Prevalence and density of sea louse (*L. salmonis* and *C. Clemensi*) infections in juvenile chum salmon (*Oncorhychus keta*) in Clayoquot Sound, 2004-2007

Prepared by
The Clayoquot Sound Sea Lice Working Group*

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Summary

- The Clayoquot Sound Sea Lice Working Group is a collaborative relationship between Ahousaht First Nations, Tla-o-qui-aht First Nations, Creative Salmon Company Ltd. and Mainstream Canada. The group is coordinated by Uu-a-thluk (Nuu-chah-nulth Tribal Council Fisheries).

- Since 2004, the Clayoquot Sound Sea Lice Working Group has worked with the objective of monitoring the prevalence and density of sea lice (*Lepeophtheirus salmonis* and *Caligus clemensi*) on salmonid smolts throughout Clayoquot Sound.

- The predominant species of fish captured and analyzed between 2004 and 2007 was chum (*Oncorhynchus keta*) (n=5509). Additionally, 676 chinook salmon (*O. tshawytscha*), 121 coho salmon (*O. kisutch*), 31 Sockeye (*O. nerka*) and 121 three-spine stickleback (*Gasterosteus aculeatus*) were sampled.

- Analyses of these data reveal a prevalence of infection on chum between 7% and 20%, and chinook between 1% and 15%. This analysis combines findings of *L. salmonis* and *C. clemensi*.

- The chum sampled include sizes similar to those described by Jones et al. (2008) in pink salmon as being at elevated risk of sea lice infection.

- With the exception of Shelter Inlet, no statistically significant changes in prevalence, abundance or density between inlets were observed:
  - Seaward sites had significantly higher prevalence of infection that mirrored local salinity conditions.

- No statistically significant change in temperature between the different sample sites or inlets was observed.

- Salinity varied significantly at different sites and inlets:
  - Herbert Inlet and Tofino Inlet had lower salinities than Bedwell Sound, Fortune Channel and Shelter Inlet.

- Prevalence and abundance of sea lice infection on chum salmon showed a stepwise increase with increasing salinity.
  - Salinities between 20 and 30‰ (parts per thousand) are known to be an important factor in sea lice survival.
  - Mean salinities of all sample sites ranged between 5.72‰ and 27‰.
  - Optimum sea lice survival has been shown to be at a salinity of 30‰ by Johnson and Albright (1991).

- It appears that salinity is a more important factor in the distribution of sea lice in the inlets in Clayoquot Sound than the location of the sample sites.

Executive Summary

In 2004, a cooperative sea lice monitoring program came together in Clayoquot Sound to monitor wild salmon sea lice levels in the area. The Clayoquot Sound Sea Lice Working Group is an innovative relationship between First Nations stewards of traditional territories (Ahousaht and Tla-o-qui-aht First Nations) and salmon farmers within those territories (Creative Salmon Inc. and Mainstream Canada). Uu-a-thluk (the Nuu-chah-nulth Tribal Council's Fisheries Department) coordinates the group. The rationale for the formation of this group was a common belief amongst partners that sea lice are a naturally occurring parasite in Clayoquot Sound, but that there was little understanding of their role in the local marine environment. Research on sea lice dynamics exists in other parts of the province, but this study was the first of its kind in Clayoquot Sound. Therefore, the objective of monitoring their prevalence and density would address a knowledge gap and help the First Nations and the farmers growing salmon in these waters better understand local conditions.

Throughout the study, finfish aquaculture has been active in Clayoquot Sound, with an average of fourteen operational sites on twenty-one tenures. The Working Group has been conducting beach seines on twenty-one to twenty-eight sites throughout Clayoquot Sound from approximately mid-March until the end of June (the out-migrating period of local juvenile salmon). The group has been recording the prevalence and density of *Lepeophtheirus salmonis* and *Caligus clemensi* species on salmonid smolts within Clayoquot Sound since 2004. Because we did not begin to identify to species chalimus stage sea lice until 2006, for the purpose of this analysis, total lice numbers were used throughout. Juvenile fish analyzed for sea lice throughout the study include chum salmon (*Oncorhynchus keta*), chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), sockeye salmon (*O. nerka*) and three-spine stickleback (*Gasterosteus aculeatus*).

Dr. Kevin Butterworth of Torran Consulting was contracted to perform the statistical analysis of the juvenile salmon sea lice data gathered from 2004-2007. The results are summarized below.

Comparison of length and weight data between 2004 and 2007

All chum sampled between 2004 and 2007 at each site were under 10 cm in length and under 10 g in weight. The mean length was 4.3 ± 0.7 cm and the mean weight was 0.8 ± 0.6 g. Hence the chum sampled included sizes similar (under 0.7 g) to those described by Jones et al. (2008) in pink salmon (*Oncorhynchus gorbuscha*) as being at elevated risk due to sea lice (*Lepeophtheirus salmonis*) infestation.

Comparison of total prevalence of infestation between years (2004-2007)

The total prevalence of infestation of both *L. salmonis* and *C. clemensi* recorded between 2004 and 2007 for chum salmon varied from a low of 7% in 2007 to a high of 20% in 2006. On Chinook salmon, prevalence varied between 1% in 2006 to 15% in 2005. No baseline information exists as to the 'natural' levels of sea lice on juvenile salmon in Clayoquot Sound.

Due to the low numbers of chinook, coho, sockeye, cutthroat trout (*O. clarki*), three-spine stickleback, and perch (*S. alutus*) captured, no further analysis of these data were conducted. Rather, all further analysis focussed on chum salmon.

Differences in prevalence and abundance of sea lice infestation on chum salmon between sampling sites for 2004-2007

There was no statistically significant change in prevalence and abundance of sea lice infestation on chum salmon in Bedwell Sound, Fortune Channel, Herbert Inlet, Tofino Inlet and Sydney Inlet between 2004 and 2007. However, Shelter Inlet showed a statistically significant increase in sea lice prevalence on chum salmon for the years 2006 and 2007 between the sample sites near the top of the inlet at Belcher Point and Megin River (401 and 402) and those on the seaward side of the inlet at Dixon Bay and Jenny's Beach (403 and 404).

Differences in density (lice.g^{-1}) of sea lice infestation on chum salmon between inlets sampling sites in each inlet between 2004 and 2007

The prevalence of sea lice on juvenile salmon was low (7-20%), but for those that were infected, mean densities at specific sites pooled across all study years were found to vary between 0.77-2.5 lice/g^{-1}. There were no statistical significant differences in density of sea lice infestation on chum salmon between the sampling sites in each inlet.

Comparison of length data at Shelter Inlet between 2006 and 2007

The mean fork lengths of the chum salmon increased significantly from the sites sampled on the landward side of the inlet when compared to those sampled at the seaward side of the inlet. It is pertinent to point out that in Shelter Inlet, the prevalence of sea lice infestation (Fig. 3), the fork lengths of the chum salmon (Fig. 4) and the salinity at each sample site appear to follow the same trend. Hence it would appear that salinity may be a significant contributing factor to these differences as discussed below.

Differences in temperature and salinity of all sampling sites in all inlets between 2004 and 2007

There was no statistically significant change in temperature between the sample sites in each inlet, or in the temperature between the different inlets. However, salinities in Bedwell Sound showed some significant increases on the seaward sample sites. There was a significant decrease in salinity in Fortune Channel between a number of the sample sites within the channel and site number 206, which is situated at the convergence of Tofino Inlet and Fortune Channel. The overall lower salinities of Tofino Inlet compared to Fortune Channel may provide a possible explanation for this difference.

The salinities in Tofino Inlet were significantly higher at sample sites 510 (Island Cove) and 511 (Gunners Inlet) than those recorded in the main body of Tofino Inlet further towards the headwaters. A possible explanation is that mixing may be occurring between the higher salinities in recorded Fortune Channel and those of Tofino Inlet in this area. The salinities recorded in Shelter Inlet showed a similar trend to those of Bedwell Sound with statistically significant increases in salinity on the sample sites seaward in the Inlet.

Differences in temperature and salinity between inlets between 2004 and 2007

Overall, Herbert Inlet and Tofino Inlet had lower salinities than Bedwell Sound, Fortune Channel and Shelter Inlet. The exceptions were the sites 510 and 511, in Tofino Inlet which had higher salinities similar to those in Bedwell Sound, Fortune Channel and Shelter Inlet (a possible explanation for this anomaly at site 510 and 511 in Tofino Inlet is discussed above).

Differences in prevalence, abundance and density of sea lice infestation on chum salmon with temperature and salinity

Both prevalence and abundance of sea lice infestation on chum salmon showed a stepwise increase with increasing salinity. However, these increases were only statistically significant between salinities of 15-17.9‰ and 24-26.9‰.

Of the 72,884 chum salmon captured, 5509 were sampled, and analysis revealed that only 636 were infected with sea lice. It is these infected chum salmon that were used in the density analyses. Therefore, although the density of infestation appeared to increase with increasing salinity, these increases were not significant. Hence it appears that salinity is a more important factor in the distribution of sea lice in the inlets on the west coast of Vancouver Island than the location of the sample sites.

Definitions

The four most common terms used to describe sea lice distribution are prevalence, abundance, intensity and density. The accepted definitions of these terms with regards to sea lice were set out by the Pacific Salmon Forum in the publication “Protocols & Guidelines: A Reference Manual for Research Involving Wild/Cultured Fish Interactions with Sea Lice.” A brief definition to define the use of these terms in this document is given below.

