Interactions between sea lice (*Lepeoptheirus salmonis* and *Caligus clemensii),* juvenile salmon *(Oncorhynchus keta and Oncorhynchus gorbuscha)* and salmon farms in British Columbia

By

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B. Sc., University of Victoria, 2000

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

Abstract

The issue of sea lice (*Lepeoptheirus salmonis* and *Caligus clemensii*) transfer from salmon farms to wild salmon is a controversial topic in British Columbia (BC). A series of sea lice epizootics (four in five years) on juvenile chum (*Oncorhynchus keta)* and pink (*O. gorbuscha*) salmon in the Broughton Archipelago (BA), an area with the highest density of salmon farms on the west coast, have caused significant concern among conservationists, local First Nations, and the general public over the possible impacts of salmon farms on wild salmon. Key to the debate has been a lack of data on ambient sea lice infection rates on juvenile salmon in the absence of the influence of salmon farms. This work represents one of the first attempts to empirically examine ambient sea lice infection rates on juvenile salmonids. Objectives were to test hypotheses including: geographic variability is a significant factor in sea lice population dynamics, ambient sea lice infection rates on juvenile salmon are low, juvenile salmon susceptibility to sea lice infection, and the influence of salmon farms on ambient sea lice infection rates.

Over a three year period, samples of juvenile chum and pink salmon (n=13,874) were collected using a beach seine net in the central coast of British Columbia (Klemtu/Bella Bella), a vast area with limited salmon farming activity, and with geography that allowed for simultaneous assessment in the same region of the natural interaction between sea lice and juvenile salmon and the influence of salmon farms on the interaction. Sampling was also conducted in other areas without salmon farms (Southern Gulf Islands: n=3847) and with salmon farms (Broughton Archipelago; n=3911). The results of the field

experiments were also used in conjunction with laboratory experiments to examine the susceptibility of juvenile chum vs. pink salmon to infection by sea lice.

The ambient lice infection rates for juvenile chum and pink salmon were up to 32% prevalence, less than one louse per fish and less than two lice per gram (prevalence: 2.0 (1.0) – 32.0 (19.0), mean lice per fish: 0.02 (0.01) – 0.67 (0.22), mean lice per gram: 0.56 (0.08) – 1.93 (0.13)). This result was found to be consistent across geographic areas with no salmon farming activity suggesting that geographic variability was not a significant factor in the natural interaction between sea lice and juvenile salmon. Salmon farms were found to strongly influence the relationship between sea lice and juvenile chum and pink salmon. Sea lice infection rates of juvenile salmon collected near salmon farms were significantly higher than non-salmon farming regions ranging from 3 – 150 times higher in the BA and from 2 – 14 times higher in the Klemtu region. Infection levels near salmon farms were variable in intensity from year to year. The extent to which the sea lice-salmon relationship was affected by salmon farms was dependent on farmed species, farm location, within year variability in fish size, and the scale of salmon farming activities within the region. The results from the laboratory and field studies demonstrate that juvenile chum salmon were more susceptible to infection by sea lice than juvenile pink salmon. However, the exact mechanism for the observed differences was not identified. Possible reasons for the observed differences could be related to genetically determined susceptibility, fish mucous differences, lethal lice infection tolerances, or other factors not examined.

The results of this study suggest that the elevated sea lice infection rates observed in the BA and other areas present a significant risk to the health of wild salmon and that salmon farms are the most likely cause based on the biology and ecology of sea lice. In order to better understand the potential for salmon farms to affect wild salmon populations, it is suggested that investigations into farm level sea lice contributions be conducted in the BA and other areas where salmon farms operate. In addition, investigation into the lethal lice infection rates for juvenile salmon at early marine life size should also be conducted.

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Acknowledgements

I would like to thank my supervisor Dr. Asit Mazumder for his support throughout this project despite its many hiccups. I would like to thank Dr. John Volpe for his unyielding confidence and support of me in this project. I would also like to thank Dr. John Nelson and Dr. John Dower for serving on my committee and Dr. Doug Bight for serving as my external examiner. I would also like to acknowledge Eleanore Blaskovich and her gracious answers to my many questions.

I would likely to thank both the Heiltsuk and Kitasoo first nations and fisheries programs for the permission and support to conduct this work in their respective traditional territories. I gratefully also acknowledge the Raincoast Conservation Foundation, the Natural Sciences and Engineering Research Council (NSERC) for an industrial fellowship, and the Science Council of British Columbia for BCARDE funding.

I would like to especially thank Josh Carpenter, Randy Carpenter, Troy Whitford, Mitch Clifton, Rick and James Nislaas, Carl Humchitt, Theresa Rothenbush, Alexandra Morton, Ian and Karen McAllister, Chris Genovali, Nicola Temple, Chris Williamson, Jean-Marc LeGuerrier, Marty Krkosek, Katrina Bennett and Adriana McMullen for GIS Maps, Brian Falconer, and Mike Jacobs for their work and support of this project. I would also like to thank all of the volunteers who helped with field sampling including: Kelly Young, Andrew Rippington, Vilaiwan Fernandes, Jennifer Gee, Nicola Eyding, Jenny Kingsley, Chris Williamson, Trina De Moyne, Tim Irvin,

Byron Horbaty, Steven Anstee, Misty MacDuffee, Brendan Connor, Leah Walberg, Spenser Hansen, Andrew Bateman, Rebecca McMackin, Chris Lowe, Bill Peet, Sheila Hagerty, Dave Gaetz, and any others who I may have missed.

For the laboratory work, I would like to thank Mike James, Simon Grant, Nicola Eyding, Andrew Rippington, and the late Gerry Horne for their hard work and support throughout the laboratory study. I would also like to thank the Little Qualicum Hatchery, the Quinsam Hatchery, and the Freshwater Fisheries society in Duncan for providing the fish used in the laboratory study. I would also like to thank all those who helped with sea lice collections including Area D gillnetters (John Stevens, Calvin Siider, Arnie Hengstler, Ken McLaren, Nick Stevens and many others), Nicola Eyding, Mike James, Jennifer Gee, Rebecca McMackin Kelly Young, Jenny Kingsley, Kate Lansley, Theresa Rothenbush, Chris Williamson, Hana Hermanek, Brian Newman, and Ryan Popowich.

Dedication

I dedicate this thesis in three parts:

The first part I dedicate to ***Theresa Rothenbush***, my wife, partner, and best friend of 10 years. This would not have been possible without your support and hard work and I will be eternally grateful and curious of what price you really paid for me to be successful in this project.

The second part I dedicate to my parents, ***Bill and Sandra Peet,*** whose undying love and unconditional support of me in whatever I do is nothing short of remarkable. Your support and sacrifice for me are never forgotten.

The third part I dedicate to the late ***Gerry Horne,*** who was a significant part of the laboratory study at every stage. Thank you Gerry, you are missed.

Introduction

Pacific salmon (*Oncorhynchus spp.)* are very important to coastal and terrestrial ecosystems. During their migrations in the open Pacific Ocean, nearshore coastal areas, and spawning rivers, salmon provide vital nutrients for many organisms including: nearshore marine fishes and mammals (Groot and Margolis 1991), terrestrial carnivores (Klinka and Reimchen 2002; Darimont *et al.* 2003), birds (Christie and Reimchen 2005), plants (Wilkinson *et al.* 2005; Nagasaka *et al.* 2006), and insects (Hocking *et al.* 2006; Hocking and Reimchen 2006). Salmon also provide vital nutrients to ocean basins (Cederholm 2000). Some have speculated that nutrient transfer from salmon carcasses to the surrounding ecosystem has an important role in the richness of Pacific Northwest ecosystems (Reimchen pers. comm.). In British Columbia (BC), Pacific salmon also provide significant economic, cultural benefits, and traditional benefits (i.e. First Nations).

The ecology of juvenile salmon

Although a great deal of information exists on the biology of adult Pacific salmon, Healey (1980) suggested that we understand less about the initial nearshore marine phase of Pacific salmon than all other phases of their life history. In early spring (February-April), juvenile salmon make their way into the open waters of lakes, rivers, and nearshore marine areas. Pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) migrate straight into the nearshore marine areas without taking up residence in streams, rivers, or lakes. These fish enter the marine environment at sizes < 5 cm and < 1 gram (Groot and Margolis 1991).

Mortality of juvenile salmon during the early marine phase is in the range of 30 - 77% (Parker 1965; Parker 1968; Healey 1980; Murphy *et al.* 1988; Groot and Margolis 1991; Cooney *et al.* 2001). Survival of juvenile salmon is ultimately dependent on size. Factors such as competition for food and stress can depress growth rate, which can lead to increased vulnerability to parasites and predators (Healey 1980; Murphy *et al.* 1988; Cooney *et al.* 2001).

Typically, pink salmon enter the marine environment at an average length of 35mm and grow to 60-100mm before migrating to offshore waters, while chum salmon enter at an average length of 36 mm and grow to 80-100mm (Healey 1980; Groot and Margolis 1991). Growth rates range from 0.4mm/day to 1.5mm/day for juvenile chum and pink salmon in the early marine phase (Murphy et al. 1988). Early sea life salmon fry (fish less than 100 days marine residence) tend to remain along the shores of bays and inlets as they make their way to the continental shelf and then into the offshore regions of the east Pacific Ocean in the late summer. The nearshore marine phase can last as long as five months (Parker 1962).

With entrance into the marine environment, embryonic food stores are quickly exhausted and salmon fry become exposed to competition with other fishes and a host of new predators (marine birds, herring, other salmon, pollock, etc.) (Parker 1962). Parasites also likely contribute to juvenile salmon mortality in the nearshore environment although their

impact on mortality rates is likely much lower than that of predators or other factors such as competition for food.

Sea Lice

Sea lice are common marine ecto-parasites of salmonids and other fishes in the northern hemisphere. Sea lice have a life cycle consisting of five phases and ten stages (Figure 1). These include two free-swimming naupliar stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two pre-adult stages, and an adult stage (Johnson and Albright, 1991). Little is known about the typical densities and dispersal ability of sea lice larvae (nauplii and copepodids) although they are highly dependent on the presence of suitable hosts. For *L. salmonis*, development from nauplii to the copepodid stage is dependent on temperature and can range from 2 – 9 days. Survival of the copepodid stage ranges from 2 – 8 days dependent on temperature and salinity. Total generation time (egg to adult) ranges from 7.5 – 8 weeks at 10 ^{o}C (Johnson and Albright 1991b).

Lice feed on skin, mucous, and blood (Kabata, 1974; Brandal *et al.,* 1976). High densities of lice on individual salmonids can cause hemorrhages, sores, and even death (White, 1940; Wooten *et al.,* 1982; Grimnes and Jakobsen 1996). High densities of lice have also been found to significantly impact the swimming performance of salmonids (Wagner *et al.* 2003) and change behaviours (Birkeland 1996; Birkeland *et al.* 1997). Under certain conditions, lice infections have been found to introduce disease into salmonid populations (Johnson *et al.,* 1996).

Three species of sea lice are known to infect juvenile salmon in the eastern Pacific. *Caligus clemensii* and *Lepeoptheirus salmonis* have been documented on juvenile salmon in the nearshore marine environment, while *Lepeoptheirus cuneifer* is thought to occur on salmon infrequently (S. Johnson pers. comm.). *C. clemensii* is considered to be a generalist species that infects many nearshore marine fishes including: salmonids of the *Oncorhynchus* genus, Pacific herring *clupea harengus pallasi*, three spine stickleback *Gasterosteus aculeatus*, greenling *Hexagrammos sp.*, Pacific ratfish *Hydorlagu colliei*, copper rockfish *Sebastes caurinus*, and walleye Pollock *Theragra chalcogramma* (Johnson and Margolis 1994). Parker and Margolis (1964) suggested that this parasite is more specific to the environment than the host, staying in sheltered coastal waters where it can colonize juvenile salmon and other nearshore marine fish species.

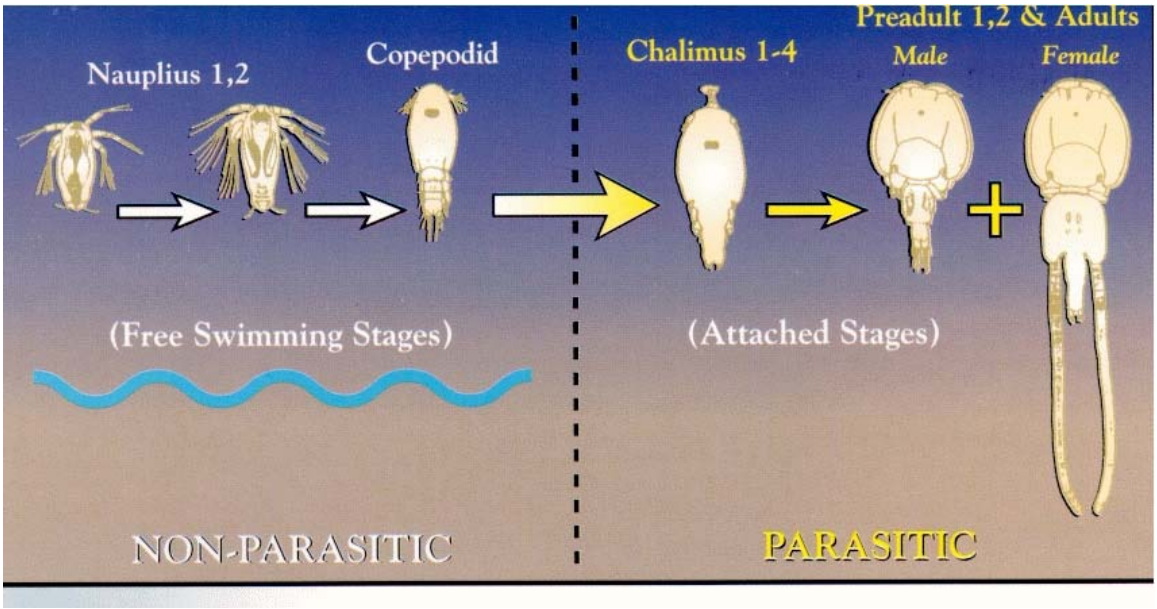


Figure 1 – General sea lice life cycle (*L. salmonis* and *C. clemensii*)
(http://www.upei.ca/~anatphys/Sea_Lice/licecycl.htm)

Lepeoptheirus salmonis is a specialist parasite to species of the Salmonidae family (*Oncorhynchus spp., Salmo spp., Salvelinus spp.*) including iterparous species such as

coast cutthroat trout and steelhead (Johnson and Margolis 1994; Pike and Wadsworth 1999). *L. salmonis* is approximately three times the size of *C. clemensii* and therefore has more pronounced effects on hosts. *L. salmonis* is known to be common in wild adult salmon populations, but occurs in abundances that result in only minor damage (Wooten *et al.,* 1982; Nagasawa , 1987; Nagasawa *et al.,* 1993; Beamish *et al.* 2005). Other hosts for *L. salmonis* have been recorded but only on rare occasions. Examples include: far east rudd *Leuciscus brandti,* flag rockfish *Sebastes rubrivinctus,* sand lance *Ammodytes hexapterus,* and white sturgeon *Acipenser transmonatanus* (Johnson and Margolis 1994). Although other hosts have been recorded for *L. salmonis*, reproductive stages have not been observed, suggesting that non-salmonid hosts offer no chance for survival and development of adult forms (Kabata 1973; Jones *et al.* 2006). *L. salmonis* is the dominant species found in cage-culture on the northern Pacific and Atlantic coasts of Canada and the USA (Smith 1998; Johnson *et al.* 2004).

Sea lice and juvenile salmon ecology

Little is known about the interactions between juvenile salmon and sea lice. Some studies have quantified lice densities on adult salmon in offshore and coastal regions (Nagasawa 1987; Nagasawa 1993; Tingley *et al.* 1997; Beamish *et al.* 2005), but very few studies have examined lice densities on early marine life juvenile salmon in the absence of anthropogenic influences (e.g. salmon farms)(Wertheimer *et al.* 2003; Morton *et al.* 2004).

adult salmon migrate from offshore into the nearshore marine environment in late summer with varying lice densities (mainly *L. salmonis*; Beamish *et al.* 2005) resulting in some magnitude of transmission to juvenile and over-wintering salmonid populations (e.g. Chinook, Coho, steelhead). As the majority of juvenile salmon will have migrated from nearshore marine areas to offshore at the time of adult salmon inmigration, the majority of transmission between adults and juveniles likely takes place over the continental shelf in the summer and fall. Given the salmon specificity of *L. salmonis*, only salmonid hosts offer reproductive habitat for survival over the winter, while this same limitation does not apply to *C. clemensii*, which can utilize numerous nearshore marine fishes as alternative hosts. Over-wintering salmonid populations disperse widely, resulting in limited habitat for sea lice to over-winter. The result is that when juvenile salmon migrate out of the nearshore environment in the spring, the potential for them to be infected by *L. salmonis* is likely low.

The balance between parasites and host

A balance, or natural feedback loop, typically exists between parasites and host. Wikel *et al.* (1994) defined a successful host-parasite relationship as a balance between limiting the parasite through host defenses and the ability of the parasite to modulate, evade, or restrict the host's responses. In other words, an interspecific evolutionary race occurs between parasites and hosts that ultimately results in a state of dynamic equilibrium.

Distribution of fish parasites within a population can maintain dynamic equilibrium. Factors contributing to over-dispersed (aggregated) parasite distributions include: genetic

differences in susceptibility, physiological differences, and age differences (Esch and Fernandez 1993). Overdispersion of the parasite on the host population acts to enhance the density-dependent regulation of both host and parasite abundance through suppression of parasite fecundity or survival or via the influence of the parasite on host survival and fecundity (Anderson and Gordon 1982). The process of over-dispersion allows the potential lethal effects of parasites (i.e. sea lice in high densities) to be isolated to only a few hosts within the population. Therefore the risk of parasite-induced host mortality is spread unevenly within the host population resulting in a low probability of an extinction event occurring due to high infestation levels (Esch and Fernandex 1993). This type of dispersion pattern has been recognized as being highly important to the population dynamics of host-parasite associations in both stabilizing and destabilizing ways (Anderson and Gordon 1982). For Pacific salmon, this means that there is some mortality due to the effects of sea lice but this mortality has not been quantified to date.

Epizootics of parasites on hosts are the result of an imbalance in the host-parasite interaction due to decreased resistance by the host due to factors such as poor nutrition, increased stress, or an increase in parasite number.

Interactions between sea lice, salmon farming, and wild salmon

In sea-farmed salmonids epizootics of sea lice are common, causing high mortality rates and disease on farmed fish if left untreated (Brandal and Egidius, 1979; Wooten *et al.,* 1982; Bravo 2003). Increased epizootics of sea lice (mainly *L. salmonis*) on wild salmon populations have been correlated with outbreaks in sea-farmed salmonids in Scotland

(Butler 2002), Ireland (Tully and Whelan 1993; Tully *et al.*, 1993; Tully *et al.*, 1999), Norway (Bjorn *et al.*, 2001; Bjorn and Finstad, 2002), and Canada (Morton and Williams 2003; Morton *et al.* 2004; Krkosek *et al.* 2005; Morton *et al.* 2005; Krkosek *et al.* 2006). Given the evidence that salmon farms can alter the natural dynamics between sea lice and salmon (i.e. increasing ambient parasite burdens), a great deal of concern has been raised about the subsequent effects to the health of wild salmon populations. In some cases, declines in wild salmon populations have been correlated with elevated sea lice infection rates and the presence of salmon farms (Gargan 2000; PFRCC 2002).

Altering the host-parasite interaction in favor of the parasite can lead to an increase in parasite-induced host mortality. Lethal sea lice infection rates for salmonids have been poorly quantified to date. Grimnes and Jakobsen (1996) examined the physiological effects of *L. salmonis* infection on post-smolt Atlantic salmon. The results showed that high intensity infections of early chalimus stages do not have severe physiological effects on the fish. However, after the moult to pre-adult, intensities above 30 lice per fish caused death in Atlantic salmon post-smolts. Similar work by Grimnes *et. al* (1996) suggests that intensities above 50 lice per fish are lethal to Arctic Char post-smolts (40 g). Bjorn and Finstad (1997) conducted a similar study on sea trout post smolts. Their results support the finding that early chalimus stages only cause minor osmoregulatory disturbance, although a heavy infection of these stages induces a primary stress response. After the lice moult to the first pre-adult stage, the infected fish were observed to experience severe osmoregulatory problems and anemia. Their results show that infection intensities above 90 salmon lice copepodids per fish may result in mortality of small sea

trout post-smolts (60 g) after the lice have developed to the pre-adult stages. Taken together, these studies suggest that the lethal sea lice infection ratio for Atlantic salmon, Arctic char, and sea trout is approximately 0.75-1.6 lice/gram. More recently, Morton and Routledge (2005) conducted lethal infection experiments on captive populations of infected juvenile chum and pink salmon from the Broughton Archipelago. Their results showed that the short-term mortality for juvenile chum and pink salmon is increased by lice infestations of 1-3 sea lice per fish.

Sea lice in British Columbia

In June 2001, an epizootic of *L. salmonis* was recorded on outmigrating juvenile pink salmon (*Oncorhynchus gorbuscha)* in the Broughton Archipelago, British Columbia (B.C.) (Morton and Williams 2003). The study revealed that 78% of juvenile pink salmon sampled near fish farms were infected at 0.75 - 1.6 lice/g. This infection rate is lethal in post smolt European salmonids (*Salmo spp.*) (Grimnes and Jakobsen, 1996; Bjorn and Finstad, 1997). The lethal infection ratio was derived for post-smolt Atlantic salmon, whose size during their outmigration to the sea is approximately 4.5-60 times larger than pink salmon, and therefore its applicability to juvenile chum and pink salmon is unclear. Subsequent epizootics were documented in the Broughton Archipelago in 2002 (Morton *et al.* 2004), 2004 (Morton *et al.* 2005), and 2005 (Peet this thesis).

The epizootics recorded in the Broughton Archipelago have been correlated with the activities of salmon farming (Morton and Williams 2003; Morton *et al.* 2004; Morton *et al.* 2005). In the salmon farming countries of Europe (Norway, Scotland, Ireland),

numerous studies have documented the correlation between increased lice levels and the presence of salmon farms (Tully *et al.* 1999; Bjorn *et al* 2001; Bjorn and Finstad 2002; Butler 2002). The literature suggests that salmon farms may be altering the natural host-parasite interaction between juvenile salmon and sea lice in ways that could be detrimental to Pacific salmon populations.

Very little information exists on the susceptibility of juvenile chum and pink salmon to infection by sea lice. Additionally, very little information exists on the ambient sea lice infection rates of sea lice and how salmon farms can influence those infection rates. There are three objectives of this thesis. Chapter 1 examines what ambient sea lice infection rates are on juvenile chum and pink salmon in an area of the BC coast with little or no salmon farming activity and examines how salmon farms change ambient infection rates. Chapter 2 examines the relative susceptibility of juvenile chum and pink salmon by comparing data from both field and laboratory experiments. Finally, chapter 3 compares data on the ambient sea lice infection rates on juvenile salmon from three separate areas of the BC coast with sea lice infection rates recorded in the Broughton Archipelago over the same time period.

Chapter 1

The impacts of salmon farms on the host-parasite relationship between sea lice (*Lepeoptheirus salmonis* and *Caligus clemensii)* and juvenile chum (*Oncorhynchus keta)* or pink (*O. gorbuscha)* salmon

Abstract

This study examined the impact of salmon farms on ambient sea lice infection rates (*Lepeoptheirus salmonis* and *Caligus clemensii)* and juvenile chum and pink salmon. Over three years, data were collected in the central coast of British Columbia where up to five active salmon farms sites currently operate. Sampling took place on either side of a large peninsula that facilitated comparison between an area with salmon farming activity and an area not exposed to salmon farms within the same geographic area. Juvenile chum and pink salmon (n=13,874; 60% chum and 40% pink) were collected using a beach seine net during the three spring migration seasons of 2003-2005. Sea lice infection rates in the < 1 km proximity category ranged from 2.2 – 14 times higher than infection rates in the > 15 km and separated categories for chum salmon and from 1.2 – 5.8 times higher for pink salmon. Ambient lice per gram infection rates were less than two lice per gram. The results of this study suggest that salmon farms can strongly influence sea lice infection rates on juvenile chum and pink salmon. The extent to which the sea lice-salmon relationship is affected is dependent on farmed species, farm location, within year variability in fish size, and salinity. This study is one of the first attempts to simultaneously assess the ambient sea lice infection rates on juvenile salmon and the influence of salmon farms on ambient infection rates within the same geographic region.

