First Report of a Sea Louse, *Lepeophtheirus salmonis,* Infestation on Juvenile Pink Salmon, *Oncorhynchus gorbuscha,* in Nearshore Habitat

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Morton, Alexandra, and Rob Williams. 2003. First report of a Sea Louse, *Lepeophtheirus salmonis,* infestation on juvenile Pink Salmon, *Oncorhynchus gorbuscha,* in nearshore habitat. Canadian Field-Naturalist 117(4): 634-641.

High infestation rates of the Sea Louse (*Lepeophtheirus salmonis*) have been reported on juvenile salmonids in Europe since 1989; however, this species has not been reported on juvenile Pacific salmonids until now. Magnitude of Sea Lice infestation was examined in 2001 on juvenile Pink Salmon (*Oncorhynchus gorbuscha*) migrating through a British Columbia archipelago. On average, the 751 juvenile Pink Salmon sampled weighed 2.25 g (± 0.039 SE), were infected with 11.3 (± 0.41 SE) Sea Lice per fish and 6.1 (± 0.24SE) Sea Lice per gram host weight. Fully 75.0% of fish were infected at loads equivalent to or higher than the lethal limit reported for much larger Sea Trout (*Salmo trutta*) post-smolts. Abundance (Kruskal-Wallis statistic = 100.95, $p<0.0001$) and intensity (KW = 70.05, $p<0.0001$) of lice, and mean number of lice/g host weight (K-W = 112.23, $p<0.0001$) were significantly higher in juvenile Pink Salmon in close proximity to salmon farms, than in Pink Salmon distant from salmon farms.

Key Words: Pink Salmon, *Oncorhynchus gorbuscha*, Sea Lice, *Lepeophtheirus salmonis*, British Columbia, aquaculture, salmon farm.

The Sea Louse, *Lepeophtheirus salmonis,* is a common caligid parasite of salmonids throughout the Northern Hemisphere (Kabata 1973, 1979). Low natural abundance and minimal host damage characterise this species (Boxshall 1974; Wootten et al. 1982; Berland 1993; Nagasawa 1987). Epizootics of *L. salmonis* on wild salmonids were rare worldwide (White 1940; Wootten et al.1982; Nagasawa 1987) until 1989, when a series of outbreaks coincident with the presence of salmon farms occurred off the coasts of Ireland (Tully et al. 1993) and Norway (Birkeland 1996).

There has never been an outbreak of *L. salmonis* reported on juvenile Pacific salmon (*Oncorhynchus* sp.). The only record of an epizootic outbreak of this species on Pacific salmon occurred on adult Sockeye Salmon (*Oncorhynchus nerka*) holding at the head of an inlet (where salmon farms existed) due to low freshwater levels (Johnson et al. 1996). Conversely, Parker and Margolis (1964) did report an infestation of the generalist, non-salmonid-specific species, *Caligus clemensi,* on juvenile Pink Salmon (*Oncorhynchus gorbuscha)*, which caused fin erosion.

The life cycle of *L. salmonis* has five phases and 10 stages (Kabata 1972; Johnson and Albright 1991a). Consequently, the approximate age of Sea Lice can be determined from first attachment until adulthood. The interval from hatching to infective capability (copepodid stage) is approximately 4 d at 10ºC and 2 d at 15°C (Johnson and Albright 1991b); therefore host attachment is more likely to occur near the site of larval release (Tully et al. 1993). On adult wild salmon, Sea Lice populations are predominantly adult phase (Pike and Wadsworth 1999); while predominantly juvenile infections are typical of infestations on wild salmonids in salmon-farmed areas (Tully et al. 1993; Bjørn and Finstad 2002).

Once attached, *L. salmonis* enters the first of four stationary chalimus stages. Next it develops into a preadult, where body shape changes into a smaller version of the final form and the louse becomes mobile (Johnson and Albright 1991a). A sudden increase in pathogenicity and host damage occurs when lice mature to the mobile, preadult stage (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997). Grimnes and Jakobsen (1996) and Bjørn and Finstad (1997) reported that 0.75 -1.6 lice/g host weight was lethal to post-smolt Sea Trout (*Salmo trutta*). *Lepeophtheirus salmonis* is intolerant of fresh water (Schram et al. 1998; Hahnenkamp and Fyhn 1985; McLean et al.1990; Johnson and Albright 1991b). When transported upriver on maturing adult salmon, this generation of lice dies, thereby reducing inshore Sea Lice populations.

