The salmon louse *Lepeophtheirus salmonis* on salmonid and non-salmonid fishes in British Columbia

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Abstract

There is a concern that farmed Atlantic salmon (*Salmo salar*) adversely influence the health and abundance of adjacent wild populations of Pacific salmon (*Oncorhynchus* spp.) in part, by acting as reservoirs of the salmon louse, *Lepeophtheirus salmonis*. The controversy surrounding this concern lies in scientific uncertainty regarding the processes that regulate the natural abundance and distribution of the parasite in addition to the susceptibility of juvenile Pacific salmon. We have developed a three-year database of *L. salmonis* on juvenile pink (*O. gorbuscha*) and chum (*O. keta*) from a coastal ecosystem in which there is a salmon aquaculture industry. We have shown that in this ecosystem: 1, there is a spatial relationship between the abundance of *L. salmonis* and another sea louse, *Caligus clemensi* and the salinity of surface seawater; 2, there is a pattern of temporal variation within each year in which increased salmon size is associated with decreased louse abundance and increased louse development; and 3, the abundance of all sea lice were significantly higher in 2004 than in either 2003 or 2005. Finally and unexpectedly, we found *L. salmonis* to be significantly more abundant on the threespine stickleback (*Gasterosteus aculeatus*) than on either salmon species in all three years. We cannot conclude that salmon aquaculture does not contribute *L. salmonis* into the local ecosystem. However, the accumulated evidence suggest that there are important natural sources of infestation and that local factors such as salinity and size of the salmon host in addition to the possible effects of salmon farms must be considered in future efforts to examine the epidemiology of these parasites.

Introduction

Sea lice (Copeopoda: Caligidae), including species of *Lepeophtheirus* and *Caligus*, are ectoparasites of marine fish. Severe infestations with *Lepeophtheirus salmonis*, a common parasite of salmon, may cause disease in farmed and wild populations. During development sea lice moult through 10 stages: 2 planktonic nauplii, a fish-infective planktonic copepodid, 4 parasitic attached (non-motile) chalimus, two motile parasitic preadults and a motile adult stage (Pike & Wadsworth, 1999). The risk of disease increases with the intensity of infestation and with development to and beyond the preadult stage. Our understanding of the epidemiology of *L. salmonis* is mainly based on host species occurring in the North Atlantic Ocean and particularly on farmed Atlantic salmon (*Salmo salar*) (Revie et al. 2002; Revie et al. 2003; Westcott et al. 2004). Since salmon farms often share coastal waterways with wild salmonids there has been considerable debate on the role of salmon farms as sources of *L. salmonis* and on impacts these parasites may have on the integrity of wild salmon populations. Open net-pens of farmed salmon on the Pacific coast of Canada occur along migratory routes used by juveniles of several species of Pacific salmon (*Oncorhynchus* spp.). Salmon lice were recently reported on juvenile pink (*O. gorbuscha*) and chum (*O. keta*) salmon migrating near salmon farms (Morton et al. 2004; Jones and Nemec 2004) and mathematical models have suggested salmon farms serve as sources of the parasite (Krkosek et al. 2005). The relatively small size of juvenile pink and chum salmon suggested increased vulnerability to the infestations however laboratory data on susceptibility of these small fish are only now being collected (S. Jones, unpublished). In addition, earlier reports of *L. salmonis* on juvenile Pacific salmon are lacking and very little is known about alternate hosts and oceanographic conditions which may also influence the distribution and abundance of the parasite in this region.

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Objectives

The objectives of the research were first, to determine the extent of spatial and temporal variations in the abundance of sea lice within a region of coastal British Columbia in which salmon farming is practised. Second, to determine the significance of certain biological and oceanographic processes as explanatory variables for any observed variation. The long-term goal is to reduce uncertainty regarding the processes that regulate the natural abundance and distribution of *L. salmonis* in order to better understand the relative role of salmon aquaculture in the ecology of the parasite. This paper summarises three-years of data obtained from pink and chum salmon in British Columbia. We show that the three-spine stickleback (*Gasterosteus aculeatus*) is a common host of *L. salmonis* and present evidence that spatial variation in louse abundance is regulated by low seawater salinity.

