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Sea lice on wild juvenile Pacific salmon and farmed Atlantic salmon in the northernmost salmon farming region of British Columbia

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Abstract

The Kitasoo/Xai'xais First Nation established a program to monitor sea lice levels on seaward migrating wild juvenile salmon in their traditional territory which contains the most northerly salmon farming region of British Columbia. A total of 12 locations were routinely sampled during the period between 2005 and 2008 to gain a better understanding of the levels and patterns of sea lice infestation on wild salmonids in the region. Over 5000 juvenile salmon were collected and examined for sea lice. Around 78% were identified as pink salmon, 18% were chum salmon and the remainder classified as 'other' salmon (coho and sockeye salmon). Two species of sea lice were observed: *Lepeophtheirus salmonis* and *Caligus clemensi*. Over 91% of all the juvenile salmon examined had no sea lice and there was no significant difference in *L. salmonis* prevalence levels among salmon species. However, chum salmon had significantly lower *C. clemensi* prevalence levels than either pink or 'other' salmon. There were significant annual and regional differences in *L. salmonis* prevalence on juvenile pink salmon; the lowest prevalence in all sampling zones occurring in 2008, while channels containing salmon farms consistently had higher levels than those without salmon farms. Mean prevalence of *L. salmonis* in the channels with salmon farms ranged from 2% to 9% which is lower than levels published for the same region in different years or for other areas without salmon farms. *C. clemensi* prevalence on wild pink salmon was associated with sampling zone and the size of pink salmon; larger juvenile fish were more likely to be infected than smaller fish. During the period of wild juvenile salmon migration, the mean abundance of motile stages of *L. salmonis* on farmed salmon ranged from 0.13 to 0.79 lice per fish but there were no significant differences among years. In comparison,

C. clemensi abundance levels on farms were significantly higher in 2005. Factors contributing to variations in these observations are discussed.

Keywords

Sea lice, juvenile Pacific salmon, farmed salmon, British Columbia, *Lepeophtheirus salmonis*, *Caligus clemensi*

1. Introduction

Sea lice are parasitic copepods that infest fish in the marine environment. There are two species of sea lice that have commonly been reported on wild and farmed salmon in British Columbia (BC): *Lepeophtheirus salmonis* commonly referred to as the “salmon louse” and *Caligus clemensi*. In farming regions in the Atlantic Ocean, *L. salmonis* infestation is considered one of the most serious marine pathogens of farmed and wild Atlantic salmon (*Salmo salar*) (Todd et al. 2000, Finstad et al. 2000, Björn et al. 2001, Holst et al. 2003, Revie et al. 2009) and there have been a number of reports suggesting that sea lice from farmed salmon can negatively affect wild salmon (Björn et al. 2001, Butler 2002, Heuch et al. 2005) and sea trout (Tully et al. 1999, Gargan et al. 2003) populations. Conversely in BC, *L. salmonis* have not been reported to be a significant health concern on salmon farms (Saksida et al. 2007, Marty et al. 2010) and recently the species of *L. salmonis* occurring in the Pacific Ocean has been demonstrated to be genetically different to that found in the Atlantic Ocean (Yasawa et al. 2008, Messmer et al. 2011). Although research has shown that wild Pacific salmon, particularly pink salmon (*Oncorhynchus gorbuscha*), are natural hosts for *L. salmonis* (Nagasawa 2001) and are highly resistant to the effects of sea lice (Fast et al. 2002, Jones et al. 2006; 2007; 2008), there has been considerable debate in the scientific press regarding the effects that sea lice (*L. salmonis*) infections of farmed Atlantic salmon (*Salmo salar*) were having on wild Pacific salmon populations in BC (Morton et al. 2004, Krkošek et al. 2007a,b, Brooks and Jones 2008, Marty et al. 2010).