Introduction

In the eastern North Pacific Ocean, two species of sea lice commonly infect salmonids. The salmon louse, *Lepeoptheirus salmonis* (Kroyer 1837), is a specialist parasite of salmonids (Johnson and Margolis 1994; Pike and Wadsworth 1999). Although *L.*

salmonis infections have been recorded on non-salmonid hosts, the infections are characterized by early development stages and not adult stages suggesting that development to reproductive stages on non-salmonid hosts is not possible (Kabata 1973; Jones *et al.* 2006). *Caligus clemensii* is a generalist species that is more specific to the environment than the host, staying in sheltered coastal waters where it can colonize juvenile salmon and other near shore marine fish species (Parker and Margolis 1964). *C. clemensii* and *L. salmonis* are distinguished from each other on the basis of morphology (Kabata 1972; Kabata 1988; Johnson and Albright 1991b).

Both species consume host mucous, epidermis, and blood (White 1942; Kabata 1974; Brandal *et al.* 1976). High densities of lice on individual salmonids can negatively affect swimming performance (Wagner *et al.* 2003), change behaviour (Birkeland 1996; Birkeland and Jakobsen 1997), cause hemorrhages and sores, and disrupt osmotic balance (White, 1940; Wooten *et al.,* 1982; Johnson and Margolis 1994; Grimnes and Jakobsen 1996; Johnson and Albright 1996; Bjorn and Finstad 1997). These factors can lead to direct mortality (determined by number and stage of lice and the size of host) or indirect mortality through secondary infection and / or increased risk of predation. Sea lice population dynamics are strongly influenced by temperature and salinity, which affect growth rates, larval settlement, and survival (Johnson and Albright 1991b; Tucker *et al.* 2000).

Sea lice are important parasites of farm-raised salmonids and have caused direct and indirect economic losses greater than US $100 million annually (Johnson *et al.* 2004). In

every country where salmon farms operate there have been cases of sea lice epizootics on farms leading to significant mortality and disease (Brandal and Egidius 1979; Wooten *et al.* 1982; Bravo 2003; Johnson *et al.* 2004). Salmon farms contain a high-density population of relatively stationary hosts that are generally under higher stress levels than wild populations. As a consequence, farmed fish are more susceptible to contracting disease and parasites and therefore positively affect conditions for sea lice production in adjacent marine areas (Bakke and Harris 1998). Increased epizootics of *L. salmonis* on wild salmon populations have been correlated with outbreaks in sea-farmed salmonids (Tully *et al.,* 1993; Tully *et al.,* 1999; Bjorn *et al.,* 2001; Bjorn and Finstad, 2002; Morton and Williams 2003; Morton *et al.* 2004; Morton *et al.* 2005; Krkosek *et al.* 2005; Krkosek *et al.* 2006). In some cases, the increased epizootics correlated with salmon farming have also been correlated with declines in adjacent populations of wild salmon (Gargan 2000; PFRCC 2002; Krkosek *et al.* 2006).

The debate over the impact of sea lice from salmon farms on the health of wild salmon populations has proven contentious. Part of the debate surrounds the lack of assessment of ambient sea lice infection rates on juvenile salmon. Reports correlating the presence of salmon farms with high sea lice infection rates on wild salmon have not established ambient sea lice infection rates in the study area due to the saturation of salmon farms in their sampling areas (Morton and Williams 2003; Morton *et al.* 2004; Morton *et al.* 2005). Thus in the absence of ambient infection rates it is not possible to establish or quantify cause and effect relationships between salmon farms and wild salmon populations.

In coastal marine areas of British Columbia's central and north coasts there are currently very few salmon farms (< 5 active sites) compared to marine areas surrounding Vancouver Island (> 80 active sites) to the south. The lack of farms on the central and north coasts offers an opportunity to assess the natural dynamics of sea lice on juvenile salmon and determine how the relationship changes with specific exposure to salmon farms. The unique geography of the Bella Bella / Klemtu, BC region allows simultaneous assessment of the ambient sea lice infection rates on juvenile salmon in the absence of salmon farms and assessment of the influence of salmon farms on the ambient infection rates within the same geographic region. This study tested the hypothesis that sea lice infection rates on juvenile chum and pink salmon depends on the proximity of the sampling site to an active salmon farm in areas of the central British Columbia coast with little or no salmon farming activity.

Methods

Juvenile chum salmon *(Oncorhynchus keta)* and pink (*Oncorhynchus gorbuscha)* were collected in coastal marine areas during spring out-migration (late-March – June) from their natal rivers in 2003, 2004, and 2005. Samples were collected near Bella Bella and Klemtu, British Columbia in all three years (Figure 1.1). In 2003, 8 sites were sampled 41 times, in 2004, 23 sites were sampled 78 times, and in 2005, 34 sites were sampled 126 times (Table 1.1).

Sample sites were selected within sheltered bays and inlets based on the following criteria: proximity to salmon producing rivers, proximity to the open ocean, and proximity to salmon farm tenures. The most important factor when selecting a sampling site was its proximity to an active salmon farm. Current, tide, and wind were not measured during the study and were treated as random factors. Over the three years of sampling, five salmon farming sites were active in the sampling area, however in any given year, no more than three salmon farms were active at the same time (see Table 1.6).

Proximity to salmon farms was separated into four categories (< 1km, 1-15 km, >15 km, and Separated). The Bella Bella and Klemtu areas are separated from each other by the presence of a long peninsula (Don Peninsula) that runs between them (Figure 1.1). Samples collected on the Bella Bella side were designated as “Separated,” while those from the Klemtu side were designated based on the distance from the nearest active salmon farm (1-15 km). The 15 km farm influence limit was selected based on local geography.

All salmon samples were collected using beach seine sampling nets. Beach seines were 30.3m long X 1.2-1.8m deep X 6.3mm bunt mesh. Our technique consistently resulted in a large number of juvenile salmon caught allowing a random sub-sample to be collected from a much larger population. A sampling crew of 2-4 used a small boat to encircle schools of wild juvenile salmon. With one crewmember anchoring the net onshore, the boat maneuvered to encircle the school between the net and shore at which point the net was brought ashore. Once fish were at the shoreline a five-gallon bucket was used to

remove as many as possible from the net. This method minimized lice-shedding abrasion. Once in the bucket fish were randomly selected and placed individually in whirl-pak™ bags and placed on ice. The number of samples collected varied from 13 to 200 fish per site. Samples were frozen at -20^{o}C as soon as possible. At each site, data for sea surface temperature (SST) and sea surface salinity (SSS) were collected using a YSI meter.

Samples were thawed and fish were individually examined for sea lice using a dissecting microscope. Sea lice were identified to stage and to species for pre-adults and adults following Johnson and Albright (1991b) and Kabata (1972). Sea lice were designated as copepodids, chalimus I, II, III, or IV, pre-adult, and adult. Maximum infection intensities (MII) were recorded. Juvenile salmon were identified to species following Phillips (1977). Weights and lengths were also recorded.

Data Analysis

The null hypothesis of interest in this study was that mean sea lice infection rates were independent of the proximity of a sampling site to an active salmon farm. Generalized linear models (univariate ANOVA) were used to allow comparison between continuous and categorical variables.

The dependent variable was the average number of lice per fish per sampling event. A replicate represents the average total lice (all sea lice life stages and species; uninfected fish included) for juvenile pink or chum salmon collected within a sampling event (n=13->200 fish) pooled to avoid pseudoreplication. Initial analyses were done by pooling all

sea lice life stages and species into a total lice variable, subsequent analyses were split for sea lice life stage (juvenile vs. adult) and sea lice species ratios (*Caligus clemensii vs. Lepeoptheirus salmonis)*. Comparisons were made between replicates (i.e. sampling events) among the different proximity categories. Other factors in the analysis included: salinity, temperature, week, proximity to active salmon farms, and average length per fish per sampling event (length was determined to be a more reliable predictor of fish size due the potential error from weighing very small fish).

The differences in yearly sampling effort and the changing dynamics of the active salmon farms (e.g. age-class, species, etc) within the region necessitated that the analyses be separated by year (2003, 2004, 2005). Additionally, the analyses were also split by species (pink vs. chum), to reduce model complexity and examine species differences.

Univariate ANOVA's (Generalized Linear Models) were fit to six data sets (chum and pink in each of the three years sampled). For each model, the data was first fit with a full model including: the main effects (week, exposure, temperature, salinity, and length), all 2-way interactions, and all three way interactions were included in the initial model (4-way and 5-way interactions were left out due to insufficient data and the difficulty associated with interpreting 4-way and 5-way interaction terms). For subsequent analyses, factors that were not significant at $\forall$=0.05 were sequentially dropped in a step-wise procedure until only the main effects remained. The change in r^2 was noted throughout the analysis procedure.

The normality assumption for univariate ANOVAs was tested using the residuals from each model. All models met the assumption with the exception of 2005 pink salmon data which was transformed using $\log_e(y + 0.5)$.

Main effects, 2-way interaction, and 3-way interaction models were assessed for explanatory power using Akaike's Information Criterion (AIC)

$$\Delta_i = AIC_i - AIC_{min}$$

For consistency, the same number of interaction terms and main effects were compared between species in the same year (Burnham and Anderson 2002). Following calculation of Δ_i the model probability or the Akaike weight (w_i) was calculated using:

$$w_i = \exp(-0.5*\Delta_i) / \Sigma \exp(-0.5*\Delta_r)$$

where Δ_r = the sum of the Δ_i for the models being compared. The model with the highest w_i was selected as the best model for the data. All analyses were conducted using S-Plus 7.0 and SPSS 11.5.

Results

During the three years of sampling, 13,874 juvenile chum and pink salmon were collected. Chum salmon made up the bulk of the catch among the three sampling years and exposure categories (62% chum, 38% pink). This catch ratio was approximated across all exposure categories and years, with the exception of the low and high exposure categories in 2003 where the ratio was reversed (60% pink and 40% chum). During their early marine phase, juvenile chum and pink salmon commonly school together therefore it is unlikely that our sampling method was biased for either species. Differences in the

total numbers of fish collected are a function of sampling effort between years due to logistical challenges (weather, access etc.)

Sea lice infections of Chum Salmon

The biological parameters of sea lice infection: prevalence (% of sample infected), average lice abundance (juvenile, adult, and total lice), and infection intensity vs. proximity to active salmon farms are presented in Table 1.2. Figure 1.2 shows the average total lice abundance in the < 1km category ranged from 1.4 to 2.3 times higher than the 1-15 km category and from 2.2 to 14 times higher than the > 15 km and separated categories across all three sampling years. Mean sea lice infection rates were the lowest (marginal difference between 1-15 km and separated in 2005) in the > 15 km and separated categories across all three years. Juvenile lice dominated the infections ranging from 72% – 89% across all years and proximity categories. Overall, lice abundance was similar in 2003 and 2004 but dropped dramatically in both the < 1km and 1-15 km categories and only marginally in the > 15 km and separated categories in 2005.

Average prevalence (mean prevalence per sampling event) in the < 1 km sites ranged from 7-18% higher than the 1-15 km sites and from 10-47% higher than the > 15 km and separated sites across all years (Table 1.2). Chum salmon prevalence ranged from 9% - 60% across all proximity categories and years. Prevalence was the lowest in the > 15 km and separated categories across all years. Overall, prevalence was the highest in 2004 and the lowest in 2005. The maximum infection intensity (M.I.I.) shows the maximum lice per fish observed in all samples per year and per proximity category. Maximum infection

intensities observed in the < 1 km (M.I.I.=18) and 1-15 km (M.I.I.=15) categories were 1.1 – 4.5 times higher than the > 15 km and separated categories (M.I.I.= 7) across all years (Table 1.2).

For all three years the main effects model (week, proximity, mean fork length, temperature, and salinity) was found to be the strongest predictor of the data as it had the highest Akaike weight (Table 1.3). The results from the 2003 chum analysis suggest that proximity category was the most important predictor of the average total lice per juvenile chum salmon (R^2=0.37, p=0.0019)(Figure 1.3A). No other factor was significant or close to significant in the model in 2003 (Table 1.4).

In 2004, proximity to salmon farms was again found to be a strong predictor of lice abundance per fish (R^2=0.37, p=0.0147)(Table 1.4). Average chum length and sampling week were also found to have a significant effect on the average total lice abundance (p=0.0135 and p=0.0173 respectively) (Figure 1.3B). In 2005, only week was found to be a significant predictor of the average total lice per chum salmon (R^2= 0.23, p=0.0027)(Table 1.4). Figure 1.3C shows a sharp increase in total lice abundance in sampling week 6 in the 1-15 km and < 1 km proximity categories.

Sea lice infections of Pink Salmon

Average total lice per fish for juvenile pink salmon was 2-3 times lower than for chum salmon across all years and proximity categories (Table 1.2). Mean total lice abundance in the < 1 km category was found to be 1.2 to 5.8 times higher than the > 15 km and

separated categories and 2.2 to 4 times higher than the 1-15 km category across all sampling years except in 2004 where the 1-15 km category was marginally higher than the < 1 km category (0.81 (0.14) and 0.66 (0.12) respectively) (Figure 1.4). Average total lice abundance was the lowest in the > 15 km and separated categories in 2003 and 2004 but not in 2005 (Table 1.2).

Overall, prevalence was approximately 10% lower for pink salmon than that observed for chum salmon (Table 1.2). Pink salmon prevalence ranged from 3% - 44% across all years and proximity categories. In 2003 and 2004, prevalence in the < 1 km category was 25% and 26% higher than the separated category respectively. Average prevalence was the lowest in all categories in 2005, with little or no difference among the proximity categories (Table 1.2). Large differences were observed in maximum infection intensities among proximity categories in 2003 (unexposed (M.I.I.=3) to high (M.I.I.=23))(Table 1.2). Conversely, in 2004 and 2005, maximum infection intensity ranged from 2 to 8 lice per fish across both years and proximity categories. The proportion of juvenile sea lice stages on juvenile pink salmon ranged from 50-84% across all years and exposure categories (Table 1.2).

Similar to the results for juvenile chum salmon, the main effects models were shown to be the best predictors of the data (Table 1.3). In 2003, proximity to salmon farms was the strongest predictor of lice abundance on juvenile pink salmon (R^2=0.0188). Figure 1.4 shows that fish collected in the < 1 km category were more heavily infected than were

fish collected in the other proximity categories across all sampling weeks. No other factor was found to be significant in any of the life stages present (Table 1.4).

In 2004 and 2005, no factor was found to be a significant predictor of the average total lice per fish (Table 1.4). In 2004, higher average lice per fish levels for fish collected in the < 1 km and 1-15 km categories were recorded when compared to the 1-15 km category across all sampling weeks (Figure 1.5 A, B, C). Very low infection levels (<0.3 lice per fish) were recorded for fish collected in all proximity categories in 2005, however a sharp rise (<0.2 lice per fish) in lice levels was recorded in week six in the < 1 km and 1-15 km categories as the study came to an end.

Salinity and Temperature

Mean salinity ranged from 19.4 (0.1) ppt to 30.4 (0.8) ppt across all proximity categories and sampling years (Table 1.5). The highest salinity was found in the < 1km and the 1-15km proximity categories (range: 24 ppt – 34 ppt) with only small differences observed between them. The lowest salinity was found in the > 15km and separated categories (range: 5 ppt – 32 ppt). In addition to having the lowest salinity, the > 15km and separated categories had at least twice the variation of the other categories for salinity and the highest average temperatures. Mean temperature ranged from 9.1(1.0)^{O}C to 12.5(0.2) ^{O}C across all proximity categories and sampling years (Table 1.5).

Mean length and weight

Mean fork length ranged from 4.53 (0.36)cm to 5.94 (0.37)cm for chum salmon and from 4.24 (0.63)cm to 5.94 (0.41)cm for pink salmon across all proximity categories and years. Mean weight ranged from 1.06 (0.40)g to 2.86 (0.35)g for chum salmon and from 0.85 (0.35)g to 2.81 (0.44)g for pink salmon across all proximity categories and years. No major differences for either mean length or mean weight were observed across all proximity categories, within years. Across years, overall mean length and mean weight (all proximity categories combined) were the highest in 2004 and the lowest in 2005.

Farm site infection rates

Sea lice infection rates for juvenile pink and chum salmon collected at the West Jackson Pass and Lochalsh Bay (sites 1 and 2 - Figure 1.1) farm sites ranged from 2.27 (0.74) lice/fish to 3.34 (1.68) lice/fish for chum salmon and from 0.76 (0.04) lice/fish to 1.23 (0.36) lice/fish in 2003 and 2004 (Table 1.6). The West Jackson Pass farm had Chinook smolts in 2003 and adult Chinook in 2004, while the Lochalsh Bay farm had adult Atlantics in 2003 and was fallowed (emptied of salmon) in 2004. In 2005, both sites were fallowed and lice infection rates dropped to near zero (< 0.05 (0.05) lice/fish) (Table 1.6).

At Arthur Island (site 4 – Figure 1.1), lice infection rates for juvenile salmon were correlated with farm production. Mean lice per juvenile chum salmon was 0.48 (0.20) lice/fish when the farm had 1 year Chinook smolts, 0.92 (0.19) lice/fish when the farm had adult Chinooks, and 0.10 (0.05) lice/fish when the farm was fallowed (Table 1.6). A similar pattern was found for pink salmon where the mean lice per fish was 0.18 (0.03)

lice/fish when the farm had 1 year Chinook smolts, 0.48 (0.15) lice/fish when the farm had 2-year Chinook, and 0.02 (0.01) lice per fish when the farm was fallow (Table 1.6). Both species infection rates were lower in intensity to fish collected at the West Jackson Pass and Lochalsh Bay farm sites.

Sea lice infection rates of juvenile pink and chum salmon were less correlated with farm production levels at the Goat Cove and Kidd Bay farm sites (site 16 and 17 – Figure 1.1). Farm salmon production started 2004 at Goat Cove and 2005 at Kidd Bay. In 2003, no samples were collected in Goat Cove and a single sample of 51 fish was collected in Kidd Bay (0.30 lice/fish (chum) and 0.39 lice/fish (pink)) (Table 1.6). In 2004, single samples of 50 fish were collected at the Goat Cove (0.83 lice/fish (chum) and 0.73 lice/fish (pink)) and Kidd Bay (2.20 lice/fish (chum) and 1.67 lice/fish (pink)) farm sites. In 2005, both sites were active and both juvenile pink and chum salmon had mean lice per fish levels of less than 1 louse per fish (0.11 (0.08) lice/fish to 0.38 (0.18) lice/fish)(Table 1.6). In 2005, lice levels began to rise within the last few weeks of the study (Figures 1.3C and 1.5C).

Lice per unit weight of juvenile chum and pink salmon

The mean lice per gram of body weight of juvenile chum and pink salmon infected with sea lice (i.e. salmon with zero lice per fish were removed) was found to be the highest in the < 1km category across all sampling years (except pink salmon in 2005 where the separated category was the highest and chum salmon 2004 where the > 15km was the highest) (Table 1.7). The mean lice per gram in the < 1 km category ranged from 1.17 (0.08) to 2.94 (0.24) for chum salmon and from 0.85 (0.11) to 3.64 (0.4) for pink salmon

across all sampling years. In 2003, the mean lice per gram for juvenile chum and pink salmon at the high exposure sites was 1.8 and 2.3 times respectively above the lethal infection limit of 1.6 lice per gram reported for some Atlantic salmon (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997). All other categories across all years had lice levels below 1.6 lice per gram (except pink salmon in 2005: 1.97 (0.19)). The mean lice per gram for the > 15 km and separted categories ranged from 0.65 (0.06) to 1.45 (0.13). For the purpose of this study, it was important to remove uninfected fish from the data set and examine only infected fish, due to unequal sample sizes and lack of control of the zero fish, which have the potential to obscure an impact assessment.

Lice species ratio

Lepeoptheirus salmonis and *Caligus clemensii* were the only lice species found on juvenile chum and pink salmon in all proximity categories and sampling years, based on pre-adult and adult sea lice counts. In 2003 and 2004, *L. salmonis* was the dominant lice species in the < 1 km proximity category for both chum and pink salmon (74.8% to 88.4% *L. salmonis* vs. *C. clemensii*) while *C. clemensii* was dominant in the separated category for chum and pink salmon (75% to 87.5 % *C. clemensii*)(Table 1.8). In 2005, *C. clemensii* dominated all proximity categories ranging from 54.5% to 88.9% *C. clemensii* for chum salmon and from 60% to 100% *C. clemensii* for pink salmon (Table 1.8).

Discussion

The results of this study suggest that salmon farms influence ambient sea lice infection rates on juvenile chum and pink salmon. The enhancement of ambient sea lice infection

rates by salmon farms and the subsequent transfer to migrating wild salmon has been widely documented (Tully *et al.* 1993; Tully *et al.,* 1999; Bjorn *et al.* 2001; Bjorn and Finstad 2002; Butler 2002; Morton and Williams 2003; Morton *et al.* 2004; Krkosek *et al.* 2005; Morton *et al.* 2005). Elevated sea lice infection rates recorded in 2003 at the < 1 km sites suggest a strong farm influence when compared to other areas more distant from salmon farms (Figure 1.2 and 1.4). Regrettably, we were unable to gain access to sea lice infection data from the farms in our study area as salmon farming companies in BC do not release their sea lice infection rate data by farm site. However, several factors suggest that sea lice infection rates were high on some farms in 2003. The Lochalsh Bay farm site in Jackson Pass contained two-sea winter Atlantic salmon. Sea lice infections are known to increase with farmed salmon sea residence time (Revie *et al.* 2002a) and increasing surface area available for attachment (Tucker *et al.* 2002). In addition, of the salmon cultured on the west coast of North America, Atlantic salmon have been found to be the most susceptible to sea lice infection (Johnson and Albright 1992; Fast *et al.* 2002). Finally, two independent reports from observers at the Lochalsh Bay salmon farm suggest that high sea lice infection rates were present on the adult Atlantic salmon during the sampling period in 2003 (pers. comm. Otto Langer at David Suzuki Foundation) and Tony Nislaas (Kitasoo Fisheries)).

Our results also show significant inter-annual variability of lice infection on juvenile chum and pink salmon. Temporal variability in sea lice infection rates has been noted in other multi-year studies (Boxaspen 1997). In 2004, elevated lice infection rates were observed for chum salmon at the < 1 km sites and for pink salmon at the < 1 km and 1-15

km sites. In 2004, Lochalsh Bay was fallow (emptied of salmon) and most of the samples in the < 1 km category were collected near the West Jackson Pass and Arthur Island farms both of which contained two-sea winter Chinook salmon. Chinook salmon have been found to be less susceptible than Atlantic salmon to infection by sea lice (Johnson and Albright 1992). Thus, it was expected that lice infection rates for fish collected near the Chinook salmon farms would be lower than those collected near Atlantic salmon farms. Our results suggest little difference among samples collected between the two types of farms. However, high salinity (> 30 ppt) is known to enhance lice settlement and survival (Johnson and Albright 1991a; Tucker *et al.* 2000). In 2004, salinity was slightly elevated (Table 1.5) and it is possible that even low-level infections could have been enhanced by the higher salinities observed in the 1-15 km and < 1 km proximity categories.

In 2005, lice infection rates were the lowest of all the years sampled. Farm sites in Jackson Pass and Arthur Island were fallowed and lice infection rates for chum and pink salmon were near zero for all three sites (Table 1.6). The results suggest that fallowing is an effective method for reducing lice infection rates that were enhanced in previous years. In addition, the results suggest little or no “seed” effect (i.e. lice do not have access to significant overwintering habitat) on sea lice populations, which is consistent with their requirements for salmonid hosts, which are not likely to be abundant all year round in the absence of salmon farms in the areas sampled. Fallowing of salmon farms has been found to be an effective way of reducing lice infection rates in near shore environments (Bron *et al.* 1993; Grant and Treasurer 1993; Morton *et al.* 2005).