Methods

In each of 2003, 2004 and 2005 juvenile salmon and sticklebacks were collected by beach and purse seines between March and July, coincident with the migration of juvenile pink and chum salmon from natal streams to the open ocean. Samples were collected from approximately 105 sites in Knight Inlet and the channels surrounding Broughton and Gilford Islands north of Vancouver Island, British Columbia (BC), Canada (Fig. 1). Sites were sampled repeatedly at biweekly or monthly intervals (collection periods). The study area was arbitrarily stratified into 11 zones containing on average 10 collection sites per zone. The first 30 specimens of each species collected from each site were individually bagged, labelled and immediately stored at -20ºC. Following receipt in the laboratory, frozen specimens were identified to species and fork length and wet weight determined. Each fish was examined under a dissecting microscope and all lice, including any remaining in the bag, were counted and the developmental stage determined. Surface seawater salinity data were obtained from most sites at each collection. Salinity was calculated from the conductivity of surface seawater samples using a calibrated salinometer.

The significance of differences in the abundance of sea lice between years and among hosts was tested using the Kruskal-Wallis test. The significance of differences in mean salinity was determined by ANOVA and Bonferroni-adjusted multiple comparison tests. Differences were considered significant when $P \leq 0.05$. The relationship between intensity of *L. salmonis* and *C. clemensi* (total and motile stages), expressed by the number of lice per individual fish, and suspected explanatory quantities (species, geographical area, collection period, ambient salinity and fish length) was analysed using a multiple Poisson regression analysis. The appropriateness of the regression was assessed by inspection of the residuals. Impact of the explanatory factors was quantified by the p-values of the Type III likelihood ratio statistics. Pearson correlations between explaining factors were used to identify highly correlated factors.

Results

A total of 5,437 sticklebacks, 16,181 chum salmon and 12,911 pink salmon were examined and sea lice were observed on all species in all three years of the survey (Table 1).

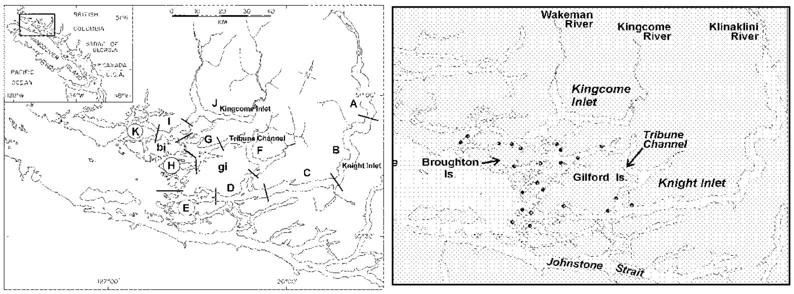

Figure 1. Map of study area showing zone subdivisions (left) and approximate locations of salmon farms (dots, right).

Lepeophtheirus salmonis and another sea louse, *Caligus clemensi*, were found on all three species of fish in all years however their proportions varied among years. *Lepeophtheirus salmonis* represented 38.1%, 92.7% and 72.0% of sea lice specimens examined in 2003, 2004 and 2005, respectively.

Table 1. Summary of host and sea lice (*Lepeophtheirus salmonis* and *Caligus clemensi*) data

Year	Fish Species	No. Fish Examined	Weight (g)	Length (mm)	Prevalence (%)	Mean Intensity (Range)	Mean Abundance
2003 March - June	Stickleback	2,720	3.5±.04*	64.1±.23	60.4	6.0±.13 (1 - 51)	3.6±.10
	Chum	10,683	2.0±.02	51.6±.16	27.3	2.2±.03 (1 – 25)	0.6±.01
	Pink	7,124	1.7±.02	48.9±.20	24.0	1.7±.02 (1 – 12)	0.4±.01
2004 May - July	Stickleback	1,419	3.6±.04	65.2±.23	83.4	20.8±.89 (1 – 294)	17.3±.77
	Chum	3,182	6.3±.12	74.4±.40	73.0	11.9±.38 (1 – 384)	8.7±.29
	Pink	1,905	4.5±.07	71.7±.34	66.5	4.8±.20 (1 – 82)	3.2±.14
2005 Mar - July	Stickleback	1,298	4.0±.03	68.5±.19	58.3	5.4±.25 (1 – 113)	3.2±.16
	Chum	2,316	8.5±.23	77.2±.67	33.2	2.9±.10 (1 – 31)	1.0±.04
	Pink	3,882	4.4±.08	64.4±.41	31.2	1.9±.05 (1 – 21)	0.6±.02