Klemtu is a small, isolated aboriginal community of approximately 400 residents located on the BC central coast within the Kitasoo/Xai'xais traditional territory (Figure 1), the area of current and past occupation and use for the Kitasoo/Xai'xais people. Around 114 salmon-bearing streams are found in this territory which is also the most northerly salmon farming region of BC. This farming region is located more than 180km from the next closest farming region near Port Hardy on Vancouver Island. There are six operating salmon farms that produce around 3,000 tonnes of fish per year. Over the last 10 years there has been a switch from primarily raising Chinook salmon (*O. tshawytscha*) to Atlantic salmon. Even though aquaculture related activities provide 40-60% of the jobs in the community, the Kitasoo/Xai'xais people consider the wild salmon an essential food item and a key element of their cultural identity. As a result of the community's desire to better understand sea lice in their region, the Kitasoo Fisheries

Program was established to monitor lice loads on seaward migrating wild juvenile salmon in the region.

The following provides a summary of sea lice levels occurring on juvenile salmon, particularly pink salmon, sampled over a four year period (2005-2008). Prevalence of lice on wild Pacific salmon is compared and differences assessed over time and between regions with and without salmon farms. Lice infestation levels on Atlantic salmon collected from farms operating in the region were evaluated as well.

2. Material and Methods

2.1 Juvenile salmon sampling

The study area is located approximately 250km north of the Broughton Archipelago described in Jones and Hargreaves (2007). Sampling locations were selected and grouped into four zones based on their relative location to the salmon farms: near farm, up the channel and down the channel from farms, and a control zone where no farms exist (Figure 1). Locations included in the near farm zone were only sampled if the farms were stocked with salmon (Figure 1; Table 1). Sampling occurred in the spring of the year during juvenile Pacific salmon emergence from the local rivers.

Juvenile salmon sampling was carried out as described in Butterworth et al. (2008) utilizing a beach seine measuring approximately 30m x 2m. Between 50 and 100 juvenile salmon were sampled from each seine and placed in a small bucket using a dip net. Individual salmon from the bucket were euthanized by a swift blow to the head, placed in a labeled Ziploc™ bag and frozen for later evaluation in the laboratory. This technique may have resulted in an underestimation of preadult/adult stages of *C. clemensi* as this species is highly mobile, often swimming off its host (Saksida et al. 2007). However, it provides a good estimation of infestation for sessile stages of both *C. clemensi* and *L. salmonis* and for motile (preadult/adult) stages of *L. salmonis*.

Frozen samples were transported to the BC Centre for Aquatic Health Sciences in Campbell River, BC and stored at -20°C. Salmon were partially thawed for examination. The juvenile salmon were identified by species (pink, chum, 'other' salmon). Fork lengths (mm) and weights (g) were measured. Condition factor ($W \cdot L^{-3}$) was calculated for each fish. Each salmon was examined under a dissecting microscope and all lice were counted. All lice were identified to stage and species using criteria outlined in Jones et al. (2006).

2.2 Farm data

Farm data were provided by Marine Harvest Canada which operates the farm sites in the Kitasoo/Xai'xais traditional territory. Sea lice abundance data were collected using the standard sampling methods for sea lice on BC salmon farms as outlined in Saksida et al. (2007): a minimum of 20 fish from each of three pens, at least once a month. The salmon farming company also provided an estimate of the total number of Atlantic salmon on the farm during each sampling.

2.3 Statistical Analysis

Over the four year period, 5,399 wild juvenile salmon were collected and examined for sea lice. However, only data collected from 5,228 fish (96.8%) are described in this report. The main reason for this is that the poor condition of a small proportion of samples hindered proper laboratory assessment. Some samples were also removed for other reasons including missing or incomplete data sheets accompanying the samples.

Due to the fact that the vast majority of samples (>99%) had either one or no lice, the decision was made to use a simple presence/absence (prevalence) approach to assess and model the wild fish lice data. A logistic regression model evaluated factors associated with the presence/absence of sea lice on pink salmon, including: year and zone as categorical variables; fish weight and length entered as continuous variables; and a single interaction term year*zone. Only the pink salmon data collected during the years when sampling occurred in all the zones were included in the logistic regression. Chi-square test was used to evaluate juvenile pink salmon sea lice prevalence data collected in 2005 which were not included in the logistic regression analysis since data had not been collected from all four zones.