The elevated lice infection rates recorded in Jackson Pass (Table 1.6) in 2003 and 2004 were significantly higher than at any of the other farming sites throughout the study (with the exception of Goat Cove and Kidd Bay in 2004 where one-time samples of 50 fish taken at each site were comparable). The proximity of the Lochalsh Bay farm to the West Jackson Pass farm (less than 4km) likely contributed to the observed lice infections in both years. In addition, the narrowness of Jackson Pass is a factor in elevating sea lice infection rates on juvenile salmon (Figure 1.1). Holst *et al.* (2000) suggested that the common practice of siting salmon farms in confined coastal waters can aggravate both farm infections and wild salmon stock collapse through sea louse amplification on farm stocks. Thus, evidence suggests that Jackson Pass and other narrow passages throughout the British Columbia coast should not be favored for farming salmon regardless of the farmed species being considered.

Our results show significant variability in sea lice infection rates for juvenile chum and pink salmon collected at different salmon farm sites. In 2005, the Kidd Bay and Goat Cove farm sites were raising adult and sub-adult Atlantic salmon and the lice infection rates recorded were much lower when compared to the lice infection rates recorded at the farm sites in Jackson Pass in 2003 and 2004 (Table 1.6). Although lice infection rates began to rise at the Goat Cove and Kidd Bay sites towards the end of the sampling period in 2005 (Figure 1.3C and 1.5C), the results suggest salmon farm effects on ambient sea lice infection rates are variable among farming locations. Factors affecting the output of sea lice from salmon farms would include: water movement through the farming sites and

its effect on sea lice larval dispersion, farmed species and age, local temperature and salinity dynamics, and interactions with wild lice hosts. In the present study, the Arthur Island, Goat Cove, and Kidd Bay farm sites were all located along the sides of large channels exposed to wind and wave action. The Jackson Pass farm sites were located inside a narrow pass and would not experience the same dispersal factors (Figure 1.1). Future studies should attempt to incorporate current, tidal, and wind data into the analysis.

It is unlikely that the differences in infection rates observed across proximity categories can be explained by differences in the abundance of natural hosts. The dominance of *L. salmonis* observed at the < 1 km sites for chum and pink salmon (Table 1.8) was expected given the salmonid specific nature of *L. salmonis* and the lack of salmonid hosts likely present in near shore marine areas during spring sampling. In addition, *L. salmonis* is the dominant lice species affecting farmed salmonids on the northern Pacific and Atlantic coasts of Canada and the USA (Smith 1998; Johnson *et al.* 2004). Revie *et al.* (2002a) observed that the abundances of *L. salmonis* and *C. elongatus* on salmon farms in Scotland were inversely correlated with each other. Possible reasons for this observation include a greater sensitivity to treatment for *C. elongatus* (treatment regimes are more intensive in the second year of production), different rates of development between the two species over the two-year production cycle, and the possibility that *L. salmonis* has a competitive advantage over *C. elongatus* based on due to bigger size (Revie *et al.* 2002a). The data from the present study suggest that salmon farms are acting as significant reservoirs for *L. salmonis* not previously available. Heuch and Mo (2001) estimated that

total *L. salmonis* egg production in salmon farming areas has increased by more than 50 times compared to the pre-farming conditions in Norway. Tully and Whelan (1993) suggested that 95% of the total production of *Lepeoptheirus salmonis* nauplii in the mid west coast region of Ireland originated from salmon farms. Krkosek *et al.* (2005) demonstrated that a single salmon farm in British Columbia raised ambient infection levels by four orders of magnitude. Given the evidence available, scenarios whereby natural hosts and natural factors would interact to produce such high infection rates are highly unlikely.

Lice infection rates were significantly higher in areas of close proximity to salmon farms vs. those more distant (Table 1.2; Figure 1.2 and 1.5). These results suggest that salmon farms were the main driver of the observed patterns, which is consistent with studies comparing lice infection rates across geographic locations and different proximity conditions (Bjorn *et al.* 2001; Bjorn and Finstad 2002; Morton *et al.* 2004). Geographic variability is often cited as a reason for lice differences observed among geographic areas, yet no published studies support this idea. Revie *et al.* (2002b) conducted a survey of lice abundance on 33 salmon farms in Scotland found that geographic location did not affect mean lice abundance. Geographic factors that could influence the observed data include: temperature and salinity differences, current movements, and the abundance of fish.

Differences in temperature and salinity were observed among proximity categories across all years of this study (Table 1.5). Temperature and salinity are important factors driving

the populations of sea lice on salmon (Johnson and Albright 1991a; Tucker *et al.* 2000). Salinity ranged 28.4 – 30.4 ppt in the < 1 km and 1-15 km categories and ranged from 19.4 – 27.6 ppt in the > 15 km and separated categories. Laboratory studies have shown that salinity near 30 ppt allows for optimal settlement and survival of the copepodid life stage; however viable copepodids can be produced at salinities above 24 ppt (Johnson and Albright 1991a; Tucker *et al.* 2000). Although it is possible that the observed salinities had some effect on lice infection rates, the results of the statistical analysis found that neither temperature nor salinity was a significant factor in the observed lice infection rates. An alternative explanation could be that the higher temperatures in the > 15 km and separated categories offset the negative effects of the lower salinity as has been found for other parasitic copepods (Kinne 1957; Lance 1963; Tucker *et al.* 2000). Additionally, mean temperature and salinity in the > 15 km and separated categories had high standard deviations, suggesting high variability within the system (Table 1.5). One possible explanation could be that the salinity data collected represents sea surface salinity (SSS) and some of the samples in the > 15 km and separated categories were collected within 1 or 2 km of a large river inflow, which resulted in some values less than 10 ppt due to the presence of a freshwater layer. In 2005, salinity profiles were collected and the results show that the fresh layer at the river mouth sites was consistently less than 2.5 m in depth (actual depth not measured). Therefore, the differences in salinity and the high variability could be due to the effect of this fresh layer on some of the samples. It is unclear what impact on lice infection rates the fresh water may have had given that juvenile salmon can be common as deep as 10 m (Healey 1980).

The mean lice per gram of infected fish (fish with zero lice excluded) was examined to determine the potential impact on juvenile salmon. Mean lice per gram has been used as a proxy for host impact by sea lice in experimental laboratory studies conducted on post-smolt European salmonids that determined 0.75 – 1.6 lice /g of fish weight to be a lethal infection ratio (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997). However, these numbers were generated from fish that were 8-60 times (40-60 g post-smolt Atlantic, sea trout (*Salmo trutta*), Arctic Char (*Salvelinus alpinus*)) larger than juvenile chum and pink salmon that enter the marine environment weighing less than 1 gram (Healey 1980; Heard 1991). Morton and Routledge (2005) have conducted the only study examining the impact of sea lice on juvenile pink and chum salmon. Their results suggest that the short-term mortality for similarly sized juvenile pink and chum salmon is significantly increased by infection of 1-3 sea lice per fish. It is, however, important to note that the results of laboratory studies will likely overestimate the effect of sea lice due to the effects of fish handling and culturing.

Mean lice per gram was above 1.6 lice per gram in 2003 in the < 1 km category where juvenile chum and pink salmon had average lice per gram infections of 2.94 (0.24) and 3.64 (0.40) respectively (Table 1.7). The bulk of the 2003 data were collected near the farms in Jackson Pass where a lice outbreak on adult Atlantic salmon was suspected. The strong signal suggests a high contribution from the farm sites given the narrowness of the pass and the unlikely contribution of other sources. Lice per gram levels greater than 6 have been reported for juvenile pink and chum salmon collected near salmon farms in

other areas of British Columbia where salmon farms operate in higher densities than the Klemtu region (Morton and Williams 2003; Morton *et al.* 2004).

Lice per gram levels at the < 1 km sites in 2004 were much lower than those in 2003 despite having comparable average lice abundances (Figures 1.3 and 1.5) and having similar average prevalence levels (Table 1.2). The average lice per gram for both chum and pink salmon were marginally higher or lower than all of the other proximity categories. Two possibilities account for the observed differences. The first difference in 2004 versus 2003 was the species and numbers of salmon being farmed in Jackson Pass. In 2003, it was 2-year-old Atlantic salmon (Lochalsh Bay) and 1-year-old Chinook (West Jackson), while in 2004 only 2-year-old Chinook salmon (West Jackson) were present (Table 1.6). Second, the average weight in 2004 was 1.02 g higher in chum salmon and 1.20 g higher in pink salmon, which, given similar average lice abundances would result in lower lice per gram rates.

This study is one of the first to examine data on the ambient sea lice infection rates on juvenile salmon and examine how salmon farms can influence that relationship within the same geographic region. The lack of information on natural lice infection rates has been at the center of the wild-farm disease interaction debate. The present study controlled for temporal and spatial variability and the results suggest that the mean lice per fish rates for juvenile pink and chum salmon ranged between 0.14 (0.05) to 0.31 (0.07) for chum salmon and 0.04 (0.01) to 0.37 (0.15) for pink salmon (Table 1.2) across all sampling years. In addition, the mean lice per gram levels for both species in the > 15 km and

separated categories were below two lice per gram for both species in across all sampling years. These data support the finding that lice levels above 2-3 lice per gram may be lethal infection levels for juvenile pink and chum salmon (Morton and Routledge 2005). Furthermore, the results of this study suggest that salmon farms can strongly influence the host-parasite relationship between sea lice and juvenile chum and pink salmon. The extent to which the sea lice-salmon relationship is affected by salmon farms is dependent on farm species, farm location, and within year variability in fish size, temperature, and salinity.

Table 1.1 – Sampling site proximity to salmon farms (<1 km, 1-15 km, >15km, Separated) 2003-2005. Blank spaces indicate no sampling took place at that site during that sampling year.

Site	2003	2004	2005
1*	<1 km	<1 km	1-15 km
2*	<1 km	1-15 km	>15km
4*	<1 km	<1 km	>15km
5	1-15 km	1-15 km	>15km
6		1-15 km	>15km
7		1-15 km	>15km
8	1-15 km	1-15 km	>15km
9	Separated	Separated	Separated
11		Separated	Separated
12	Separated	Separated	Separated
13	Separated	Separated	Separated
14		Separated	Separated
15		Separated	Separated
16*		1-15 km	<1 km
17*		<1 km	<1 km
18		>15km	1-15 km
19		>15km	>15km
20			>15km
21		>15km	>15km
22		>15km	>15km
23		>15km	>15km
24		>15km	>15km
25			>15km
26			>15km
27		>15km	>15km
28			Separated
29			Separated
30		1-15 km	1-15 km
31			Separated
32			Separated
33			Separated
34			Separated
36			Separated
37			>15km

***** Salmon farm site

Table 1.2 – Average prevalence (% of population infected with sea lice), average lice per fish (total lice, juvenile lice, adult lice), and maximum infection intensity (M.I.I.) for samples of juvenile chum and pink salmon collected in the Bella Bella / Klemtu region between 2003-2005. Numbers reflect averages for sampling events (including uninfected fish) pooled into proximity categories.

	Proximity to salmon farm	N	M.I.I.	Prevalence (%)	Juvenile (lice/fish)	Adult (lice/fish)	Total Lice (lice/fish)
Chum							
2003	<1km	16	18	56.0 (8.0)	1.59 (0.34)	0.40 (0.13)	1.99 (0.43)
	1-15km	13	15	38.0 (7.0)	0.76 (0.18)	0.09 (0.05)	0.85 (0.22)
	Separated	13	4	9.0 (3.0)	0.11 (0.05)	0.03 (0.01)	0.14 (0.05)
2004	<1km	11	14	60.0 (6.0)	1.37 (0.45)	0.40 (0.11)	1.77 (0.54)
	1-15km	24	13	53.0 (4.0)	0.92 (0.20)	0.35 (0.06)	1.27 (0.21)
	>15km	7	7	19.0 (6.0)	0.20 (0.07)	0.11 (0.05)	0.30 (0.11)
	Separated	28	5	20.0 (3.0)	0.25 (0.07)	0.06 (0.01)	0.31 (0.07)
2005	<1km	10	7	20.0 (6.0)	0.27 (0.09)	0.06 (0.04)	0.33 (0.11)
	1-15km	6	9	10.0 (7.0)	0.12 (0.07)	0.03 (0.02)	0.14 (0.09)
	>15km	45	6	10.0 (2.0)	0.13 (0.03)	0.02 (0.01)	0.15 (0.04)
	Separated	39	5	9.0 (2.0)	0.13 (0.05)	0.02 (0.01)	0.15 (0.05)
Pink							
2003	<1km	16	23	34.0 (6.0)	0.51 (0.17)	0.19 (0.05)	0.70 (0.18)
	1-15km	13	12	21.0 (5.0)	0.27 (0.09)	0.05 (0.18)	0.32 (0.10)
	Separated	13	3	9.0 (4.0)	0.09 (0.05)	0.02 (0.01)	0.12 (0.05)
2004	<1km	11	5	39.0 (6.0)	0.34 (0.08)	0.32 (0.09)	0.66 (0.12)
	1-15km	25	8	44.0 (5.0)	0.44 (0.14)	0.37 (0.06)	0.81 (0.14)
	>15km	7	6	31.0 (14.0)	0.19 (0.14)	0.19 (0.11)	0.37 (0.15)
	Separated	27	3	13.0 (4.0)	0.13 (0.05)	0.05 (0.01)	0.18 (0.05)
2005	<1km	10	3	10.0 (3.0)	0.08 (0.03)	0.04 (0.02)	0.12 (0.04)
	1-15km	6	2	3.0 (3.0)	0.02 (0.03)	0.01 (0.01)	0.03 (0.03)
	>15km	48	6	3.0 (1.0)	0.02 (0.01)	0.02 (0.01)	0.04 (0.01)
	Separated	31	4	8.0 (2.0)	0.05 (0.03)	0.05 (0.02)	0.10 (0.04)

Table 1.3 – Model comparisons using Akaike's Information Criterion (AIC) used to assess the factors that predict the average total lice per fish for chum and pink salmon collected in the Bella Bella / Klemtu area in 2003-2005 **(Δ_I =** AIC difference, **w_i =** Akaike weight).

	Model Description	Parameters	AIC	Δ_i	w_i
Chum					
2003	Full	22	197.37	46.55	0.00
	13 int + Main	18	185.05	34.23	0.00
	1 int + Main	6	155.65	4.83	12.61
	Main effects	5	150.82	0.00	141.15
2004	Full	22	273.27	62.05	0.00
	13 int + Main	18	230.47	19.25	0.01
	1 int + Main	6	212.75	1.54	63.27
	Main effects	5	211.21	0.00	136.59
2005	Full	22	213.86	154.81	0.00
	13 int + Main	18	162.54	103.49	0.00
	1 int + Main	6	64.76	5.71	25.05
	Main effects	5	59.05	0.00	435.28
Pink					
2003	Full	22	181.83	89.99	0.00
	13 int + Main	18	159.85	68.02	0.00
	1 int + Main	6	98.11	6.28	11.72
	Main effects	5	91.83	0.00	270.87
2004	Full	22	252.65	119.84	0.00
	13 int + Main	18	210.47	77.66	0.00
	1 int + Main	6	139.05	6.24	14.83
	Main effects	5	132.81	0.00	335.91
2005	Full	22	151.70	166.23	0.00
	13 int + Main	18	115.87	130.39	0.00
	1 int + Main	6	2.86	17.38	0.09
	Main effects	5	-14.52	0.00	517.70

Table 1.4 – Results for the generalized linear models (univariate ANOVA) for juvenile chum and pink salmon for the total lice vs. proximity, week, temperature, salinity, and average length per fish (*significant $p < 0.05$).

Year	Dependent Variable	Effect	df	F	p-value	R^2
Chum						
2003	Total Lice	Week	1	0.01	0.9449	0.370
		Proximity	2	8.10	0.0019*	
		Temperature	1	1.42	0.3730	
		Salinity	1	0.05	0.7690	
		Length	1	0.48	0.4963	
2004	Total Lice	Week	1	5.97	0.0173*	0.370
		Proximity	2	5.95	0.0147*	
		Temperature	1	0.03	0.0847	
		Salinity	1	0.50	0.2882	
		Length	1	5.92	0.0135*	
2005	Total Lice	Week	1	9.66	0.0027*	0.230
		Proximity	2	0.87	0.2892	
		Temperature	1	0.24	0.6485	
		Salinity	1	0.67	0.4446	
		Length	1	1.41	0.2750	
Pink						
2003	Total Lice	Week	1	0.19	0.5205	0.250
		Proximity	2	3.17	0.0188*	
		Temperature	1	0.10	0.8611	
		Salinity	1	0.00	0.9251	
		Length	1	0.02	0.7669	
2004	Total Lice	Week	1	0.02	0.7400	0.330
		Proximity	2	0.95	0.1008	
		Temperature	1	0.34	0.5771	
		Salinity	1	1.26	0.0744	
		Length	1	2.76	0.0988	
2005	Total Lice	Week	1	0.18	0.6579	0.210
		Proximity	2	1.42	0.1408	
		Temperature	1	0.52	0.4433	
		Salinity	1	0.01	0.9652	
		Length	1	1.46	0.2473	

Table 1.5 – Average sea surface temperature (SST) (+/- SE), average sea surface salinity (SSS) (+/- SE), and standard deviations (SD) for areas of different proximity to salmon farms in the Bella Bella / Klemtu region between 2003-2005.

	Exposure	N	SST (^{O}C)	SST / SD	SSS (ppt)	Range	SSS / SD
2003	<1km	16	11.0 (0.31)	1.25	29.2 (0.40)	24.9-30.8	1.61
	1-15km	13	10.8 (0.34)	1.23	28.6 (0.58)	24.0-30.9	2.10
	Separated	13	12.3 (0.70)	2.54	25.0 (1.32)	16.9-32.0	4.77
2004	<1km	11	11.2 (0.14)	0.48	30.4 (0.82)	26.0-34.0	2.73
	1-15km	24	11.7 (0.13)	0.63	30.0 (0.60)	24.0-34.0	2.92
	>15km	7	11.5 (0.41)	1.08	19.4 (3.30)	5.0-29.0	8.73
	Separated	28	12.5 (0.18)	0.96	25.1 (0.86)	16.0-31.0	4.57
2005	<1km	10	09.9 (0.70)	2.21	28.7 (0.71)	25.5-31.4	2.24
	1-15km	6	09.1 (0.96)	2.34	28.4 (0.74)	26.2-30.2	1.82
	>15km	45	09.8 (0.39)	2.60	27.6 (0.64)	10.0-31.7	4.31
	Separated	39	11.2 (0.44)	2.78	24.8 (0.72)	8.3-30.0	4.50

Table 1.6 –Mean lice per fish (+/- SE) for juvenile chum and pink salmon and age class and species of salmon being farmed at farm locations in the Klemtu, BC region (2003-2005).

Year	Farm Site	Production	Chum (lice/fish)	N	Pink (lice/fish)	N
2003	*W. Jackson**	1 yr Chinook	3.30 (0.62)	144	0.87 (0.33)	156
	*Lochalsh Bay**	2 yr Atlantic	2.27 (0.74)	103	1.22 (0.40)	91
	Arthur Island	1 yr Chinook	0.48 (0.20)	81	0.18 (0.03)	220
	Kidd Bay	did not exist	0.30	33	0.39	18
	Goat Cove	did not exist	N/a	0	N/a	0
2004	*W. Jackson**	2 yr Chinook	3.34 (1.67)	91	0.76 (0.05)	104
	*Lochalsh Bay**	Fallow	2.77 (0.65)	53	1.23 (0.36)	105
	Arthur Island	2 yr Chinook	0.92 (0.18)	192	0.48 (0.15)	98
	Kidd Bay	did not exist	2.20	35	1.67	15
	Goat Cove	1 yr Atlantic	0.83	35	0.73	15
2005	*W. Jackson**	Fallow	0.00 (0.00)	51	0.00 (0.00)	59
	*Lochalsh Bay**	Fallow	0.05 (0.05)	82	0.05 (0.00)	71
	Arthur Island	Fallow	0.10 (0.05)	296	0.02 (0.01)	151
	Kidd Bay	1 yr Atlantic	0.28 (0.16)	282	0.11 (0.08)	163
	Goat Cove	2 yr Atlantic	0.38 (0.18)	233	0.12 (0.05)	190

* *Jackson Pass Farm Sites*

Table 1.7 – Lice per gram (+/- SE) for infected juvenile chum and pink salmon (fish with zero lice excluded) collected in the Bella Bella / Klemtu region in 2003-2005.

	Year	Proximity	N	Mean
Chum	**2003**	<1km	202	2.94 (0.24)
		1-15km	105	1.29 (0.11)
		Separated	31	1.06 (0.13)
	2004	<1km	214	1.17 (0.08)
		1-15km	293	0.96 (0.05)
		>15km	68	1.40 (0.11)
		Separated	184	1.08 (0.07)
	2005	<1km	122	1.20 (0.18)
		1-15km	26	0.58 (0.11)
		>15km	213	0.66 (0.04)
		Separated	131	0.87 (0.06)
Pink	**2003**	<1km	152	3.64 (0.40)
		1-15km	84	1.54 (0.18)
		Separated	31	1.43 (0.19)
	2004	<1km	99	1.19 (0.12)
		1-15km	245	1.32 (0.18)
		>15km	27	0.72 (0.13)
		Separated	63	1.12 (0.14)
	2005	<1km	37	0.85 (0.11)
		1-15km	4	0.37 (0.09)
		>15km	54	0.65 (0.06)
		Separated	84	1.97 (0.19)

Table 1.8 – Lice species ratios (% *Caligus vs. % Lepeoptheirus)* for samples of juvenile chum and pink salmon infected with adult sea lice (non-infected fish removed) collected in the Bella Bella and Klemtu regions (2003-2005).

		Chum		Pink	
Year	**Region**	***Caligus*/ motile lice**	**% *Caligus***	***Caligus*/ motile lice**	**% *Caligus***
2003	<1km	17/146	11.6	13/82	15.8
	1-15km	2/3	66.7	3/9	33.3
	Separated	7/8	87.5	6/8	75.0
2004	<1km	28/135	20.7	28/111	25.2
	1-15km	49/187	26.2	42/126	33.3
	>15km	12/52	23.1	19/52	36.5
	Separated	48/60	80.0	23/29	79.3
2005	<1km	28/45	62.2	10/16	62.5
	1-15km	24/27	88.9	9/15	60.0
	>15km	6/11	54.5	3/3	100
	Separated	32/44	72.7	13/20	65.0

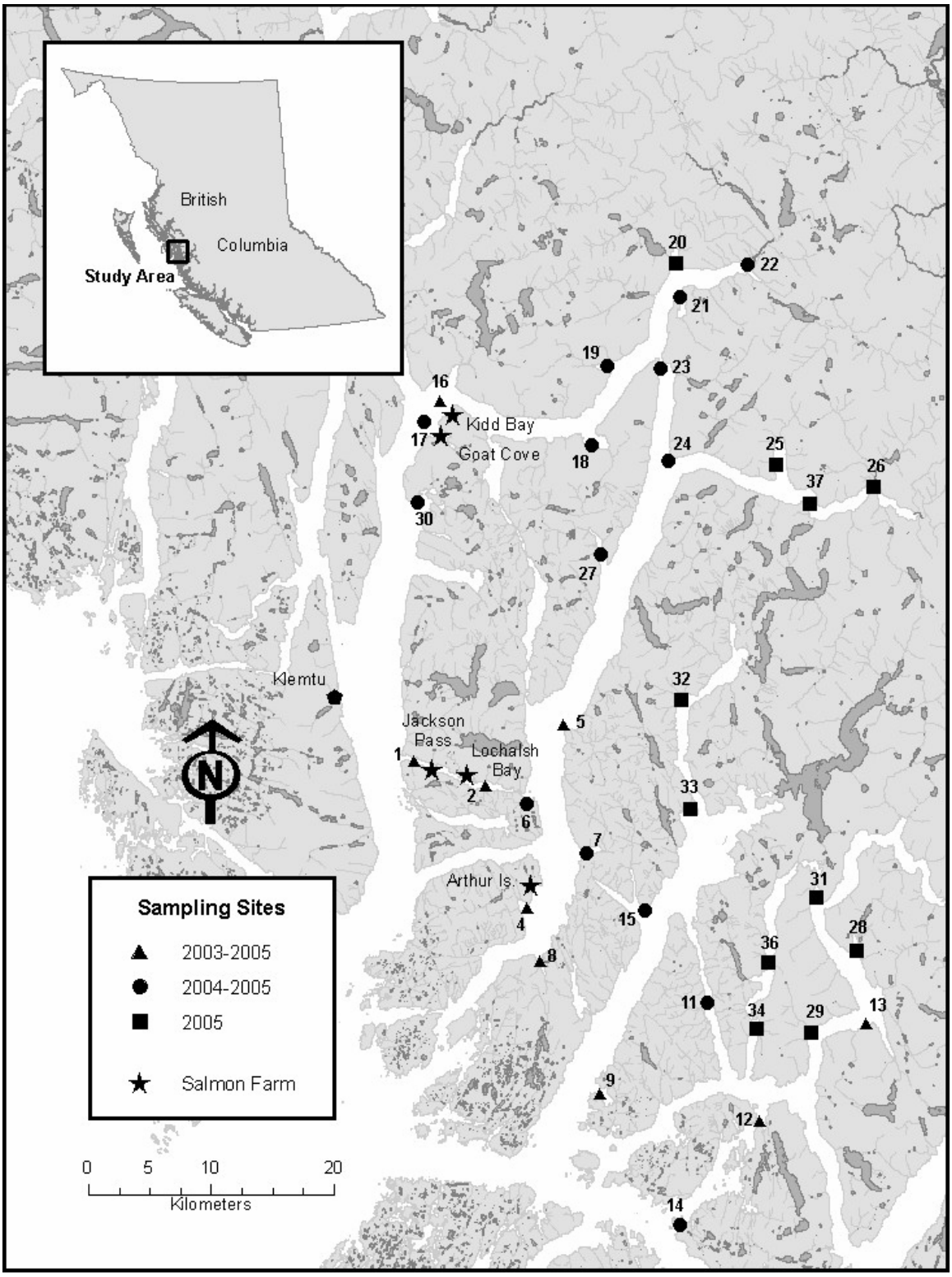


Figure 1.1 – Map of sampling sites and salmon farm locations for field collections in 2003 - 2005 near Bella Bella and Klemtu, BC.