The abundance of sea lice on all species was significantly higher in 2004 than in 2003 or 2005 ($P<0.05$) (Table 1). In addition, the mean abundance of sea lice on sticklebacks was significantly higher than on either salmon species in all three years ($P<0.05$). In all years, the abundance of *L. salmonis* on chum salmon was significantly greater than on pink salmon ($P<0.05$). In 2004 and 2005 there was significant spatial variation in the abundance of *L. salmonis* on all three hosts and in the salinity of surface seawater (Fig. 2). In all years salinity was greatest in zones E, H, and K and least in zones A, B, C and J. In 2004 and 2005, temporal variations in the size of salmon coincided with a reduction in abundance and maturation of *L. salmonis* (Fig. 3) and in these years, a higher proportion of *L. salmonis* developed to motile stages on pink compared with chum salmon.

Poisson regression analysis was used to determine for the 2005 data, the probability that sea louse intensity could be explained by salmon species, zone, collection period or salinity (Table 2).

Table 2. Summary of multiple Poisson regression analysis of *L. salmonis* and *C. clemensi* intensity data on pink and chum salmon in 2005

Response Variables	Explanatory Variables (P-values)			
	Species	Zone	Collection Period	Salinity
Total *L. salmonis*	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Motile *L. salmonis*	0.3212	< 0.0001	< 0.0001	0.0853
Total *C. clemensi*	<0.0001	<0.0001	<0.0001	<0.0001
Motile *C. clemensi*	0.8211	0.0003	<0.0001	0.0005

The zones A, B, C and J were excluded from the analysis. For each of *L. salmonis* and *C. clemensi*, the response variables were "total lice" and "total motile stages". With respect to total *C. clemensi* and *L. salmonis* total stages, intensity was significantly dependent on host species, zone, collection period and salinity. The intensity of motile *L. salmonis* was significantly dependent on zone and collection period whereas motile *C. clemensi* was significantly dependent on zone, period and salinity. The variables "collection period" and "fish length" were highly correlated ($r = 0.92$, $p < 0.0001$), suggesting that the effect of "collection period" is due to "fish length".

Discussion

Given the dependency of sea louse development and survival on temperature and salinity, Brooks (2005) has argued that local oceanography will play an important role in the distribution of larval sea lice developmental stages in this region. The study area is a coastal ecosystem in which the oceanography is dominated by an estuarine pattern of circulation (Foreman et al. 2006). A seaward flow of surface water is driven by seasonally-variable precipitation and snow melt from coastal mountains. Thus surface salinities tend to be lowest and temperatures highest during the summer months. The salinity data shown in the present study are consistent with an estuarine circulation in which salinities are lowest in bodies of water furthest from the open ocean. Predictions made from the movements of neutrally buoyant particles in the study area suggest planktonic sea lice larvae will drift several kilometres seaward before they mature to infective stages (D. Stucchi, personal communication). Thus juvenile salmon leave natal streams and move through this complex mixture of salinities, temperatures and currents during their seaward migration. During this time the fish are growing rapidly and are exposed to infective stages of sea lice belonging to two species. The exact relationship between the timing and locations of these exposures is predicted to be dependent on the oceanographic features described above. Other studies have suggested salmon farms serve as the sources of sea lice in this region and that levels of infections on migrating juveniles are highest among samples collected near to farms (Morton et al. 2004; Krkosek et al. 2005).