Virtually all research on *L. salmonis* pertains to Sea Trout, Atlantic Salmon (*Salmo salar*) and Arctic Charr (*Salvelinus alpinus*); however, Pink Salmon have a markedly different life cycle than these species. Pink Salmon have a fixed two-year life span (Heard 1991) and enter the marine environment in spring (March – May) at approximately 3.5 cm fork length (Heard 1991) and 0.24 g (Bailey et al. 1975). Pink Salmon thus enter seawater four to five times shorter than Atlantic salmon (Scott and Crossman 1973) and with a much lower mass. Atlantic Salmon enter seawater at approximately 30 g (Poole et al. 2003). Pink Salmon disperse rapidly from estuarine environments (Pearcy 1992), forming dense surface schools (Parker 1965) in the top few centimetres of the water column (Healey 1980). They exhibit strong shoreward orientation (Healey 1967,

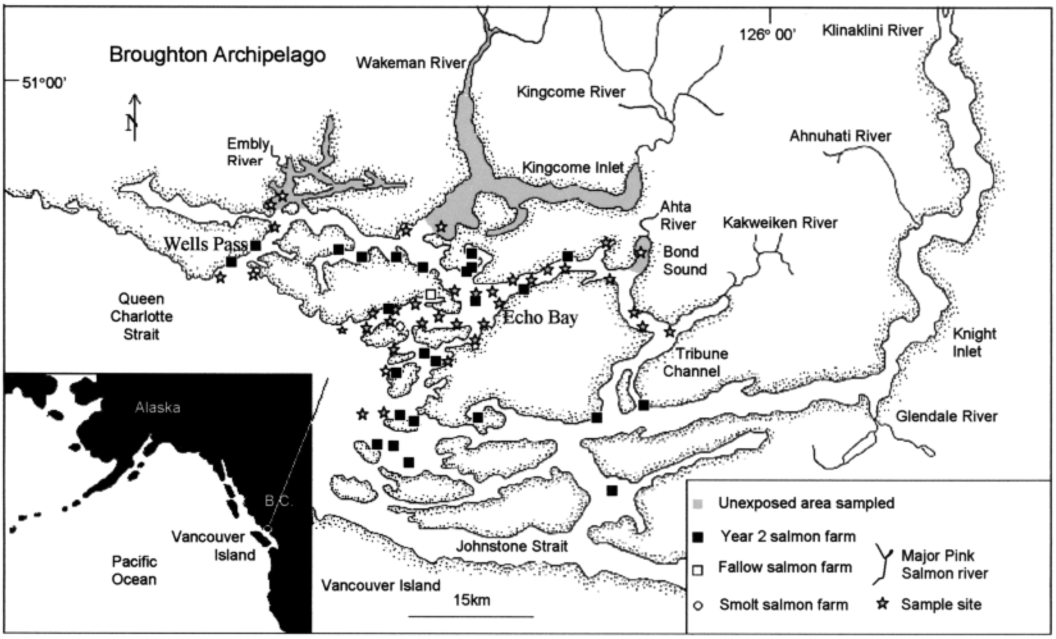

FIGURE 1. Map of the Broughton Archipelago, British Columbia, study area showing the eight major Pink Salmon-producing rivers, location of salmon farms and general areas where one or more sampling sites were located.

1980) and remain nearshore until they grow to approximately 6.0-7.0 cm fork length (Cooney et al. 1978*). Once reaching a length of 6.0-7.0 cm, Pink Salmon move offshore (LeBrasseur and Parker 1964; Cooney et al. 1978*). This transition, accompanied by a shift to lower lipid levels (Parker and Vanstone 1966), is considered "smolting" in Pink Salmon. Hereafter, Pink Salmon fry and smolts will be referred to collectively as juveniles.