- **Prevalence** is the number of hosts infected with one or more sea lice divided by the number of hosts examined. Hence if 10 salmon were sampled and three had 2 sea lice each, then 3/10 salmon were infected giving a prevalence of 30%.

- **Abundance** is the total number of sea lice divided by the total number of hosts examined. Therefore for the example above, if 10 salmon were sampled and three had 2 sea lice each, then 3 salmon x 2 sea lice = 6 sea lice total. Or, an abundance of 6/10 or 0.6.

- **Intensity** is the number of sea lice on a single salmon. Therefore for the same sample the three salmon that had 2 sea lice each had an intensity of infection of 2. NOTE: Intensity is not used throughout the report because the fish collected during the study were of varying sizes. The effect of 1 louse on a 1 gram fish will be greater than the effect of 1 louse on a 10 gram fish. Instead we used density throughout the report in order to level the playing field and provide a better understanding of what was happening with the small fish (which are at higher risk if infected).

- **Density** is the number of sea lice on a single salmon divided by the weight of the salmon. Hence if a salmon weighing 1 gram has 2 sea lice the density is 2 lice.g^{-1}. However, a salmon with 2 sea lice weighing 10 grams would have a density of 0.2 lice.g^{-1}. Hence density measurements take into account the size of the salmon when comparing sea lice numbers between salmon.

- **Statistical significance** is a statistical term used when a result is statistically significant and is unlikely to have occurred by chance. When reporting on statistical significance, statisticians usually refer to p-values. The p-value indicates the probability that the result obtained in a statistical test is due to chance rather than a true relationship between measures. Small p-values indicate that it is very unlikely that the results were due to chance. Therefore, if the p-value is small, statisticians would be confident that the result obtained is "real." When p is less than 0.05 ($P<0.05$)- meaning that there is a less than 5% chance that the relationship is due to chance - statisticians usually conclude that the relationship is strong enough that it is probably not just due to chance. A p-value of 0.05 or less is the commonly used standard to determine that a relationship between variables is significant and is the value used throughout this report.

Background

In British Columbia, much research and public concern has focused on the Broughton Archipelago. However, data on sea lice incidence on wild Pacific salmon from other areas can provide valuable baseline data (where no salmon farms exist) as well as comparative data from areas with existing salmon farms, such as Clayoquot Sound on the west coast of Vancouver Island (WCVI).

In 2003, the two salmon farm companies operating in Clayoquot Sound (Creative Salmon Company Ltd. and Mainstream Canada Ltd.) began sampling for sea lice on wild juvenile salmon, and in 2004 the Working Group was struck, incorporating the two local First Nations groups. The salmon farming companies, in approaching the First Nations communities, have respected the governance structures of the traditional territories within which the companies operate. From inception, the group has worked closely with DFO on sampling methodology and on the choice of appropriate seining sites.

Since that time, the salmon farming companies have been working with the local First Nations to foster the relationship between these parties. Creative Salmon grow only Chinook salmon and operate in the traditional territories of Tla-o-qui-aht First Nations in the Tofino Inlet / Fortune Channel area. Sampling with Tla-o-qui-aht Fisheries focuses mainly on emigrating salmon fry/smolts from the Kennedy Watershed. Mainstream Canada, which operates in Ahousaht territory and farms Atlantic salmon, focuses their sampling efforts along with Ahousaht Fisheries in Bedwell Sound (migration route for salmon from the Bedwell/Ursus Rivers). Uu-a-thluk (Nuu-chah-nulth Tribal Council Fisheries), works with all parties to sample other salmon routes within Ahousaht territory where Mainstream has farms; namely Herbert Inlet (migration route for salmon from the Moyaha River), and Shelter Inlet (migration route for salmon from the Megin River). The team sends the frozen captured juvenile salmon to a consultant at Mainstream Biological Consulting Inc., for analysis. In 2008, Dr. Kevin G. Butterworth conducted the statistical analyses of the 2004-2007 data, and the sampling is on-going in 2009.

Collaboration

In the BC Pacific Salmon Forum's Final Report (2009), the authors noted that "everyone calls for facts and science-based processes, but every contending group offers its own 'facts' and the science itself is often contradictory and not always agenda free." That same report (PSF 2009) also states that "industry distrusts the findings of research originating from conservation groups; ENGOs distrust studies carried out by scientists connected to government or business. Too little science is seen as agenda-free."

The Clayoquot Sound Sea Lice Working Group sought to avoid such personal agendas when conducting sea lice monitoring even though this group brought together industry and First Nation environmental stewards that don't always agree on all aspects of the salmon aquaculture industry. The group follows a pre-established "Code of Behaviour" when participating in the research in order to ensure that participation in the research would meet the demands and scrutiny of the main stakeholder groups. All participating parties voluntarily joined the group, and understood at inception that joint sampling is one method for increasing capacity in both the First Nations and the salmon farm companies in fostering technical skills and for increasing mutual understanding about the issues and concerns of all parties.

Methodology

Field work methodology

Sea lice sampling of juvenile salmonids in Clayoquot Sound occurs from approximately March 15 through to June 30 using established beach seine procedures that conform to DFO protocols.

Figure 1 shows all the beach seine sample sites used throughout the course of the study. On average, about 25 sites were sampled on a regular, systematic basis each year. The selection of beach seining sites was based on physical conditions, such as having a beach conducive to beach seining as well as distance from fish bearing streams and creeks. Sites were initially chosen based on their suitable characteristics at a low tide, which provides the best access to near shore eelgrass beds. However, it was not always logistically possible to conduct the sampling at low tides

Figure 1: Map of Clayoquot sound depicting all potential samples sites for Bedwell Sound, Fortune Channel, Herbert Inlet, Shelter Inlet, Tofino Inlet, and Sydney Inlet

For the study, juvenile fish are caught using a beach seine (2 beach seines are used, both are 150’ long and 12’ deep. One seine has a mesh size of ½”, the other is finer at 5/16”). To conduct the seine, the sampling boat approaches a beach, and allows one person to jump off of the bow of the boat holding the line from one end of the beach seine. A second person drives the boat while a third carefully deploys the net from the bow of the boat. The boat is driven near shore and the bow person jumps off with the line from the other end of the net while the boat driver secures the boat and takes the water quality parameters in the top six inches (fifteen centimeters) of water (DO, salinity, percent saturation, temperature). The beach seine is carefully pulled in, evenly from each end (Fig. 2). As the net begins to shallow, the lead line is pulled in carefully to ensure that fish do not escape from the sides of the net. If the net is hung up on a rock or submerged log, the boat driver tries to walk out to the snag, or use the boat, to carefully pull on the nets to slip it over the hang-up.

Figure 2: Beach seine is carefully pulled in while boat driver records water quality parameters (DO, temperature, salinity, percent saturation)

The fish in the net are transferred to a white bucket using a dip net (Fig. 3). At times, less than thirty fish are caught. Other times, thousands of fish are bagged in the seine. Up to thirty juvenile salmon of each species are individually bagged (Fig. 4) and placed in a cooler. When the crew returns to shore, the fish are frozen until laboratory analysis.

Figure 3: Fish are transferred from the seine net to a white bucket using a dip net.

Figure 4: Chum salmon, (*Oncorhynchus keta*), *individually bagged then frozen for future analysis.*

As noted above, not all fish captured were analyzed. Table 1 shows the number of juvenile salmon of each species and stickleback that were captured between 2004 and 2007. The table also notes the number of beach seines conducted throughout the study period of approximately mid-March to the end of June of each sampled year.

Species	**2004**		**2005**		**2006**		**2007**	
	N	**# of seines**	**N**	**# of seines**	**N**	**# of seines**	**N**	**# of seines**
Chum (*O. keta*)	25,261	159	2,547	139	5,908	109	14,560	86
Chinook (*O. tshawytscha)*	216	159	175	139	137	109	117	86
Coho (*O. kisutch*)	32	159	42	139	38	109	2	86

Sockeye (*O. nerka*)		159	31	139		109		86
Stickleback (*G. aculeatus*)	*NA*	159	*NA*	139	257	109	41	86

Table 1: Number of fish captured, and the number of beach seines conducted, in each sample year.

Laboratory Analysis: Sea lice identification

Mainstream Biological Consulting of Campbell River, BC, was selected by the Clayoquot Sound Sea Lice Working Group to conduct the sea lice identification, morphometric measurements, and to enter all data into a database. Mainstream Biological Consulting is in no way associated with Mainstream Canada.

The frozen fish were thawed, counted and identified to species. They were then scanned under a stereoscopic dissection microscope for the presence of sea lice. The microscope was set at a magnification of 20X for the preliminary survey of each fish sample, but magnification was occasionally increased to 40X during individual lice identification. Individual bags were inspected after the removal of the specimen for the presence of pre-adult or adult lice that may have become dislodged from the fish specimen. These “loose” lice were recorded on the data sheet with the corresponding specimen’s data and it was assumed that the lice had come from that individual.

At the beginning of the study, the sea lice on the thawed juvenile wild salmon collected by the Clayoquot Sound Sea Lice Working Group were identified as being in the non-motile or motile stage of their life history. Non-motile lice were identified as chalimus and were not identified to genus. Motile lice (pre-adults and adults) were identified as either *Lepeophtheirus sp.* or *Caligus sp.* and as male or female specimens. The motile lice were not identified to species, but have been assumed to be either *L. salmonis* or *C. clemensi* due to the lack of documented infections of Pacific salmon by other lice species.

In 2007, the sea lice identification methodology was slightly revised, identifying the species and the developmental stage of each louse. Motile lice were also distinguished as male or female.