Sea lice abundance data from farms were examined using ANOVA to determine whether there was significant annual variation in motile *L. salmonis* and *C. clemensi* levels on the farmed salmon over the same period that most of the juvenile wild salmon sampling took place (April to June). Multiple comparisons were carried out using the Tukey HSD test. T-tests were used to assess whether there was any significant difference between motile *L. salmonis* and *C. clemensi* levels on the farms during the same period. Analysis was carried out on log-transformed data to correct for the positive skewness in the sea lice data (Saksida et al. 2007).

3. Results

Table 1 provides a summary of sampling periods and locations for the years examined in this study. The sampling season in 2005 was the shortest, and included the fewest locations (n=7); no sites located up the channel from the farms were sampled. During that year, the selected locations were sampled one to three times (mean= 1.4, SD= 0.8). In 2006, samples were collected from nine locations representing all four zones. The selected locations were sampled an average of 2.2 times (SD= 1.0; range 1 - 4). Samples

collected during 2007 and 2008 were the most similar both in terms of sites (n=9) and period sampled (late May to mid July). Sampling was also more frequent during these two years with the selected locations sampled on average 3 times (mean= 2.9, SD=1.2 and mean=3.3, SD=1.0 respectively; range 1-5). Fish were collected from all locations sampled.

Of the 5,228 fish examined, 78% (n=4,062) were identified as pink salmon, 17.5% (n=919) were chum salmon, while the remaining 4.7% (n=247) were classified as 'other' salmon. These juvenile salmon were not identified to species but were most likely coho (*O. kisutch*) and sockeye salmon (*O. nerka*) as there are no major Chinook salmon runs in the region. There were significant differences in both their weights and lengths, with chum being the smallest and the 'other' salmon grouping the largest (Table 2). The number of pink salmon collected ranged between 138 and 402 per zone per year with the mean annual sample total for all sites averaging 1,015 (range 758-1,442). Pink salmon were the most abundant species captured in each seine set.

Both *L. salmonis* and *C. clemensi* were observed on the wild juvenile salmon. Although all stages of both species were observed, motile (preadult/adult) stages predominated. Motile stages of *L. salmonis* accounted for between 67% (in 2005) and 95% (in 2008) of all lice observed (mean for all years was 80%). For *C. clemensi*, the percentage of motile stages ranged between 54% (2006) and 95% (in 2008) of all lice (mean=78%). Across all of the juvenile salmon species, around 9% were infected with one or more lice (Table 3). Single louse species infections predominated with only 13 (0.3%) pink salmon, three (0.3%) chum and three (1.2%) 'other' salmon having mixed louse species infections. There was no significant difference in *L. salmonis* prevalence levels among salmon species. However, chum salmon had significantly lower *C. clemensi* prevalence levels than either pink or 'other' salmon (while these two do not have significantly different prevalence levels from one another). The pink salmon were observed to have significantly higher levels of *C. clemensi* than *L. salmonis*, while no such differences were observed for chum salmon or for the 'other' salmon. Of the 4,062 pink salmon sampled, only 35 fish (i.e. less than 1%) had more than a single louse, providing justification for the use of a logistic regression approach to analyzing the data.

Since there was no sampling up the channel from farms during 2005, only data collected during 2006-2008 was evaluated using logistic regression. The generalized logistic model for *L. salmonis* showed year and zone were significantly associated with the presence of *L. salmonis* (Table 4) with the control zone consistently having the lowest *L. salmonis* prevalence levels in all three of the years examined. In 2008, the reporting prevalence was lowest for all zones (Figure 2A). The interaction variable (Year*Zone), pink salmon weight, and length did not contribute significantly to the model.

When *C. clemensi* on pink salmon were examined using a similar model, zone and year, they were found to be significantly associated with lice prevalence, but in this instance, fish weight was significant also (Table 5). Once again, the lowest prevalence values in all

zones were seen in 2008 (Figure 2B). The substitution of fish length in place of weight indicated that this factor was also strongly associated with the presence of *C. clemensi* and caused 'year' to drop from the model as a significant factor. The juvenile pink salmon sampled during 2008 not only had the lowest sea lice prevalence but also were significantly smaller in length than those sampled during the other years although their condition factor was higher than those sampled in either 2005 or 2006 (Table 6). In 2008, there were approximately 107 pink salmon weighing less than 1g (7% of total) while in the other years there were never more than two fish that weighed less than 1g. Thus, fish weight (and indeed length) is most likely to be confounded in some way with the variable year.

