 doi:10.1111/j.1365-2761.2008.00933.x

Early development of resistance to the salmon louse, *Lepeophtheirus salmonis* (Krøyer), in juvenile pink salmon, *Oncorhynchus gorbuscha* (Walbaum)

S Jones, E Kim and W Bennett

Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada

Abstract

This study examined the effect of fish weight on the susceptibility of post-emergent pink salmon to *Lepeophtheirus salmonis* (Krøyer). Three trials were conducted, each with two stocks of pink salmon, *Oncorhynchus gorbuscha* (Walbaum), at starting weights of *c.* 0.3, 0.7 and 2.4 g, respectively. In each trial, duplicate tanks of fish were exposed to 0, 25 (only in Trial 1), 50 or 100 copepodids per fish. Mortality in Trial 1 was *c.* 37%, regardless of stock following exposures to 50 or 100 copepodids. Mortalities occurred up to 26 days after exposure, and more than 80% of the lice on the dead fish were chalimus stages. Infections with adult or preadult lice were observed on *c.* 35% of fish surviving to 37 days after exposure. Mortality was 5% in Trial 2 and there was no mortality in Trial 3. The abundance of *L. salmonis* was lower in Trial 3 compared with Trials 1 or 2. Histological changes in the skin coincident with fish growth included a thickening of the epidermis, infiltration of the dermis with fibroblasts by the end of Trial 1 and the first evidence of scales by the end of Trial 2; scales were evident throughout Trial 3. These results showed that the previously reported innate resistance to *L. salmonis* displayed by pink salmon develops in fish heavier than 0.3 g and appears to be functional by 0.7 g. This resistance coincided with changes to the epidermis and dermis, including the formation of scales. The present results indicate that elevated risk associated with *L. salmonis* infection among migrating post-emergent pink salmon may occur during a relatively brief period before the fish reaches 0.7 g.

Keywords: laboratory exposure, *Lepeophtheirus salmonis, Oncorhynchus gorbuscha,* resistance, size.

Correspondence *Dr S Jones, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia, V9T 6N7, Canada*
(e-mail: joness@pac.dfo-mpo.gc.ca)

Introduction

The salmon louse, *Lepeophtheirus salmonis* (Krøyer), is a parasite of marine salmonids in the northern hemisphere and is found on the skin and occasionally on the gills and within the buccal cavity. The life cycle includes three planktonic stages (two nauplii and an infective copepodid) and eight parasitic stages (the copepodid, four chalimus, two preadult and one adult). Salmon lice cause damage to the host at the site of attachment by feeding on host cells and blood (Johnson, Treasurer, Bravo, Nagasawa & Kabata 2004). Secretion of bioactive salivary compounds, including proteases and prostaglandins (Fast, Burka, Johnson & Ross 2003; Fast, Ross, Craft, Locke, Mackinnon & Johnson 2004; Fast, Johnson, Eddy, Pinto & Ross 2007), may further promote disease. Disease associated with *L. salmonis* has been reported in farmed Atlantic salmon, *Salmo salar* L. (Johnson *et al.* 2004), and less frequently in wild salmon (Johnson, Blaylock, Elphink & Hyatt 1996). While mortality during laboratory infections of Atlantic salmon and sea trout, *S. trutta* L., has been associated with the larger and motile preadult and adult stages (Bjørn & Finstad 1998; Finstad, Bjørn, Grimnes & Hvidsten 2000), clinical signs have also been associated with the earlier developmental stages. The physiological consequences of infection include a stress response, immunological dysfunction and

an inability to osmoregulate resulting in changes to blood electrolytes and anaemia (Grimnes & Jakobsen 1996; Bjørn & Finstad 1997; Bowers, Mustafa, Speare, Conboy, Brimacombe, Sims & Burka 2000; Wagner, McKinley, Bjørn & Finstad 2003).