Individual fish specimens were measured (fork length for salmonids, total length for non-salmonids) in millimeters and weighed to the nearest tenth of a gram.

In order to ensure the accuracy of the sea lice identification and to maintain strict scientific principles to increase public confidence in this research, a randomly selected sub-sample of fish specimens (10% of the total sample) was sent to a second technician to undergo similar analysis. The results from the two independent analyses were compared and a confidence level for the accuracy of the initial sea lice analysis was determined.

Results and Discussion

Comparison of length and weight data between 2004 and 2007

Between 2004 and 2007, a range of species were captured during sampling including chum, (*Oncorhynchus keta*), chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), sockeye (*Oncorhynchus nerka*) and cutthroat trout (*Oncorhynchus clarki*). Non salmonids included the three-spine stickleback (*Gasterosteus aculeatus*), and pacific perch (*Sebastes alutus*). The predominant salmon sampled was chum, with 5509 individuals examined for sea lice, followed by Chinook with 676 examined. Very low numbers of coho**,** sockeye, cutthroat trout, three-spine stickleback, and perch were captured.

Due to the high numbers of chum sampled, the length (mm) and weight (g) relationships for the chum salmon are presented graphically for ease of comparison (Fig. 5). Not all sample sites were sampled in each inlet for each of the study years. Bedwell Sound, Fortune Channel and Tofino Inlet were sampled each year between 2004 and 2007. Herbert Inlet and Shelter Inlet were sampled in 2006 and 2007. Sydney Inlet was sampled in 2006.

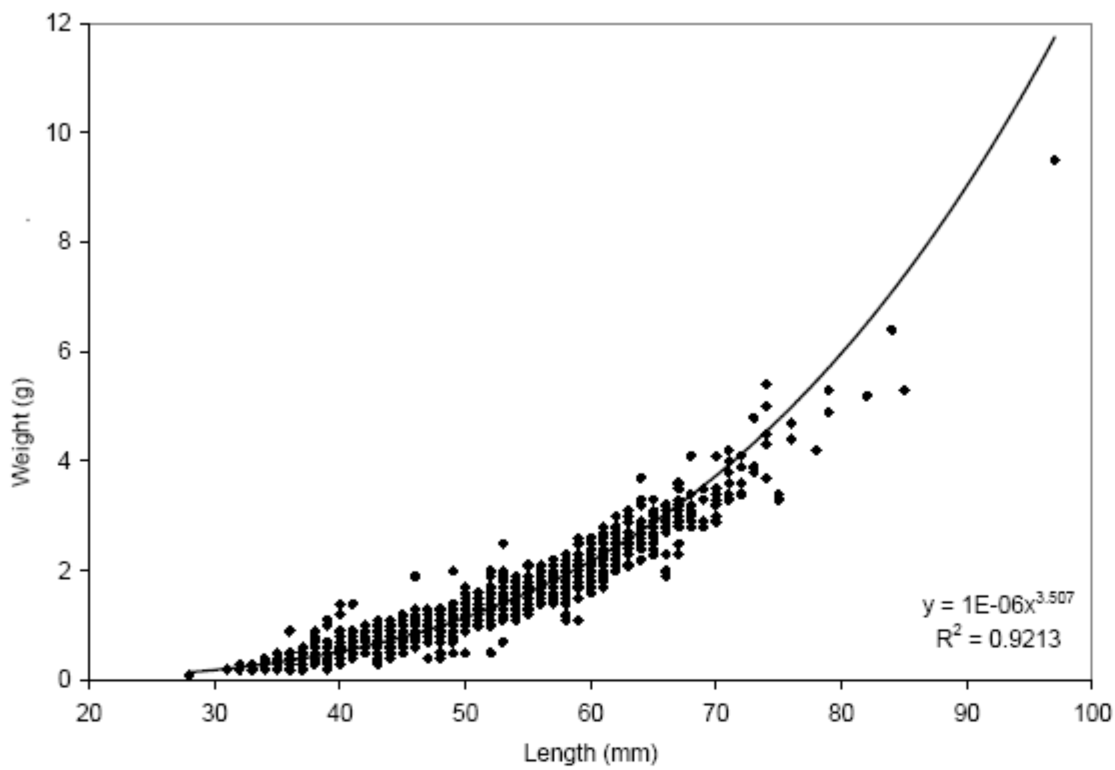


*Figure 5: Comparison of weight (g) and length (mm) of chum (*O. keta*) between 2004 and 2007 in Bedwell Sound, Fortune Channel, Herbert Inlet, Shelter Inlet, Tofino Inlet and Sydney Inlet. The trend line, equation and R^2 values are included.*

All of the chum sampled between 2004 and 2007 at each site were under 10 cm in length and under 10 g in weight. The mean length was 4.3 ± 0.7 cm and the mean weight was 0.8

± 0.6 g. Hence the chum sampled included sizes similar (under 0.7 g) to those described by Jones et al. (2008) in pink salmon (*Oncorhynchus gorbuscha*) as being at elevated risk of physiological impacts as a result of sea lice (*Lepeophtheirus salmonis*) infection.

Comparison of total prevalence of infestation between years (2004-2007)

Using the definitions above, the prevalence of infection (%) was calculated and compared to the total number of fish examined each year (Table 2).

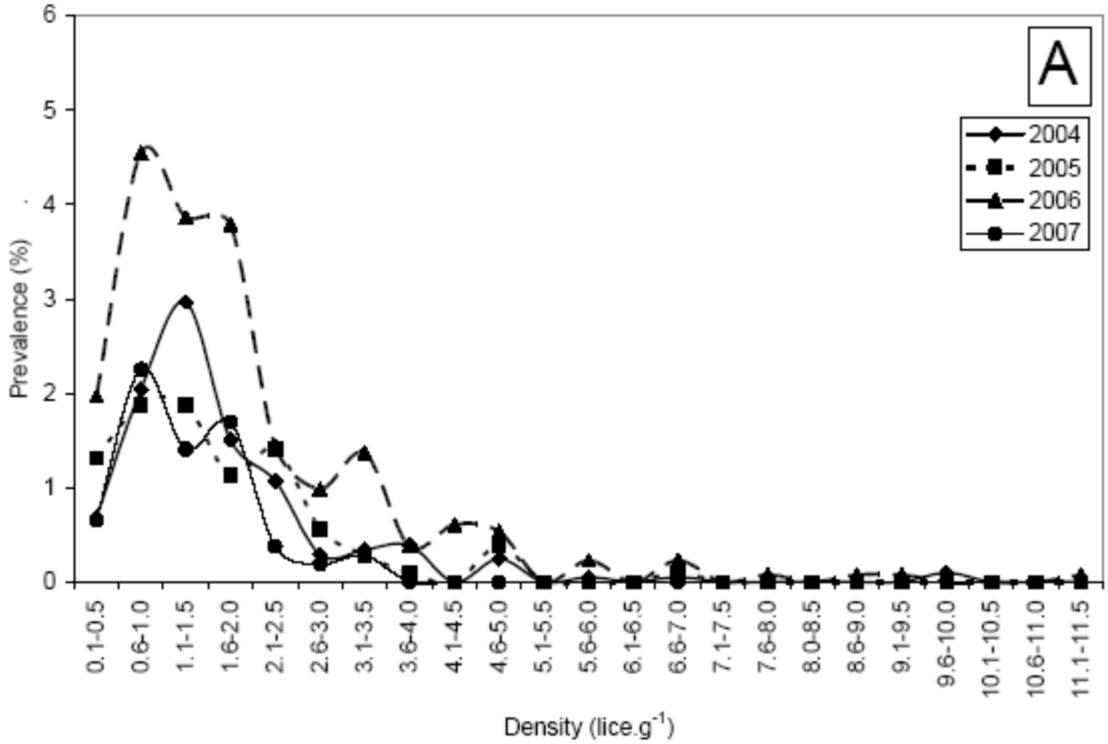
Table 2: Species, total numbers and prevalence of sea lice on fish sampled between 2004 and 2007 in Sydney Inlet, Shelter Inlet, Herbert Inlet, Bedwell Sound, Fortune Channel and Tofino Inlet.

Species	**2004**		**2005**		**2006**		**2007**	
	N	**Prevalence (%)**	**N**	**Prevalence (%)**	**N**	**Prevalence (%)**	**N**	**Prevalence (%)**
Chum (*O. keta*)	2057	10%	1065	9%	1321	20%	1066	7%
Chinook (*O. tshawytscha*)	315	4%	173	15%	137	1%	51	14%
Coho (*O. kisutch*)	32	12.50%	42	0%	31	3%	16	0%
Sockeye (*O. nerka*)	0		31	12.90%	0		0	
Stickleback (*G. aculeatus*)	11	18%	0		68	9%	42	7%

The total prevalence of infestation of both *L. salmonis* and *C. clemensi* recorded between 2004 and 2007 for chum salmon varied from a low of 7% in 2007 to a high of 20% in 2006. On Chinook salmon, prevalence varied between 1% in 2006 to 15% in 2005. No baseline information exists as to the 'natural' levels of sea lice on juvenile salmon in Clayoquot Sound. The Clayoquot prevalence findings are higher than the 4.5% prevalence of sea lice on chum in 2007 which was reported in the Bella Bella region (Raincoast Conservation Foundation, 2007), but similar to those reported for the Mathieson and Finlayson Channels on the Central coast where a mean prevalence of *L. salmonis* was 18.4% (Butterworth et al., 2008). However, Clayoquot prevalence findings are lower than that cited in Jones and Hargreaves (2007) and Beamish et al (2006), both of whom studied juvenile salmon infection in the Broughton Archipelago. Jones and Hargreaves (2007) found a prevalence of *L. salmonis* on chum in 2004 of 58.6% and of 23.1% in 2005. Beamish et al (2006) found a 24% prevalence of combined *L. salmonis* and *C. clemensi* on pink salmon in 2003. Note that both Butterworth et al (2008) and Jones and Hargreaves (2007) reported on *L. salmonis* infection, whereas Clayoquot data reports on combined infection from *L. salmonis* and *C. clemensi*.