The 2005 data were analyzed separately. Chi-square test found no significant difference in *L. salmonis* prevalence among control, near farm and downstream zones (mean prevalence = 0.03, $p=0.33$). However, *C. clemensi* levels were significantly different among zones ($p<0.001$) with the prevalence of *C. clemensi* in both near farm and downstream zones being higher than that observed in the control zone (see also Figure 2B).

There were a total of six different Atlantic salmon farms in operation at some point during 2005 to 2008. The number of farms operating in the spring of each year (i.e. April to July) ranged from two to four: two in 2005, three in 2007 and four in both 2006 and 2008. The estimated total number of farmed salmon in June of each year ranged from 1.5M (2005) to 1.9M (2008). Figure 3 shows the average abundance of motile *L. salmonis* and *C. clemensi* on all farmed salmon in the area by month. A seasonal variation in *L. salmonis* levels was seen on the farm, with levels increasing over the summer before peaking towards the end of the year. This pattern was reasonably consistently from 2005 to 2007. In contrast, no such pattern was reported in 2008 when *L. salmonis* motile levels remained low throughout the year. Levels during the wild juvenile salmon migration period (April to June) were very low and did not vary significantly across years (mean motile stages abundance = 0.23; $p=0.58$).

The farm data show that *L. salmonis* is more abundant than *C. clemensi* on farmed Atlantic salmon in the fall and winter (September to January). However, during the spring (April to June), motile *L. salmonis* abundance levels were not significantly different than *C. clemensi* with the exception of 2005 when they were significantly lower (means of 0.16 and 0.88 respectively, $p= 0.03$). Mean monthly abundance levels of *C. clemensi* remained low (< 2 motile lice) on farmed salmon with no evidence of a seasonal pattern (Figure 3B). However, a significant annual difference in abundance levels of this species of louse during the wild juvenile salmon migration (April to June) was observed with higher *C. clemensi* levels seen during the odd years (2005 and 2007) than the even years (2006 and 2008) ($p < 0.001$).

All farms operating in 2005 and 2007 were treated for sea lice during November/December using emamectin benzoate (SLICE®). Treatments on the four

farms operational in 2006 occurred around a month earlier, in October. By the end of 2008, only two of the four farms in operation that year had treated for sea lice both in September of that year.

4. Discussion

The study was designed to monitor sea lice levels on juvenile salmon in the Kitasoo/Xai'xais territory located on the mid coast of BC. Although the sampling methodology was not designed to specifically target pink salmon, this species was captured in every seine set and was the most abundant salmon species captured. Pink salmon are the most abundant Pacific salmon species and have the simplest life cycle of all the Pacific salmon species (*Oncorhynchus sp.*) with a fixed two year life cycle. As a result, pink salmon spawning in even and odd years are reproductively isolated and have developed into genetically different lines. This unique characteristic makes them a less complicated group to evaluate in terms of population dynamics. As a result, assessing the effects that certain factors, such as sea lice infection, might have on these populations is more straightforward.

L. salmonis were found on the juvenile pink salmon as well as the other juvenile Pacific salmon species examined with no significant difference in levels of prevalence observed among the species. The sea lice prevalence levels on juvenile pink salmon in our study were considerably lower than those reported from other regions of BC: some of which have Atlantic salmon farms present (Jones and Hargreaves 2007), but others which come from regions with no salmon farming activity such as the southern BC area reported in Beamish et al. (2009). Intensity was also low with most infected fish hosting just a single louse. Both motile and non-motile lice were observed, though there was a predominance of motile stages of *L. salmonis*. Jones and Hargreaves (2007) reported a trend towards parasite maturation over the sampling season in the Broughton Archipelago, with a predominance of non-motile stages in March/April shifting to a predominance of motile stages by June. The larger proportion of motile stages observed in this study suggests that emergence from the rivers and exposure to sea lice likely occurred some weeks (or even months) prior to sampling.