Laboratory studies have shown that the pattern of *L. salmonis* infection on Pacific salmon, *Oncorhynchus* spp., differs from that observed on salmon of the genus *Salmo* (Johnson & Albright 1992a; Johnson 1993; Fast, Ross, Mustafa, Sims, Johnson, Conboy, Speare, Johnson & Burka 2002), in a way that suggests Pacific salmon possess an elevated resistance to the parasite. In addition, this innate resistance varies among *Oncorhynchus* spp. Thus, coho salmon, *O. kisutch* (Walbaum), are more resistant than Chinook salmon, *O. tshawytscha* (Walbaum) (Johnson & Albright 1992a). Similarly, pink salmon, *O. gorbuscha* (Walbaum), reject the parasite more quickly than chum salmon, *O. keta* (Walbaum), allowing the former to avoid clinical signs of disease (Jones, Kim & Dawe 2006; Jones, Fast, Johnson & Groman 2007). The mechanisms by which juvenile *Oncorhynchus* spp. avoid the consequences of severe *L. salmonis* infection are not well understood, but appear to be associated with inflammation at the site of attachment and the local and systemic elaboration of proinflammatory cytokines (Johnson & Albright 1992a; Fast, Ross, Muise & Johnson 2006; Jones *et al.* 2006, 2007; Jones, Fast & Johnson 2008). The more susceptible Atlantic salmon does not develop an inflammatory reaction at the site of infection. Furthermore, coho salmon injected with hydrocortisone fail to develop this reaction and *L. salmonis* infections are more severe on cortisol-injected salmon (Johnson & Albright 1992b).

Little is known about the effect of host size on resistance to *L. salmonis*. Apparently healthy adult pink salmon maintain heavy burdens of salmon lice as they migrate into coastal waters prior to spawning (Beamish, Neville, Sweeting & Ambers 2005; Beamish, Neville, Sweeting, Jones, Ambers, Gordon, Hunter & MacDonald 2007). Similarly, no mortality was reported among 3- and 20-g pink salmon following laboratory exposure to as many as 735 copepodids per fish (Jones *et al.* 2006, 2007, 2008). Following emergence from the gravel, pink salmon fry of *c.* 300 mg migrate directly to an estuarine environment (Heard 1991) and acquire infections with *L. salmonis* shortly after entry into sea water (Morton *et al.* 2004; Jones & Hargreaves 2007). However, the consequences of lice infections on salmon during this early marine phase are poorly understood. The purpose of the present study was to evaluate the effects of body size and the severity of the copepodid challenge on post-emergent pink salmon fry.

Materials and methods

Collection and maintenance of salmon

Pink salmon were obtained in April 2007 as fry from two stocks: the Quinsam River, Campbell River, British Columbia (BC) and the Glendale River, on the central coast of BC. The Quinsam fry were reared in hatchery trays, whereas the Glendale fry were collected from in-river rotary screw traps and were derived from a naturally spawned, gravel-reared population. The fry were transported in oxygenated ambient fresh water to the Pacific Biological Station (PBS) where they were maintained in 400-L stock tanks supplied with a flow-through mixture of dechlorinated fresh water and sand-filtered sea water. Salinity and temperature were monitored daily using a refractometer and digital thermometer, respectively. No pathogenic bacteria or viruses were detected in a routine health screen of 30 fish from each stock, and fish were monitored twice daily for mortality. The salmon were fed a crumble (0 and 1) ration (Ewos, Canada Ltd., Surrey, BC, Canada) at an average daily rate of 1.2% body weight. The mean weight was determined weekly.