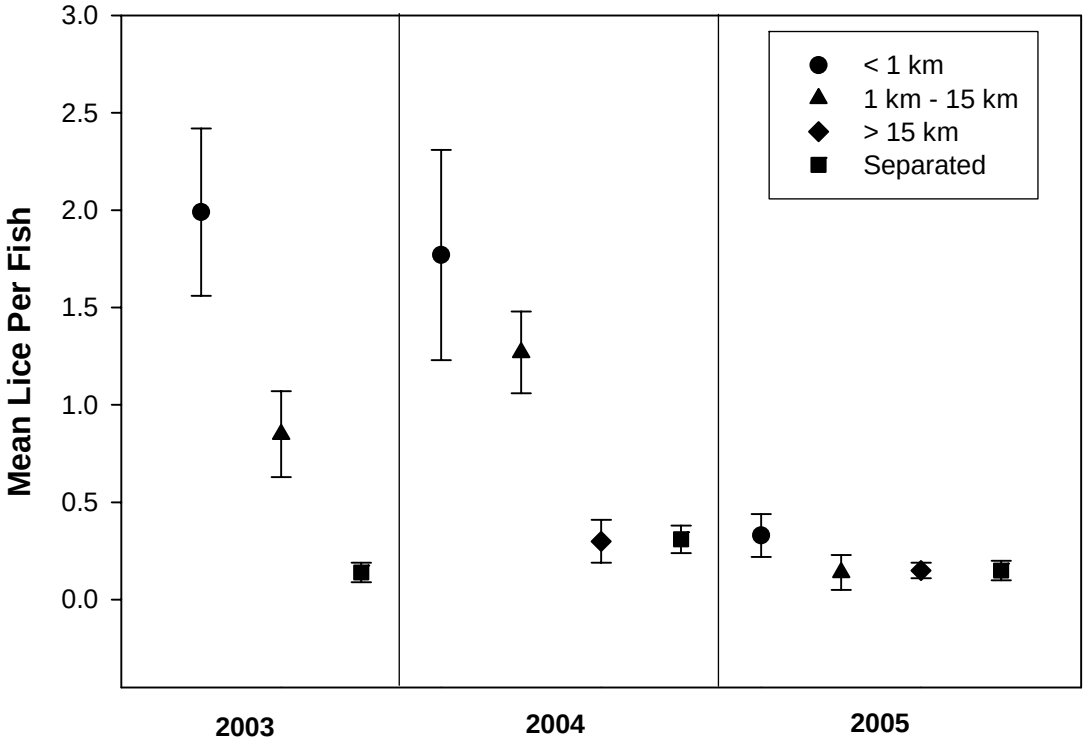
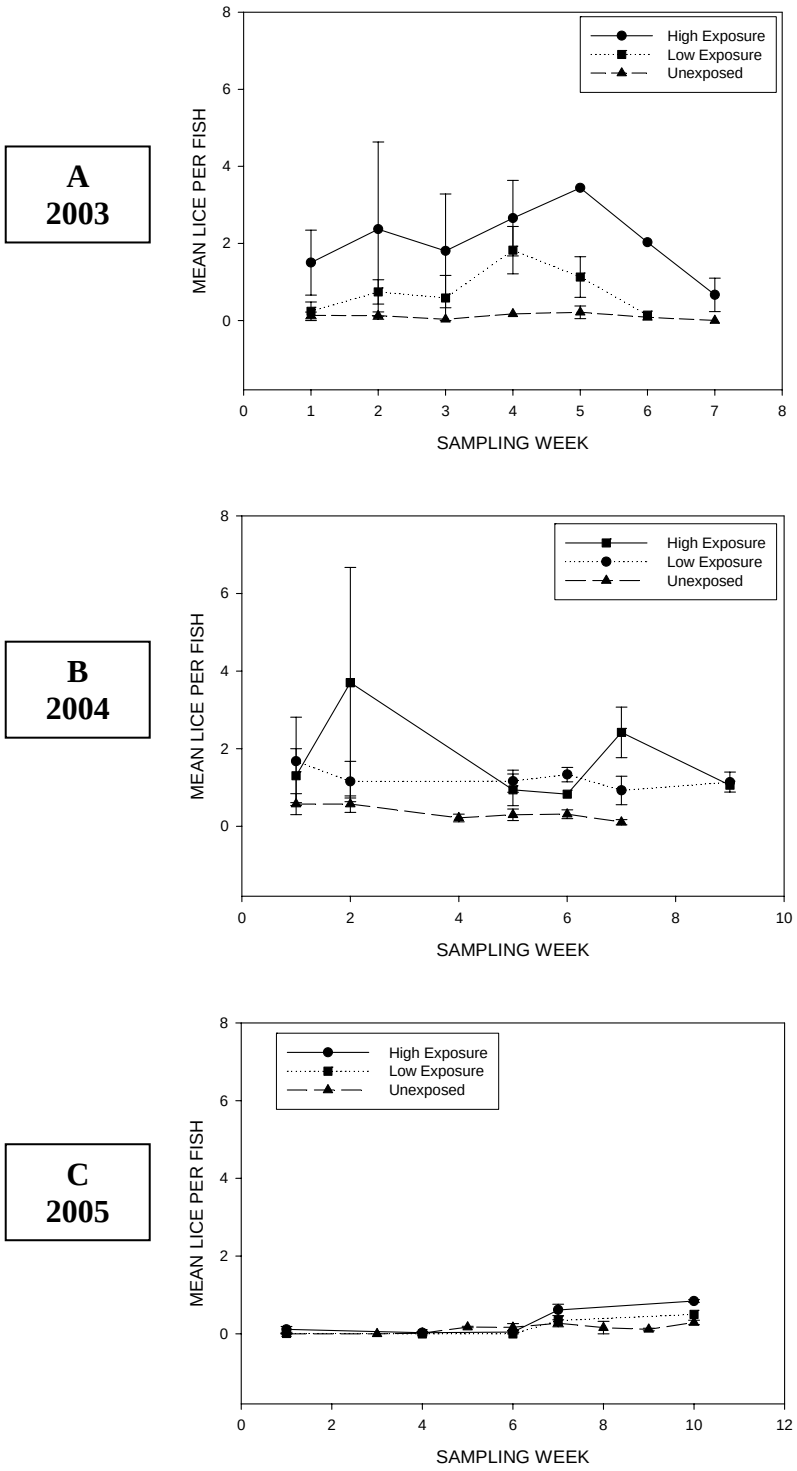


Figure 1.2 – Mean lice abundance (+/- SE) for juvenile chum salmon collected in areas with different proximities to active salmon farms in the Bella Bella and Klemtu areas between 2003 – 2005.



Figures 1.3 A, B, C - The mean lice per fish per sampling week for juvenile chum salmon collected in 2003, 2004, 2005 in areas of different proximity to active salmon farms in the Bella Bella / Klemtu region.

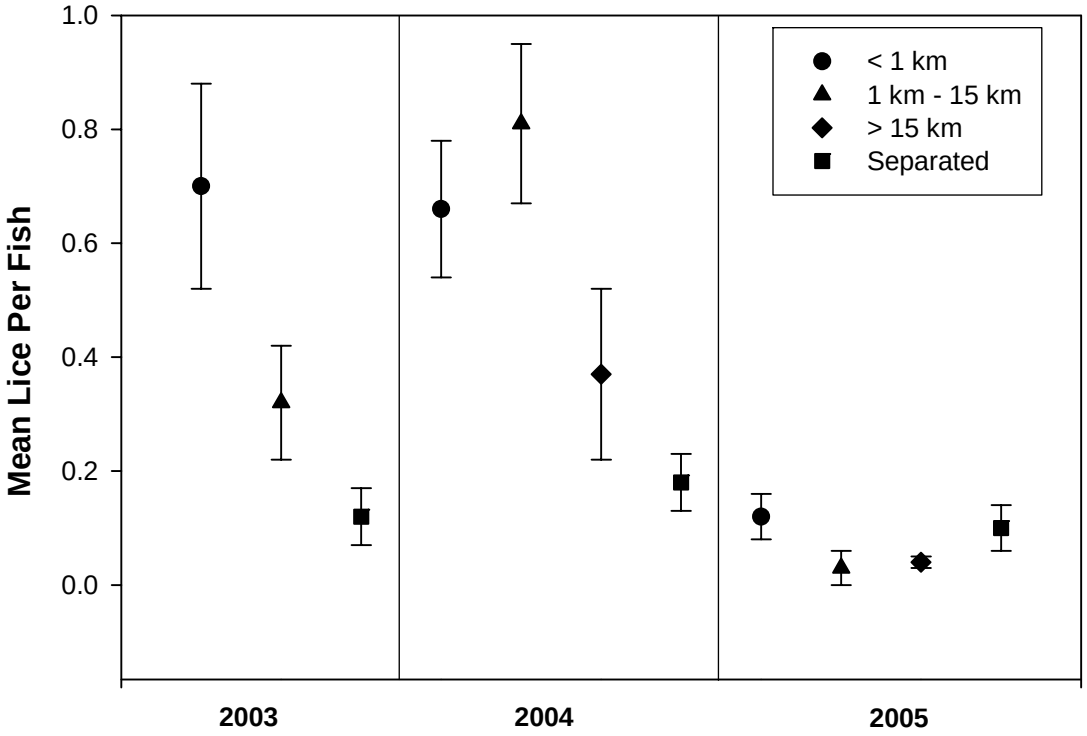


Figure 1.4 – Mean lice per fish (+/- SE) for juvenile pink salmon collected in areas with different proximities to active salmon farms in the Bella Bella and Klemtu areas between 2003 – 2005.

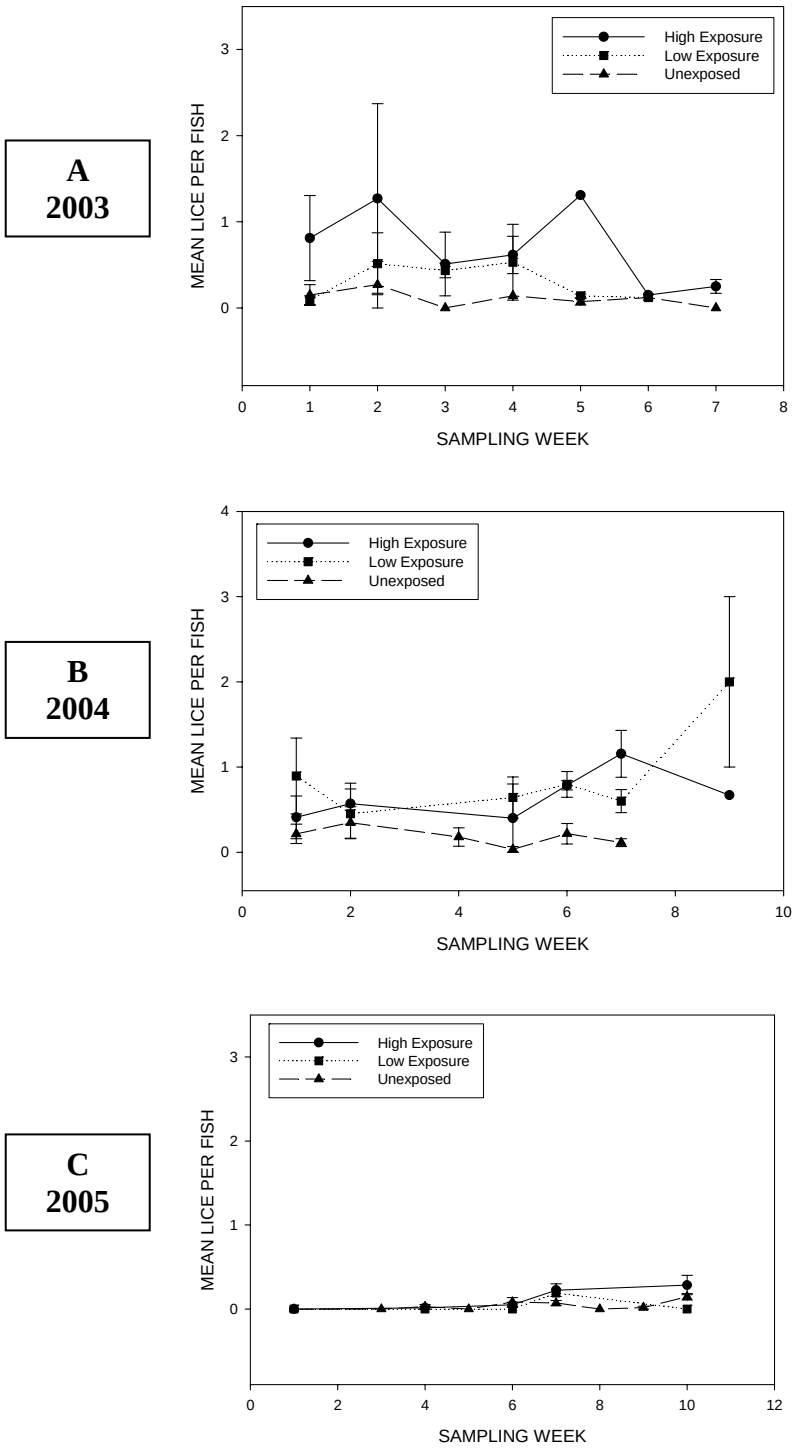


Figure 1.5 A, B, C - The mean lice per fish per sampling week for juvenile pink salmon collected in 2005 in areas of different proximity to active salmons in the Bella Bella / Klemtu region.

Chapter 2

Relative susceptibility of juvenile chum (*Oncorhynchus keta*) and pink (*Oncorhynchus gorbuscha*) salmon to infection by sea lice (*Lepeoptheirus salmonis* and *Caligus clemensii*)

Abstract

The relative susceptibility of juvenile chum and pink salmon to infection by sea lice was tested in laboratory and field experiments. Laboratory experiments were conducted by artificially infecting juvenile chum and pink salmon with sea lice collected and cultured from adult chum salmon collected from commercial fisheries. Juvenile chum salmon were significantly more infected (44.11 (18.13) lice/fish to 87.45 (2.69) lice/fish) than juvenile pink salmon (0.05 (0.05) lice/fish to 1.52 (0.79) lice/fish) under the same conditions. A similar pattern was observed for field data where samples of juvenile chum and pink salmon were collected in areas of the central coast of British Columbia. Over three years, 13,874 juvenile chum and pink salmon were collected with the use of a beach seine net in areas of different proximity to active salmon farms (< 1 km, 1-15 km, > 15 km). Over all sampling years and proximity categories juvenile chum salmon were significantly more infected than juvenile pink salmon (up to 4.67 times higher). The results of this study suggest a difference in the relative susceptibility of juvenile chum and pink salmon to infection by sea lice. However, the exact mechanism for the observed differences was not identified. Possible reasons for the observed differences could be related to genetically determined susceptibility, mucous differences, lethal lice infection tolerances, or other factors.

Introduction

Sea lice, *Lepeoptheirus salmonis* and *Caligus clemensii,* are common marine ecto-parasites of salmonids in the northern hemisphere. Sea lice cause serious losses for the salmon farming industry with annual losses due to sea lice infestations estimated to be

greater than US $100 million annually (Johnson *et al.* 2004). In every country where salmon farms operate there have been cases of sea lice epizootics on salmon farms leading to significant mortality and disease (Brandal and Egidius 1979; Wooten *et al.* 1982; Bravo 2003; Johnson *et al.* 2004).

In addition to causing problems for farmed salmon, there have been epizootics of sea lice on wild salmon in coastal marine areas where salmon farms operate (Tully *et al.*, 1993; Tully *et al.*, 1999; Bjorn *et al.*, 2001; Bjorn and Finstad, 2002; Morton and Williams 2004; Morton *et al.* 2004; Krkosek *et al.* 2005; Morton *et al.* 2005). Salmon farms can disrupt the natural balance that defines a successful host-parasite relationship (Wikel *et al.* 1994) between sea lice and juvenile salmon. The relationship is altered in such a way that salmon lice can become heavily favored resulting in individual salmon becoming infected with sea lice at rates (e.g. intensity) and frequencies (e.g. prevalence) much higher than would occur naturally. These amplified sea lice infection rates have been linked to declines in populations of wild salmon (Gargan 2000; PFRCC 2002; Krkosek *et al.* 2006).

Recently in British Columbia, declines in pink salmon stocks in the Broughton Archipelago (a region with the highest concentration of salmon farms on the west coast of North America) have coincided with an unprecedented series of sea lice epizootics on juvenile chum and pink salmon (4 epizootics in 5 years). Existing studies have correlated these observations with the presence of salmon farms (Morton and Williams 2003; Morton *et al.* 2004; Morton *et al.* 2005). Controversy has ensued over the risk posed to

wild salmon populations due to enhanced sea lice infection rates produced by salmon farms. While the scientific community generally accepts that sea lice from salmon farms affect the dynamics between sea lice and wild salmon, the impacts these enhanced sea lice rates have on salmon population dynamics is not clear. Part of the reason for this uncertainty is due to the lack of comprehensive data on the susceptibility of juvenile chum or pink salmon to infection by sea lice.

The susceptibility of salmonids to sea lice infection has been found to vary inter-specifically based on several factors including: genetically determined susceptibility (Mustafa and MacKinnon 1999; Glover *et al.* 2003), stage of the fish's life cycle, and overall health of the fish (MacKinnon 1998). Susceptibility of juvenile salmonids could also be affected by the alteration of lice development rates due to a host response (Johnson and Albright 1992; Johnson 1993; Fast *et al.* 2002), the release of antibodies (Dawson et. al 1997), variable swimming speed, depth and distribution, and in the suitability of the skin as a site of infection (Nagasawa *et al*. 1991).

Numerous studies have examined differences in susceptibility in numerous species of salmonids including, chinook salmon *(Oncorhynchus tshawytscha),* coho salmon *(O. kisutch),* Atlantic salmon *(Salmo salar),* sea trout *(Salmo trutta),* rainbow trout (*O. mykiss),* and brown trout *(Salmo trutta)* (Johnson and Albright 1992; Johnson 1993; Dawson *et al.* 1997; Fast *et al.* 2002; Glover *et al* 2003). These studies have all been conducted on adults or post-smolts. Susceptibility is host-size dependent, and therefore

may change with size and may not be similar for adult and juvenile stages. To date, no studies have examined the susceptibility of juvenile salmonids to *L. salmonis* infection.

Understanding the relative susceptibility of juvenile chum and pink salmon to sea lice infection is key to making predictions about the impact from salmon farms enhancing the ambient sea lice infection rates in coastal British Columbia. We investigated the relative susceptibility of juvenile chum and pink salmon to sea lice infection and tested the hypothesis that susceptibility among juvenile Pacific salmonids varies inter-specifically. The relative susceptibility of juvenile chum and pink salmon was determined both from controlled laboratory experiments and field observations during spring migrations.

Methods

Laboratory Studies

Juvenile chum (*Oncorhynchus keta)* and pink *(Oncorhynchus gorbuscha)* salmon were collected from local Vancouver Island hatcheries in late March 2004. Fish were collected as fresh water fry to minimize variability due to inherited genetically determined resistance factors. Fish went through smoltification in 400-litre tanks at the University of Victoria (UVic) Aquatics Facility. After smolting, juvenile salmonids were reared for three months and then placed into the experimental systems where they were acclimatized for two months. Mortality rates were recorded on a daily basis.

Fish were fed a commercial pellet diet. Feeding frequencies, growth rates, and water quality data (temperature, salinity, dissolved oxygen) were recorded and carefully

monitored as part of an overall fish health-monitoring program (Table 2.1). Fish were maintained in tanks with a natural photoperiod, flowing filtered seawater, water temperature of 8-12^{o}C, and ambient salinity of 26-32 ppt.

Gravid salmon lice (*Lepeoptheirus salmonis*) were collected from adult chum salmon during local commercial fisheries in October 2004 in the Johnstone Strait and Qualicum river fishing areas (DFO management areas 13 and 14). Lice collected in the field were maintained in 5-gallon aquaria with dead and moribund individuals removed continuously. Water was changed at least once a day. Temperature and salinity were maintained at 6-13 ^{o}C (temperature was stabilized once back in the laboratory) and >30 ppt respectively. Newly hatched nauplii were removed and placed in separate aquaria and allowed to develop to the infective copepodid stage. Infectious copepodids were held for 24 hours as post-moult before artificial infection. Artificial infection took place under conditions of darkness, no water flow, or aeration for a period of 7.5-12 hours after which water flow and aeration was restored to normal (Johnson and Albright 1992; Dawson *et al.* 1997).

Susceptibility was measured at three different infection levels (control, low, and high infection probability). Infection probability was quantified in units of approximate number of infective copepodids per 120 L tank. Although infection intensities were based on the literature, field measurements in 2002 and 2003, and from the susceptibility pilot experiments in 2003, the limited lice supply and unknowns associated with artificial infections (e.g. mortality rates of copepodids in experimental tanks) necessitated limited

infection options. For this study, infection intensities are defined as follows: low infection probability as < 3000 infective copepodids / 120 L and high infection probability as > 3000 infective copepodids / 120 L (Table 2.2). Given the lack of data on natural sea lice infection rates, the exposure rates used in the lab experiments were determined based on pilot experiments.

Infectious copepodids were placed into a 700ml container and counted prior to artificial infection. Tank infection intensities were calculated by taking a 10 ml sub-sample of the 700ml container and counting the number of copepodids present in the sub-sample with a dissecting microscope. Five sub-samples were counted per 700ml container to obtain an estimate (mean / 10 ml X 700 ml) of the total number of copepodids per 700ml container.

Experiments took place in 120 L tanks and 25 chum or pink salmon were placed into each tank. In total 18 experimental tanks were used (9 chum and 9 pink). For each species, three tanks were allocated as controls (no lice introduced), three tanks were allocated for low exposure (< 3000 infective copepodids / 120 L) and three tanks were allocated as high exposure (> 3000 infective copepodids / 120 L). Fish were randomly allocated to each tank using protocols designed to minimize stress. Chum and pink salmon susceptibility (# of lice/ fish per unit time) was sampled at 1, 7, 14, 21, and 28 days post-infection. Five fish were sampled per time interval without replacement at each interval.

