), a smolt salmon farm site, and the grower sites.

Infection rate	Exposure category		
	Control	Smolt exposed	Grower exposed
Pink			
Under 1.6 lice·g ⁻¹	279	6	63
Over 1.6 lice·g ⁻¹	90	5	375
Total	369	11	438
Proportion over	0.244	0.455	0.856
Chum			
Under 1.6 lice·g ⁻¹	132	9	3
Over 1.6 lice·g ⁻¹	36	17	50
Total	168	27	53
Proportion over	0.214	0.654	0.943

number of farms has increased since that time, and more recently, stocking densities have also increased to over one million Atlantic salmon per farm at some sites (Naylor et al. 2003). The number of lice larvae released by salmon farms has been calculated to increase with the number of salmon per farm (Heuch and Mo 2001).

While European sea lice studies benefit from access to sea lice counts on farm salmon, salmon farm companies do not release their sea lice infection rates by farm site in B.C. Therefore, we could not compare infection rates between adjacent wild and farmed salmon stocks; we could only look at how the number of sea lice varied near to and distant from salmon farms.

The differences in mean lice numbers between locations and over time were not merely artefacts of confounding with salinity and temperature differences. While *L. salmonis* survival is optimal at a salinity of 30‰ (Johnson and Albright 1991b) and copepodids avoid water with less than 20‰ (Heuch 1995), we found the highest numbers of lice in 28.6‰ and one of the lowest levels in water at 33.6‰. Although this study was not designed specifically to test the impact of salinity, its potential to confound the effects of proximity to fish farms can be evaluated. When salinity, temperature, and exposure categories were included in the statistical model, only the exposure category remained significant. Therefore, partial confounding with salinity and temperature cannot explain the increased abundance of lice caught near the fish farms. The overall low salinity values recorded in this study (less than 30‰) suggest that lice abundance could have been higher if salinity values had reached levels optimal for *L. salmonis* survival.

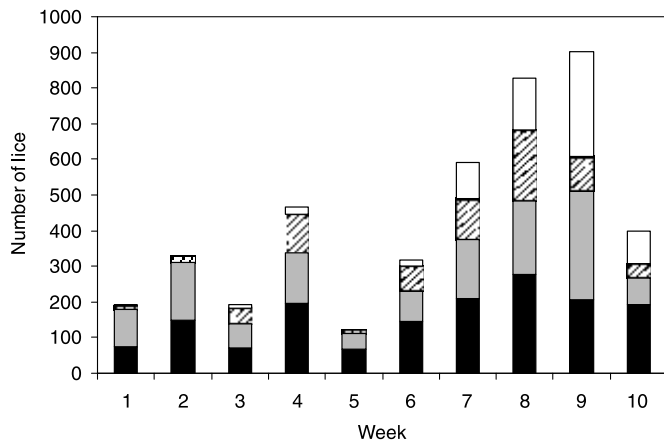
The sustained large number of juvenile-stage lice in this study provides evidence of continuous larval lice production somewhere within range of the fish we sampled. At 10.4 °C, sea lice would progress rapidly from newly hatched nauplii through the critical stage at which they must find a host (6–10 days). These observations are closely analogous to sea lice demographics near salmon farms elsewhere (Tully et al. 1993; Bjørn and Finstad 2002). Persistent lice production could not occur in the absence of a substantial salmonid host

Table 4. Summary of model fits to *Lepeophtheirus salmonis* abundance data.

Dependent variable	Model	Effect	Degrees of freedom			
			Numerator	Denominator	F	p
Total	Full	Exposed	2	2.42	22.9	0.0267
		Week	9	44.8	6.61	<0.0001
		Week × exposed	10	47.9	2.59	0.0134
		Species	1	1112	0	0.8908
		Salinity	1	8.12	0.56	0.474
		Temperature	1	29.4	0.26	0.616
Total	Reduced	Exposed	2	5.84	26.58	0.0012
		Week	9	50.3	9.5	<0.0001
		Week × exposed	10	48.4	2.75	0.009
Adult	Reduced	Exposed	2	8.27	4.17	0.0559
		Week	9	40.4	4.4	0
		Week × exposed	10	40.6	1.3	0.2625
Juvenile	Reduced	Exposed	2	2.74	30.84	0.0132
		Week	9	52.6	8.61	<0.0001
		Week × exposed	10	50.6	2.72	0.009

Note: The full model is presented only for the totals. Because none of the three factors (species, salinity, or temperature) was ever significant in these models (smallest *p* value = 0.39), only reduced model results are presented for adults and juveniles.