Lepeophtheirus salmonis and *Caligus clemensi* were common parasites of juvenile pink and chum salmon and during the study several patterns of variation in their abundance were demonstrated. Spatial variations in abundance observed among study zones coincided with similar patterns in the salinity of surface seawater. Zones of low salinity coincided with low abundances of sea lice on all three host species. Furthermore, a relationship with salinity and the intensity of all but motile stages of *L. salmonis* (and all stages of *C. clemensi*) was confirmed by the Poisson regression analysis. This is consistent with other observations that showed early and infective developmental stages to be most sensitive to low salinity (Johnson and Albright 1991; Tucker et al. 2000). Temporal variations in salmon size, louse abundance and louse development were observed. Thus salmon growth coincided with development of *L. salmonis* to more mature motile stages, particularly on pink salmon. The apparent reduced rate of louse development on chum relative to pink salmon observed in 2005 may be related to later or ongoing exposures of the chum salmon to infective stages. It is unlikely that the reduced proportion of mature stages was due to death or morbidity of infested chum salmon as abundances approximately 10-fold higher with equal or more advanced developmental stages were observed a year earlier on chum salmon. Nevertheless, the abundance of *L. salmonis* on chum salmon in 2004 was up to 5-times greater than on pink salmon suggesting that distinct relationships exist between *L. salmonis* and these salmon species. Finally, significantly more lice were observed on all species in 2004 compared with 2003 or 2004. At present we do not understand why sea lice levels were elevated in 2004. Morton et al. (2005) suggested that the reduced lice levels observed in 2003 relative to 2002 and 2004 were the result of the fallowing of salmon farms along Tribune Channel in 2003. However, these farms were stocked again in 2005 and the overall biomass of salmon throughout the study area did not change markedly between 2002 and 2005 (http://www.agf.gov.bc.ca/ahc/fish_health/Sealice/Prod_Fallowing_BA.pdf). In addition, there are few differences in the annual proportions of first- and second-year farmed salmon in this region between 2003 and 2005 (S. Saksida, personal communication). Thus it is unlikely that the significant annual variations in the abundance of *L. salmonis* and *C. clemensi* observed on wild juvenile salmon was due entirely to fallowing activities undertaken by salmon farms.

The salmon louse *L. salmonis* has only rarely been observed on non-salmonid hosts. The greater abundance of *L. salmonis* on threespine sticklebacks than on juvenile salmon throughout this study was therefore, a novel and unexpected finding (Jones et al. 2006). Although future epidemiological studies in this region must take into account the significant abundance of *L. salmonis* on the stickleback, further work is necessary to determine the role played by this host in the local ecology of this parasite. Detailed data from salmon farms were not available for this study, therefore we cannot comment on the role of salmon farms in interpreting the spatial variations in sea lice abundance observed on the wild salmon. The significant relationship between salinity, fish length and sea louse intensity however, underscores the need for a more sophisticated approach, including detailed oceanographic and biological data, to understanding the epidemiology of *L. salmonis* in this region occupied by wild and captive stocks of salmonids.

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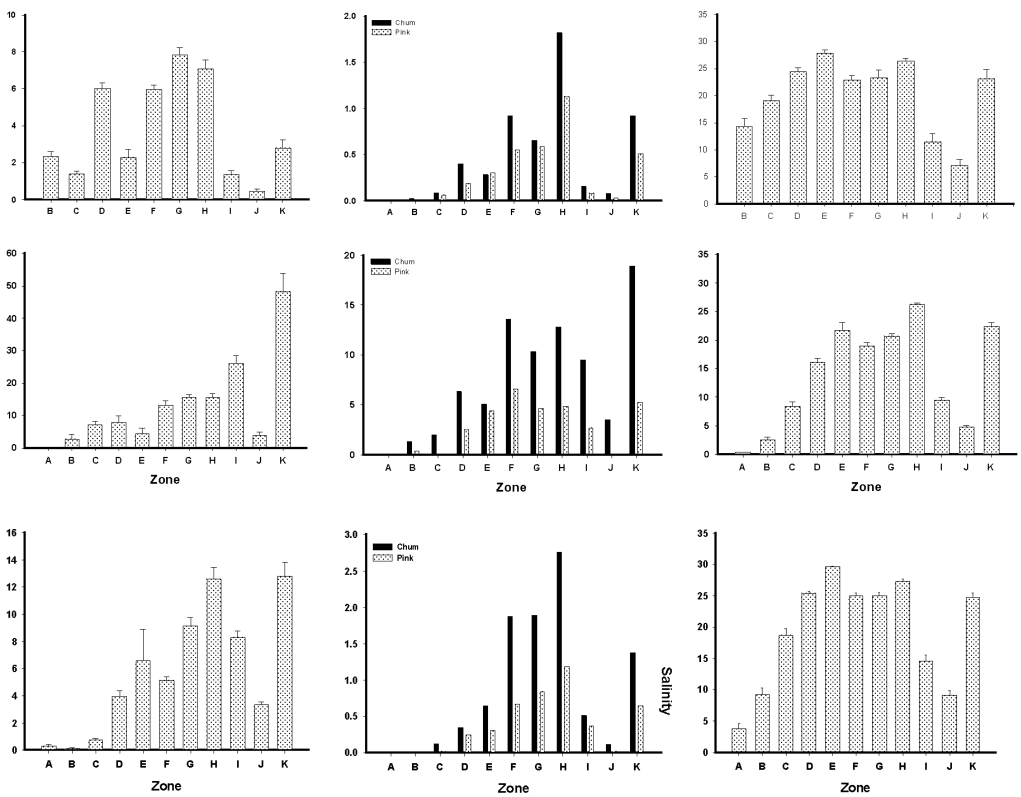