Analysis indicated significant annual and regional differences in *L. salmonis* prevalence levels on juvenile pink salmon with higher lice levels seen in those zones having salmon farms than those without. The annual variation appears to be supported by earlier work, where Morton et al. (2004) found no lice on the limited number of fish sampled (n=36) in the farming area in 2002, while Butterworth et al. (2008) reported *L. salmonis* prevalence rates of between 12% and 26% in areas overlapping those represented by the up the channel from, near and down the channel from farms locations in the current study. Marty et al. (2010) reported that sea lice abundance in the spring on farmed salmon in another farming region in BC (the Broughton Archipelago) was highly correlated with the number of wild adult pink salmon returning to the area the previous

autumn and that farmed salmon are the main source of *L. salmonis* for juvenile salmon migrating out in the spring. In our study area, the pink salmon that return in the odd numbered years (e.g. 2005, 2007) are the dominant runs. For example, in 2007 there were 153,000 pink salmon that returned to the rivers in the area of the salmon farms; while in 2006 just over 15,000 fish returned (Greba, unpublished data). The autumn lice counts on the salmon farms do show considerable variation among years; however, little lice level variation was observed during the spring sampling period suggesting that the lice management practices employed by the farms are effective at controlling sea lice on farmed salmon. The lack of annual variation in *L. salmonis* on the farmed salmon during the spring suggests that there may be other factors which give rise to the inter-annual variations observed on the wild salmon in the region including environmental conditions (i.e. temperature, salinity) or other sources of lice (other fish).

All species of juvenile Pacific salmon examined also hosted *C. clemensi* with the lowest prevalence levels found on chum salmon. In 2005, higher levels of *C. clemensi* observed on the salmon farms were concurrent with higher levels of this species of louse on the wild juvenile pink salmon captured around farms and at locations down the channel from farms. An apparent decline in *C. clemensi* levels on the farms operating in the area occurred over the summer without the need for therapeutic treatment. *Caligus clemensi* is very mobile and it has been generally accepted that with its wide host range, which consists of both salmon and non-salmon species (Johnson et al. 2004, Beamish et al. 2005), salmon farms are unlikely to be the primary source (Krkošek et al. 2007a). Beamish et al. (2009) suggested that a possible source of *C. clemensi* could be Pacific herring (*Clupea harengus pallasii*) in their study of sea lice on wild juvenile salmon on the south coast of BC. The logistic regression analysis indicated that size (length) of juvenile pink salmon was significantly associated with *C. clemensi* infections with larger fish more likely to be infected. This may be associated with risk of exposure - larger juvenile pink salmon have likely been in seawater longer. This may also provide an explanation as to why the chum salmon, which are generally smaller, had significantly lower prevalence levels of *C. clemensi* than those seen on pink or 'other' salmon.

Overall, the Kitasoo/Xai'xais territory *L. salmonis* and *C. clemensi* lice prevalence levels reported, even near farms, were actually closer to the levels suggested by Krkošek et al. (2007a) as natural background levels (2-3% and 8-20% respectively) than they were to levels reported in the Broughton Archipelago (Jones et al. 2007). This includes the significantly higher levels of *C. clemensi* observed near farms in 2005. The Kitasoo/Xai'xais territory is situated closer to the sampling site described in Krkošek et al. (2007a) than it is to the Broughton Archipelago. Therefore, it may be that "background" levels depend more on the local environment and ecology, including the relative abundances of wild fish species, than simply the presence or absence of salmon farms.

Modifications have been made to improve the wild fish monitoring program which includes sampling earlier in the season in order to assess smaller pink salmon (<1g)

when they are more likely to be susceptible to infection. Controlled laboratory studies (Jones et al. 2006; 2008) could not induce mortality in pink salmon weighing more than 1g even when exposed to very high sea lice copepodid challenges. Measuring temperature and salinity at time of sampling has now been added to the sampling protocols as these factors were found to be very important not only for development of sea lice but also survival (Johnson and Albright 1991). It is hoped that the knowledge gained through the development of long-term, integrated programmes such as the one described in this report will lead to a better understanding of such inter-dependencies.