As prevalence only shows the percentage of infected salmon versus uninfected salmon, it doesn't give an idea as to the distribution of sea lice amongst the salmon sampled. Hence by comparing prevalence and density, a more comprehensive view can be obtained (Fig. 6).

The mean densities at specific sites pooled across all study years were found to vary between 0.77-2.5 lice.g^{-1}. However, densities of over 1.6 lice.g$^{\square}$ were observed on between 0.8 % (2007) and 6% (2006) of chum sampled and between 0% (2006) and 2.5% (2004) percent of chinook sampled. These are similar densities to those reported for the Mathieson and Finlayson Channels on the Central coast by Butterworth et al. (2008) of 0.42%. In comparison, densities of over 1.6 lice.g$^{\square 1}$ were reported on 90% of juvenile pink and chum salmon sampled in the Broughton Archipelago (Morton et al., 2004).



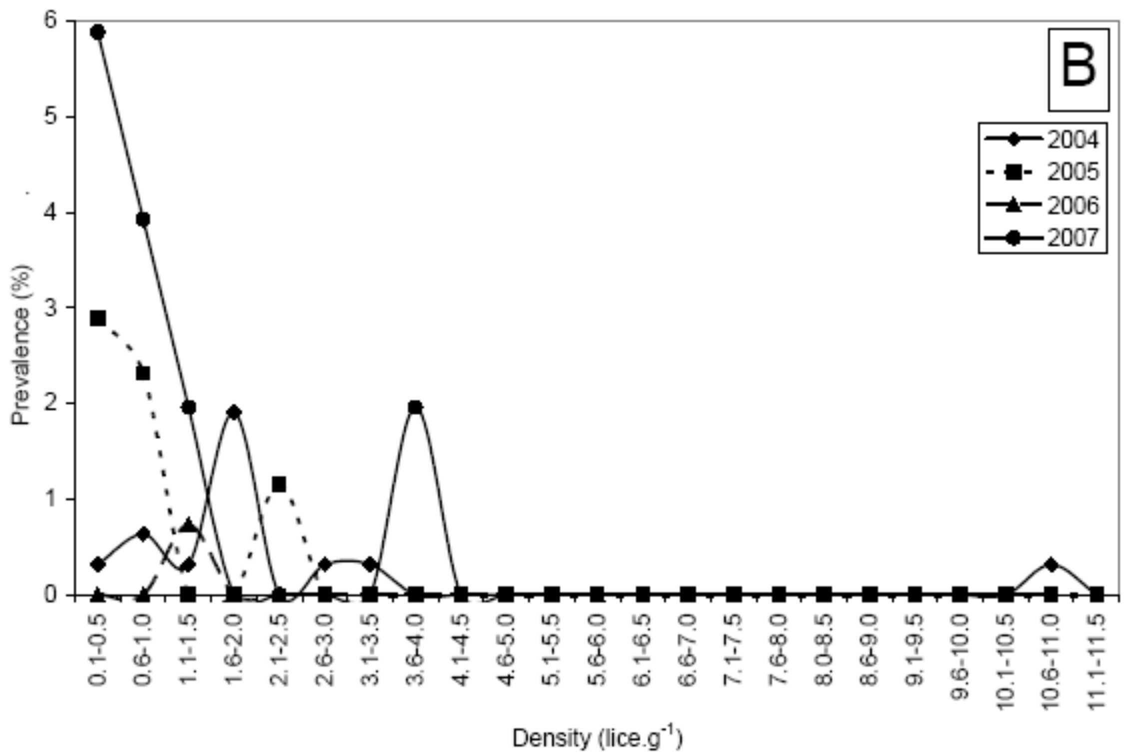


*Figure 6: Comparison of total prevalence and density of infestation for **A** chum (*O. keta*) and **B** chinook (*O. tshawytscha*) between 2004 and 2007 in Sydney Inlet, Shelter Inlet, Herbert Inlet, Bedwell Sound, Fortune Channel and Tofino Inlet. Values are mean ± standard error.*

Due to the low numbers of chinook, coho, sockeye, cutthroat trout, three-spine stickleback, and perch captured, no further analysis of these data were conducted. Rather all further analysis was focussed on chum.

Differences in prevalence and abundance of infestation between sampling sites for 2004-2007

The mean prevalence and abundance for each sample site from 2004-2007 was calculated and compared to the other sample sites in the same inlet.

Bedwell Sound

These data showed a normal distribution (0.882≤normal scores≤1.000 and 0.903≤normal scores≤1.000). Additionally, there was no significant heterogeneity of variance for abundance (Levene's test = 1.13, p = 0.386) and prevalence (Levene's test = 0.8, p = 0.618). An ANOVA revealed that there was no significant difference in abundance (f_{10} = 0.98, p = 0.49) and prevalence (f_{10} = 1.29, p = 0.295) of sea lice on chum sampled from the sites in Bedwell Sound between 2004 and 2007.

Fortune Channel

These data showed a normal distribution (0.96≤normal scores≤1.000 and 0.892≤normal scores≤1.000). Additionally, there was significant heterogeneity of variance (Levene's test = 4.94, p = 0.027) for abundance, but not for prevalence (Levene's test = 3.53, p = 0.062). A Kruskal-Wallis Test revealed that there was no significant difference in abundance (H_7 = 9.42, P = 0.224), and an ANOVA revealed that there was no significant difference in prevalence (f_7 = 1032, p = 0.343) of sea lice on chum sampled from the sites in Fortune Channel between 2004 and 2007.

Herbert Inlet

Only site number 301 has been sampled for more than one year in Herbert Inlet. It is necessary for there to be a measure of temporal variation to avoid pseudoreplication[1]. Hence, it is not possible to examine the prevalence and abundance data for Herbert Inlet for statistical significance at this time.

Shelter Inlet

These data showed a normal distribution (normal scores =1.000). Additionally, there was no significant heterogeneity of variance for abundance (Levene's test = 4.82, p = 0.186) and prevalence (Levene's test = 5.33, p = 0.149). An ANOVA revealed that there was no significant difference in abundance (f_3 = 2.41, p = 0.207) of sea lice on chum sampled from the sites in Shelter Inlet between 2006 and 2007. However, there were significant difference in prevalence (f_3 = 18.86, p = 0.008) of sea lice on chum sampled from the sites in Shelter Inlet between 2006 and 2007. Post-Hoc testing (Tukey's Test) revealed that the sample sites 401 and 402 near the top of the inlet had significantly lower prevalence of sea lice infestation on chum than those sampled at sample sites 403 and 404 on the seaward side of the inlet (Fig. 7).

[1] Pseudoreplication is defined by Hurlbert (1984) as the use of inferential statistics to test for treatment effects with data from an experiment where either the treatments were not replicated or the experimental units are not statistically independent.

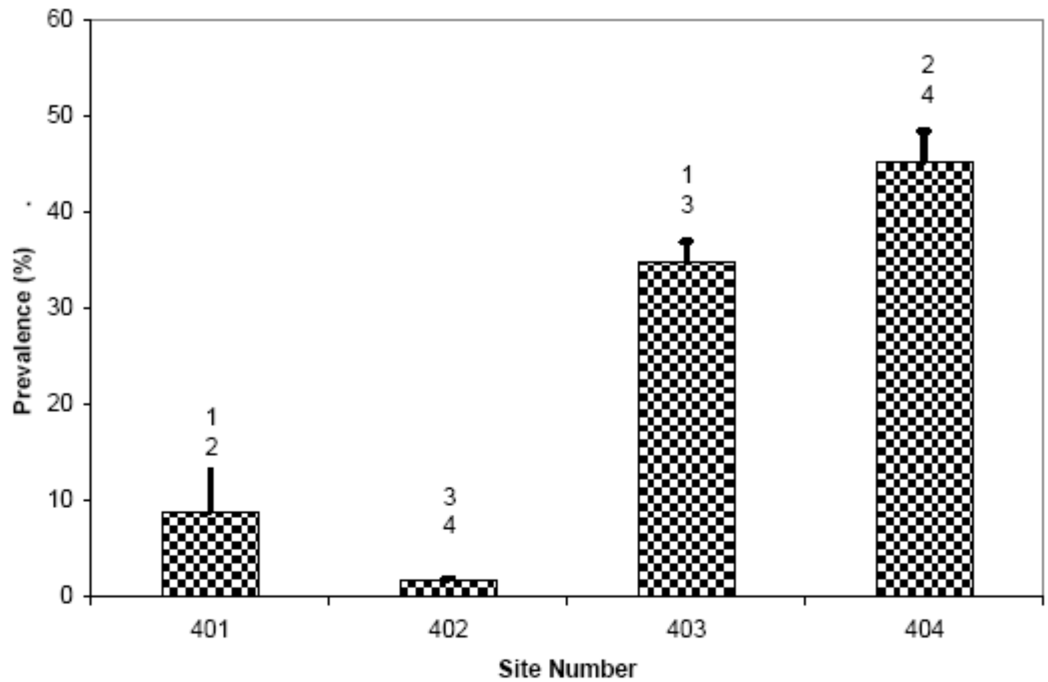


*Figure 7: Mean prevalence of sea lice on chum (*O. keta*) sampled in Shelter Inlet between 2006 and 2007. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different.*

Tofino Inlet

These data showed a normal distribution (normal scores =1.000). Additionally, there was no significant heterogeneity of variance for abundance ((Levene's test = 1.02, p = 0.455) and prevalence (Levene's test = 1.16, p = 0.388). An ANOVA revealed that there was no significant difference in abundance (f_6 = 1.41, p = 0.287) and prevalence (f_6 = 1.71, p = 0.203) of sea lice on chum sampled from the sites in Tofino between 2004 and 2007.