Figure 2. Spatial distribution in the abundance of sea lice on sticklebacks (left column) and salmon (centre column) and in surface seawater salinity (right column) in 2003 (upper), 2004 (middle) and 2005 (lower) rows.

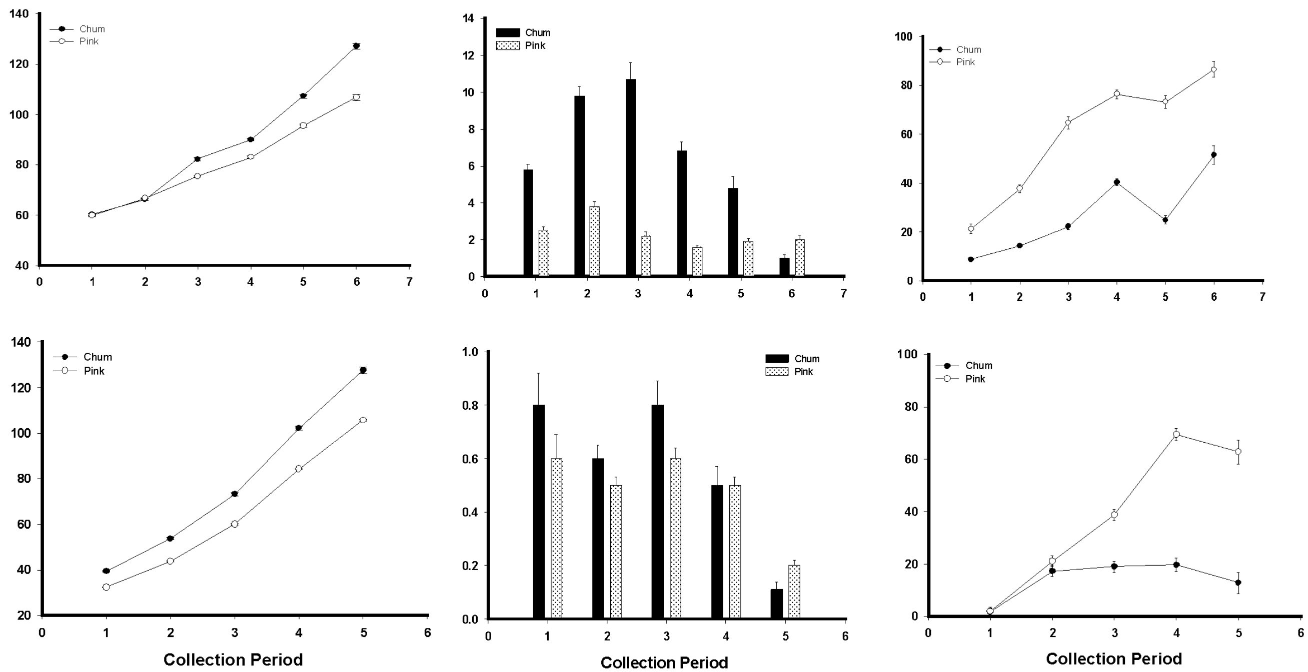

Figure 3. Temporal changes in the size of pink and chum salmon (fork length (mm), left) and in the abundance (middle) and development (percent motile stages, right) of *Lepeophtheirus salmonis* in 2004 (upper) and 2005 (lower)