In early June 2001, a local fisherman delivered a 5 cm juvenile Pink Salmon to us that was heavily infested with *L. salmonis*. Our response was this preliminary survey of juvenile Pink Salmon throughout the Broughton Archipelago to document the occurrence of this unusual infection. Here we report the results of a spontaneously organized survey of seaward-migrating Pink Salmon juveniles in the marine nearshore habitat of the Broughton Archipelago.

Methods

This study was conducted in the Broughton Archipelago, a ca. 400 km^2 network with 90 km of narrow passages and 200 km of inlets flushed by a 3.6 m mean tidal exchange. The Broughton Archipelago is a major natural production area for all native Pacific salmon species (*Oncorhynchus* sp.), except Sockeye. The eight major Pink Salmon rivers in the Broughton Archipelago collectively produce the fish referred to by Fisheries and Oceans Canada as the Area 12 Mainland Pink stock. This stock is a highly productive portion of even-year cycle Pink Salmon in southern British Columbia. Adult Pink Salmon returns to these eight rivers during 2000 totalled 3.6 million and over one-half of all Pink Salmon spawning escapement in southern British Columbia (PFRCC 2002*).

There were 26 Atlantic Salmon farm sites located in the Broughton Archipelago in 2001 (Figure 1). Sea Lice infestation of wild salmonids has occurred at some interval following major salmon farming activity in most salmon farmed areas (Anonymous 1997*). Epizootics on wild salmonids are often concurrent with Sea Lice outbreaks on farmed fish (Mackinnon 1997) and the effect of Sea Lice on wild salmonids in the vicinity of salmon farms seems particularly acute within long fjord/inlet-type environments (Holst et al. 2002*). Contrary to reporting requirements in other countries, the number of Sea Lice on farm salmon is not available in British Columbia.

Juvenile Pink Salmon were collected from schools spotted along the shoreline, using a 45 cm diameter dip net, fit with 5 mm knotless mesh on a 2.45 m pole, from a shallow draft 7.5 m boat (as per Bailey et al. 1975). This gear-type had the advantage of being available for a quick response to the observed infestation and allowed access and capture of juvenile Pink Salmon in the shallow waters where this species is found during its most juvenile life-stages. A dip net could have introduced bias by selecting for weaker, more heavily infested fish; however, while some bias can be assumed with any gear type, it remained constant over time and among all exposure categories. Sampling took place between 12 June and 16 August 2001 at 46 sites, which were selected to provide reasonable geographic coverage of the study area, and to intercept all potential salmon migrating out from rivers in Knight Inlet, Kingcome Inlet, Tribune Channel and Wells Pass (Figure 1).

Reports of epizootics of *L. salmonis* on wild juvenile Atlantic salmonids (Sea Trout and Atlantic Salmon) specifically near fish farms (Tully et al. 1993; Birkeland 1996) prompted us to classify our sample sites post-hoc into three categories – *unexposed* (3-5.5 km "upstream" from all salmon farms, relative to direction of the juvenile salmon migration – 5 sites); *exposed* (within 60 m of Year 2 Atlantic Salmon pens, or where fish could only have swum past pens – 38 sites); and *smolt* (near a Year 1 farm where Atlantic Salmon had been in the saltwater for less than 6 months – 3 sites). Unexposed sites were comparable (e.g., not brackish) to exposed and smolt sites except for proximity to farms. The samples were collected in such a way as to provide good spatial coverage of the study area (Figure 1), in order to assess whether the infestation was restricted to the one fish presented to us. Our subsequent classification, to test whether variation in lice infestation could be explained partly due to proximity to fish farms, created an unbalanced sampling design for this aspect of the pilot study. This imbalance in number of sites between categories occurred for two reasons. Most of the Broughton Archipelago falls into the *exposed* category due to the high density of farms in the region. Secondly, this spontaneous survey was initiated after the juvenile Pink Salmon migration was well underway, with very few fish remaining in the more eastern, *unexposed* regions closer to the rivers. Collection sites were dispersed through the archipelago to include migration routes from all eight major Pink Salmon-producing rivers (Embly, Wakeman, Kingcome, Ahta, Kakweikan, Glendale, Ahnuhati and Klinaklini) (Figure 1), the waters adjacent to 21 Atlantic salmon farm sites and the seaward edge of the archipelago at Queen Charlotte Strait.