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Tables

- Table 1 Summary of sampling period and locations by year
- Table 2 Summary of mean and 95% confidence intervals for weight (g), length (mm), and condition factor of fish by Pacific salmon species [“Other” = coho and sockeye salmon]
- Table 3 Summary of the number of salmon (as % of total sampled) with various levels of *L. salmonis* and *C. clemensi* lice infestation [“Other” = coho and sockeye salmon; blank cells indicate n=0]
- Table 4 The results from the generalized logistic model on factors tested for association with the presence of *L. salmonis* on juvenile pink salmon
- Table 5 The results from the generalized logistic model on factors tested for association with the presence of *C. clemensi* on juvenile pink salmon
- Table 6 Mean and 95% confidence limits for weight (g), length (mm) and condition factor of juvenile pink salmon sampled between 2005 and 2008

Table 1 Summary of sampling period and locations by year

	Sampling Period	Sampling Locations
2005	June 1 - 20	1 - 4,6,7,11
2006	April 30 - June 23	3 - 5, 7-12
2007	May 29 - July 10	3 - 11
2008	May 22 - July 11	3 - 11

Table 2 Summary of mean and 95% confidence intervals for weight (g), length (mm) and condition factor of fish by Pacific salmon species ["Other" = coho and sockeye salmon]

Species	Mean weight (g) [95% CI]	Mean length (mm) [95% CI]	Mean condition factor [95% CI]
Chum	4.5 [4.3 - 4.7]	68.1 [67.0 - 69.2]	1.36 [1.32 - 1.40]
Pink	4.9 [4.8 - 5.0]	71.5 [71.1 - 71.9]	1.24 [1.23 - 1.26]
Other	15.9 [14.6 - 17.0]	99.9 [96.9 - 102.9]	1.49 [1.43 - 1.56]

Table 3 Number of salmon (and % of total sampled) with various levels of *L. salmonis* and *C. clemensi* lice infestation ["Other" = coho and sockeye salmon; blank cells indicate n=0]

	Pink (n=4062)		Chum (n=919)		Other (n=247)	
	<i>L. salmonis</i>	<i>C. clemensi</i>	<i>L. salmonis</i>	<i>C. clemensi</i>	<i>L. salmonis</i>	<i>C. clemensi</i>
no lice	96.3%	94.4%	97.0%	96.9%	94.4%	92.3%
1 louse	3.5% (n=142)	5.0% (n=204)	2.8% (n=26)	3.0% (n=28)	4.8% (n=12)	5.6% (n=14)
2 lice	0.2% (n=7)	0.6% (n=24)	0.2% (n=2)	0.1% (n=1)	0.4% (n=1)	1.2% (n=3)
3 lice	<0.1% (n=1)	<0.1% (n=2)			0.4% (n=1)	0.4% (n=1)
4 lice	<0.1% (n=1)					0.4% (n=1)

Table 4 The results from the generalized logistic model on factors tested for association with the presence of *L. salmonis* on juvenile pink salmon

	Degrees of freedom	Wald statistic	p
Intercept	1	15.2	< 0.001
Zone	3	15.9	0.001
Year	2	12.1	0.002
Length	1	0.40	0.527
Weight	1	0.19	0.663
Year*Zone	6	10.6	0.100

Table 5 The results from the generalized logistic model on factors tested for association with the presence of *C. clemensi* on juvenile pink salmon

	Degrees of freedom	Wald statistic	p
Intercept	1	303.2	< 0.001
Zone	3	20.1	< 0.001
Weight	1	4.6	0.032
Year	2	6.1	0.048
Year*Zone	6	3.1	0.799
Model re-run to incorporate fish length			
Intercept	1	64.7	< 0.001
Zone	3	19.9	< 0.001
Length	1	4.9	0.028
Year	2	4.1	0.128
Year*Zone	6	2.9	0.822

Table 6 Mean and 95% confidence limits for weight (g), length (mm) and condition factor of juvenile pink salmon sampled between 2005 and 2008