Collection and culture of *Lepeophtheirus salmonis*

Gravid female lice were collected from Atlantic salmon following harvest from commercial net pens near Vancouver Island. The lice were transported in ice-cold sea water to the PBS where groups of dissected egg strings were placed into 3-L beakers containing aerated sea water with a mean salinity of 32.5‰. The sea water was filtered to 1 μm and ultraviolet irradiated prior to use. The beakers were incubated in a bath of flowing sea water with an average temperature of 8.9 °C. Following evidence of hatching, three 5-mL samples were collected daily from each beaker and the number of nauplii and copepodids was determined by direct microscopic examination. An inoculum containing a known number of copepodids was prepared by pooling the contents of the beakers when the number of copepodids was greatest, typically by day

7 or 8 of incubation. Separate inocula were prepared from new batches of eggs for each of the three consecutive trials.

Experimental design

Three exposure trials, each including Quinsam and Glendale stock pink salmon, were initiated with fish weighing *c.* 0.3 g (Trial 1), 0.7 g (Trial 2) and 2.4 g (Trial 3). For 7 days prior to exposure, the salmon were acclimatized to sea water with salinity and temperature as described above. Exposure trials were conducted in 33-L tanks at a density of 20 fish per tank. In Trial 1, copepodid densities of 0, 25, 50 and 100 per fish were used, whereas in Trials 2 and 3, densities of 0, 50 and 100 per fish were used. Duplicate tanks were used for each exposure density. To initiate the exposure, water flow was stopped and the fish were sedated by dissolving 0.07 mg L^{-1} metomidate·HCl (Syndel, Qualicum Beach, BC, Canada) in the tank water. The water volume in each tank was reduced to 3 or 4 L, supplemental aeration provided and the required number of copepodids was added. Water flow was resumed after 2 h. Exposures to copepodids were conducted in darkness and thereafter, the daily photoperiod was maintained at 12 h light and 12 h dark. The unexposed controls were treated the same way with the exception that a volume of sea water alone, equal to that of the copepodid inoculum, was added to the tank during the sedated exposure. A mean temperature of 8.9 °C (range, 7.7–9.6 °C) and a mean salinity of 32.5‰ (range, 28–34‰) was maintained throughout all three trials.

Following exposure, fish were fed and examined twice daily for mortality. At six and 12 days post-exposure (dpe), all fish were sedated and five were randomly removed from each tank and killed in an overdose of MS-222 (Syndel). Length, weight and salmon lice number for each fish were measured and the developmental stage of all lice was determined by microscopic examination. Each fish was bisected immediately anterior to the dorsal fin and the anterior portion flash frozen in liquid nitrogen for gene expression studies, to be reported elsewhere. The posterior portion was fixed in 10% neutral buffered formalin. At 14 dpe, fish in the duplicate tanks were consolidated into a single tank per treatment and monitored as before until the end of the trial 36 dpe (37 dpe in Trial 1), at which time fish were killed in MS-222 and sampled as described above.

Histological examination

The fixed samples were sectioned at 5 μm, mounted on glass slides and stained with haematoxylin and eosin (H & E). Five to 15 transverse serial sections from the same site anterior to the anal fin and posterior to the dorsal fin were obtained from ten fish per treatment. Stained sections were examined using an Axio Imager A1 compound microscope and digital images were obtained using the AxioCam MRc (Carl Zeiss, Canada Ltd., Toronto, ON, Canada). Changes to the skin associated with scale development and the attachment of salmon lice at or near the lateral line were documented.

Statistical analysis

Prevalence, mean abundance and mean intensity were calculated according to Bush, Lafferty, Lotz & Shostak (1997). Cumulative mortality was calculated as a percentage of the population that died before the first (n = 40), second (n = 30) and third (n = 20) sample days. The significance of differences in mean salmon weight was compared using 2-sample t-tests. The Kruskal–Wallis test was used to test the significance of differences in mean intensity (or abundance) of *L. salmonis*. Chi-squared tests were used to test for the significance of differences in mortality, in the prevalence of *L. salmonis* and in the proportion of parasite developmental stages. In all the cases, $P \leq 0.05$ was considered significant.