Each salmon was placed in a Whirl-pak™ specimen bag, and put on ice. Fish were weighed and measured (fork length). The species, number and age class of lice (using Johnson and Albright 1991a) were recorded using a 30× stereoscope after Bjørn and Finstad (1998). Specimens of the non-salmon-specific Sea Louse, *Caligus clemensi*, were distinguished from *L. salmonis* using the taxonomic key provided by Kabata (1972), counted, but were rare and not used in the analyses presented here.

We compared prevalence (percentage of fish with Sea Lice, *L. salmonis*), abundance (louse count per fish), and intensity (louse count per infected fish) (Margolis et al. 1982) among exposure categories. Box plots were produced in S-Plus 2000 (Release 3, MathSoft Inc.) to contrast the variation in lice counts (per fish and per gram host mass) with respect to exposure category. If any Kruskal-Wallis (K-W) test showed that the response differed among exposure category at the p=0.05 level, then Dunn's post-test was conducted to determine which pairs of exposure categories differed (Zar 1996).

Results

We sampled 751 juvenile Pink Salmon in waters that averaged 12.1 ºC (±0.066SE), collecting mass, and length and Sea Lice counts. Overall, on average the fish hosted 11.3 (± 0.41SE) Sea Lice per fish and 6.1 (± 0.24SE) lice per gram host weight. Mean length was 5.9 cm (±0.034SE; range 2.8 to 10.4 cm) and mass was 2.3 g (±0.039SE; range 0.21 to 10.78 g) (Table 1). Fully 75.0% were infected at loads equivalent to or higher than 1.6 Sea Lice per gram of host mass.

We counted 8206 *L. salmonis*, with counts per fish ranging from 0 to 69. The most juvenile (copepodid

TABLE 1. Abundance, intensity, length and mass were compared using Kruskal-Wallis tests (the non-parametric equivalent of a one-way ANOVA). Results of Dunn's post-tests (Zar, 1996) for pairwise comparisons are given in the text.

	Exposed to Year-two salmon farm	Exposed to Year-one smolt farm	Unexposed to farms	K-W test (p-value)
prevalence (%)	98.4	90.6	68.4	
Abundance	12.3	4.2	1.9	100.95 (<0.0001)
(SE)	(0.45)	(0.61)	(0.33)	
Intensity	12.5	5.2	2.7	70.05 (<0.0001)
(SE)	(0.45)	(0.65)	(0.38)	
% of fish with ≥ 1.6 lice/g body weight	81.2	39.6	15.7	
mean length (cm)	5.9	6.2	6.4	14.89 (0.0006)
(SE)	(0.036)	(0.13)	(0.15)	
mean mass (g)	2.2	2.7	2.9	40.683 (<0.0001)
(SE)	(0.041)	(0.13)	(0.17)	
n (fish)	660	53	38	–