Sydney Inlet

As Sydney Inlet was only sampled in 2006, it is not possible to examine the prevalence and abundance data for Sydney Inlet for statistical significance at time (see Herbert Inlet above for a more detailed explanation).

Differences in density (lice.g^{-1}) of sea lice infestation on chum salmon at sampling sites in each inlet between 2004 and 2007

As discussed above, density is the number of sea lice on a single salmon divided by the weight of the salmon. Hence, the graphs in this section only pertain to those chum salmon that were infected with sea lice. Therefore, of the 5509 chum salmon sampled, 636 were infected with sea lice. It is these infected chum salmon that were used in the following analyses.

Bedwell Sound

These data showed a normal distribution (0.809≤normal scores≤0.988). Additionally, there was no significant heterogeneity of variance (Levene's test = 0.93, p = 0.499). An ANOVA revealed that there was no significant difference density (f_9 = 1.75, p = 0.082) at the sites in sampled in Bedwell Sound between 2004 and 2007.

Fortune Channel

These data showed a normal distribution (0.814≤normal scores≤1.000). Additionally, there was no significant heterogeneity of variance (Levene's test = 0.37, p = 0.872). An ANOVA revealed that there was no significant difference density (f_6 = 0.52, p = 0.795) at the sites in sampled in Fortune Channel between 2004 and 2007.

Herbert Inlet

Only 5 sea lice infested chum salmon were captured in Herbert Inlet. Hence no further analysis was conducted due to insufficient data.

Shelter Inlet

These data showed a normal distribution (0.761≤normal scores≤1.000). Additionally, there was no significant heterogeneity of variance (Levene's test = 0.05, p = 0.985). An ANOVA revealed that there was no significant difference density (f_3 = 0.24, p = 0.868) at the sites in sampled in Shelter Inlet between 2006 and 2007.

Tofino Inlet

These data showed a normal distribution (0.915≤normal scores≤1.000). Additionally, there was no significant heterogeneity of variance (Levene's test = 0.51, p = 0.605). An ANOVA revealed that there was no significant difference density (f_3 = 0.24, p = 0.842) at the sites in sampled in Tofino Inlet between 2004 and 2007.

Sydney Inlet

Only one sea lice infested chum salmon was captured in Sydney Inlet. Hence no further analysis was conducted due to insufficient data.

Comparison of length data at Shelter Inlet between 2006 and 2007

The only significant differences in prevalence, abundance and density of sea lice infection was found in Shelter Inlet between 2006 and 2007, where there was a significant increase in prevalence, but not in abundance or density of sea lice infection. This is interesting as logically one would expect density of infestation to increase as prevalence increases. However, this has proven to not be the case at the sample sites in Shelter Inlet between 2006 and 2007. To further examine potential causes of this result, the fork lengths of the chum salmon sampled from Shelter Inlet were compared for 2006 and 2007.

These data showed a normal distribution for 2006 (0,933≤normal scores≤0.957) and 2007 (0.836≤normal scores≤0.954). Additionally, there was heterogeneity of variance in the length data for 2006 (Levene's test = 9.81, $p<0.001$) and 2007 (Levene's test = 7.77, $p<0.001$). Kruskal-Wallis Tests revealed that there were significant differences in length between the sample sites in Shelter Inlet (H_3 = 52.87, P < 0.001; H_3 = 97.54, P < 0.001) for 2006 (H_3 = 52.87, P < 0.001) and 2007 (H_3 = 97.54, P < 0.001).

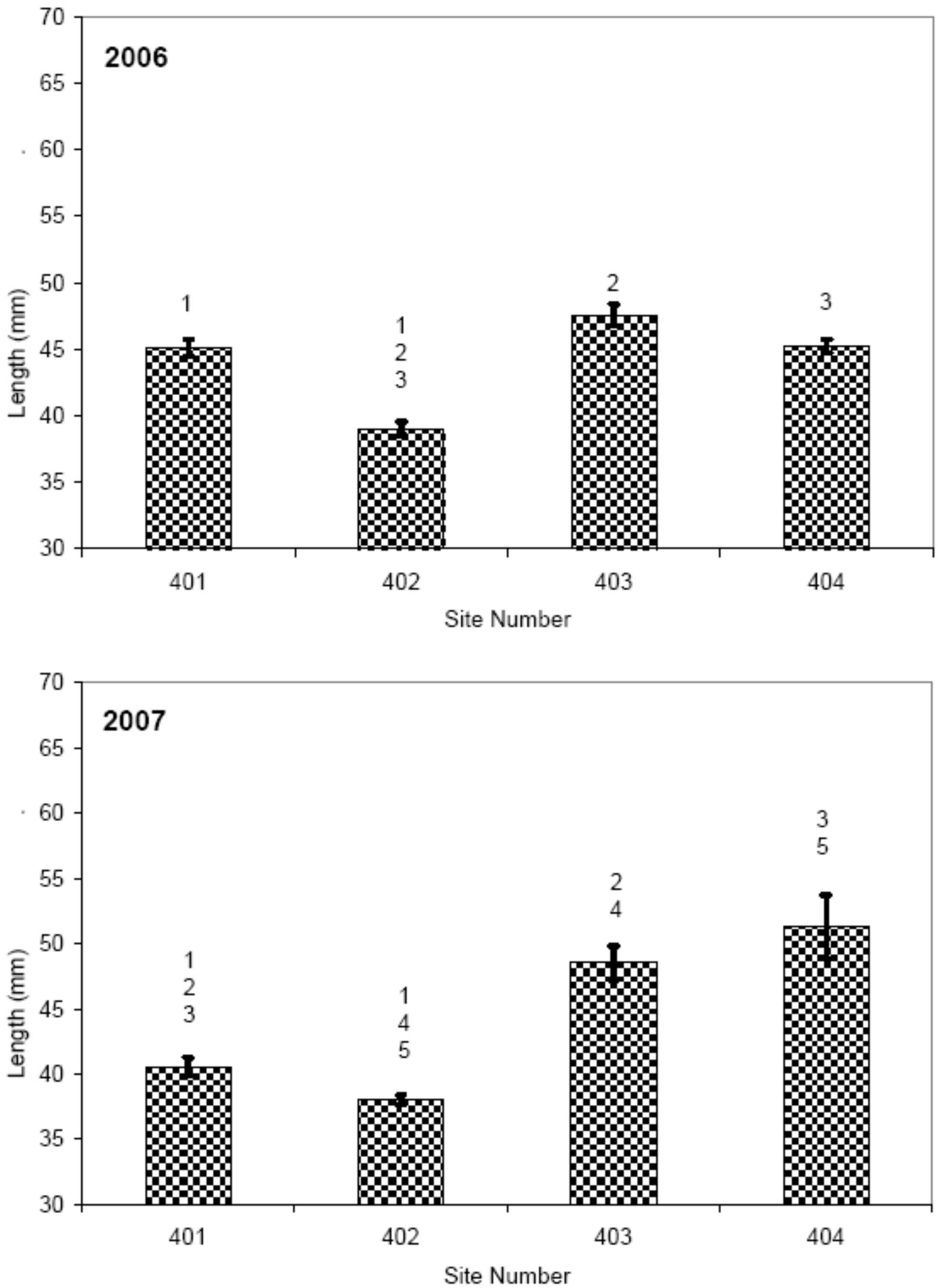


Figure 8: Mean length (mm) of chum salmon sampled in Shelter Inlet for 2006 and 2007. Values are mean ± standard error. Significant differences are numerically denoted, i.e. columns with the same number are significantly different.

Post-Hoc testing (Tamhane's Test) revealed that significantly smaller chum were sampled from sample sites 401 and 402 than at 403 and 404 for 2006 and 2007 (Fig. 8). Additionally, the chum sampled at site 402 were also significantly smaller than those sampled at site 401. Hence the mean fork lengths of the chum salmon increased significantly from the sites sampled on the landward side of the inlet when compared to those sampled at the seaward side of the inlet.

It is pertinent to point out that in Shelter Inlet, the prevalence of sea lice infestation (Fig. 7), the fork lengths of the chum salmon (Fig. 8) and the salinity at each sample site all appear to follow the same trend. Hence it would appear that salinity may be a contributing factor to both the prevalence of sea lice infection and the lengths of the salmon sampled at each site.

Differences in temperature and salinity of all sampling sites in all inlets between 2004 and 2007

The mean temperature and salinity for each sample site from 2004-2007 was calculated and compared to the other sample sites in the same inlet.