Results

Trial 1

Mean weights (±SE) at the onset of the trial were 0.31 ± 0.01 g (range, 0.16–0.59 g) for Quinsam salmon and 0.25 ± 0.01 g (range, 0.12–0.49 g) for Glendale salmon ($P < 0.01$). At 37 dpe, Quinsam salmon exposed to 100 copepodids weighed less (P = 0.02) than unexposed salmon (Table 1). At the end of the trial 37 dpe, Quinsam salmon (1.29 ± 0.04 g) continued to weigh more than Glendale salmon (0.99 ± 0.03 g) ($P < 0.01$).

Salmon lice were observed on Quinsam and Glendale salmon following exposures to 25, 50 or 100 copepodids per fish (Table 1). At neither 6 nor 12 dpe was there a significant difference among exposure levels in the prevalence of *L. salmonis* on Quinsam salmon. However, at both days, the prevalence was lower on Glendale salmon exposed

Table 1 Infection with *Lepeophtheirus salmonis* on Quinsam and Glendale River pink salmon, following laboratory exposure at four copepodid densities (Trial 1)

Stock	Day[a]	Density[b]	*n*[c]	Weight[d] (g)	Prev. (%)	Intensity[e] (range)	Mortality[f] (%)
Quinsam	6	0	10	0.57 ± 0.04	0	0	–
		25	10	0.52 ± 0.03	80.0	1.4 ab (1–2)	–
		50	10	0.47 ± 0.04	90.0	3.4 (1–7)	–
		100	10	0.53 ± 0.04	100.0	4.3 (2–7)	1/40 (2.5)
	12	0	10	0.63 ± 0.03	0	0	–
		25	10	0.63 ± 0.04	70.0	1.4 ab (1–2)	–
		50	10	0.65 ± 0.05	90.0	3.7 (1–6)	–
		100	10	0.56 ± 0.02	100.0	4.7 (1–15)	1/29 (3.3)
	37	0	10	1.33 ± 0.08	0	0	–
		25	19	1.35 ± 0.06	0	0	1/20 (5.0)
		50	13	1.37 ± 0.08	23.1	2.0 (1–3)	7/20 (35.0)
		100	12	1.05 ± 0.08 c	41.7	2.0 (1–3)	6/18 (33.3)
Glendale	6	0	10	0.40 ± 0.04	0	0	1/40 (2.5)
		25	10	0.35 ± 0.02	60.0 b	2.2 b (1–4)	–
		50	10	0.30 ± 0.04	90.0	3.3 (1–6)	–
		100	10	0.33 ± 0.03	100.0	4.9 (1–8)	1/40 (2.5)
	12	0	10	0.42 ± 0.05	0	0	–
		25	10	0.48 ± 0.01	60.0 b	2.8 b (1–5)	–
		50	10	0.39 ± 0.02	90.0	2.4 b (1–6)	3/30 (10.0)
		100	10	0.49 ± 0.02	100.0	6.3 (1–11)	3/29 (10.3)
	37	0	10	1.00 ± 0.04	0	0	–
		25	18	1.07 ± 0.08	27.8	1.4 (1–2)	2/20 (10.0)
		50	12	0.88 ± 0.06	50.0	1.2 (1–2)	5/17 (29.4)
		100	11	0.98 ± 0.08	36.4	1.3 (1–2)	5/16 (31.3)

Prev. = prevalence.
[a]Days after exposure, [b]copepodids per fish, [c]sample size, [d]mean weight at sampling (±SE), superscript indicates significantly different from 0 exposure, [e]mean intensity, [f]cumulative mortality within 0–6, 7–12 and 13–37 day intervals: i.e. number dead/population size at the beginning of interval (per cent). Online: a = significantly different from 50 copepodids per fish, b = significantly different from 100 copepodids per fish, c = significantly different from 0 copepodids per fish.

to 25 compared with 100 copepodids ($P = 0.03$ in both cases) (Table 1). At no exposure level was there a significant difference in the prevalence of *L. salmonis* between Quinsam and Glendale River pink salmon at 6, 12 or 37 dpe.