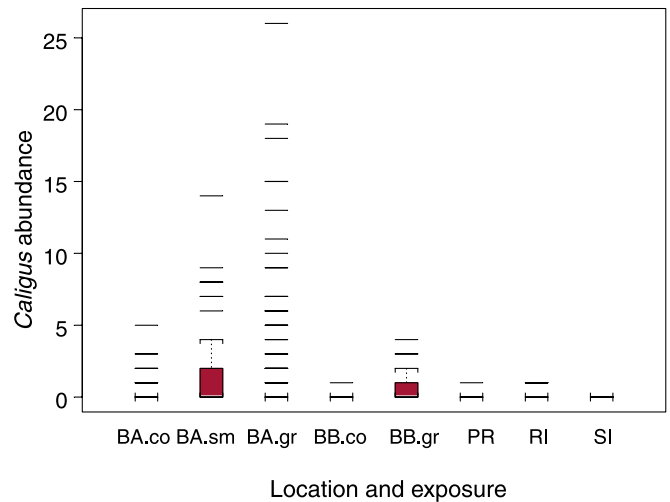
Fig. 7. The presence of life stages of sea lice (*Lepeophtheirus salmonis*) found each week in the Broughton Archipelago: copepodid (solid), chalimus I–II (shaded), chalimus III–IV (hatched), adult (open).



population within the Broughton Archipelago throughout the 10 weeks of this study. While there are some yearling chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon, steelhead (*Salmo gairdneri*), and cutthroat trout (*Salmo clarkia*), no reports of large wild salmonid populations could be found in this area (Glen Neidrauer, Department of Fisheries and Oceans patrolman, P.O. Simoom Sound, BC V0P 1S0, Canada, personal communication).

High numbers of a *Caligus* species in areas where farm salmon had been placed more recently (i.e., Bella Bella and the smolt site in the Broughton) correspond to what others have found (Hogans and Trudeau 1989). This non-salmon-specific, more generalist fish parasite may already be present in the new-farm environment as the farm stock arrive, and therefore may be capable of taking advantage of new hosts. *Lepeophtheirus salmonis* might only oc-

Fig. 8. Presentation of abundance of *Caligus* lice on juvenile salmon sampled in all five areas for all exposure categories. BA.co, Broughton Archipelago control (distant from salmon farm); BA.sm, Broughton Archipelago smolt site; BA.gr, Broughton Archipelago grower sites; BB.co, Bella Bella control (distant from salmon farm); BB.gr, Bella Bella grower sites; PR, Prince Rupert (no salmon farm in region); RI, Rivers Inlet (no salmon farm in region); SI, Smith Inlet (no salmon farm in region). See Fig. 3 for interpretation of box plots.



cur in pulses during the wild salmon spawning migrations in summer and fall.

There is evidence that sea trout, which inhabit the nearshore environment longer than Atlantic salmon (Johnstone et al. 1995), are more susceptible to sea lice in areas stocked with farm salmon than Atlantic salmon (R.S. McKinley, The University of British Columbia, 4160 Marine Drive, Vancouver, BC V7V 1N6, Canada, personal communication). Since pink salmon are also known to utilize the

nearshore marine environment for a number of months (Bailey et al. 1975; Cooney et al. 1978), it is reasonable to expect this behaviour to similarly exacerbate susceptibility of pink salmon to sea lice.

Pink and chum salmon are much smaller than salmonids in the Atlantic studies at seawater entry, and this might be important in regards to degree of lethal impact from sea lice. For the first 3 weeks of this study, the fish sampled adjacent to salmon farms weighed substantially less than 1 g, but averaged $3.4 \text{ lice} \cdot \text{fish}^{-1}$. The only lethal lice level known for salmonids is $0.75\text{--}1.6 \text{ mobile lice} \cdot (\text{g host mass})^{-1}$ (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997). Not all lice on these very young pink and chum salmon had reached their mobile stages, but by week 7 of the study, the mean mass was 0.9 g and the load was $1.8 \text{ mobile lice} \cdot (\text{g host mass})^{-1}$ (SD = 0.56). More research on sea lice pathogenicity to Pacific salmonids is paramount, but these numbers would seem solid ground for enacting a precautionary approach until the dynamic between sea lice and juvenile Pacific salmon is more fully understood.

In conclusion, heavy infestations of sea lice have been observed on juvenile pink salmon in the Broughton Archipelago in 2001 (Morton and Williams 2004) and pink and chum salmon in 2002. The evidence from both this study and European research points to a sea lice source in the vicinity of the fish farms. In the absence of any B.C. studies directed on sea lice in the salmon farms and therefore any means to relate lice on farm salmon to lice on wild salmon, there do not seem to be any direct tests possible for the concern that salmon farming is involved in this cycle of infections.