Bedwell Sound

These data showed a normal distribution (0.9≤normal scores≤0.988 and 0.843≤normal scores≤0.996). Additionally, there was no significant heterogeneity of variance for temperature (°C) (Levene's test = 0.478, p = 0.885) and salinity (‰) (Levene's test = 1.076, p < 0.389). An ANOVA revealed that there was no significant difference in temperature (f_9 = 1.814, p = 0.077) at the sites sampled in Bedwell Sound between 2004 and 2007. However, there were significant differences in salinity (f_9 = 3.947, p < 0.001) at the sites in sampled in Bedwell Sound between 2004 and 2007. Post-Hoc testing (Tukey's Test) revealed that the sample site 104 had a significantly lower salinity than those recorded at sample sites 107, 109 and 112 on the seaward side of the inlet (Fig. 9).

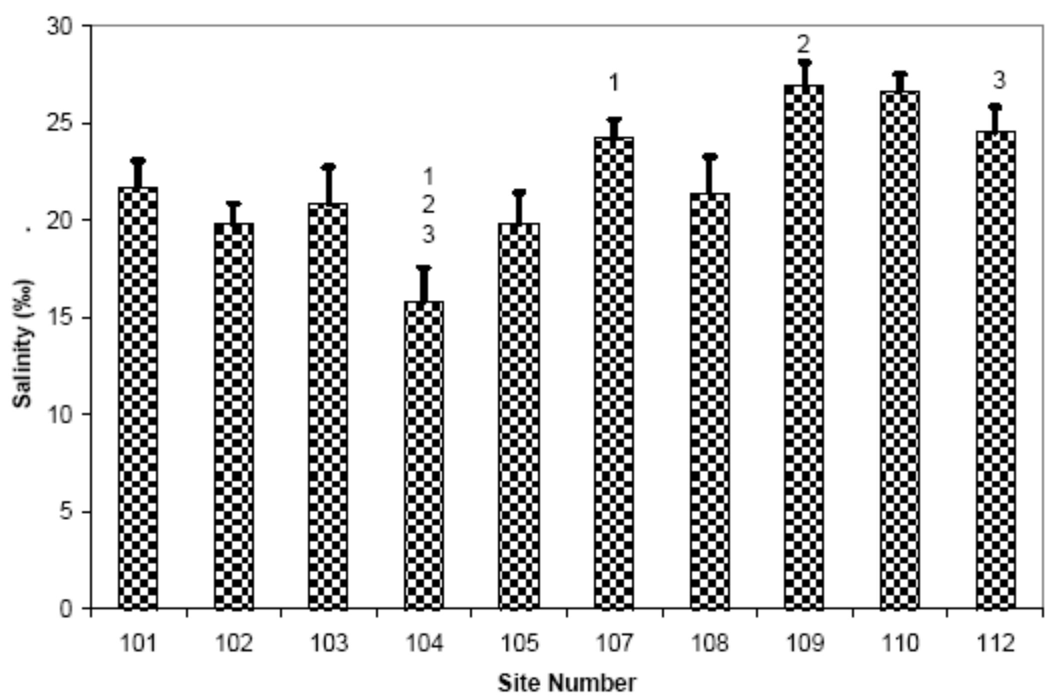


Figure 9: Mean salinity (‰) sampled in Bedwell Sound between 2004 and 2007. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different.

Fortune Channel

These data showed a normal distribution (0.955≤normal scores≤0.992 and 0.864≤normal scores≤0.986). Additionally, there was no significant heterogeneity of variance for temperature (°C) (Levene's test = 0.639, p = 0.699) and salinity (‰) (Levene's test = 1.999, p = 0.072). An ANOVA revealed that there was no significant difference in temperature (f_6 = 0.726, p = 0.629) at the sites in sampled in Fortune Channel between 2004 and 2007. However, there were significant differences in salinity (f_6 = 5.594, $p < 0.001$) at the sites in sampled in Fortune Channel between 2004 and 2007. Post-Hoc testing (Tukey's Test) revealed that the sample site 206 had a significantly lower salinity than those recorded at sample sites 201, 202, 203 and 205 further towards the headwaters of the channel (Fig. 10).

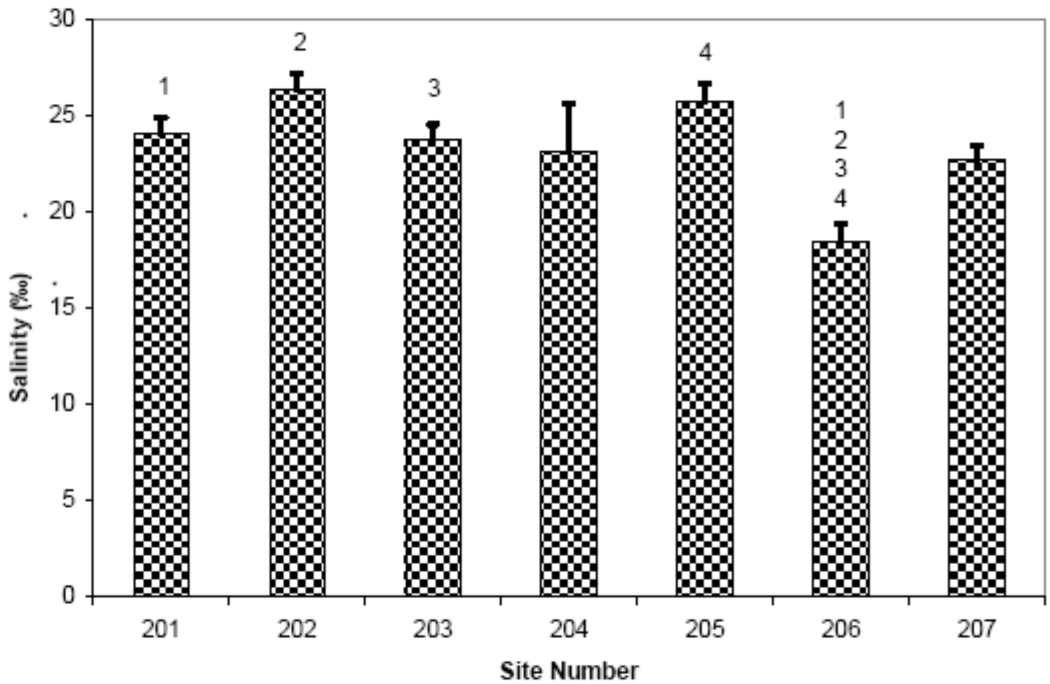


Figure 10: Mean salinity (‰) sampled in Fortune Channel between 2004 and 2007. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different.

Herbert Inlet

These data showed a normal distribution (0.966≤normal scores≤0.992 and 0.941≤normal scores≤0.953). Additionally, there was no significant heterogeneity of variance for temperature (°C) (Levene's test = 0.342, p = 0.573) and salinity (‰) (Levene's test = 2.264, p = 0.167). An ANOVA revealed that there was no significant difference in temperature (f_1 = 1.034, p = 0.366), and salinity (f_1 = 1.018, $p < 0.339$) at the sites in sampled in Herbert Inlet between 2006 and 2007.

Shelter Inlet

These data showed a normal distribution (0.96≤normal scores≤0.98 and 0.787≤normal scores≤0.963). Additionally, there was no significant heterogeneity of variance for temperature (°C) (Levene's test = 1.63, p = 0.208) and salinity (‰) (Levene's test = 0.218, p = 0.883). An ANOVA revealed that there was no significant difference in temperature (f_3

= 0.9, p = 0.455) at the sites in sampled in Shelter Inlet between 2004 and 2007. However, there were significant differences in salinity (f_3 = 11.672, $p < 0.001$) at the sites in sampled in Shelter Inlet between 2006 and 2007. Post-Hoc testing (Tukey's Test) revealed that the sample site 402 near the top of the inlet had a significantly lower mean salinity than sample sites 403 and 404 on the seaward side of the inlet (Fig. 11).

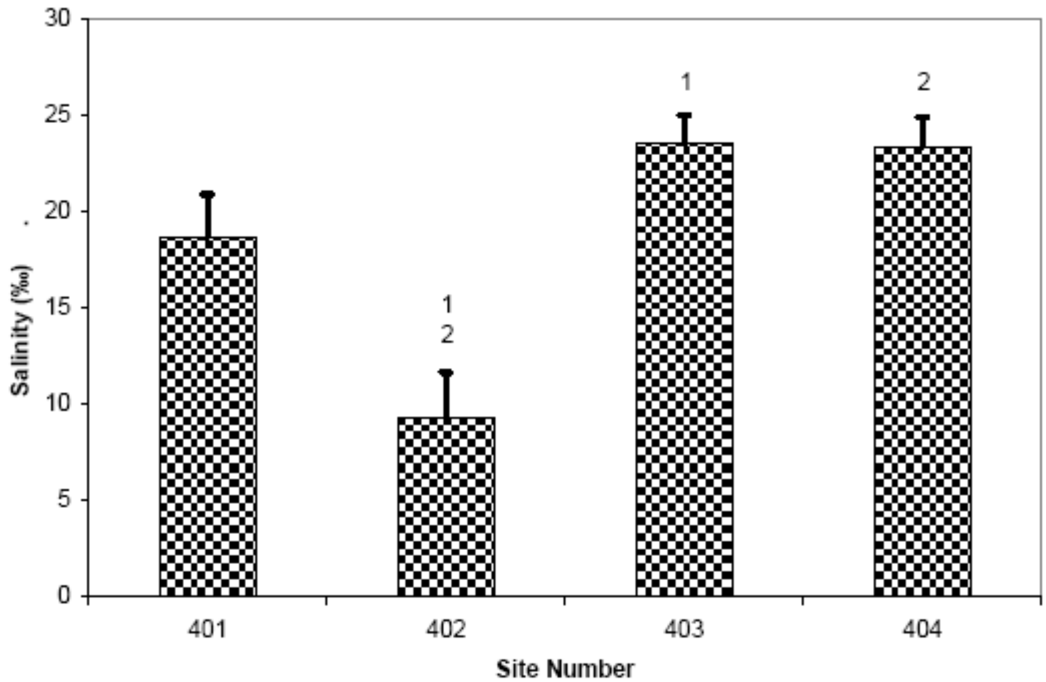


Figure 11: Mean salinity (‰) sampled in Shelter Inlet between 2006 and 2007. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different from each other.