At 6 and 12 dpe, the mean intensity was higher among salmon exposed to 100 compared with 25 copepodids, both for Quinsam ($P = 0.00$ and 0.01, respectively) and Glendale salmon ($P = 0.01$ and 0.03, respectively) (Table 1). At these times, the intensity of lice on Quinsam salmon exposed to 50 copepodids was higher than on fish exposed to 25 copepodids ($P = 0.00$ and 0.01, respectively). Compared with Quinsam salmon, the intensity on Glendale salmon was higher at 12 dpe in fish exposed to 25 copepodids ($P = 0.05$) and lower at 37 dpe in fish exposed to 50 copepodids ($P = 0.04$). No copepods were observed on unexposed control salmon. Only copepodids were observed at 6 dpe, and chalimus I and II predominated at 12 dpe (Table 4). Differences in the proportion of developmental stages between stocks were not significant ($P < 0.05$).

During the trial, a total of 36 salmon died, 16 from the Quinsam and 20 from the Glendale stocks. One Quinsam salmon died during each of the first two intervals (0–6 and 7–12 dpe) (Table 1). During the third interval (13–37 dpe), mortality was higher in fish exposed to 50 or 100 copepodids compared with those exposed to 25 copepodids ($P = 0.02$ in both cases) (Table 1). A total of two Glendale salmon died during the first interval, one following exposure to 100 copepodids and one unexposed control. During the second interval, six salmon died and there was no difference in mortality between those exposed to 50 or 100 copepodids ($P < 0.01$). Overall mortality during the second interval was higher among exposed Glendale compared with exposed Quinsam salmon ($P = 0.05$). During the third interval, there was no difference in mortality among Glendale salmon exposure groups ($P > 0.10$) and there was no difference in mortality between stocks ($P = 0.85$). The total cumulative mortality among Quinsam and Glendale salmon was *c.* 37% following exposure to 50 or 100 copepodids per fish and 7.5% following exposure to 25 copepodids per fish (Fig. 1). No mortalities occurred after 26 dpe in either stock, and 80.9% of lice on dead fish were the chalimus IV stage or earlier. Preadult (10.3%) and adult (89.7%) lice

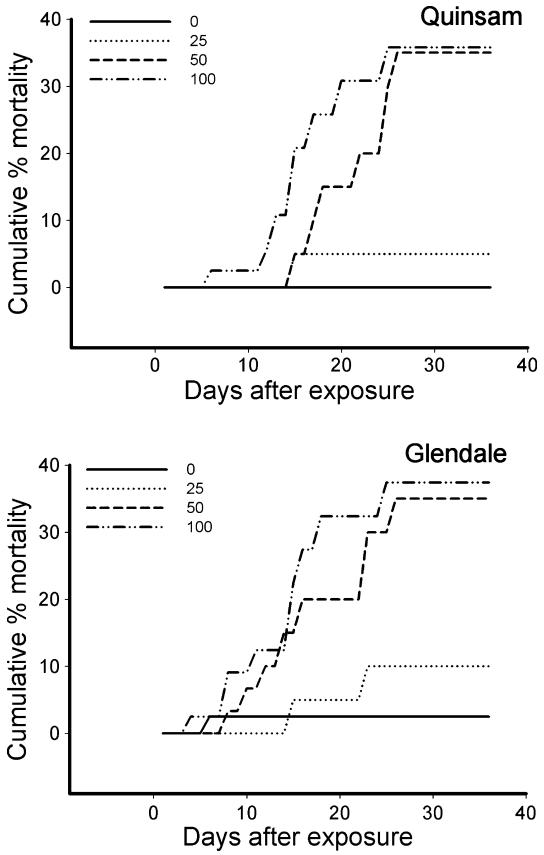

Figure 1 Mortality among Quinsam River and Glendale River pink salmon, following laboratory exposures to four densities of *Lepeophtheirus salmonis* (Trial 1).

were observed on 32% of the 25 surviving Quinsam salmon (50 and 100 exposure groups) and on 37% of the 41 surviving Glendale salmon (25, 50 and 100 exposure groups) (Table 4).