A small dip net (20cm X 15.5 cm modified with 100um Nitex® mesh to retain any sea lice dislodged) was used to haphazardly sample individuals which were killed with a blow to the end and then frozen until analysis. Individuals were assayed with a dissecting microscope for lice, fish weight, and fish fork length. Total lice counts were categorized according to lice development stage (Johnson and Albright 1991).

Field Sampling

Juvenile chum salmon *(Oncorhynchus keta)* and pink (*Oncorhynchus gorbuscha)* were collected in coastal marine areas during spring out-migration (late-March – June) from their natal rivers in 2003, 2004, and 2005. Samples were collected near Bella Bella and Klemtu, British Columbia in all three years (Figure 2.1). In 2003, eight sites were sampled 41 times, in 2004 23 sites were sampled 78 times, and in 2005 34 sites were sampled 126 times.

Sample sites were selected based on its proximity to an active salmon farm. Over the three years of sampling, five salmon farming sites were active in the sampling area, however in any given year, no more than three salmon farms were active at the same time. Site proximity was separated into three categories (< 1 km, 1- 15 km, and > 15 km). The Bella Bella and Klemtu areas are separated from each other by a long narrow peninsula (Figure 2.1). Samples collected on the Bella Bella side were designated as "> 15 km," while those from the Klemtu side were designated based on their distance to active salmon farm tenures (< 1 km, 1-15 km, > 15 km) (Costelloe 2006).

Sites were sampled on a weekly basis as much as possible to provide adequate replication for temporal variability. Factors preventing continuous weekly samples included: adverse weather conditions, non-cooperation from farm authorities, and lack of necessary resources. All salmon samples were collected using a beach seine (30.3m long X 1.2-1.8m deep X 6.3mm bunt mesh). The technique consistently resulted in a large number of juvenile salmon caught allowing a random sub-sample to be collected from a large sample.

A sampling crew of 2-4 used a small boat to encircle schools of wild juvenile salmon. With one crewmember anchoring the net onshore the boat maneuvered to encircle the school between the net and shore at which point the net was brought ashore.
Once fish were at the shoreline a five-gallon bucket was used to remove as many as possible from the net. This method minimized lice-shedding abrasion. Once in the bucket fish were randomly selected and placed individually in a whirl-pak™ bag and placed on ice. The number of samples collected varied from 13 to 200 per site. Samples were frozen at -20^{o}C as soon as possible. At each site, data for sea surface temperature (SST) and sea surface salinity (SSS) were recorded using a YSI meter.

Samples were thawed and fish were individually examined for sea lice using a dissecting microscope. Sea lice were identified to life stages for copepodids, chalimus I, II, III, or IV, pre-adult, and adult and to species for pre-adults and adults using Johnson and Albright (1991b) and Kabata (1972). Juvenile salmon were identified to species following Phillips (1977). Fish weight and fish fork length were also recorded.

Data Analysis

The null hypothesis of interest in this study was that there was no difference in mean sea lice infection rates among juvenile chum and pink salmon.

A generalized linear model was applied to the laboratory data, which included exposure (high and low) and species (pink or chum salmon) as factors. The dependent variable was the average number of lice per fish per sampling event (all sea lice life stages and species). The normality assumption for the univariate ANOVAs was verified using the residuals from the model.

For the field data, a generalized linear model was applied to the data, which included the factors proximity (< 1 km, 1-15 km, > 15 km), species (chum or pink salmon), and mean fish length as a covariate. The analysis was separated by year to reduce model complexity and to set up three discrete tests of susceptibility. The dependent variable was the average number of lice per fish per sampling event. The normality assumption for univariate ANOVAs was verified using the residuals from the model.

Results

Susceptibility under controlled laboratory experiments

Significantly higher *L. salmonis* infection rates were recorded on juvenile chum salmon than on juvenile pink salmon in laboratory experiments. Mean infection rates for chum salmon were 44.11 (18.13) lice/fish and 87.45 (2.69) lice/fish in the low and high

exposure categories, respectively. In contrast, mean infection rates for pink salmon were 0.05 (0.05) lice/fish and 1.52 (0.79) lice/fish (Table 2.2).

The results of the univariate analysis of variance suggest that the average abundance of sea lice per fish is significantly influenced by salmon species and exposure category ($p<0.0001$ and $p=0.040$ respectively). Additionally, the interaction of salmon species and exposure was found to influence sea lice levels ($p=0.052$) on juvenile pink and chum salmon (Table 2.3).

Chum salmon were found to be smaller than pink salmon during laboratory experiments. The mean fork length of pink salmon (control fish) was 14.23 (0.09)cm and the mean weight was 30.89 (0.09)g. Chum salmon (control fish) had a mean length of 13.04 (0.11)cm and a mean weight 24.44 (0.40)g (Table 2.2). These numbers are the mean size of control fish sampled at the end of the experiments. For chum salmon, daily observations of poor feeding behavior and erratic swimming behavior increased with increased exposure. These effects are seen in the increased differences in the final mean weight vs. control across the exposure categories (Table 2.2). None of the same effects were observed in the infected pink salmon. At the time of hatchery collection, both species were less than 5 cm in length and weighed less 0.5g. Mean growth rates over the course of the 244 days of husbandry were 0.41 (0.11) mm/day and 0.10 (0.03) g/day for chum salmon, and 0.46 (0.15) mm/day and 0.12 (0.07) g/day for pink salmon (Table 2.1).

Differences were observed in the total mortalities of pink and chum salmon both 30 days pre and 30 days post infection. Pink salmon had lower mortalities than chum salmon across all exposure categories and both pre and post infection (Table 2.4). In the low and high exposure treatments the post infection mortalities of chum were 2 times and 5 times higher than 30 days pre-infection, while no significant differences was observed for pink salmon (Table 2.4).

Susceptibility under natural field conditions

During the three years of this study, 13,874 juvenile chum and pink salmon were collected. Chum salmon made up the bulk of the catch among the three sampling years and exposure categories (62% chum, 38% pink). This catch ratio was consistent across all proximity categories and years, with the exception of the < 1 km and 1-15 km proximity categories in 2003 where the ratio was reversed (60% pink and 40% chum). The differences in species abundances were not due to a bias in sampling technique. Differences in the total numbers of fish collected were a function of sampling effort between years due to logistical challenges (weather, access etc.)

Juvenile chum experienced significantly greater sea lice infection than pink salmon across all proximity categories and across all sampling years (Figures 2.2 A, B, C). On average, (all proximity categories each year), juvenile chum salmon were infected with sea lice approximately 2.5x (range 1.17 - 4.67) higher than juvenile pink salmon. The univariate analysis of variance found that sea lice infection was strongly influenced by species identity across all sampling years ($p<0.0001$ for all years) (Table 2.5). Proximity

to salmon farms was also found to be highly significant in 2003 and 2004 ($p<0.0001$), but not in 2005 ($p=0.731$). In addition, the interaction between species and proximity was found to be significant in 2003 ($p=0.0009$) and 2004 ($p=0.0013$), but not in 2005 ($p=0.1354$) (Table 2.5).

Mean length was not found to be significant in the model in either 2003 ($p=0.2974$) or 2004 ($p=0.0897$), but was highly significant in 2005 ($p<0.0001$). Juvenile chum ranged from 0.02 to 0.38 cm longer and from 0.05 to 0.43 g heavier than juvenile pink salmon across all proximity categories and sampling years (Table 2.6).

Discussion

The results from this study strongly suggest a difference in the susceptibility of chum and pink salmon to infection by sea lice. Differences in susceptibility to sea lice infection has been previously documented in both field and laboratory settings for several species of salmonids including: Atlantic salmon, Coho salmon, Chinook salmon, sea trout, Arctic char, and rainbow trout (Dawson *et al.* 1997; Glover *et al.* 2001; Johnson and Albright 1992; Johnson 1993; Nagasawa 2001; Fast *et al.* 2002). Johnson and Albright (1992) suggested that host nutritional factors and non-specific immune responses may be involved in resistance mechanisms to salmon louse infections. More specific reasons cited for the differences in susceptibility include: genetically determined suceptibility, differences in cell-based reactions which affect sea lice settlement (Johnson and Albright 1992), mucous differences among salmonids that may prevent the use of certain enzymes by sea lice (Fast *et al.* 2003), nutritional factors, differences in antibody responses, and

behavioral differences such as swimming speed and swimming depth distribution (Nagasawa *et al.* 1991).

In the present study, the results from laboratory trials suggest a strong difference in the susceptibility of juvenile chum and pink salmon to infection by *L. salmonis*. The mortality data collected pre and post infection suggest that pink salmon may have been healthier than chum salmon (Table 2.4) and thus the differences observed were more related to health rather than differences in susceptibility. However, fish health, feeding habits, and water quality factors were carefully controlled throughout the course of the husbandry and experimental periods. Average growth rates of 0.4 mm/day were recorded for both species, which compare to the lower end of growth rates reported for wild juvenile pink (0.9-1.5mm/day) and chum salmon (0.4-1.5 mm/day)(LeBrasseur and Parker 1964; Murphy *et al.* 1988). Chum salmon were slightly smaller than pink salmon (comparing control fish), however, it is unlikely that the difference in susceptibility could be solely due to differences in size. It is more likely that there was an interaction effect between fish health, fish size, and innate differences in susceptibility that produced the observed results. Future studies should consider testing susceptibility to lice infection by mixing chum and pink salmon in the same experimental tank and try to more carefully control for fish size.

The results from the field samples suggest that species was the most consistent predictor of the data across all three years and strongly suggest a difference in susceptibility to infection between chum and pink salmon. Chum salmon were significantly more infected

with sealice across all spatial (exposure to active salmon farms) and temporal scales (year)(Figure 2.2 A, B, C) fish size was not a significant predictor of infection in either 2003 or 2004 and although it was significant in 2005, the differences were marginal (Table 1.6). Differences in infection rates between chum and pink salmon have been recorded in the offshore regions of the Pacific Ocean (Nagasawa 1987; Nagaswa *et al.* 1993). Nagasawa (2001) documented prevalence (% of fish infected by sea lice) rates for adult chum salmon during surveys over seven years ranging from 25.5% to 46.6% and intensity levels ranging from 1.77 to 2.33 lice per fish. Pink salmon had greater prevalence (75% to 100%) and intensity rates (4.63 to 8.67 lice per fish). These infection rates suggest that pink salmon are less susceptible than chum salmon, but they are opposite to the results observed in the present study, which suggests that the dynamics of sea lice infection between adult and juvenile life stages are substantially different.

One possible explanation for the observed differences could be due to mucous differences, which have been cited as a possibility for differences in susceptibility in other species (Fast *et al.* 2003). The mucus layer is the first site of interaction between sea lice and salmon and is therefore, the first line of defense. Fast *et al.* (2003) suggested that there was variation in the enzymes released by sea lice in response to the mucous of different salmonids, which suggests that the mucous composition of salmonids may itself be variable. The mucous of coho can block the enzymes secreted by sea lice (Fast *et al.* 2003). One observation made throughout the course of the lab study was the difference in mucous between the laboratory-cultured chum and pink salmon. Whenever pink salmon were handled (e.g. during transfer pre experiment), the dip net would remain free of

mucous even after continual use. When handling chum salmon, the dip net would repeatedly clog with mucous and would require cleaning after the handling of only a few fish. These mucous differences were not quantified in the lab study nor were they observed in the field collections because chum and pink salmon were collected simultaneously. However, the difference was quite apparent in the laboratory and should be considered in future studies on chum and pink salmon susceptibility to sea lice infection.

Although the field data suggest that pink salmon are less susceptible to sea lice infection than chum salmon to infection by sea lice, alternative explanations must be considered. One key factor not controlled for in the field studies were the lethal sea lice infection rates. Experimental laboratory studies conducted on post-smolt European salmonids have determined that 0.75 – 1.6 lice /g of fish weight is a lethal infection ratio (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997). However, these numbers were generated from fish that were from 8-60 times (40-60 g post-smolt Atlantic, sea trout (*Salmo trutta*), Arctic Char (*Salvelinus alpinus*)) larger than juvenile chum and pink salmon that enter the marine environment weighing less than 1 gram (Healey 1980; Heard 1991). More recently, Morton and Routledge (2005) found that the short-term mortality for juvenile chum and pink salmon is increased by lice infestations of 1-3 sea lice per fish. Given these data, it is possible that chum salmon are able to handle higher sea lice infection rates than juvenile pink salmon and thus the samples are biased towards chum salmon with higher lice and fewer pink salmon because pink salmon experience higher mortality per lice infection and are therefore removed from the sampling pool. The average level of

sea lice infection per pink salmon across all years and proximity categories did not exceed 0.5 lice per fish. Given that stress responses can be triggered by sea lice infections (Nolan *et al.* 1999; Bowers *et al.* 2000), it is possible that given the small size of pink salmon at the time of marine entry (< 5cm and < 0.5 g)(Heard 1991) even low infection rates could have significant impacts on juvenile pink salmon. Further research into lethal sea lice infection rates should be conducted to further understand the observed difference.

In summary, this study has identified that juvenile pink salmon are less susceptible than juvenile chum salmon to infection by sea lice. In field and laboratory studies, juvenile chum salmon were significantly more infected than juvenile pink salmon. The exact mechanism for the observed differences was not quantified but could be related to genetic differences, mucous differences, or differences in lethal lice infection tolerances. The results suggest that further investigation into the lethal infection levels for early marine life juvenile salmon (especially pink salmon) are required to better understand the consequences of increased sea lice infection on the health of wild salmon populations.

Table 2.1 – Growth rates (over 244 days of husbandry) and water quality data (temperature (^{o}C), salinity (ppt), and DO (% sat) collected from experimental systems containing juvenile chum and pink salmon at the University of Victoria aquatics facility from June-December 2004.

	Growth Rate (mm / day)	**Growth Rate (g / day)**	**Temperature (^{o}C)**	**Salinity (ppt)**	**Dissolved Oxygen (% saturation)**
Chum	0.41 (0.11)	0.10 (0.03)	10.97 (0.13)	28.95 (0.15)	99.79 (0.73)
Pink	0.46 (0.15)	0.12 (0.07)	11.04 (0.07)	29.10 (0.09)	100.70 (0.70)

Table 2.2 – Time of infection (T.O.I.-hours), number of copepodids introduced per tank (+/- SE), mean lice per fish, average fork length (+/- SE), and average weight (+/- SE) for artificial infections of juvenile chum and pink salmon.

	T.O.I.	**Tanks**	**Cope / 120 L**	**Lice / fish**	**Length (cm)**	**Weight (g)**
Chum						
Control	7.5	3	0.00 (0.00)	0.00 (0.00)	13.04 (0.11)	24.44 (0.40)
Low Exposure	7.5	3	1900 (889.2)	44.11(18.13)	12.68 (0.12)	19.25 (1.86)
High Exposure	7.5	3	4545 (916.4)	87.45 (2.69)	12.47 (0.10)	17.74 (0.25)
Pink						
Control	12.5	3	0.00 (0.00)	0.00 (0.00)	14.23 (0.09)	30.89 (0.63)
Low Exposure	12.5	3	1190 (337.5)	0.05 (0.05)	13.46 (0.19)	30.54 (0.93)
High Exposure	12.5	3	3486 (225.3)	1.52 (0.79)	13.57 (0.08)	30.26 (0.37)

Table 2.3 – Results of the univariate Anova for total lice per fish, species (juvenile chum or pink salmon), exposure (low and high) for artificial *L. salmonis* infections at the UVic aquatic facility in 2004 ($R^2 = 0.885$).

Dependent Variable: total lice per fish

Effect	df	F-statistic	p-value
Species	1	50.186	<0.0001
Exposure	1	5.964	0.0400
Exposure:Species	1	5.208	0.0520

Table 2.4 – Experimental mortalities of juvenile pink and chum salmon during the susceptibility trials 30-days pre and post infection with *Lepeoptheirus salmonis*

	Pink			Chum		
	control	low	high	control	low	high
Pre-infection	3	2	0	7	15	10
Post-infection	2	1	1	10	33	48

Table 2.5 – Results of the univariate analysis of variance separated by year (2003, 2004, 2005) for total lice per fish, species (juvenile chum or pink salmon), proximity (< 1 km, 1-15 km, and > 15 km) to active salmon farms.

Year	Dependent	Effect	df	F	Sig.	R^2
2003	Total Lice	Length	1	1.10	0.2974	0.472
		Proximity	2	11.59	<0.0001	
		Species	1	23.16	<0.0001	
		Proximity*Species	2	7.73	0.0009	
2004	Total Lice	Length	1	2.92	0.0897	0.392
		Proximity	2	15.55	<0.0001	
		Species	1	44.97	<0.0001	
		Proximity*Species	2	6.96	0.0013	
2005	Total Lice	Length	1	23.61	<0.0001	0.220
		Proximity	2	2.65	0.0731	
		Species	1	11.25	0.0010	
		Proximity*Species	2	2.02	0.1354	

Table 2.6 – Means (+/- SE) for length, weight, and total lice per fish (all life stages and species) for samples of juvenile chum and pink salmon collected in the Bella Bella / Klemtu region between 2003-2005. Numbers reflect averages for sampling events pooled into proximity categories.

Year	Proximity	N	Length (cm)		Weight (g)	
			Chum	Pink	Chum	Pink
2003	<1 km	16	5.28 (0.29)	4.95 (0.29)	1.80 (0.31)	1.41 (0.25)
	1-15 km	13	5.25 (0.24)	4.88 (0.22)	1.73 (0.23)	1.30 (0.19)
	>15 km	13	5.35 (0.24)	5.18 (0.23)	1.71 (0.21)	1.38 (0.17)
2004	<1 km	11	5.94 (0.37)	5.95 (0.41)	2.82 (0.55)	2.61 (0.49)
	1-15 km	24	5.91 (0.25)	5.93 (0.29)	2.86 (0.35)	2.81 (0.44)
	>15 km	36	5.47 (0.16)	5.47 (0.21)	2.08 (0.20)	1.98 (0.21)
2005	<1 km	10	4.53 (0.36)	4.43 (0.40)	1.30 (0.40)	1.27 (0.42)
	1-15 km	6	4.61 (0.59)	4.24 (0.63)	1.06 (0.40)	0.85 (0.35)
	<15 km	86	4.95 (0.14)	4.57 (0.15)	1.78 (0.18)	1.35 (0.14)

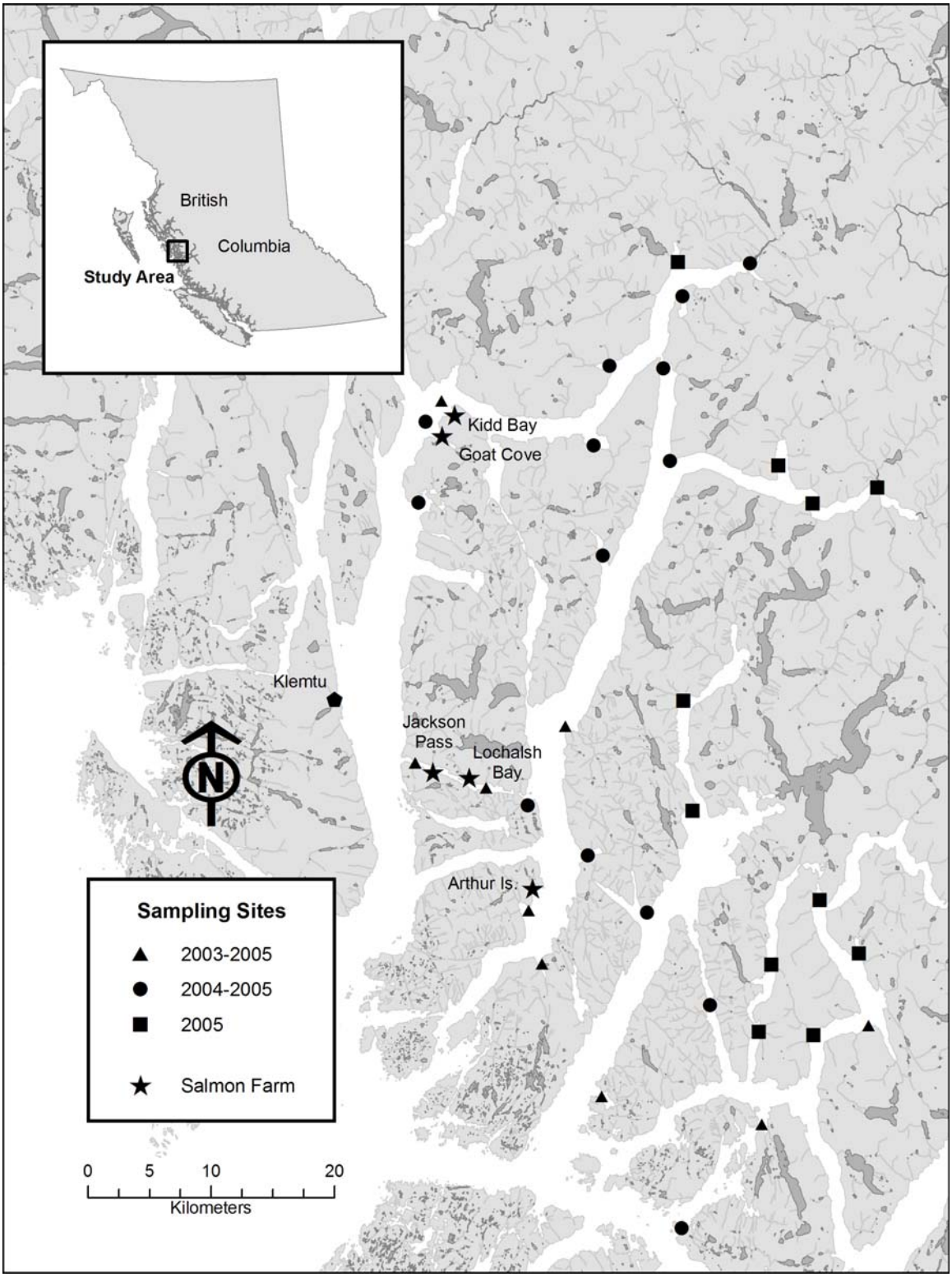


Figure 2.1 – Map of sampling sites and salmon farm locations for field collections in 2003 - 2005 near Bella Bella and Klemtu, BC.

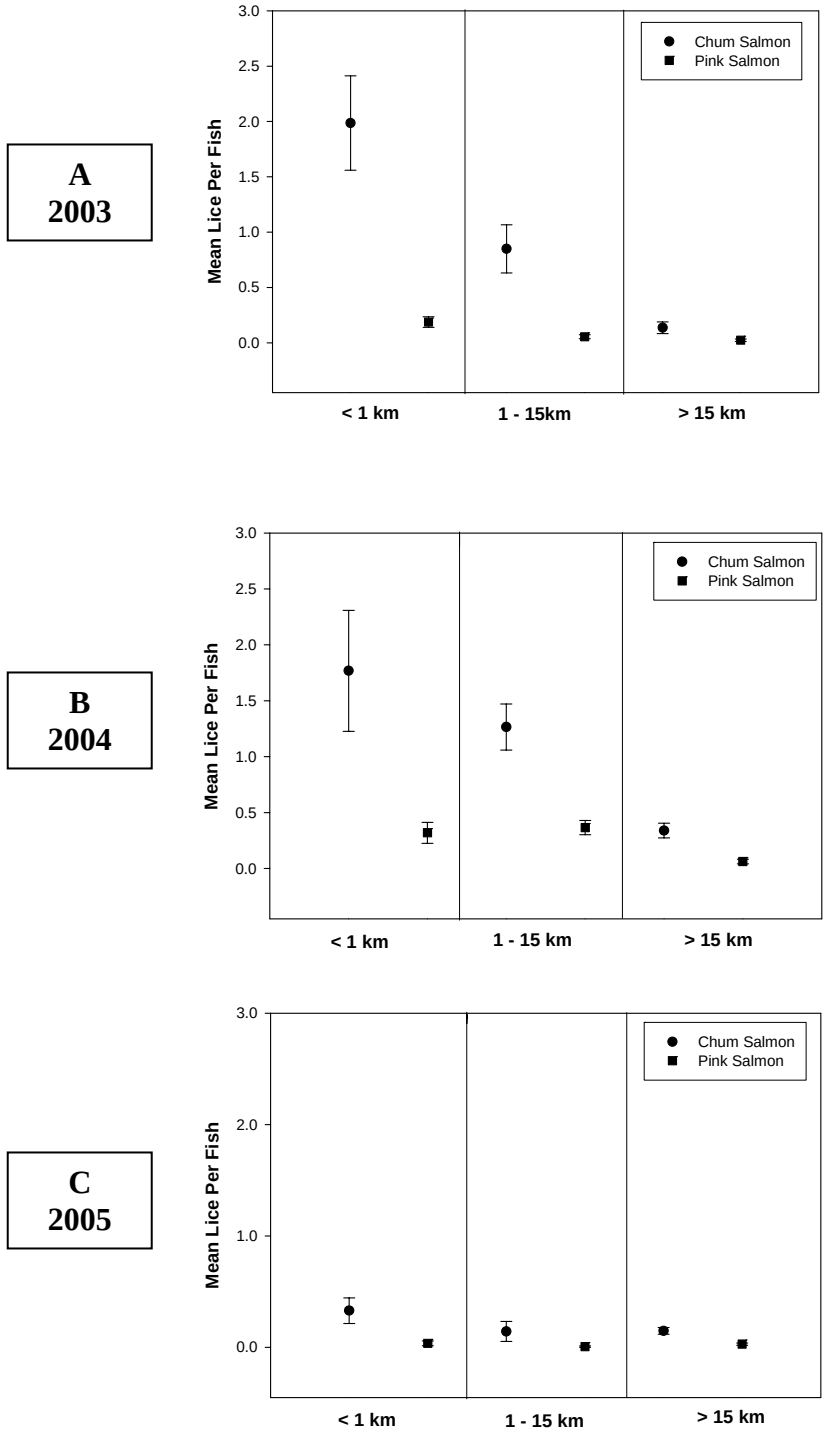


Figure 2.2 A, B, C – Mean lice per fish (+/-SE) per sampling event for juvenile chum and pink salmon collected in areas of different proximity to active salmon farms in the Bella Bella / Klemtu region (2003 – 2005).