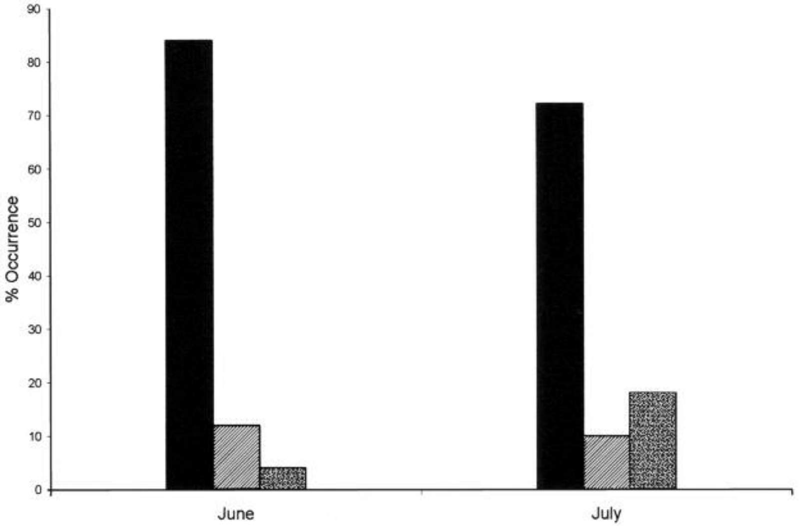

FIGURE 2. The percent occurrence of copepodid/chalimus lice (black), pre-adult (diagonal lines), and adult (spotted) per month.

and chalimus I-IV) stages dominated the lice population throughout June and July. These were only nine samples from August and so were not included (Figure 2). Adult lice were observed beginning on Day 21 of our study.

Lice burdens varied significantly among exposure categories (Table 1, Figure 3). Variation in median louse abundance was greater among the three exposure categories than would be expected by chance (Kruskal-Wallis (KW) test statistic 100.95; $p<0.0001$). Dunn's post-tests indicated that all three pairwise comparisons showed significant differences. Louse abundance on juvenile Pink Salmon sampled near farms holding adult Atlantic Salmon was significantly higher than that seen on fish sampled at unexposed sites (Mean Rank Difference (MRD) 183.5; $p<0.001$) or at farm sites holding Atlantic Salmon smolts (MRD 306.06; $p<0.001$). Fish from smolt-only sites had significantly higher louse abundance than fish from unexposed sites (MRD 122.47; $p<0.05$).

Of infected fish, median louse intensity varied more among exposure categories than one would expect by chance (KW 70.05; $p<0.0001$). Dunn's post-tests indicated that intensity was higher in fish sampled near farms containing adult Atlantic Salmon than in fish sampled near smolt-only sites (MRD 172.68; $p<0.001$) or unexposed sites (MRD 272.9; $p<0.001$). However, the difference in intensity between fish sampled near smolt-only and unexposed sites was not significantly different (MRD 100.22; $p>0.05$).

The number of lice per gram host weight (another response variable commonly used to report the magnitude of Sea Louse infestation) varied significantly among exposure categories (Figure 3; KW 112.23, $p<0.0001$). The median value of 4.7 lice per gram host mass of fish sampled near farms housing adult Atlantic Salmon was significantly higher than that of fish sampled near smolt farms (median 1.3 lice per gram host mass; MRD 203.59, $p<0.001$) and unexposed sites (median 0.6 lice per gram host mass; (MRD 315.18, $p<0.001$). Median values of smolt-only and unexposed samples were significantly different from one another ((MRD 111.59, $p<0.05$).

The size of juvenile Pink Salmon also varied among exposure categories (Table 1). Median length of juvenile Pink Salmon showed higher variation among exposure categories than one would expect by chance (KW 14.89; $p=0.0006$). Fish exposed to farms housing adult Atlantic Salmon were shorter than fish sampled at unexposed sites (MRD -119.28; $p<0.01$), but they were not significantly shorter than fish sampled near smolt farms (MRD -67.446; $p>0.05$). Smolt-only samples did not differ significantly in length than those sampled at unexposed sites (MRD -51.836; $p>0.05$). Variation in median mass of fish also showed higher variation among exposure categories than one would predict by chance (KW 40.683; $p<0.0001$). Fish sampled near adult Atlantic Salmon farms weighed significantly less than either fish sampled near smolt sites (MRD -131.18; $p<0.001$) or unexposed sites (MRD -181.99; $p<0.001$). No significant difference was found in mass of fish sampled near smolt farms or unexposed sites (MRD -50.809; $p>0.05$). The mass of salmon carrying <1.6 lice/g (mean 3.1 g ± 0.10SE; median 2.7 g; range 0.82 to 10.78 g) was significantly higher than that of salmon carrying ≥1.6 lice/g host mass (mean 2.0g ± 0.031SE; median 1.89 g; range 0.21 to 7.38 g) (Mann-Whitney U-statistic = 25004, $p<0.0001$). This effect is confounded by the non-identical distribution of fish lengths in the two samples; however, fish