Trial 2

Mean weights at the onset of the trial were 0.71 ± 0.02 g (0.31–1.39 g) for Quinsam salmon and 0.69 ± 0.02 g (0.36–1.06 g) for Glendale salmon ($P = 0.36$). At 12 dpe, Glendale salmon exposed to 100 copepodids weighed less than unexposed salmon ($P = 0.04$) (Table 2). At 36 dpe, Quinsam salmon exposed to 100 copepodids weighed less than unexposed salmon ($P = 0.05$). At the end of the trial 36 dpe, Glendale salmon (2.66 ± 0.09 g) weighed more than Quinsam salmon (2.42 ± 0.08 g) ($P = 0.05$).

Salmon lice were observed following exposures to 50 or 100 copepodids per fish. At 6 dpe, there was no significant difference in prevalence of lice between those exposed to 50 or 100 copepodids, regardless of stock (Table 2). At 12 and 36 dpe, the prevalence was lower on Quinsam salmon exposed to 50 compared with 100 copepodids ($P = 0.01$ and 0.02, respectively). On Glendale salmon, there was no significant difference in prevalence between the 50 and 100 copepodid exposure groups at 12 or 36 dpe. The prevalence on Quinsam salmon was lower than that on Glendale salmon 12 days after exposure to 50 copepodids ($P = 0.03$).

At 6 dpe, the intensity was lower on Quinsam salmon exposed to 50 compared with 100 copepodids ($P = 0.01$) (Table 2). However, there were no other significant differences in intensity because of exposure level, regardless of time or stock, nor were there differences in intensity between stocks. No lice were observed on unexposed control salmon.

The pattern of parasite development was very similar to that described in Trial 1 and there were no differences in the proportion of developmental stages ($P < 0.05$) between stocks (Table 4).

Two fish died during the 36-day trial, representing a cumulative mortality of *c.* 5% in both stocks (Table 2). The dead fish were infected with 13 (eight chalimus III and five chalimus IV) and zero lice, respectively. No unexposed controls died.

Trial 3

Mean weights at the onset of the trial were 2.46 ± 0.06 g (1.16–4.82 g) for Quinsam salmon and 2.37 ± 0.04 g (1.45–3.50 g) for Glendale salmon ($P = 0.18$). At 36 dpe, Quinsam salmon exposed to 100 copepodids weighed more than unexposed salmon ($P = 0.01$) (Table 3). At the end of the trial 36 dpe, the weight of Quinsam salmon (5.75 ± 0.18 g) was not significantly different from that of Glendale salmon (5.45 ± 0.16 g) ($P = 0.22$).

Salmon lice were observed on salmon following exposures to 50 and 100 copepodids per fish. At 6 dpe, the prevalence was lower on Quinsam and Glendale salmon exposed to 50 compared with 100 copepodids ($P = 0.00$ in both cases) (Table 3). By 36 dpe, one louse was observed on one fish in each of the exposure groups in both stocks. Differences in prevalence between stocks were not significant. No lice were observed on control salmon.