Chapter 3

Ambient sea lice infection rates (*Lepeoptheirus salmonis* and *Caligus clemensii)* on juvenile chum and pink salmon (*Oncorhynchus spp.)* in relation to sea lice infection rates observed in the Broughton Archipelago: the effects of salmon farms

Abstract

Interactions between sea lice (*Lepeoptheirus salmonis* and *Caligus clemensii*) and juvenile chum (*Oncorhynchus keta)* and pink salmon *(Oncorhynchus gorbuscha*) were investigated in three regions of the British Columbia (BC) coast with little or no salmon farming activity over a three year period. Results were compared with similar data from the Broughton Archipelago (BA), a region with the highest density of salmon farms on the west coast of North America, where elevated lice infection rates on juvenile salmon have been correlated with the activities of salmon farms in recent years. In total, 15,285 juvenile chum and pink salmon were collected and analyzed for sea lice infection rates. The results suggest that ambient sea lice infection rates on the early marine phase of juvenile chum and pink salmon are less than one louse per fish and less than two lice per gram in the absence of anthropogenic influences like salmon farms. Sea lice infection rates in the BA were significantly higher (0.56 (0.07) to 9.01 (1.72) lice/fish; 2.65 (0.25) to 8.03 (0.42) lice/g) than in the non-salmon farming regions and were variable in intensity from year to year. Only marginal differences were observed between lice infection rates among the non-salmon farming regions, suggesting that geographic variability is not an important factor for sea lice population dynamics. The results of this study suggest that the sea lice infection rates observed in the BA from 2001-2005 are well above ambient sea lice infection rates and may present a significant risk to the health of wild salmon.

Introduction

Sea lice are common ecto-parasites of marine fishes. Two species, *Lepeoptheirus salmonis* (Kroyer 1837) and *Caligus clemensii* (Parker and Margolis 1964), frequently infect salmonids in the northern hemisphere. Both species have life cycles consisting of five phases and ten developmental stages. These include two free-swimming naupliar stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two pre-adult stages, and an adult stage (Parker and Margolis 1964; Kabata 1972; Johnson and Albright, 1991a).

C. clemensii is a generalist species that infects many nearshore marine fishes including salmonids of the *Oncorhynchus* genus (Johnson and Margolis 1994). Parker and Margolis (1964) suggested that *C. clemensii* is more specific to the environment than the host, staying in sheltered coastal waters where it can colonize juvenile salmon and other nearshore marine fish species. *L. salmonis* is a specialist parasite to species of the Family Salmonidae, including iterparous species such as coastal cutthroat trout and steelhead (Johnson and Margolis 1994; Pike and Wadsworth 1999). Although other hosts have been recorded for *L. salmonis*, non-salmonid hosts offer no chance for survival and development of adult forms (Kabata 1973; Jones *et al.* 2006a; Jones *et al.* 2006b). *L. salmonis* is the dominant cage-culture species on the northern Pacific and Atlantic coasts of Canada and the USA (Smith 1998; Johnson *et al.* 2004).

Sea lice feed on host tissues, mucous, and blood (White 1942; Kabata 1974; Brandal *et al.* 1976). Attachment and feeding activities are responsible for any primary disease that

develops. Severity of disease is related to the number of parasitic copepods and is dependent on the size and age of the fish (host), the general state of fish health, and the species of copepod and the development stages present (Johnson *et al.* 2004). High densities of lice on individual salmonids can impact swimming performance (Wagner *et al.* 2003), change behaviour (Birkeland 1996; Birkeland and Jakobsen 1997), cause hemorrhages, sores, disrupt osmotic balance, or cause death due to secondary bacterial infections (White, 1940; Wooten *et al.*, 1982; Johnson and Margolis 1994; Grimnes and Jakobsen 1996; Johnson *et al.* 1996; Bjorn and Finstad 1997). Sea lice population dynamics are strongly influenced by temperature and salinity, which affect growth rates, larval settlement, and survival (Johnson and Albright 1991b; Tucker *et al.* 2000).

Sea lice are important parasites of farm-raised salmonids and have caused direct and indirect economic losses greater than US $100 million annually (Johnson *et al.* 2004). In every country where salmon farms operate there have been cases of sea lice epizootics on salmon farms leading to significant mortality and disease on the farmed fish (Brandal and Egidius 1979; Wooten *et al.* 1982; Bravo 2003; Johnson *et al.* 2004). Bakke and Harris (1998) called open, net pen salmon farms “pathogen culturing facilities” because of the lack of control over microorganisms flowing in and out of adjacent ecosystems. Increased epizootics of *L. salmonis* on wild salmon populations have been correlated with epizootics in sea-farmed salmonids (Tully *et al.*, 1993; Tully *et al.*, 1999; Bjorn *et al.*, 2001; Bjorn and Finstad, 2002; Morton and Williams 2003; Morton *et al.* 2004; Krkosek *et al.* 2005). This effect of “pathogen spillover” has been documented in other industries as well (e.g. bumble bee production) (Colla *et al.* 2006).

The debate over the enhancement and transfer of sea lice by farmed salmon and its potential effects on wild salmon populations is controversial. In BC, sea lice epizootics on juvenile chum and pink salmon have been correlated with the activities of salmon farms in the BA over the last five years beginning in 2001 (Morton and Williams 2003; Morton *et. al* 2004; Morton *et. al* 2005). Consecutive parasite epizootics are not consistent with general parasite biology (Wikel *et al.* 1994) and a great deal of concern has been raised by local residents and scientists over what impact these epizootics may be having on the health of young chum and pink salmon populations in the BA.

Little is known about the ambient sea lice infection rates on juvenile salmon, which makes point source impact assessments of salmon farms difficult. In Europe, opportunities for the collection of ambient infection rates are limited due to the density of salmon farm operations and the lack of abundance of wild salmon (e.g. in Norway there are 100 times more farmed salmon than wild salmon in coastal waters (Heuch *et al.* 2005)). However, in BC, substantial portions of the coast still remain free of salmon farming and have a relative abundance of wild salmon, creating opportunities to examine ambient sea lice infection rates on juvenile salmon. To date, only two studies (Wertheimer *et al.* 2003 and Morton *et al.* 2004) have examined ambient sea lice infection rates on juvenile chum and pink salmon on the west coast of North America. Both found sea lice infection rates on juvenile salmon to be near zero. However, the informative value of these two studies was limited by a one-year time frame and a study

design that did not address the temporal and spatial scales affecting sea lice interactions with juvenile salmon.

In this study, we compare ambient sea lice infection rates (*Lepeoptheirus salmonis* and *Caligus clemensii*) on juvenile chum and pink salmon in areas of the BC coast that have had little or no development of salmon farms, to sea lice infection rates observed in the BA where there is intensive salmon farming. We test the hypothesis that sea lice infection rates on juvenile chum and pink salmon are low and variable in areas with little or no salmon farms relative to areas where salmon farms are abundant. In addition, we also tested the hypothesis that sea lice infection rates vary over geographic scales and that temperature and salinity are significant factors in the interaction between sea lice and juvenile salmon.

Methods

Juvenile chum salmon *(Oncorhynchus keta)* were collected in coastal marine areas during spring (late-March – June) in 2003, 2004, and 2005. Samples were collected near Bella Bella, Klemtu, the Southern Gulf Islands (near Saltspring Island), and the Broughton Archipelago, British Columbia in all three years (Figures 3.1, 3.2, 3.3). In 2003, 10 sites were sampled 75 times, in 2004, 21 sites were sampled 100 times, and in 2005, 30 sites were sampled 119 times among all sampling regions. Collections of chum and pink salmon did not occur in 2003 (due to lack of access) and collections for pink salmon did not occur in the Gulf Islands in 2003 or 2005 (odd year adult pink salmon only).

Sample sites were selected within sheltered bays and inlets based on the following criteria: proximity to salmon producing rivers, proximity to the open ocean, and proximity to salmon farm tenures. In the Klemtu, Bella Bella, and the southern Gulf Islands regions, all sample sites were located a minimum of 15 km from active salmon farming tenures based on geography. In the Klemtu region, five salmon farming sites were active in the sampling area, however no more than three salmon farms were active at the same time (Figure 3.1). In the Bella Bella region, no salmon farms were located in the study area (Figure 3.1), while in the southern Gulf Islands region, one salmon farm was active in 2003 and 2004 but not in 2005 (Figure 3.2).

In the Broughton Archipelago, a dip net (45 cm diameter, of 5 mm knotless mesh) on a 2.45 m pole (Bailey et al. 1975) was used in 2003 to capture the fish from a 6.7 m shallow-draft vessel (Morton and Williams 2003; Morton et al. 2004). A 15.2m x 1.8m, 0.63cm mesh beach seine was used in 2004 and 2005 to further ensure that healthy fish were not omitted from our sample. In all years, 20 – 30 pink and/or chum salmon were collected once a week, per site, for ten weeks from 16 April through 22 June.

In 2003 all Broughton salmon farm sites (collected within 1 km of a salmon farm tenure) were empty (fallow), in 2004 all sites were restocked and in 2005 site #1 was fallow while sites #2 and 3 were stocked (Figure 3.3). There are 26 salmon farm tenures in the Broughton Archipelago, with an average of 22 farms stocked at any time. There were active salmon farms encircling our study area even in 2003 when all study sites were fallowed. A passively suspended particle can travel 10 km during one tidal cycle (6h) in

the Broughton Archipelago (Dario Stucchi, DFO, Pacific Biological Station, Nanaimo, BC V9R5K6, Canada, personal communication), therefore at no time were our samples beyond the range of free-swimming planktonic stage farm-origin sea lice.

All salmon were collected using beach seines 30.3m long X 1.2-1.8m deep X 6.3mm bunt mesh. This technique consistently resulted in the capture of a large number of juvenile salmon, allowing a random sub-sample to be collected from a large population. A sampling crew of 2-4 used a small boat to encircle schools of wild juvenile salmon. With one crewmember anchoring the net onshore the boat maneuvered to encircle the school between the net and shore at which point the net was brought ashore. Once fish were at the shoreline a five-gallon bucket was used to remove as many as possible from the net. This method minimized lice-shedding abrasion. Once in the bucket fish were randomly selected and placed individually in whirl-pak™ bag and placed on ice. The number of samples collected varied from 13 to 200 per site. Samples were frozen at -20^{o}C as soon as possible. At each site, data for sea surface temperature (SST) and sea surface salinity (SSS) were collected using a YSI meter.

Samples were thawed and fish were individually examined for sea lice using a dissecting microscope. Sea lice were identified to life stage and to species for pre-adults and adults following Johnson and Albright (1991a) and Kabata (1972). Sea lice were designated as copepodids, chalimus I, II, III, or IV, pre-adult, and adult stages. Juvenile salmon were identified to species following Phillips (1977). Weights and lengths were also recorded.

Data Analysis

The hypotheses of interest in this study were that mean sea lice infection rates on juvenile salmon were low in areas where little or no salmon farming takes place and that sea lice infection rates were not influenced by geographic location.

Generalized linear models (univariate ANOVA) were used to facilitate comparison between continuous and categorical variables. The dependent variable analyzed was average number of lice per fish per sampling event. A replicate represents the average total lice (all sea lice life stages and species) per juvenile chum or pink salmon collected within a sampling event (n=13->200 fish) pooled to avoid pseudoreplication. Other factors in the analysis included: region, salinity, temperature, year, and average fish length per sampling event (length was determined to be a more reliable predictor of fish size than weight due to the potential error from weighing very small fish).

Univariate ANOVA's (Generalized Linear Models) were fit to two data sets (chum and pink salmon). For each model, the data were first fit with a full model including: the main effects (region, year, temperature, salinity, and length), all 2-way interactions, and all three way interactions were included in the initial model (4-way and 5-way interactions were excluded due to insufficient data and the difficulty associated with interpreting 4-way and 5-way interaction terms). For subsequent analyses, factors that were not significant at p=0.05 were sequentially dropped in a step-wise procedure until only the main effects remained. The change in r^2 was noted throughout the analysis procedure.

The normality assumption for the univariate ANOVAs was tested using the residuals from each model. All analyses were conducted using S-Plus 7.0 and SPSS 11.5.

Results

In total, 15,285 juvenile Pacific salmon (64% chum, 36% pink) were collected during the spring migrations of 2003, 2004, and 2005 in areas of the BC central coast (Klemtu (KL), Bella Bella (BB), Broughton Archipelago) and the southern Gulf Islands (GI)(near Saltspring Island)(Table 3.1).

The mean number of lice per fish was significantly higher in the BA than all other sampling regions in all three sampling years (Figures 3.4 and 3.5). The difference between the BA and the non-salmon farming regions was lowest in 2003 (2.8 – 4.9X higher in the BA). In 2004 and 2005, chum and pink salmon collected in the BA were infected 6-34X and 16-150X higher, respectively than were chum and pink salmon collected in the other sampling regions (Table 3.2). The highest mean lice per fish in the non-salmon farming sampling regions occurred in the GI region in 2005 (0.67 (0.22) lice/fish), while the highest mean lice per fish recorded in the BA was 9.01 (1.72) lice/fish (chum 2004). All other sampling regions and sampling years recorded mean number of lice per fish less than 0.5 (Figures 3.4 and 3.5; Table 3.2).

The mean prevalence of infection (% of the sample infected with lice) was highest in the BA across all sampling years, ranging from 75.0 (3.0) to 78.0 (5.0) % in 2004 and 2005 and from 37.0 (5.0) to 39.0 (4.0) % in 2003. Mean prevalence in the BA ranged from 4 –

18.5 times higher than prevalence in non-salmon farming regions (prevalence ranged from 9.0 (2.0) to 31.0 (6.0) % across all regions and sampling years, Table 3.2). The maximum infection intensity per fish observed in the BA ranged from 7-80 lice/fish and from 1-10 lice/ fish across the non-salmon farming regions (Table 3.2).

Salinity in the BA was the highest among all regions in 2004 (30.8 (0.35) ppt) and 2005 (28.8 (0.51) ppt) and was marginally lower than the highest value observed in the GI in 2003 (30.7 (0.49) ppt) (Table 3.3). Salinity in the non-salmon farming regions ranged from 20.7 (3.15) ppt to 30.73 (0.49) ppt among all sampling years. The highest variability in salinity was observed for samples taken from the BB and KL regions where the salinity varied from 15.0 – 25 ppt. In the GI, salinity varied from 6 – 15 ppt, while in the BA variability in salinity was the lowest (9 – 11 ppt). Temperatures ranged from 9.59 (0.66) ^{o}C to 12.51 (0.72) ^{o}C (Table 3) in the non-salmon farming regions and from 9.86 (0.15) ^{o}C to 11.1 (0.37) ^{o}C in the BA.

Length differences among both species across all sampling years and regions were less than 1.4 cm. Mean length ranged from 4.39 (0.17) cm to 4.96 (0.28)cm in the BA and from 4.43 (0.30) cm to 5.74 (0.24) cm in all other sampling regions over all sampling years. Mean weight from ranged from 0.85 (0.06) g to 1.84 (0.32) g in the BA and from 1.27 (0.19) g to 2.68 (0.37) g in all other sampling years (Table 3.4).

The results from the univariate analysis of variance for juvenile chum salmon (R^2=0.659) determined that region was the strongest predictor of the mean total lice per fish

(p=0.030). Temperature was also significant (p=0.046), while length was marginally non-significant (p=0.056). Highly significant interaction terms between region and year (p=0.015) and region, year, and length (p<0.0001) were also observed (Table 3.5).

For juvenile pink salmon (R^2=0.615), the results of the univariate analysis of variance determined that both year (p<0.0001) and temperature (p=0.050) were strong predictors of the mean total lice per fish. The interaction between region, year, and length was also found to be highly significant (p<0.0001) (Table 3.5).

Mean lice per unit of fish weight

The mean lice per gram of fish in the BA ranged from 2.65 (0.25) to 8.03 (0.42), which was up to 14 times higher than the mean lice per gram recorded in the non-salmon farming regions which ranged from 0.56 (0.06) to 1.90 (0.13) (Table 3.6). The highest lice per gram level in the non-salmon farming regions was observed in the GI region in 2004 and 2005 (1.70 (0.13) and 1.90 (0.13) respectively). The mean lice per gram for all other non-salmon farming regions and sampling years were below 1.30 lice per gram.

Lice species ratio

The percentage *C. clemensii* vs. *L. salmonis* was calculated from samples infected with pre-adult or adult lice stages based on salmon species, region, and year. The percentage *C. clemensii* was consistently the lowest in the BA ranging from 0 – 15% among all sampling years. In the BB and GI regions, the percentage *C. clemensii* ranged from

66.7% - 92.3% over all sampling years. In the Klemtu region, *C. clemensii* was found to be 27.3 - 58.1% in 2004 and 54.5 - 100% abundant in 2005 (Table 3.7).

Discussion

This study suggests that the ambient sea lice infection rates during the early marine phase of juvenile chum and pink salmon are less than one lice per fish and less than two lice per gram in coastal British Columbia (BC) (Figure 3.4 and 3.5, Table 3.6). These data indicate that the observed sea lice infection rates from the BA in 2001 (11.3 (0.41) lice/fish, 6.10 (0.24) lice/g; Morton and Willams 2003), 2002 (6.78 (0.27) lice/fish; Morton *et al.* 2004), 2004 (chum = 9.01 lice/fish, pink = 5.81 lice/fish; Morton *et al.* 2005), and 2005 (chum = 4.15 lice/fish, pink = 3.00 lice/fish) are well above ambient infection rates and are a cause for concern as to the potential effects of elevated sea lice infection rates on wild salmon populations. In 2003, many of the farms in the BA were fallowed (emptied of salmon), resulting in lower infection rates on juveniles and lower lice production from the fallowed salmon farms (Morton *et al.* 2005; Orr 2007). These data suggest that salmon farms are the most likely source of the observed high infection rates in the BA, given the life history strategies of sea lice, the density of farms in the BA, the documented history of salmon farms amplifying and transferring natural parasites to adjacent wild stocks, and the lack of analysis demonstrating the contribution of salmon farms to lice infection rates in the BA and other areas of the BC coast. Data from other areas with high levels of salmon farming activities (e.g. Quadra Island) show similar results (2005: 4.5 lice/fish; Morton *et al.* unpublished data) to the BA and thus the situation in the BA should not be considered an isolated phenomenon.

Increased epizootics of sea lice (mainly *L.* salmonis) on wild salmon populations have been correlated with outbreaks in sea-farmed salmonids in Scotland (Butler 2002), Ireland (Tully *et al.*, 1993; Tully *et al.*, 1999), Norway (Bjorn *et al.*, 2001; Bjorn and Finstad, 2002), and Canada (Morton and Williams 2003; Morton *et al.* 2004; Krkosek *et al.* 2005; Morton *et al.* 2005; Krkosek *et al.* 2006). Given the evidence that salmon farms can alter sea lice infection rates on juvenile salmon (i.e. increasing ambient parasite burdens), a great deal of concern has been raised about the subsequent effects on the health of wild salmon populations. In some cases, declines in wild salmon populations adjacent to salmon farming operations have been recorded after increased sea lice levels correlated with the presence of salmon farms were documented (Gargan 2000; PFRCC 2002; Krkosek *et al.* 2006).

The highest sea lice infection rates in the non-farmed regions occurred in the Gulf Islands at two sites in Sansum Narrows (Figure 2) in 2004 and 2005 (maximum infection level ten lice per fish). Only one salmon farm was operating in the Gulf Islands region throughout the study (2003: active, 2004: small number of broodstock, 2005: fallow) but was at least 19 km from the Sansum Narrows sampling sites. This suggests that the observed infection rates were of natural source. One possible explanation is that Sansum Narrows is a popular spot for Chinook fishing at all times of year and recreational fishing boats were continuously noted during sampling. This suggests that the lice source could have originated from wild salmon. In addition, narrow bodies of water have been found to be a factor in elevating sea lice infection rates on juvenile salmon (Holst *et al.* 2000).

It is possible that the combination of a narrow water way and a reservoir of natural hosts (perhaps increased from previous years) explain the observed infection rates.

Geographic variability in sea lice infection rates was only observed in the BA, where infection levels were significantly higher (3-150 times) than in any other sampling region across all sampling years (Figures 3.4 and 3.5). For chum salmon, both region ($p=0.030$) and the region*year interaction ($p=0.015$) were significant predictors of the data, while for pink salmon only year was found to be significant ($p<0.0001$). Significant yearly differences in infection rates were observed in the BA. Temporal variability in sea lice infection rates has been noted in other multi year studies (Boxaspen 1997). For the non-salmon farming regions, only marginal differences among regions and among years were observed suggesting that geographic variability is not a significant factor in lice infections as has been suggested (McVicar 2004), although no empirical data support the geographic variability hypothesis. Revie *et al.* (2002) conducted a survey of lice abundance on 33 salmon farms in Scotland found that geographic location did not affect mean lice abundance.

The region*year*length interaction was significant for both chum and pink salmon ($p<0.0001$ and $p<0.0001$). Size is known to be an important factor for resisting sea lice infections (Tucker *et al.* 2002), with larger fish known to be more resistant. Although significant differences were observed among some regions and within and among years, difference in length over all regions and sampling year was less than 1.4 cm. It is unclear whether this difference is biologically significant or reflects random variability in

juvenile salmon population dynamics. Future studies should consider more detailed analysis on the length vs. infection.

Salinity was not a significant predictor of sea lice infections rates for either juvenile chum or pink salmon ($p=0.925$ and $p=0.621$ respectively). Laboratory studies have shown that salinity near 30 ppt allows for optimal settlement and survival of the copepodid life stage; however viable copepodids can be produced at salinities above 24 ppt (Johnson and Albright 1991b; Tucker *et al.* 2000). Salinity was consistently the highest in the BA (exception: GI 2003), and temperature was consistently the lowest (Table 3.3). The higher temperatures in the non-salmon farming regions could offset the possible negative effects of the lower salinity as has been found for other parasitic (Kinne 1957; Lance 1963; Tucker *et al.* 2000). Additionally, mean salinity in some of the non-farming regions had high standard deviation values suggesting high variability within the system. One explanation is that the salinity data collected only represents sea surface salinity (SSS) and some of the samples in the non-salmon farming regions were collected within 1 or 2 km of large river outflows, which resulted in some salinities less than 10 ppt due to the presence of a freshwater lens. In 2005, salinity profiles (0 – 10 m) were collected and the results show that the freshwater lens at river mouth sites was consistently less than 2.5 m in depth (actual depth not measured). Therefore, the differences and variability in salinity could be due to the effect of this fresh layer whose actual biological impact on lice infection levels may be minimal given that juvenile salmon are common as deep as 10 m (Healey 1980).