Year	Mean weight (g) [95% CI]	Mean length (mm) [95% CI]	Mean condition factor [95% CI]
2005	5.4 [5.2 – 5.6]	76.8 [76.2 – 77.4]	1.16 [1.13 - 1.19]
2006	5.1 [5.0 – 5.3]	76.5 [75.7 – 77.3]	1.06 [1.05 - 1.07]
2007	6.5 [6.3 – 6.7]	75.1 [73.3 – 74.8]	1.58 [1.54 - 1.62]
2008	3.5 [3.4 – 3.6]	63.0 [62.3 – 63.7]	1.25 [1.24 - 1.27]

Figures

- Figure 1 Shows the location and type of each sampling site. The dashed lined outlines the boundary of the Kitasoo/Xai'xais traditional territory.
- Figure 2 The prevalence (mean +/- SE) of wild juvenile pink salmon infected with *L. salmonis* (A) and *C. clemensi* (B) by year and sampling zone (control - no farms, up the channel from, near and down the channel from farms).
- Figure 3 Monthly abundance (mean +/- SE) of motile stages of *L. salmonis* (A) and *C. clemensi* (B) on Atlantic salmon farms for each year from 2005 to 2008. (Note that the scale differs on the Y- axis between graphs.)

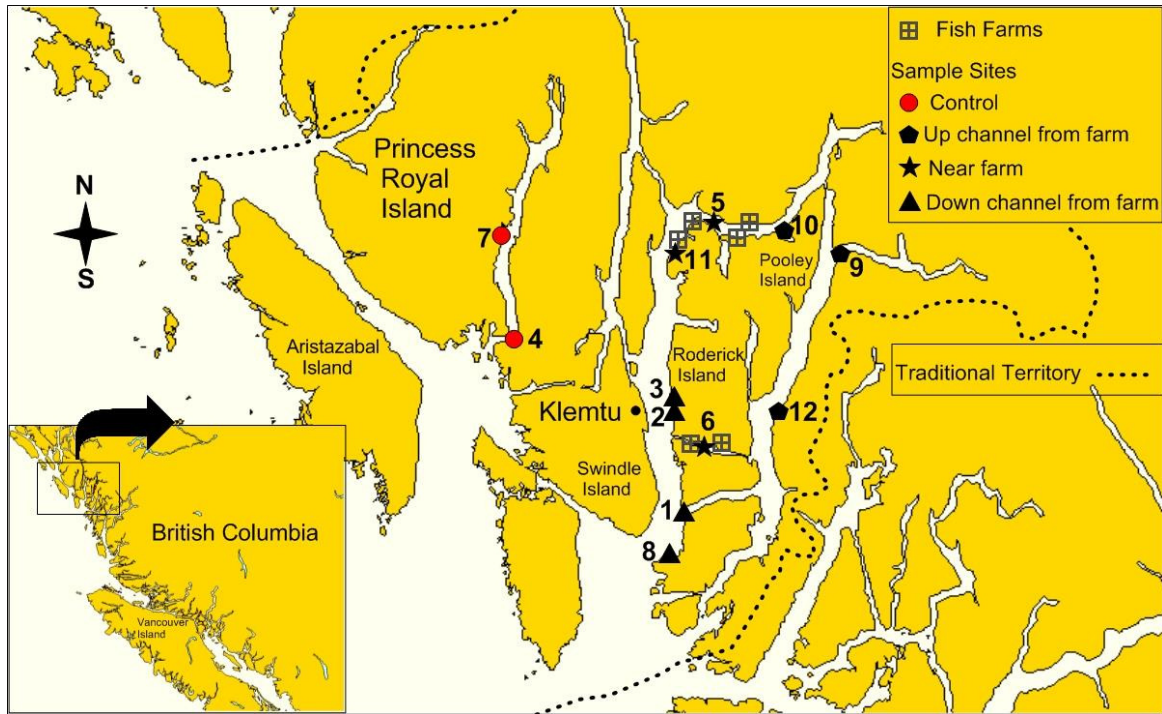


Figure 1 Location and type of each sampling site. The dashed lined outlines the boundary of the Kitisnoo/Xai'xais First Nation traditional territory.