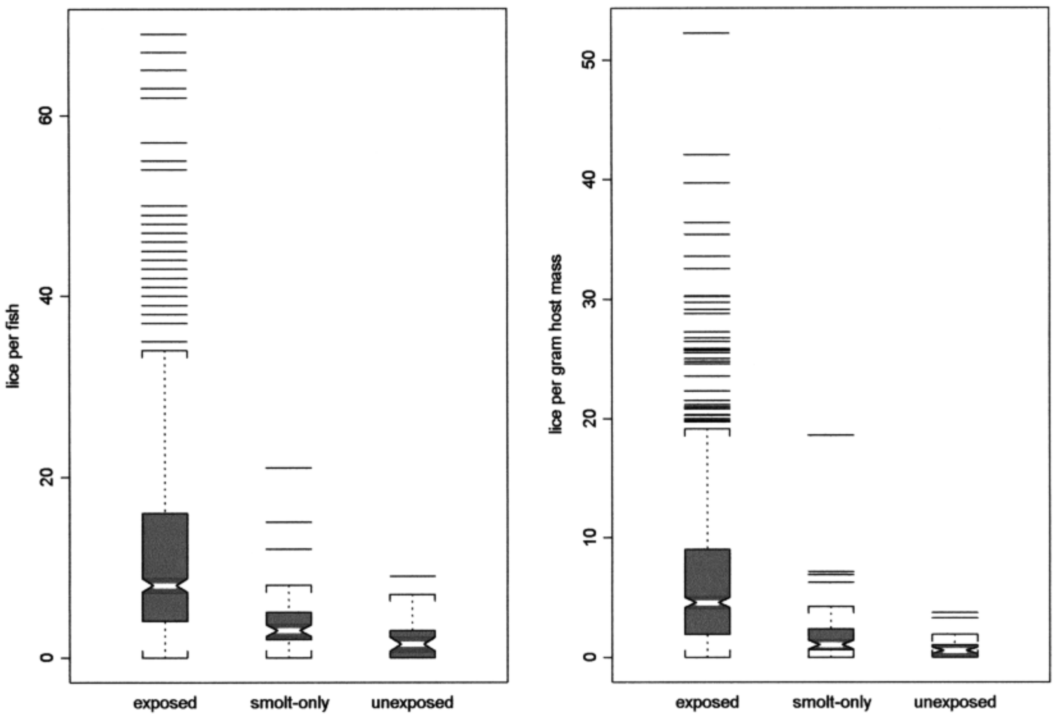

FIGURE 3. Boxplots showing range of lice abundance (left) and number of lice/g host weight (right) by exposure category. The line at the notch represents the median. Whiskers are drawn to 1.5 × (Inter-Quartile Range). Values beyond this are identified individually by horizontal lines. The skew indicated by these boxplots shows the need for non-parametric analyses.

carrying ≥1.6 lice per gram host mass weighed less, on average, than fish of the same length that carried less than 1.6 lice per gram host mass (Figure 4).

Discussion

This is the first report of *L. salmonis* infection on juvenile Pink Salmon. Our data suggest the infection was related to the presence of net-pen salmon farms in the immediate area (Table 1, Figure 3). The rate of infestation almost certainly reduced survivorship of the infected fish by compromising growth (Figure 4). The large percentage of infected fish raises concern for the health of the stock (Table 1).

Most research on *L. salmonis* infestation of juvenile wild salmonids pertains to Sea Trout, Atlantic Salmon and Arctic Charr. Finstad et al. (2000) estimated that juveniles of these species can tolerate up to 10 Sea Lice per fish. Observations on 3000 post-smolt Atlantic Salmon, in open oceanic waters from 1991 to present, did not find a single fish with more than 10 adult lice (Holst et al. 1992*). Among these, fish with close to 10 lice were physiologically compromised (reduced growth and low hematocrit values) (Holst, personal communication).