Tofino Inlet

These data showed a normal distribution (0.896≤normal scores≤0.983 and 0.941≤normal scores≤0.989). Additionally, there was no significant heterogeneity of variance (Levene's test = 0.296, *p* = 0.938) for temperature (°C). However, there was significant heterogeneity of variance (Levene's test = 17.706, *p* < 0.001) for salinity (‰). An ANOVA revealed that there was no significant difference in temperature (f_6 = 0.358, p = 0.904) at the sites in sampled in Tofino Inlet between 2004 and 2007. However, a Kruskal-Wallis Test revealed that there were significant differences in salinity (H_6 = 15.18, $p < 0.019$). Post-Hoc testing (Tamhane's Test) revealed that the sample sites 503 and 504 nearer the top of the inlet had a significantly lower mean salinity than sample sites 510 and 511 on the seaward side of the inlet (Fig. 12).

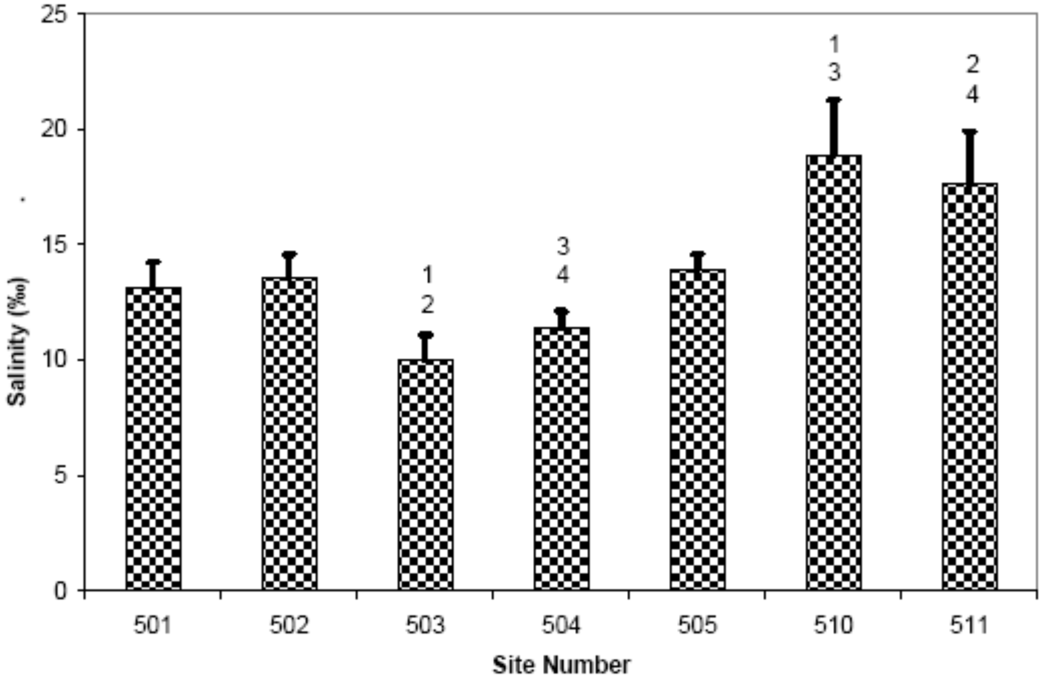


Figure 12: Mean salinity (‰) sampled in Tofino Inlet between 2004 and 2007. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different from each other.

<u>Sydney Inlet</u>

No temperature or salinity data was available for Sydney Inlet between 2004 and 2007.

Differences in temperature and salinity between inlets between 2004 and 2007

Mean temperatures ranged between 10.78°C and 13.96°C, and mean salinities ranged between 5.72‰ and 27‰.

At temperatures below 7°C, sea lice in the free-swimming copepodid stage are less able to take the next step and settle onto a host than when the water temperature is warmer (Tucker *et al*., 2000). The impact of temperature on the overall generation time for sea lice is equally pronounced. At 7.5°C, the generation time is 106 days, but at 14°C, this decreases to 36 days (Tully, 1992). Such temperature-dependant growth rates can significantly impact population densities of the copepodid life stage of *L. salmonis* in shallow coastal waters, which are prone to warming. As a result, warmer summers are likely to result in an increased number of sea lice copepods in these waters.

Salinity plays a very important part in the life cycle of sea lice. Successful development of the copepodid stage has been reported to occur only at salinities above 30‰ in the sea louse *L. salmonis* (Pike and Wadsworth, 1999). However, in British Columbia, successful copepodid development and subsequent host settlement have been achieved at salinities as low as 28‰ (Butterworth, 2005, pers obs). Once at this stage, copepodids actively avoid sea water with salinities below 20‰ (Heuch, 1995) and their optimal survival is at 30‰ (Johnson and Albright, 1991).

Hence, lower salinities such as those reported above could have a significant effect on reducing sea lice distribution and population sizes.

The differences in temperature (°C) and salinity (‰) from 2004 to 2007 were compared between the inlets. These data showed a normal distribution (0.896≤normal scores≤0.992 and 0.787≤normal scores≤0.996). Additionally, there was significant heterogeneity of variance for temperature (°C) (Levene's test = 1.59, p = 0.03) and salinity (‰) (Levene's test = 4.133, p < 0.001). Kruskal-Wallis Tests revealed that there were no significant differences in temperature (H_{29} = 54.45, p = 0.39).

However, there were significant differences in salinity (H_{26} = 214.06, p < 0.001). Post-Hoc testing (Tamhane's Test) revealed that Herbert Inlet and Tofino Inlet overall had lower salinities than Bedwell Sound, Fortune Channel and Shelter Inlet. The exceptions were the sites 510 and 511 in Tofino Inlet which had higher salinities similar to those in Bedwell Sound, Fortune Channel and Shelter Inlet and site 402 in Shelter Inlet which had lower salinities similar to those found in Herbert Inlet and Tofino Inlet.

Due to the large number of significant differences trends in significance are displayed using ovals in Fig. 13 to highlight differences.

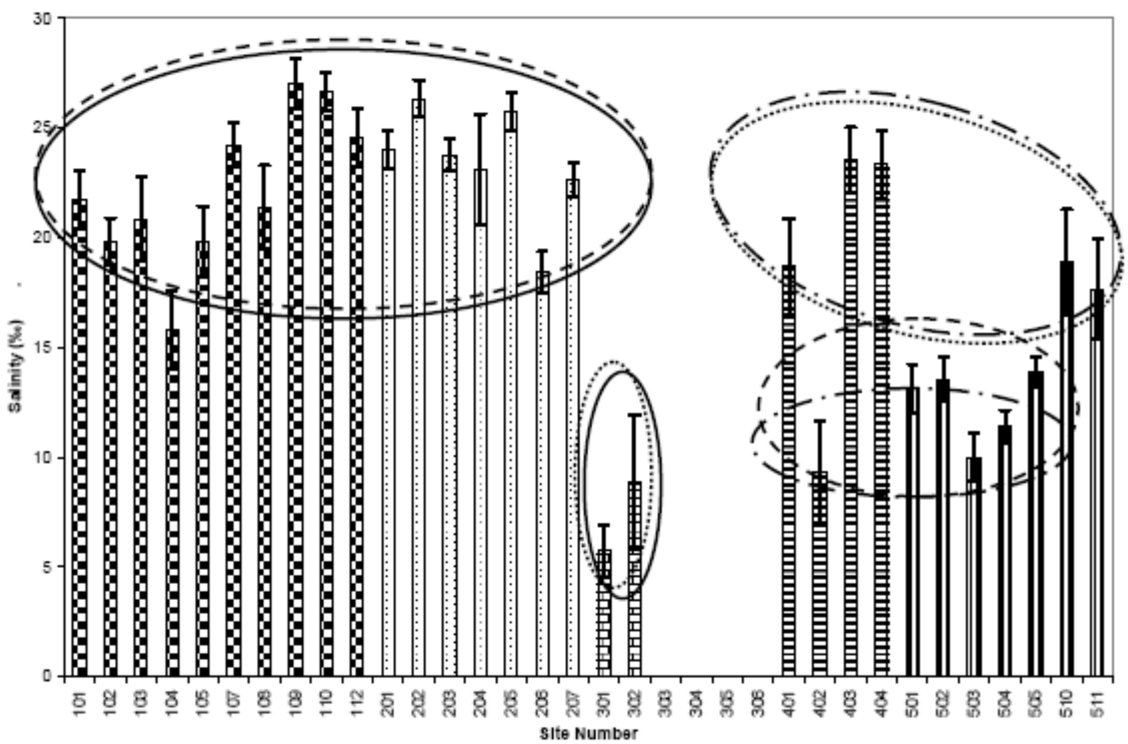


Figure 13: Mean salinity at each site in all inlets between 2004 and 2007. Values are mean ± standard error. Columns are displayed as follows: Bedwell Sound ; Fortune Channel ; Herbert Inlet ; Shelter Inlet ; Tofino Inlet . Trends in significant difference are denoted using ovals, i.e. columns within ovals displaying a similar pattern are significantly different.