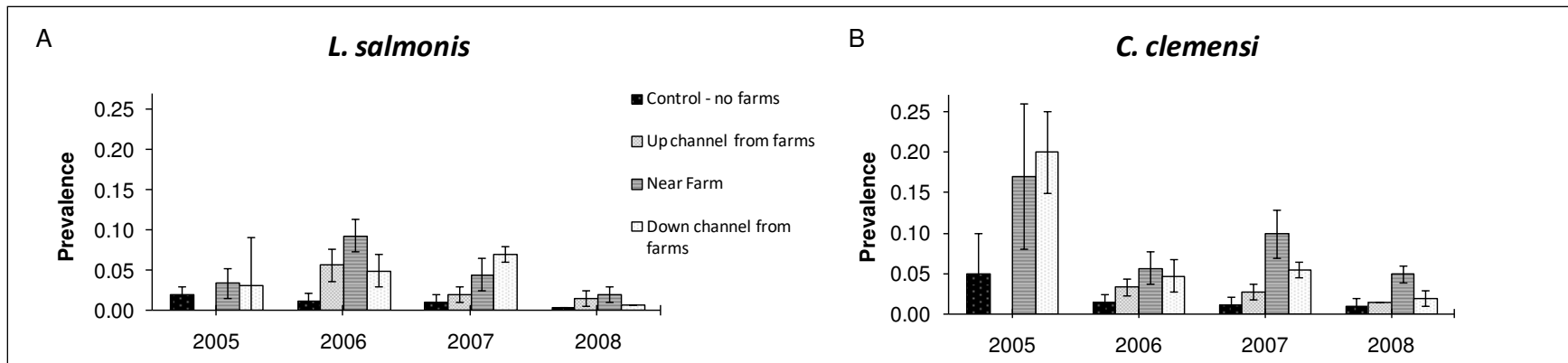


Figure 2 The prevalence (mean \pm SE) of wild juvenile pink salmon infected with *L. salmonis* (A) and *C. clemensi* (B) by year and sampling zone (control - no farms, up the channel from, near and down the channel from farms).

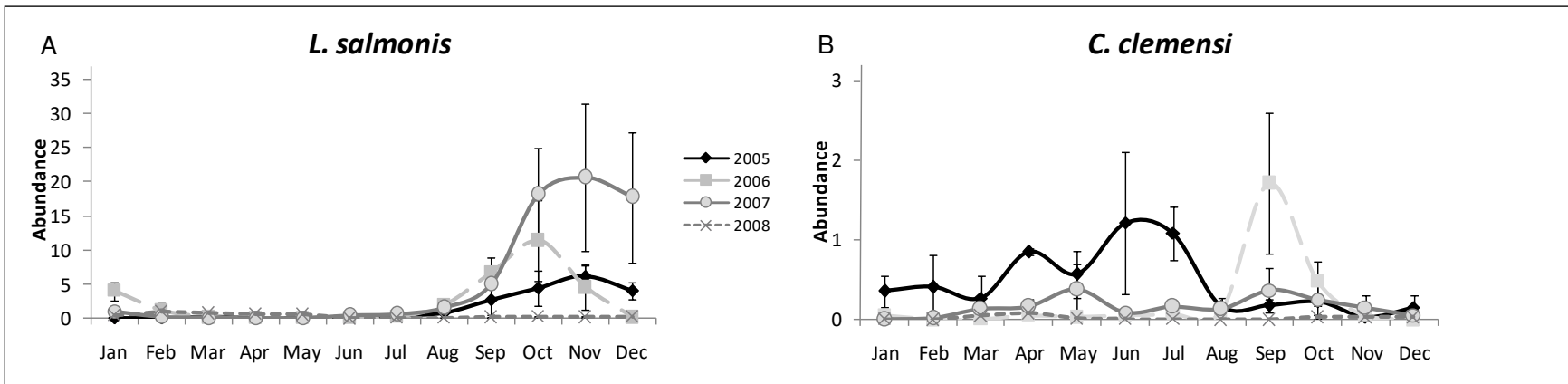


Figure 3 Monthly abundance (mean \pm SE) of motile stages of *L. salmonis* (A) and *C. clemensi* (B) on Atlantic salmon farms for each year from 2005 to 2008. (Note that the scale differs on the Y-axis between graphs.)