While wild Atlantic salmonids spend a year or more in fresh-water and the Atlantic Salmon, for example, enters sea water at approximately 30 g (Poole et al. 2003), Pink Salmon go to sea at approximately 0.24 g (Bailey et al. 1975). Given that experimental results show that young salmonids can tolerate approximately one louse per gram of body weight (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997), it is reasonable to conclude that Pink Salmon of the weights we recorded were imperilled by the lice loads we report here (Figure 3). Note that this study took place well after the peak months of saltwater entry (Heard 1991). On average, the Pink Salmon we sampled were an order of magnitude heavier than the reported mass at which Pink Salmon leave fresh water. If the lice we report were present when the fish first entered the marine environment, the number of lice per gram host weight would have been proportionally larger. Thus, the figures we present may be underestimates of the magnitude of the infestation. Pink Salmon fry are subject to such heavy predation by yearling Coho when the Pink Salmon first put to sea that their margin of survival at this life-phase is considered slight and achieved only through rapid growth (Parker and LeBrasseur 1974). Thus any impact reducing growth could be considered adverse.

Local fishermen also brought in three infected Chum (*O. keta*) smolts (average, 51 Sea Lice/g), three in-

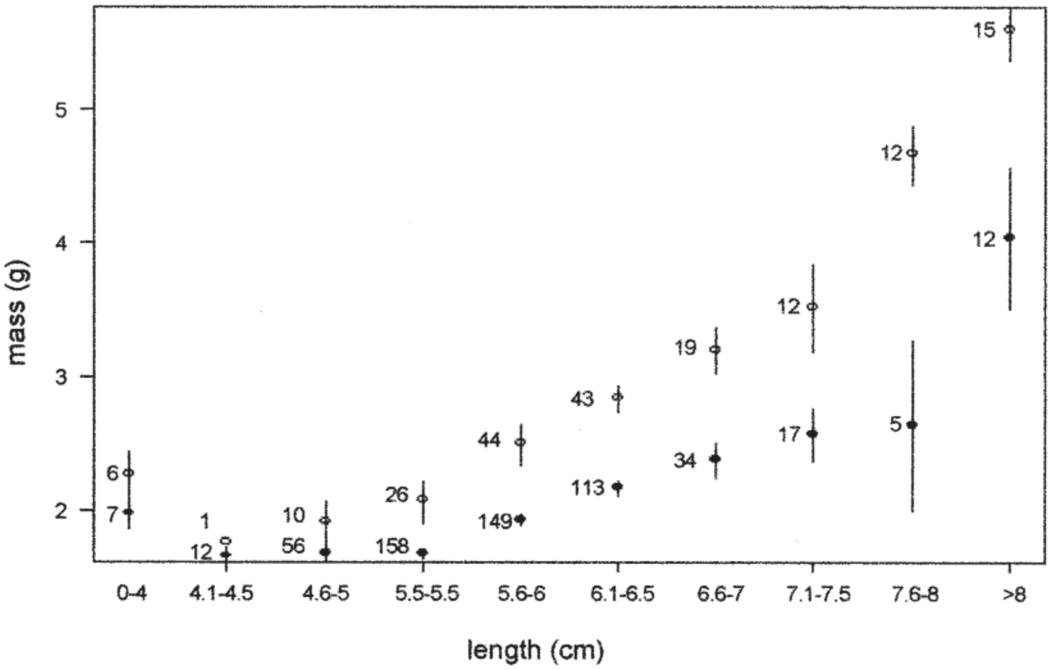

FIGURE 4. Scatterplot showing masses of fish of varying lengths. Empty circles represent fish with <1.6 lice per gram host mass, and filled circles represent fish with ≥1.6 lice per gram host mass, with standard error bars superimposed. The numbers to the left of the data points indicate the number of fish in each sample.

fected Coho (*O. kisutch*) (average, 24 Sea Lice/g) and two Chinook (*O. tshawytscha*) Salmon smolts (average, 29 Sea Lice/g), suggesting that this infestation was not limited to Pink Salmon. Unfortunately, our collection permit restricted species and gear-type, therefore we were unable to collect additional samples to quantify the extent of infestation among other salmonid species. However, this anecdotal evidence indicates that Sea Lice also were infecting other ecologically and economically important salmon species.