Temperature was a significant predictor of infection levels in both chum and pink salmon ($p=0.046$ and $p=0.050$ respectively). Temperature is known to affect the rate of sea lice growth and development on salmon (Johnson and Albright 1991b). The results of this study show that in all regions, temperature declined slightly per sampling year with differences of less than 2 ^{o}C among regions within each year (Table 3.3) (exception: BA 2003). Temperatures in the BA correlated well with the observed lice infection rates with the highest temperature and infection rate recorded in 2004. However, its importance in driving sea lice dynamics is not clear from this data set. In future studies, full water column profiles of both temperature and salinity should be collected to allow for a more thorough assessment of the role of temperature and salinity in sea lice population dynamics. However, it should be noted that a small temperature change is unlikely to have a physiological effect.

The mean lice per gram per infected fish (fish with zero lice excluded) was found to be less than 2.0 across all non-salmon farming regions and sampling years (Table 3.6). In the BA, lice per gram levels ranged from 2.65 to 8.03. For this analysis, fish with zero lice were removed to allow the impact of sea lice on juvenile salmon to be assessed. Lice per gram has been used as a measure of the impact of sea lice on their hosts based on experimental laboratory studies conducted on post-smolt European salmonids, which determined that 0.75 – 1.6 lice /g of fish weight is a lethal infection ratio (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997). More recently, Morton and Routledge (2005) found that the short-term mortality for juvenile chum and pink salmon is increased by lice infestations of 1-3 sea lice/fish. These are the only data available to interpret the results of

this study from the perspective of lice impact on juvenile salmon. However, it is important to note that the results of laboratory studies will likely result in some degree of underestimation of the natural tolerance of salmon to infection by sea lice due to the effects of fish handling and culturing. This study is consistent with the findings of Morton and Routledge (2005) and suggests that the observed mean lice per gram rates from the BA are cause for substantial conservation concern, while lice per gram rates in areas without salmon farms are well below mortality thresholds.

This study demonstrates that *Caligus clemensii* dominates in areas without salmon farms, while *Lepeoptheirus salmonis* dominates in areas with salmon farms (Table 3.7). It was predicted in advance that because of the difference in life history strategy between the two species, sea lice infection rates on juvenile salmon would be low in nearshore marine waters and would be dominated by *Caligus clemensii.* The generalist nature of *Caligus clemensii* (Parker and Margolis 1964) suggests that it is more likely to be ubiquitous in the nearshore marine environment, while the specialist nature of *Lepeoptheirus salmonis* (Johnson and Margolis 1994; Pike and Wadsworth 1999) suggests that it is only likely to be found near host sources (e.g. salmon farms, overwintering salmonids, etc). One of the most important impacts that salmon farms can have on the ecology of sea lice and juvenile salmon is to provide a previously unavailable overwintering habitat to *L. salmonis*. This change in the interaction between sea lice and juvenile salmon can have dramatic effects, as it creates scenarios whereby juvenile salmon (< 5cm, <1 g) can be exposed to lice larval concentrations against which they have little or no defense (i.e. their natural defenses are overwhelmed by the substantial increase in infection potential).

This creates a serious conservation concern related to the potential impact of sea lice transfer from salmon farms to wild salmon populations. Recently, Krkosek *et al.* (2006) estimated that salmon farms can induce 9 - 95% mortality on juvenile salmon in adjacent marine waters.

A useful conceptual framework within which to consider the impact of salmon farms on the health of wild salmon populations is their potential to enhance ambient infection rates of sea lice and disrupt the equilibrium between sea lice and juvenile salmon. Wikel *et al.* (1994) defined a successful host-parasite relationship as a balance between limiting the parasite through host defenses and the ability of the parasite to modulate, evade, or restrict the host's responses. In other words, an interspecific arms race occurs between parasites and hosts that ultimately results in a state of dynamic equilibrium. Epizootics of parasites (e.g. sea lice) on hosts (e.g. wild salmon) are a result of an imbalance in the host-parasite interaction due to decreased resistance by the host due to factors such as poor nutrition, increased stress, or an increase in parasite number. In the case of salmon farms, numerous studies have quantified their output of lice larvae (ranging in the billions) into adjacent marine areas (Tully and Whelan 1993; Heuch and Mo 2001; McKibben and Hay 2004; Penston *et al.* 2004; Orr 2007). The disruption of the dynamic equilibrium between sea lice and juvenile salmon by salmon farms is the most likely explanation to the lice infection rates in the BA. This study supports this explanation and suggests that an analysis of the magnitude of sea lice contributions from all salmon farms in the BA is needed if proper mitigation measures are to be developed.

A counter hypothesis to the this discussion is the “overwintering sea lice hypothesis”, which states that sea lice egg strings drop off returning adult salmon in estuaries and lie dormant until reactivated by temperature cues which coincide with the marine entry of juvenile salmon in the following spring (Costelloe *et al.* 1998). This hypothesis has been suggested as an alternative to salmon farms as the source of the epizootics in the BA. This study was designed to capture the early marine life interaction between sea lice and juvenile salmon. Over all three years of sampling and in all regions sampled (some of which represent areas of the highest salmon returns on the BC coast), no evidence (i.e. no high infection rates of *L. salmonis* under natural conditions) was found to support the overwintering hypothesis as a reasonable explanation for the lice rates observed in the BA. In fact, the dominance by *C. clemensii* and the low rates of infection suggest that this type of strategy is not being utilized by *L. salmonis.* Additionally, Costelloe (2006) suggested that *L. salmonis* eggstrings do not fall off their hosts until in fresh water for some days and their eggs do not hatch or survive in freshwater (Mclean *et al.* 1990; Johnson and Albright 1991b; Finstad *et al.* 1995).

In summary, this study suggests that ambient lice infection rates for juvenile chum and pink salmon range from 0.56 (0.08) to 1.93 (0.13) lice/fish, prevalence ranges from 2.0 (1.0) % to 32.0 (19) %, and the maximum infection intensity observed was ten lice/fish. These results are significantly lower than the infection rates observed in the BA in recent years and help to frame the situation in the BA in terms of the contribution of salmon farms to the observed lice infection rates. Additionally, the results from this study do not identify geographic variability as an important factor in sea lice population dynamics.

Table 3.1 – Total catches of juvenile chum and pink salmon collected in Bella Bella, Klemtu, southern Gulf Islands, and the Broughton Archipelago, BC (2003-2005).

	2003	**2004**	**2005**	**Total**
Klemtu				
Chum	0	531	1271	**1802**
Pink	0	168	775	**943**
Bella Bella				
Chum	331	941	2131	**3403**
Pink	273	451	655	**1379**
Gulf Islands				
Chum	1408	1164	1126	**3698**
Pink	0	149	0	**149**
Broughton				
Chum	309	540	67	**916**
Pink	367	546	2082	**2995**
Total	2688	4490	8107	**15285**

Table 3.2 – Mean total lice per fish, prevalence (% of sample infected), and maximum infection intensity (MII) for samples of juvenile chum and pink salmon collected in Bella Bella, Klemtu, southern Gulf Islands, and the Broughton Archipelago, BC (2003-2005).

			Chum			Pink		
Year	**Region**	**N**	**MII**	**Prevalence**	**Total Lice**	**MII**	**Prevalence**	**Total Lice**
2003	Bella Bella	12	4	9.0 (3.0)	0.13 (0.06)	3	8.0 (4.0)	0.10 (0.05)
	Gulf Islands	30	8	14.0 (2.0)	0.20 (0.04)	n/a	n/a	n/a
	Broughton	33	9	37.0 (5.0)	0.56 (0.07)	7	39.0 (4.0)	0.68 (0.11)
2004	Klemtu	8	6	24.0 (7.0)	0.43 (0.16)	4	32.0 (19.0)	0.36 (0.20)
	Bella Bella	28	7	20.0 (3.0)	0.31 (0.07)	6	13.0 (4.0)	0.18 (0.05)
	Gulf Islands	25	6	17.0 (3.0)	0.26 (0.05)	2	9.0 (2.0)	0.10 (0.02)
	Broughton	39	73	77.0 (5.0)	9.01 (1.72)	80	78.0 (5.0)	5.81 (0.90)
2005	Klemtu	21	6	10.0 (3.0)	0.15 (0.05)	1	2.0 (1.0)	0.02 (0.01)
	Bella Bella	40	6	9.0 (2.0)	0.15 (0.05)	6	7.0 (2.0)	0.08 (0.03)
	Gulf Islands	14	10	31.0 (6.0)	0.67 (0.22)	n/a	n/a	n/a
	Broughton	27	26	77.0 (6.0)	4.15 (0.77)	28	75.0 (3.0)	3.00 (0.27)

Table 3.3 – Average sea surface temperature (SST) and sea surface salinity (SSS) for samples of juvenile chum and pink salmon collected in the Bella Bella, Klemtu, southern Gulf Islands, and the Broughton Archipelago, BC.

Year	Region	N	SST (^{o}C)	Range	SSS (ppt)	Range
2003	Bella Bella	12	12.51 (0.72)	10.3 – 19.4	24.70 (1.39)	16.9 – 32.0
	Gulf Islands	30	12.20 (0.25)	9.8 – 13.0	30.73 (0.49)	25.0 – 34.0
	Broughton	33	9.86 (0.15)	8.5 – 12.0	29.97 (0.36)	25.0 – 34.0
2004	Klemtu	8	11.56 (0.36)	10.0 – 13.0	20.75 (3.15)	5.0 – 30.0
	Bella Bella	28	12.50 (0.18)	11.0 – 14.3	25.14 (0.86)	16.0 – 31.0
	Gulf Islands	25	10.77 (0.18)	9.5 – 13.0	22.24 (0.70)	16.0 – 31.0
	Broughton	39	11.10 (0.37)	8.3 – 16.0	30.87 (0.35)	26.0 – 35.0
2005	Klemtu	21	9.59 (0.66)	3.5 – 13.9	26.12 (1.22)	10.0 – 30.2
	Bella Bella	40	11.31 (0.44)	2.9 – 17.7	24.77 (0.70)	8.3 – 30.0
	Gulf Islands	14	11.64 (0.31)	10.0 – 13.5	25.57 (0.53)	22.0 – 28.0
	Broughton	27	9.90 (0.35)	7.0 – 13.0	28.85 (0.51)	21.0 – 32.0

Table 3.4 – Mean length and mean weight for juvenile chum and pink salmon collected near Bella Bella, Klemtu, the southern Gulf Islands, and the Broughton Archipelago, BC (2003-2005).

Year	Region	N	Chum		Pink	
			Length (cm)	Weight (g)	Length (cm)	Weight (g)
2003	Bella Bella	12	5.48 (0.22)	1.71 (0.21)	5.29 (0.21)	1.38 (0.17)
	Gulf Islands	30	5.74 (0.24)	2.68 (0.37)	n/a	n/a
	Broughton	33	4.39 (0.17)	0.85 (0.06)	4.39 (0.21)	1.24 (0.07)
2004	Klemtu	8	5.23 (0.30)	1.83 (0.37)	5.53 (0.48)	2.20 (0.49)
	Bella Bella	28	5.53 (0.19)	2.15 (0.24)	5.46 (0.23)	1.98 (0.21)
	Gulf Islands	25	4.55 (0.17)	1.32 (0.19)	4.81 (0.25)	1.38 (0.22)
	Broughton	33	4.83 (0.22)	1.27 (0.05)	4.76 (0.23)	1.61 (0.05)
2005	Klemtu	21	4.76 (0.19)	1.51 (0.20)	4.43 (0.30)	1.27 (0.19)
	Bella Bella	40	5.24 (0.21)	2.07 (0.29)	4.74 (0.22)	1.35 (0.14)
	Gulf Islands	14	5.13 (0.28)	1.93 (0.35)	n/a	n/a
	Broughton	27	4.96 (0.28)	1.84 (0.32)	4.69 (0.22)	1.24 (0.04)

Table 3.5 – Results for the generalized linear model (univariate ANOVA) for mean total lice vs. region, temperature, salinity, and mean length per fish (*significant $p < 0.05$) for juvenile chum and pink salmon.

Species	Variable	Effect	df	F	Sig.	R^2
Chum	Total Lice	REGION	3	3.04	0.030*	0.659
		YEAR	2	2.15	0.119	
		TEMP	1	4.02	0.046*	
		SALINITY	1	0.01	0.925	
		LENGTH	1	3.70	0.056	
		REGION * YEAR	5	2.87	0.015*	
		REGION * YEAR * LENGTH	10	15.38	<0.0001*	
Pink	Total Lice	REGION	3	0.78	0.506	0.615
		YEAR	2	8.51	<0.0001*	
		TEMP	1	3.90	0.050*	
		SALINITY	1	0.24	0.621	
		LENGTH	1	2.25	0.135	
		REGION * YEAR * LENGTH	8	12.70	<0.0001*	

Table 3.6 – Lice per gram (+/- SE) for infected juvenile chum and pink salmon (fish with zero lice excluded) collected in the Bella Bella, Klemtu, southern Gulf Islands, and the Broughton Archipelago, BC (2003-2005).

		Chum		Pink	
	Region	**N**	**Mean**	**N**	**Mean**
2003	Bella Bella	25	0.81 (0.09)	26	1.19 (0.16)
	Gulf Islands	206	1.02 (0.07)	n/a	n/a
	Broughton	93	2.78 (0.23)	151	2.65 (0.25)
2004	Klemtu	85	1.26 (0.10)	46	0.56 (0.08)
	Bella Bella	184	1.08 (0.07)	63	1.00 (0.14)
	Gulf Islands	194	1.76 (0.14)	18	0.83 (0.15)
	Broughton	399	8.03 (0.42)	467	5.62 (0.26)
2005	Klemtu	135	0.90 (0.10)	87	1.88 (0.18)
	Bella Bella	105	0.81 (0.06)	13	0.78 (0.27)
	Gulf Islands	336	1.93 (0.13)	n/a	n/a
	Broughton	49	4.16 (0.52)	1549	6.65 (0.19)

Table 3.7 – Lice species ratios (% *Caligus vs. % Lepeoptheirus)* for samples of juvenile chum and pink salmon infected with sea lice (non-infected fish removed) collected in the Bella Bella, Klemtu, southern Gulfs Islands, and the Broughton Archipelago regions (2003-2005).

		Chum		Pink	
	Region	**Caligus/ motile lice**	**Mean**	**Caligus/ motile lice**	**Mean**
2003	Bella Bella	7/8	87.5	6/8	75.0
	Gulf Islands	49/56	87.5	n/a	n/a
	Broughton	12/80	15.0	16/135	11.8
2004	Klemtu	9/33	27.3	18/31	58.1
	Bella Bella	43/53	81.1	22/27	81.5
	Gulf Islands	24/26	92.3	5/7	71.4
	Broughton	14/209	7.0	21/291	7.2
2005	Klemtu	6/11	54.5	3/3	100
	Bella Bella	29/40	72.5	12/18	66.7
	Gulf Islands	43/53	81.1	n/a	n/a
	Broughton	0/17	0.0	37/727	5.1

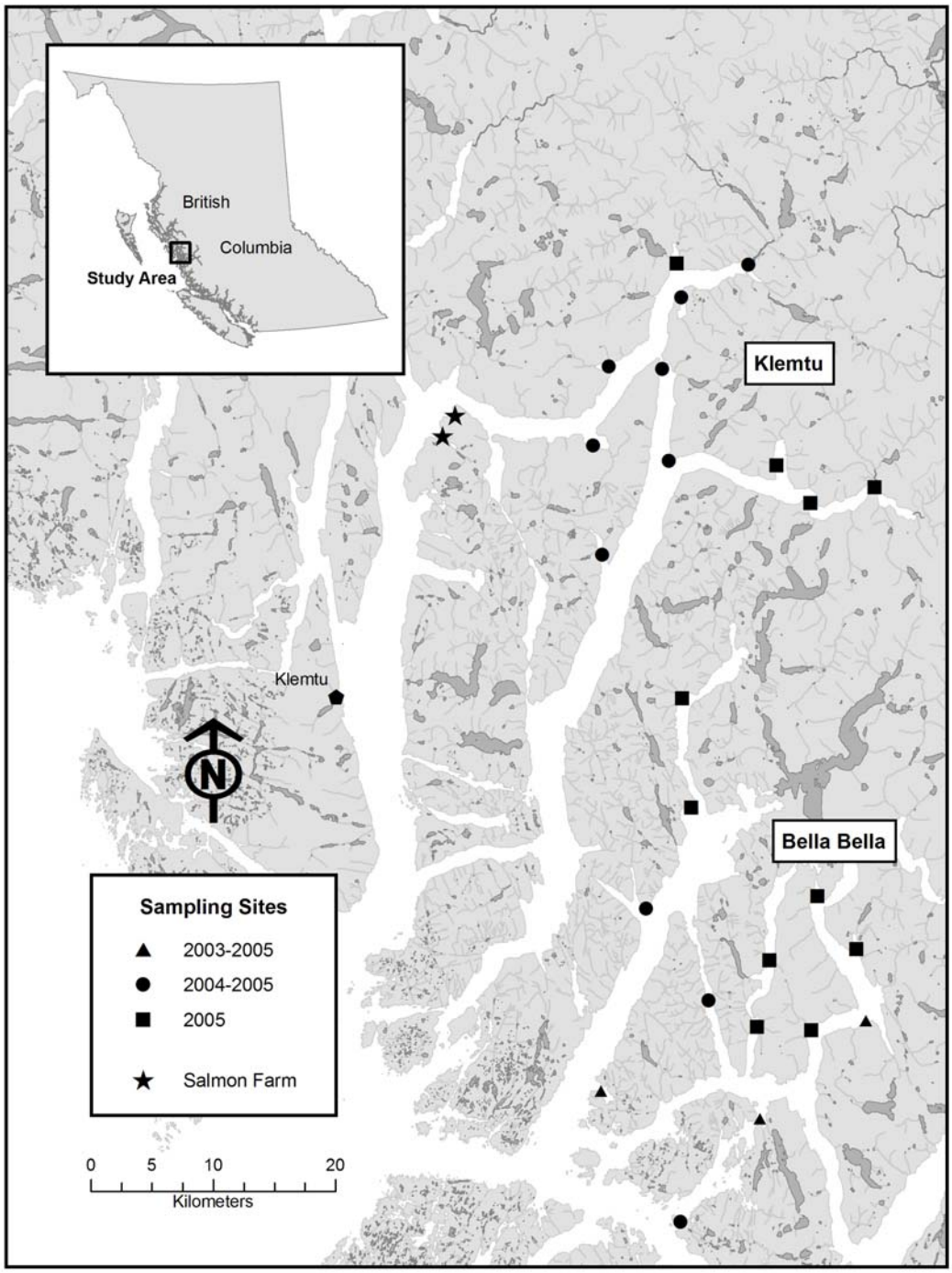


Figure 3.1 – Map of sampling sites and salmon farm locations for field collections near Bella Bella and Klemtu, BC (2003 - 2005).

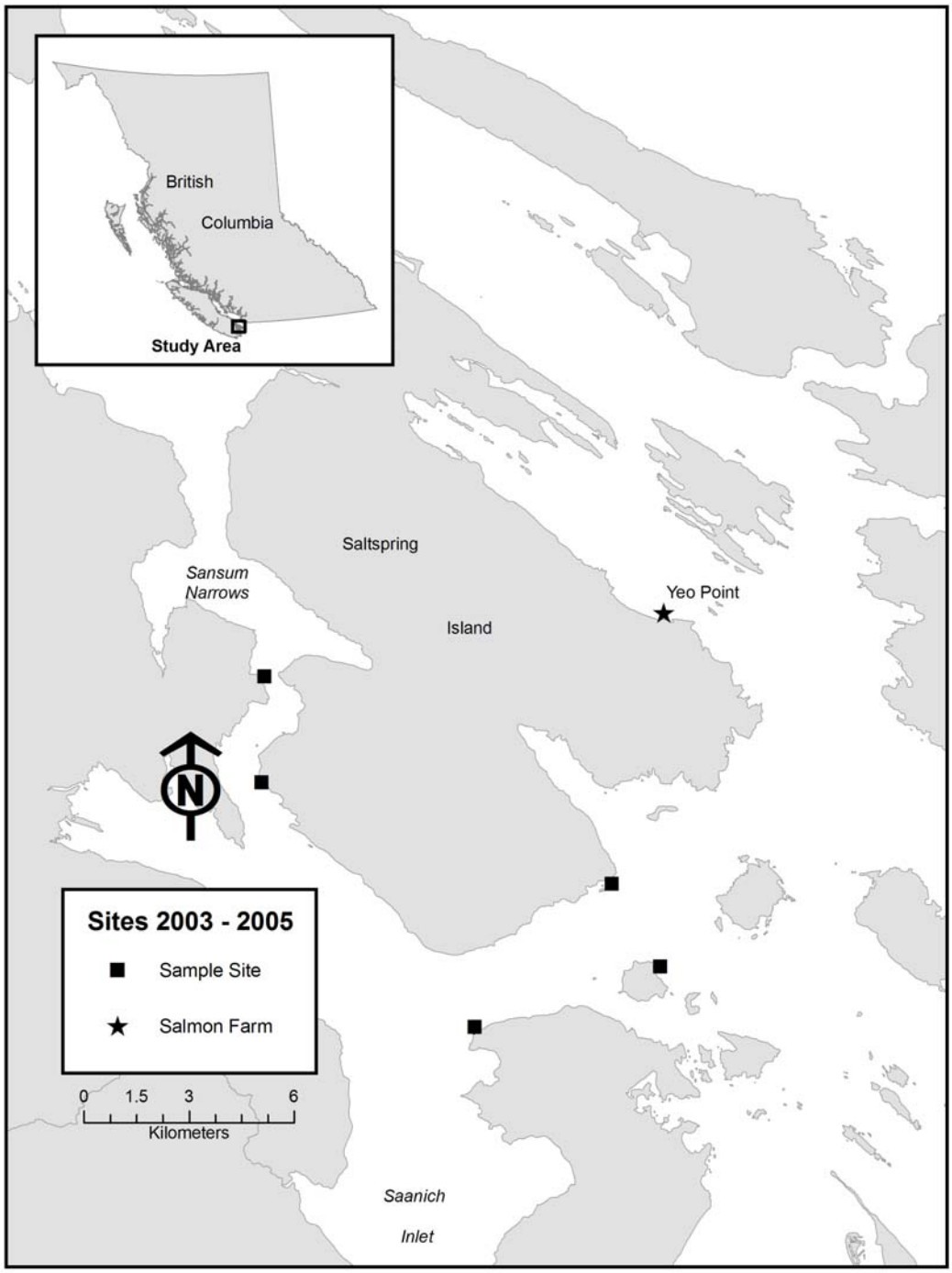


Figure 3.2 – Map of sampling sites and salmon farm locations for field collections in the Southern Gulf Islands, BC (near Saltspring Island) (2003 - 2005).