When the pink salmon stock examined in this study returned to spawn, their numbers were 87% lower than the parental stock (Gordon McEachen, Department of Fisheries and Oceans, Rm 150 – 1260 Shoppers' Row, Campbell River, BC V9W 2C8, Canada, personal communication). Declines in pink salmon returns in 2002 and 2003, known to have been heavily infected by sea lice as juveniles, point to serious concern for the continuance for some salmon populations in the Broughton Archipelago. Further research concerning sea lice dynamics in the Broughton Archipelago ecosystem and lethality of *L. salmonis* on juvenile salmonids is clearly an immediate priority.

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References

Anonymous. 1995. Report of the sea trout working group 1994. Report to the Minister for the Marine, Ireland.

- Anonymous. 1997. Report of the workshop on the interactions between salmon lice and salmonids, Edinburgh, U.K., 11–15 November 1996. ICES CM 1997/M:4,Ref.F.
- Bailey, J.E., Wing, B.L., and Mattson, C.R. 1975. Zooplankton abundance and feeding habits of fry of pink salmon, *Oncorhynchus gorbuscha*, and chum salmon, *Oncorhynchus keta*, in Traitors Cove, Alaska, with speculations on the carrying capacity of the area. Fish. Bull. (US), **73**: 846–861.
- Bax, N.J. 1983. The early marine migration of juvenile chum salmon (*Oncorhynchus keta*) through Hood Canal — its variability and consequences. Ph.D. thesis, University of Washington, Seattle, Wash. In Pacific salmon life histories. Edited by C. Groot and L. Margolis. UBC Press, Vancouver, B.C. pp. 121–230.
- Birkeland, K. 1996. Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeophtheirus salmonis* Krøyer); migration, growth, and mortality. Can. J. Fish. Aquat. Sci. **53**: 2808–2813.
- Bjørn, P.A., and Finstad, B. 1997. The physiological effects of salmon lice infection on sea trout post smolts. Nord. J. Freshw. Res. **73**: 60–72.
- Bjørn, P.A., and Finstad, B. 2002. Salmon lice, *Lepeophtheirus salmonis*, (Krøyer), infestation in sympatric populations of Arctic char, *Salvelinus alpinus* (L.) and sea trout, *Salmo trutta* (L.), in areas near and distant from salmon farms. ICES (Int. Council. Explor. Sea) J. Mar. Sci. **59**: 1–9.
- Boxaspen, K. 1997. Geographical and temporal variation in abundance of salmon lice (*Lepeophtheirus salmonis*) on salmon (*Salmo salar*). ICES (Int. Council. Explor. Sea) J. Mar. Sci. **54**: 1144–1147.
- Cooney, R.T., Urquhart, D., Nevé, R., Hilsinger, J., Clasby, R., and Barnard, D. 1978. Some aspects of the carrying capacity of Prince William Sound, Alaska for hatchery released pink and chum salmon fry. Alaska Sea Grant Report 78-4; University of Alaska Institute of Marine Research, IMS R78-3.
- Costelloe, M., Costelloe, J., and Roche, N. 1996. Planktonic dispersion of larval salmon-lice, *Lepeophtheirus salmonis* associated with cultured salmon, *Salmo salar*, on the west coast of Ireland. J. Mar. Biol. Assoc. U.K. **76**: 141–149.
- Grimnes, A., and Jakobsen, P.J. 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. J. Fish Biol. **48**: 1179–1194.
- Håstein, T., and Bergsjø, T. 1976. The salmon lice *Lepeophtheirus salmonis* as the cause of disease in farmed salmonids. Rivista Italiana di Piscicoltura e Ittiopatologia, **A11**: 3–4.
- Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. In Salmonid ecosystems of the North Pacific. Edited by W.J. McNeil and D.C. Himsworth. Oregon State University Press, Corvallis, Ore. pp. 203–229.
- Heard, W. 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*). In Pacific salmon life histories. Edited by C. Groot and L. Margolis. UBC Press, Vancouver, B.C. pp. 121–230.
- Heuch, P.A. 1995. Experimental evidence for aggregation of salmon louse copepodids, *Lepeophtheirus salmonis*, in step salinity gradients. J. Mar. Biol. Assoc. U.K. **75**: 927–939.
- Heuch, P.A., and Mo, T.A. 2001. A model of salmon louse production in Norway: effects of increasing salmon production and public management measures. Dis. Aquat. Org. **45**: 145–152.
- Hogans, W.E., and Trudeau, D.J. 1989. *Caligus elongates* (Copepoda: Caligoida) from Atlantic salmon (*Salmo salar*) cultured in marine waters of the Lower Bay of Fundy. Can. J. Zool. **67**: 1080–1082.
- Johannessen, A. 1978. Early stages of *Lepeophtheirus salmonis* (Copepoda Caligidae). Sarsia, **63**: 169–176.