As we counted the Sea Lice, we noted extensive damage to the skin of the host juvenile Pink Salmon. Common marks included paired, pin-prick perforations through the skin. There were areas where the skin was removed and chalimus-stage lice affixed to the exposed flesh. Black spots were visible corresponding to reports of darkened localized cellular response of accumulated melanocytes (Pike and Wadsworth 1999). Bleeding at the base of the fins was observed frequently.

We infer that the source of the Sea Lice must have been located within the Broughton Archipelago for several reasons. First, the fish sampled were moving generally west, from rivers deep within the archipelago's inland reaches (Figure 1) and thus were unlikely to have been exposed to larval lice beyond the archipelago. Secondly, such young salmon, averaging 5.9 cm and 2.25 g, but some with yolk sacs still visible, would seem unlikely to have migrated from outside the 400 km^2 study area. Finally, Sea Lice infestations that are dominated by the short-lived juvenile life-history stages have been shown to be indicative of a local source of gravid female lice (Costelloe et al. 1996). Throughout this study, 78% of the lice we sampled were juveniles (copepodid and chalimus), which endure only a matter of days (Johnson and Albright 1991b) (Figure 2). Thus, gravid female Sea Lice had to be present locally and continuously. Since *Lepeophtheirus salmonis* is salmon–specific this can only mean a large, host population of salmon existed in the Broughton throughout this study. No large wild salmon population could be identified in the study area during this time period (Neidrauer, personal communication). While there are no reports made public on the number of farm salmon in the Broughton Archipelago, there were 26 salmon farms, some with over one million stationary Atlantic Salmon per farm (Nayler et al.).

The inability of *L. salmonis* to tolerate freshwater suggests that a mechanism has evolved whereby young salmon do not encounter this parasite until farther offshore, when they have attained a more robust body size than that observed in this study. If the Broughton Archipelago is now providing over-wintering habitat for Sea Lice in its nearshore environment, then the efficacy of that natural safety mechanism is compromised. In 2002, when the juvenile salmon examined in this study matured, 98% failed to return to the rivers to spawn (PFRCC 2002*). This Area 12 Mainland Pink Salmon stock collapsed amidst good to excellent Pink Salmon returns elsewhere coast-wide throughout British Columbia (PFRCC 2002*).

While the cause of this crash has not been identified with certainty, the PFRCC (2002*) could not find any fresh water or open-ocean factors that could have affected all of the Broughton Archipelago Pink Salmon runs, and indeed, only the Broughton Archipelago Pink Salmon runs. The available evidence pointed to factors confined to the Broughton Archipelago's nearshore marine environment (PFRCC 2002*). This nearshore environment is both critical Pink Salmon rearing habitat and heavily used by the salmon farming industry. Clearly, identifying the source of the Lice with greater certainty requires additional experimental research. A preliminary step would be to require farmers to report the number of Sea Lice on farm salmon, as farmers in other countries are required to do. However, given that damaging Sea Lice outbreaks have occurred on juvenile wild salmon in many places where salmon farms exist, it would seem reasonable to consider precautionary management of the primary suspect source of the infection, the gravid female lice on farm salmon. Such action might avert potentially irreversible harm to the Area 12 Mainland Pink Salmon stock as science and policy mature to manage this young industry in British Columbia.

Acknowledgments

We thank Jens Christian Holst, Institute of Marine Research (Bergen, Norway), Bill Heard and Alan Walker for their contributions, and Simon Northridge, Sascha Hooker, and several colleagues and three anonymous reviewers who improved earlier drafts of this paper. Thank you also to Glen Neidrauer, (Fisheries and Oceans Canada patrolman, Simoom Sound, BC V0P 1S0) for answering our questions regarding presence of wild salmon stocks. This work was in part supported by Tides Canada. RW was supported by the Jane Marcher Foundation. Collection permit MMM 2001-MMM 2002 was granted to this project.

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Received 30 June 2003
Accepted 8 January 2004