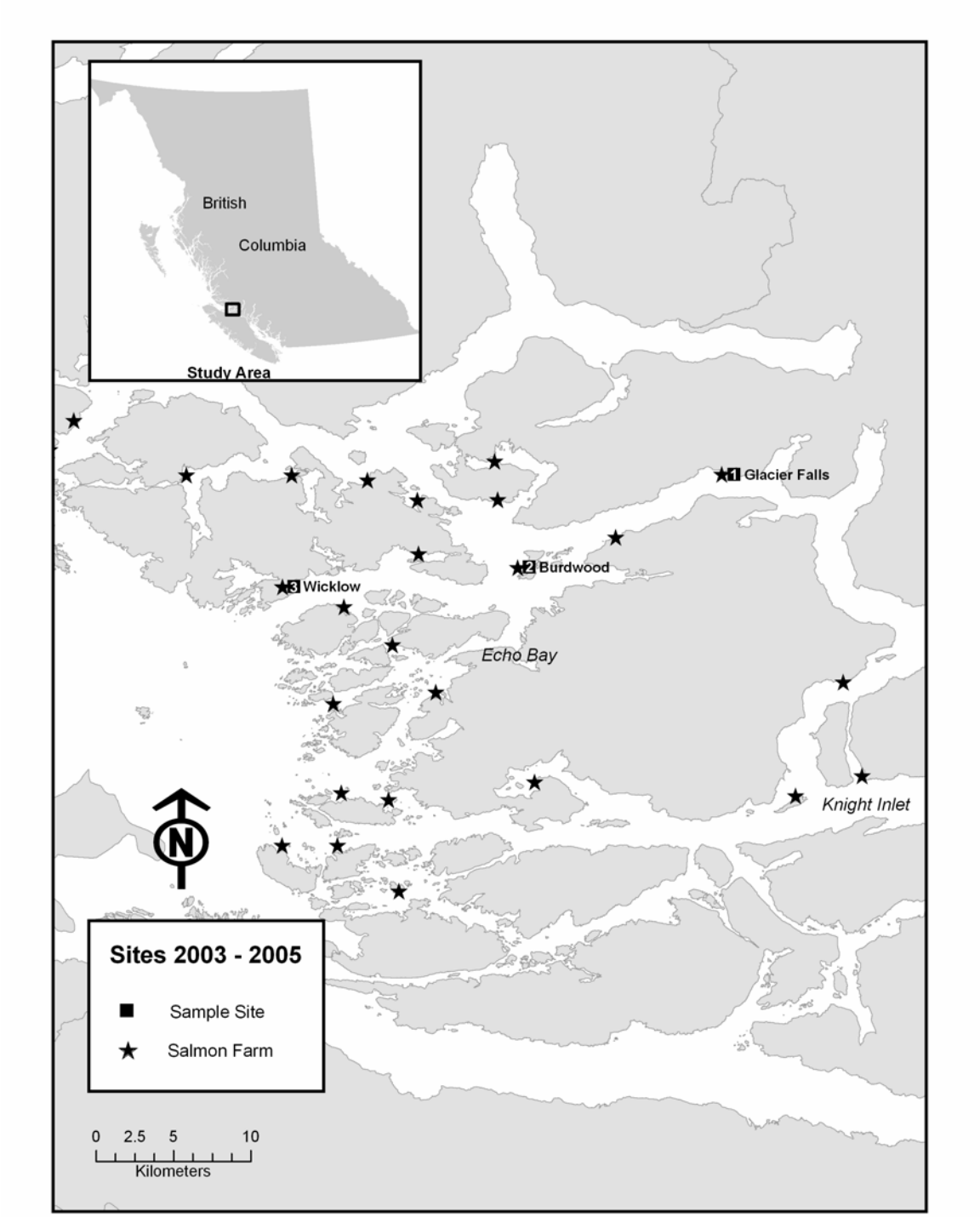


Figure 3.3 – Map of sampling sites and salmon farm locations for field collections in the Broughton Archipelago, BC (2003 - 2005).

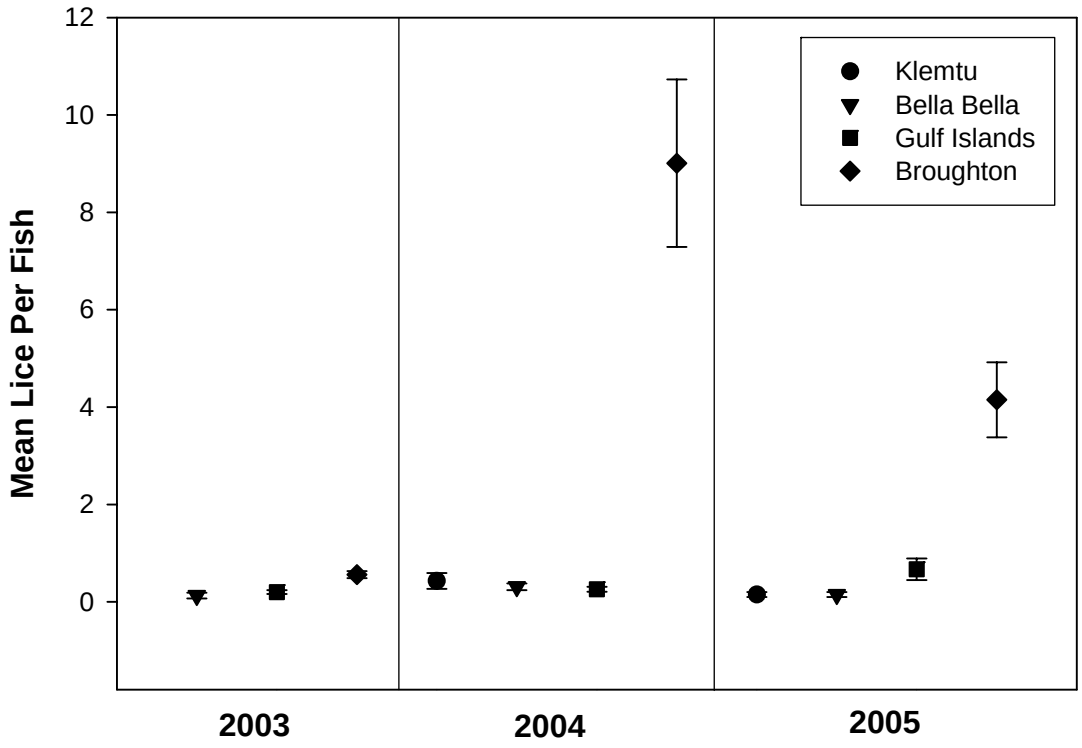


Figure 3.4 – Mean lice abundance (+/- SE) for juvenile chum salmon collected in nearshore marine areas near Klemtu, Bella Bella, the southern Gulf Islands, and the Broughton Archipelago (2003 – 2005).

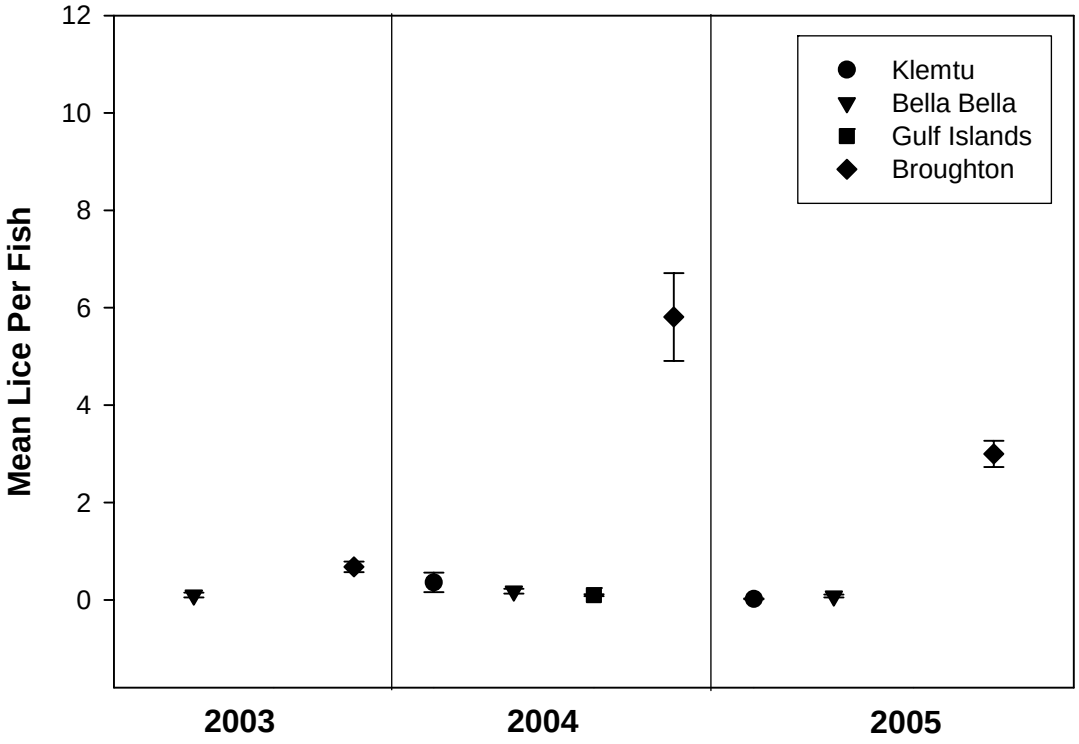


Figure 3.5 - Mean lice abundance (+/- SE) for juvenile pink salmon collected in nearshore marine areas near Klemtu, Bella Bella, the southern Gulf Islands, and the Broughton Archipelago (2003 – 2005).

General Discussion

The commercial culture of salmon is currently the largest agriculture food export in British Columbia (BC) and is poised to further expand production. One of the major hurdles for the expansion of the salmon farming industry has been its environmental impact on coastal ecosystems and wild salmon populations. One of the major areas of concern surrounds the question of whether salmon farms are amplifying ambient densities of sea lice causing migrating juvenile salmon to become infected at rates that would increase their mortality and affect the health of wild salmon.

The Broughton Archipelago (BA) is home to the largest density of salmon farms on the west coast of North America. Epizootics of sea lice on juvenile chum and pink salmon were recorded in 2001 (11.3 (0.41) lice/fish, 6.10 (0.24) lice/g; Morton and Williams 2003), 2002 (6.78 (0.27) lice/fish; Morton *et al.* 2004), 2004 (chum = 9.01 lice/fish, pink = 5.81 lice/fish; Morton *et al.* 2005), and 2005 (chum = 4.15 lice/fish, pink = 3.00 lice/fish). Despite strong scientific literature (theoretical and applied) demonstrating that salmon farms amplify ambient sea lice infection rates, which then infect wild juvenile salmon migrating in adjacent waters at rates higher than they would normally encounter, the problem has not been adequately acknowledged nor addressed by either the BC and federal governments or the salmon farming industry. A key argument used to discount the role of salmon farms in the elevated infection rates in the BA is the lack of data on the ambient sea lice infection rates for migrating juvenile salmon.

The present work represents one of the first attempts to empirically examine the ambient sea lice infection rates on juvenile salmon over relevant temporal and geographical scales. Ambient lice rates for juvenile chum and pink salmon were less than one louse per fish and less than two lice per gram across all sampling years. This result was found to be consistent across geographic areas (with no salmon farming activity), suggesting that geographic variability was not a significant factor in the natural interaction between sea lice and juvenile salmon. These results are consistent with the findings of Morton and Routledge (2005), the only study on the effects of sea lice on the short-term mortality of early sea life salmon, which found that infestations above 1-3 lice per fish are lethal.

Salmon farms were found to strongly influence ambient sea lice infection rates on juvenile chum and pink salmon. Infection rates of juvenile salmon collected near salmon farms were higher than non-salmon farming regions; ranging from 3 – 150 times higher in the BA and from 2 – 14 times higher in the Klemtu region. Infection rates near salmon farms varied in intensity from year to year. The extent to which the sea lice-salmon relationship was affected by salmon farms was dependent on farm species, farm location, within year variability in fish size, temperature, salinity, and the scale of salmon farming activities within the region. In every country where salmon farms operate there have been cases of sea lice epizootics on salmon farms leading to significant mortality and disease on the farmed fish (Brandal and Egidius 1979; Wooten *et al.* 1982; Bravo 2003; Johnson *et al.* 2004). Additionally, epizootics of *L. salmonis* on wild salmon populations have been correlated with epizootics in sea-farmed salmonids in other salmon farming

countries such as: Ireland (Tully *et al.,* 1993; Tully *et al.,* 1999), Scotland (Butler 2002), and Norway (Bjorn *et al.,* 2001; Bjorn and Finstad).

The results from the susceptibility experiments suggest a strong difference in susceptibility between juvenile chum and pink salmon. The field data suggests that pink salmon are less susceptible than chum salmon to infection by sea lice, which was consistent with the results from the laboratory study; however one key factor not controlled for in the field studies were the lethal infection rates. Morton and Routledge (2005) found that the short-term mortality for juvenile chum and pink salmon is increased by lice infestations of 1-3 sea lice. Given these data, it is possible that chum salmon are able to handle higher sea lice rates than juvenile pink salmon and thus the field samples are biased towards chum salmon with higher lice and fewer pink salmon because pink salmon experience higher mortality per lice infection and are therefore removed from the sampling pool. The average rate of sea lice infection per pink salmon across all years and proximity categories did not exceed 0.5 lice per fish. Given that stress responses can be triggered by sea lice infections (Nolan *et al.* 1999; Bowers *et al.* 2000), it is possible that given the small size of pink salmon at the time of marine entry (< 5cm and < 0.5 g)(Heard 1991) even low infection rates have significant impacts on juvenile pink salmon. Determining the lethal infection rates for juvenile salmon at sizes less than 5 cm and 1 -2 g will likely prove to be very challenging given the difficulties in securing reliable lice sources in the months of March and April, when the fish of small size need to be tested. It is important to note that dynamics of susceptibility likely change as size increases and therefore the results from experiments on larger fish may not be applicable.

In order to properly frame the results of this study it is useful to consider the impact of salmon farms on the health of wild salmon populations from the potential to enhance ambient sea lice infection rates and disrupt the equilibrium between sea lice and juvenile salmon. Wikel *et al.* (1994) defined a successful host-parasite relationship as a balance between limiting the parasite through host defenses and the ability of the parasite to modulate, evade, or restrict the host's responses. In other words, an interspecific arms race occurs between parasites and hosts that ultimately results in a state of dynamic equilibrium. Epizootics of parasites (e.g. sea lice) on hosts (e.g. wild salmon) are a result of an imbalance in the host-parasite interaction due to decreased resistance by the host due to factors such as poor nutrition, increased stress, or an increase in parasite number. In the case of salmon farms, numerous studies have quantified their output of lice larvae (ranging in the billions) into adjacent marine areas (Tully and Whelan 1993; Heuch and Mo 2001; McKibben and Hay 2004; Penston *et al.* 2004; Orr 2007 (in press)). Bakke and Harris (1998) called open net pen salmon farms "pathogen culturing facilities" because of the lack of control over microorganisms flowing in and out of adjacent ecosystems. The disruption of the dynamic equilibrium between sea lice and juvenile salmon by salmon farms is the most likely explanation to the observed lice infection rates in the BA.

Some have suggested that the high lice rates on juvenile salmon are normal, given that adult salmon in coastal waters and offshore waters have been found to have up to several hundred lice per fish and close to 100% prevalence (Nagasawa 1987; Nagasawa 1993; Tingley *et al.* 1997; MacKenzie *et al.*, 1998; Bjorn and Finstad, 2002; Beamish *et al.*

2005). However, these data need to be considered from the perspective of how they represent the equilibrium between lice and salmon. For example, if the upper limit of the lethal infection ratio of 1.6 lice per gram quantified for postsmolts (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997) were applied to adult salmon, a 10lb salmon would require over 7200 sea lice per fish in order to kill it. The results from current studies are nowhere close to this estimate. In this context, the lice infection rates recorded on adult salmon represent the state of equilibrium between adult salmon and sea lice. This is further evidenced by the lack of overall effect that sea lice rates (< 100 per fish) have on adult on adult salmon (personal observation). It is therefore concluded that it is not appropriate to suggest that high lice rates on juvenile salmon are normal due to those observed on adult salmon.

The results of this study suggest that it is highly unlikely that the epizootics in the BA are of natural source, given that salmon specific life history requirement of *L. salmonis* and the lack of suitable reproductive overwintering habitat in the absence of salmon farms. These results suggest that the next step in the debate is to understand the population dynamics of sea lice on salmon farms, especially given the ease with which such data can be collected and analyzed. This will require the industry to be much more transparent than it has been to date, as raw data on the prevalence and intensity of sea lice per pen per salmon farm in addition to relevant abiotic factors (i.e. temperature, salinity, DO, etc.) will need to be made available. Currently, data are made available by the industry only in the form of averages for areas, which does not allow for an accurate analysis of sea lice dynamics per farm.

Government and industry have both cited counter hypotheses, such the overwintering hypothesis and the alternative host hypothesis, as better explanations for the observed high lice infection levels in the BA. The overwintering sea lice hypothesis states that sea lice egg strings drop off returning adult salmon in estuaries and lie dormant until reactivated by temperature cues which coincide with the marine entry of juvenile salmon in the following spring (Costelloe *et al.* 1998). The present work was designed to capture the early marine life interaction between sea lice and juvenile salmon. Over all three years of sampling and in all regions sampled (some of which represent areas of the highest salmon returns on the BC coast), no evidence was found to support the overwintering hypothesis as a reasonable explanation for the lice rates observed in the BA. In fact, the dominance by *C. clemensii* and the low infection rates suggest that this type of strategy is not being utilized by *L. salmonis.* Additionally, Costelloe (2006) suggested that *L. salmonis* eggstrings do not fall off their hosts until in fresh water for some days and their eggs do not hatch or survive in freshwater (Mclean *et al.* 1990; Johnson and Albright 1991; Finstad *et al.* 1995).

The alternative host hypothesis states that three-spine sticklebacks (*Gasterosteus aculeatus*) are providing significant overwintering habitat for *Lepeoptheirus salmonis*, which has led to the epizootics. This hypothesis comes from observations from government studies where *L. salmonis* was found in high abundance (prevalence 83.6%, intensity 18.3) on three-spine stickleback (Jones *et al.* 2006a). Previous to this study, *L. salmonis* had not been documented on three-spine stickleback. However, Kabata (1973)

reported that non-salmonid hosts offer no chance of survival of development to reproductive stages for *L. salmonis*. Jones *et al.* (2006a) found that 97% of the *L. salmonis* found on three-spine sticklebacks were non-motile stages (copepodids and chalimus). In addition, Jones *et al.* (2006b) reported that motile stages of *L. salmonis* were not observed to develop in artificially infected three-spine stickleback. It is not possible for stickleback to be an overwintering source of *L. salmonis* if they cannot support motile and reproductive stages. A better explanation is that the environment in the BA has become saturated with sea lice larvae that sticklebacks have become "sink" for lice rather than a source for *L. salmonis.*

To conclude, I would like to share a personal observation. During my experience in this project I was exposed to a significant amount of political interference affecting both my work and the work of my colleagues. It is clear that both the BC and federal governments are set on the development of salmon farming and appear willing to discount the good work of good scientists if it does not suit their agenda. Based on my experience in this project, I would suggest that not only has neither government responded appropriately to the possible risks that salmon farms pose to wild salmon, but actions such as countering peer-reviewed science in the public forum with non-published counter-hypotheses threatens to erode the credibility of the scientific process in the public eye. I suggest that it is this issue and not the lack of credible science, which poses the biggest threat to wild salmon in British Columbia.

Conclusion

This work represents one of the first attempts to empirically examine ambient sea lice infection rates on juvenile salmonids. The ambient lice infection rates for juvenile chum and pink salmon were less than one louse per fish and less than two lice per gram. Salmon farms were found to strongly influence the host-parasite relationship between sea lice and juvenile chum and pink salmon. The extent to which the sea lice-salmon relationship was affected by salmon farms was dependent on farm species, farm location, within year variability in fish size, temperature, salinity, and the scale of salmon farming activities within the region. The results from the laboratory and field studies on susceptibility found that juvenile chum were more susceptible than juvenile pink salmon to infection by sea lice; however, the exact mechanism for the observed differences was not identified. Possible reasons for the observed differences could be related to genetically determined susceptibility, mucous differences, lethal lice infection tolerances, or other factors not examined.

The results of this study suggest that the elevated infection rates observed in the BA and other areas present a significant risk to the health of wild salmon. It is suggested that investigations into farm level sea lice contributions be conducted in the BA and other areas where salmon farms operate. In addition, investigation into the lethal lice infection levels for juvenile salmon (especially pink salmon) at early marine life size should also be conducted.

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Appendix 1

Techniques for the field collection and culture of sea lice (*Lepeoptheirus salmonis*)

Field Collection - Sources

Field collections of sea lice were made from commercial fisheries due to the potential for securing large quantities of sea lice. Very small quantities were also collected from recreational fisheries (especially for pink salmon). Commercial seine boats were not a good source of sea lice because the captured salmon spend a lot of time crushed against each other (i.e. while bridling) as well as jumping around in the capture bin, which damaged the sea lice and resulted in high mortality of adult lice.

Commercial gill net boats were the best source of sea lice. Although gill nets do scrape lice some lice off the fish, the ability to take lice of immobile adult salmon that have only been out of the water a very short time results in very little adult lice mortality and good lice larvae production. The only drawback was the time required to collect as gillnet boats only brought in limited numbers per fishery. Pink or chum fisheries were the best source to maximize collection effort. Sockeye or Chinook fisheries were not found to be abundant in sea lice.

Commercial troll boats would also likely be a good source given that they pull fish right out of the water and into the boat. Attempts to collect lice from troll boats were not attempted due to the difficulty of accessing their boats when they are fully fishing (i.e. many lines are deployed from the boat).

Field Collection Techniques

Collection in the field was done with a set of tweezers. One side of the tweezers was gently placed between the gravid female lice and the fish to gently separate them without closing the tweezers. The lice were immediately placed into a collection cooler that was gently aerated with a battery operated aquarium pump and maintained at a temperature between 6 – 13 ^{o}C and a salinity of 30 – 32 ppt. Temperature in the field was maintained using ice but care was taken to not let the temperature fluctuate too much nor drop too low (below 5^{o}C). Signs of healthy lice included swimming and response to changes in light with the opening and closing of the collection cooler.

Transport

Transport of collected lice was accomplished by placing the collection cooler inside larger coolers and regulating the temperature with ice. The larger coolers were placed on something soft (e.g. lifejackets, etc) during road or boat transport as the jarring has been suggested to affect lice health (S. Johnson pers. comm.). If travel time exceeded more than 6 hours, dead individuals were removed regularly. It was noted that live lice often appear dead (i.e. floating in the water, not immediately responding to touch, etc.) when they are not. Dead individuals can be identified by discoloration from their normal hues and ragged appearance (e.g. dark lice will appear light purple). Water changes were conducted every 24 hours in the field or the lab.

Lab Culture

Temperature was stabilized above 10 ^{o}C and salinity must be maintained between 30 – 32 ppt. Dead gravid females were removed continuously and their eggstrings were clipped with a pair of scissors and allowed the opportunity to hatch. Not all clipped eggstrings hatched. Adult females that drop their eggstrings (which happened quite frequently when disturbed) were removed along with the dead ones. The colour of the eggstrings was indicative of how close they were to hatching. The darker the string the closer they are to hatching. Dark eggstrings were always clipped while discretion was used about how many white eggstrings should be allowed per aquarium due to concerns about water quality and nauplii collection.

Every 24 hours collection tanks were water changed and the newly hatched nauplii were removed. Newly hatched nauplii liked to aggregate in the corners of the clear glass aquaria. To remove them, a filter (a piece of 5” diameter PVC with mesh attached) with 100-micrometer mesh was used in conjunction with a siphon. It was very helpful to attach pieces of Styrofoam to the filter for flotation. One end of the siphon was placed in the filter so that water free of nauplii can be siphoned out of the aquaria. Siphoning was stopped once the water level reached a level that could be easily poured into a 700 ml container. The sides of the aquaria were gently sprayed with a spray bottle to ensure all nauplii were collected. Once the remaining water with nauplii was placed into a smaller container, all eggstrings and lice were removed and placed back into the main collection tank. Only the nauplii were placed into a separate tank to develop. No water changes were required for the nauplii tank, nor was aeration. Both of these factors were found to

disturb the development of the nauplii and copepodids. At 10-14 ^{o}C, nauplii development took 2-4 days (Johnson and Albright 1991b). Small sub-samples were collected with a pipette and examined under a dissecting microscope to ensure that all nauplii had hatched into copepodids before use for artificial infection. Copepodids were identified using Johnson and Albright 1991a).

Counting sea lice

Once the nauplii had moulted to the copepodid stage, they were removed from the aquaria and placed into a small container as done previously for the nauplii. Once in the container, a gentle stir created a uniform distribution of lice within the 700ml container. A 10 ml glass pipette was used to sample the 700 ml container. The sub-sample was placed in a small plastic cup. Using a dissecting microscope the number of copepodids in the cup was estimated. This procedure was replicated five times and then the mean of the replicates was taken to get an average per 10 ml sample. The average was then multiplied by the volume of the container (700ml) to get an estimate of the total number of copepodids in the container sampled.

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