- Johnson, S.C., and Albright, L.J. 1991a. The development stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Can. J. Zool.* **69**: 929–950.
- Johnson, S.C., and Albright, L.J. 1991b. Development, growth and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *J. Mar. Biol. Assoc. U.K.* **71**: 425–436.
- Johnstone, A.D.F., Walker, A.F., Urquhart, G.G., and Thorne, A.E. 1995. The movement of sea trout smolts, *Salmo trutta* L., in a Scottish west coast sea loch determined by acoustic tracking. Scottish Fisheries Research Report 56.
- Kabata, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). *J. Fish. Res. Board Can.* **29**: 1571–1593.
- Kleinbaum, D.G., Kupper, L.L., Muller, K.E., and Nizam, A. 1998. Applied regression analysis and other multivariable methods, 3rd ed. Duxbury, Pacific Grove, Calif.
- Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M., and Schad, G.A. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of parasitologists). *J. Parasitol.* **68**: 131–133.
- McCulloch, C.E., and Searle, S.R. 2001. Generalized, linear, and mixed models. John Wiley & Sons Inc., New York.
- Miller, R.G., Jr. 1981. Simultaneous statistical inference, 2nd ed. Springer-Verlag New York Inc., New York.
- Morton, A.B., and Williams, R. 2004. First report of the sea louse, *Lepeophtheirus salmonis*, infestation on juvenile pink salmon *Oncorhynchus gorbuscha*, in nearshore habitat. *Can. Field-Nat.* **118**. In press.
- Nagasawa, K. 1987. Prevalence and abundance of *Lepeophtheirus salmonis* (Copepod: Caligidae) on high-sea salmon and trout in the North Pacific Ocean. *Bull. Jpn. Soc. Sci. Fish.* **53**: 2151–2156.
- Nagasawa, K., Ishida, Y., Ogura, M., Tadokora, K., and Hiramatsu, K. 1993. The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on six species of Pacific salmon in offshore waters of the North Pacific Ocean and Bering Sea. In Pathogens of wild and farmed fish: sea lice. Edited by G.A. Boxshall and D.D. Defaye. Ellis Horwood, New York. pp. 166–178.
- Naylor, R.L., Eagle, J., and Smith, W.L. 2003. Salmon aquaculture in the Pacific Northwest a global industry with local impacts. *Environment*, **45**: 18–39.
- Parker, R.R. 1965. Estimation of sea mortality rates for the 1961 brood-year pink salmon of the Bella Coola area, British Columbia. *J. Fish. Res. Board Can.* **22**: 1523–1554.
- PFRCC (Pacific Fisheries Resource Conservation Council). 2002. 2002 advisory: the protection of Broughton Archipelago pink salmon stocks. Report to the Minister of Fisheries and Oceans, and B.C. Minister of Agriculture, Food and Fisheries. Available from http://www.fish.bc.ca/reports/pfrcc_broughton_advisory.pdf [cited 11 February 2004; updated November 2002].
- Piasecki, W. 1996. The developmental stages of *Caligus elongates* von Nordmann, 1832 (Copepoda: Caligidae). *Can. J. Zool.* **74**: 1459–1478.
- Pike, A.W., and Wadsworth, S.L. 1999. Sealice on salmonids: their biology and control. *Adv. Parasitol.* **44**: 234–337.
- Revie, C.W., Gettinby, G., Treasurer, J.W., Rae, G.H., and Clark, N. 2002. Temporal, environmental and management factors influencing the epidemiological patterns of sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon (*Salmo salar*) in Scotland. *Pest. Manag. Sci.* **58**: 576–582.
- Salo, E.O. 1991. Life history of chum salmon (*Oncorhynchus keta*). In Pacific salmon life histories. Edited by C. Groot and L. Margolis. UBC Press, Vancouver, B.C. pp. 121–230.
- Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada (reprinted 1990). *Bull. Fish. Res. Board Can.* No. 184.
- Steel, R.G.D., and Torrie, J.H. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, New York.
- Stuart, R. 1990. Sea lice, a maritime perspective. *Aquaculture Association of Canada Bulletin* **90**: 18–24.
- Tully, O., Poole, W.R., and Whelan, K.F. 1993. Infestation parameters of *Lepeophtheirus salmonis* (Krøyer) (Copepod: Caligidae) parasitic on sea trout, *Salmo trutta* L., off the west coast of Ireland during 1990 and 1991. *Aquac. Fish. Manag.* **24**: 545–555.
- White, H.C. 1940. “Sea Lice” (*Lepeophtheirus*) and death of salmon. *J. Fish. Res. Board Can.* **5**: 172–175.
- Wootton, R., Smith, J.W., and Needham, E.A. 1982. Aspects of the biology of parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongates* on farmed salmonids, and their treatment. *Proc. R. Soc. Edinb. Sect. B (Biol.)*, **81**: 185–197.