Differences in prevalence, abundance and density of sea lice infestation on chum salmon with temperature and salinity

Environmental factors that have the greatest impact on the development and settlement success of the infective larval stage (the copepodid stage) are temperature and salinity. Depending on the combination, these two factors can either promote swift growth and survival of sea lice, or retard their development and severely reduce their survival.

<u>Prevalence</u>

These data showed a normal distribution for temperature (0.875≤normal scores≤1.000) and salinity (0.86≤normal scores≤1.000). Additionally, there was significant heterogeneity of variance (Levene's test = 3.554, *p* = 0.004) for prevalence with temperature, but not for prevalence with salinity (Levene's test = 1.908, *p* = 0.091). Kruskal-Wallis Tests revealed that there were no significant differences in prevalence with temperature (H_8 = 11.06, p = 0.199).

However, an ANOVA revealed that there were significant differences in prevalence with salinity (f_8 = 2.25, p = 0.041). Post-Hoc testing (Tukey's Test) revealed that prevalence of sea lice infestation is significantly lower at salinities between 15-17.9 ‰ than at salinities between 24-26.9 ‰ (Fig. 14).

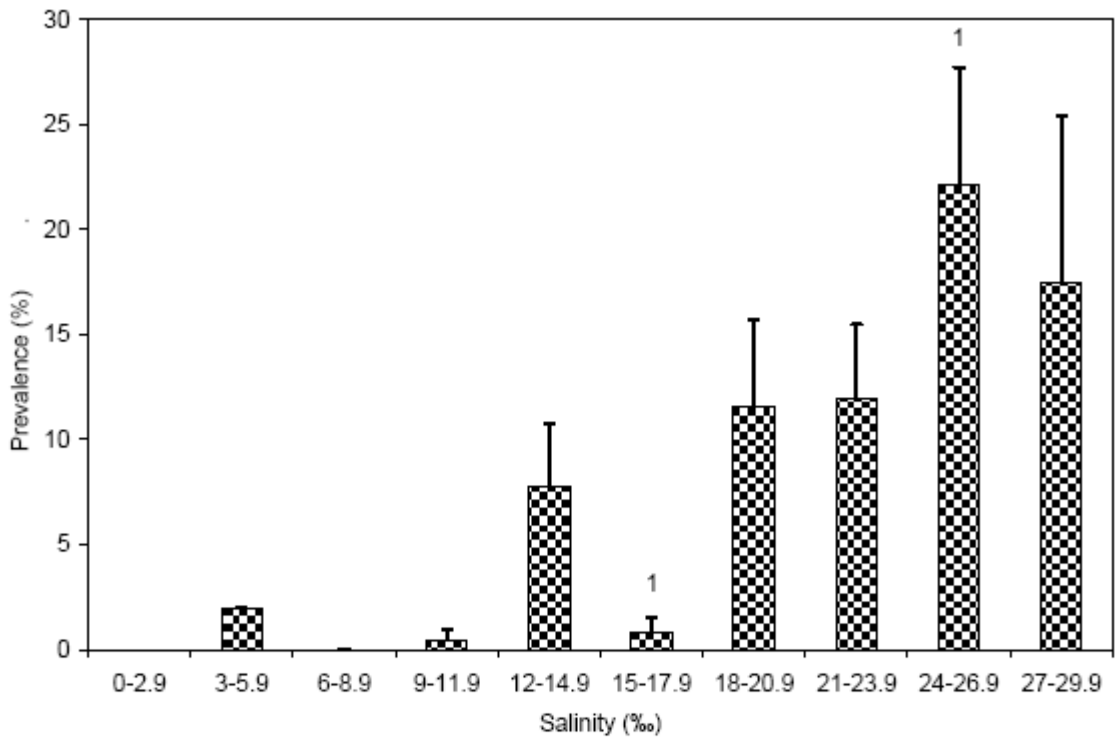


Figure 14: Mean prevalence with increasing salinity for all inlets. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different.

Abundance

These data showed a normal distribution for temperature (0.77≤normal scores≤1.000) and salinity (0.789≤normal scores≤1.000). Additionally, there was significant heterogeneity of variance; for abundance with temperature (Levene's test = 2.566, p = 0.026) and salinity (Levene's test = 2.251, p = 0.048). Kruskal-Wallis Tests revealed that there were no significant differences in sea lice abundance on chum salmon with increasing temperature (H_8 = 9.79, p = 0.28).

However, there were significant differences in abundance with increasing salinity (H_8 = 21.8, p = 0.005). Post-Hoc testing (Tamhane's Test) revealed that the abundance of sea lice infestation was significantly lower at salinities between 15-17.9 ‰ than at salinities between 24-26.9 ‰ (Fig. 15).

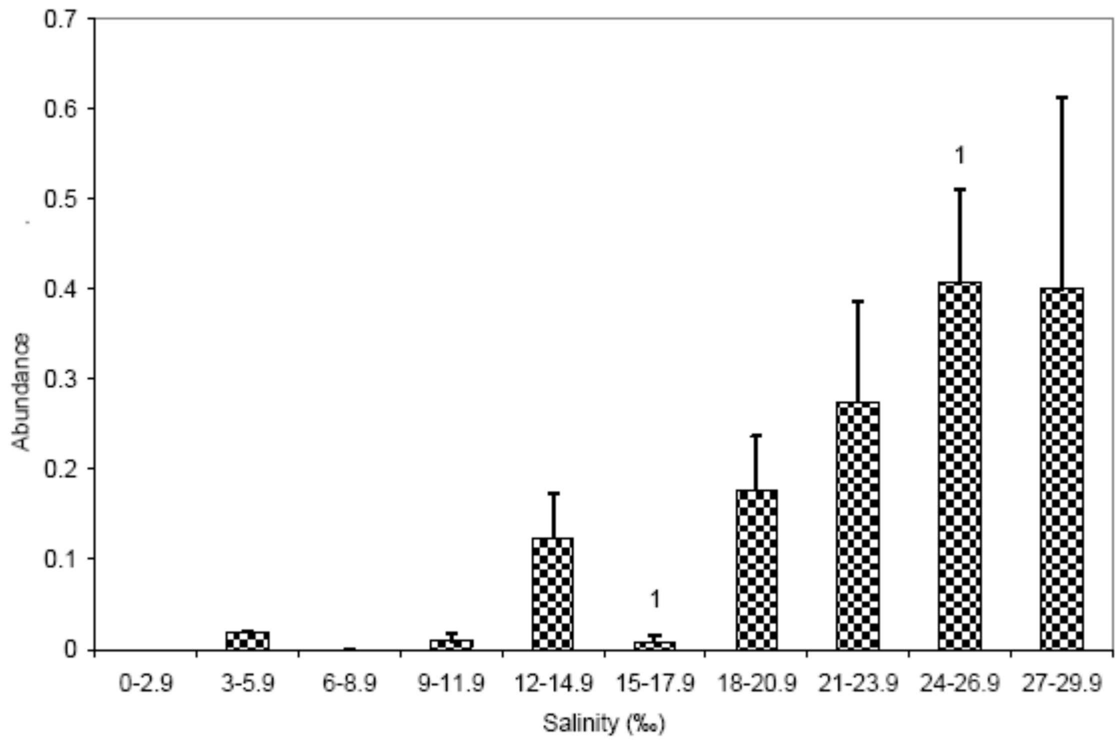


Figure 15: Mean abundance with increasing salinity for all inlets. Values are mean ± standard error. Significant differences are numerically denoted i.e. columns with the same number are significantly different.

Density

These data showed a normal distribution for temperature (0.694≤normal scores≤0.958) and salinity (0.817≤normal scores≤0.934). Additionally, there was no significant heterogeneity of variance for density with increasing temperature (Levene's test = 1.72, p = 0.081). However, there was significant heterogeneity of variance for density with increasing salinity (Levene's test = 3.97, p = 0.001).

An ANOVA revealed that there were no significant differences in sea lice density on chum salmon with increasing temperature (F_8 = 1.13, p = 0.34). A Kruskal-Wallis Test revealed that there were no significant differences in sea lice density on chum salmon with increasing salinity (H_8 = 14.46, p = 0.07).

Study limitations

Like all field research, limitations were present in this study. Figure 1 depicts all possible beach seine locations that were identified in the original site reconnaissance. On average 25 of these sites, distributed throughout Tofino Inlet, Fortune Channel, Bedwell Inlet, Herbert Inlet and Shelter Inlet, were regularly sampled each year. Some sites were found not to be suitable beach seining sites since they were only accessible on very low tides (which, as mentioned in the Methodology section, were not always possible to access). Furthermore, even within the main 25 sites, some were periodically missed because the wind had picked up substantially throughout the course of the day, or the tides made access impossible. Additionally, because of the collaborative nature of the team, regular sampling was not always possible because of weather, deaths in the community, or boat problems. In an ideal study, samples would have been taken on a regular regime, but circumstances did not always permit this.

Difficulties arose when attempting to compare our data to that in other areas. Many researchers differentiate between infection from *L. salmonis* and *C. clemensi*. It is important to note that our early data (2004-05) did not differentiate to species at the chalimus stage, and so our combined prevalence of *L. salmonis* and *C. clemensi* may look larger than those of other researchers who report only on prevalence of *L. salmonis*. Future analyses of our data will focus on that from 2006 onward, where species are differentiated.

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