At 6 and 12 dpe, the intensity was lower on Glendale salmon exposed to 50 compared with 100 copepodids ($P = 0.03$ and 0.05, respectively). The

Table 2 Infection with *Lepeophtheirus salmonis* on Quinsam and Glendale pink salmon, following laboratory exposure at three copepodid densities (Trial 2)

Stock	Day[a]	Density[b]	*n*[c]	Weight[d] (g)	Prev. (%)	Intensity[e] (range)	Mortality[f] (%)
Quinsam	6	0	10	0.92 ± 0.04	0	0	–
		50	10	0.97 ± 0.07	90.0	1.6 (1–5)	–
		100	10	0.95 ± 0.05	100.0	4.1 a (1–8)	– b
	12	0	10	1.19 ± 0.09	0	0	–
		50	10	1.10 ± 0.07	30.0	1.7 (1–2)	–
		100	10	1.30 ± 0.07	90.0 a	2.6 (1–4)	–
	36	0	20	2.72 ± 0.15	0	0	–
		50	20	2.25 ± 0.11	5.0	1.0 (-)	–
		100	17	2.30 ± 0.12 c	35.3 a	1.3 (1–3)	1/18 (5.6)
Glendale	6	0	10	1.08 ± 0.06	0	0	–
		50	10	1.04 ± 0.06	80.0	3.0 (1–10)	–
		100	10	0.88 ± 0.08	100.0	3.0 (1–5)	–
	12	0	10	1.22 ± 0.06	0	0	–
		50	10	1.37 ± 0.12	80.0	1.9 (1–3)	–
		100	10	1.03 ± 0.06 c	60.0	2.5 (1–4)	–
	36	0	20	2.79 ± 0.17	0	0	–
		50	19	2.82 ± 0.15	21.1	1.0 (-)	1/20 (5.0)
		100	20	2.37 ± 0.14	35.0	1.3 (1–2)	–

Prev. = prevalence.
[a]Days after exposure, [b]copepodids per fish, [c]sample size, [d]mean weight at sampling (±SE), superscript indicates significantly different from 0 exposure, [e]mean intensity, [f]cumulative mortality within 0–6, 7–12 and 13–37 day intervals: i.e. number dead/population size at the beginning of interval (per cent). Online: a = significantly different from 50 copepodids per fish, b = two fish died on day of exposure, c = significantly different from 0 copepodids per fish.

Table 3 Infection with *Lepeophtheirus salmonis* on Quinsam and Glendale River pink salmon, following laboratory exposure at three copepodid densities (Trial 3)

Stock	Day[a]	Density[b]	*n*[c]	Weight[d] (g)	Prev. (%)	Intensity[e] (range)	Mortality[f] (%)
Quinsam	6	0	10	2.69 ± 0.18	0	0	–
		50	10	2.89 ± 0.12	20.0	1.5 (1–2)	–
		100	10	2.70 ± 0.17	90.0 a	2.7 (1–4)	–
	12	0	10	3.31 ± 0.20	0	0	–
		50	10	3.64 ± 0.30	30.0	1.0 (–)	–
		100	10	2.85 ± 0.16	50.0	1.2 (1–2)	–
	36	0	19	5.13 ± 0.28	0	0	– b
		50	20	5.89 ± 0.32	5.0	1.0 (–)	–
		100	19	6.23 ± 0.31 c	5.3	1.0 (–)	– b
Glendale	6	0	10	2.86 ± 0.24	0	0	–
		50	10	2.46 ± 0.16	30.0	1.0 (–)	–
		100	10	2.29 ± 0.16	100.0 a	2.8 a (1–8)	–
	12	0	10	3.17 ± 0.20	0	0	–
		50	10	3.23 ± 0.21	20.0	1.0 (–)	–
		100	10	2.77 ± 0.25	40.0	2.8 a (2–4)	–
	36	0	20	5.35 ± 0.25	0	0	–
		50	20	5.72 ± 0.27	5.0	1.0 (–)	–
		100	20	5.28 ± 0.29	5.0	1.0 (–)	–

Prev. = prevalence.
[a]Days after exposure, [b]copepodids per fish, [c]sample size, [d]mean weight at sampling (±SE), superscript indicates significantly different from 0 exposure, [e]mean intensity, [f]cumulative mortality within 0–6, 7–12 and 13–37 day intervals: i.e. number dead/population size at the beginning of interval (per cent). Online: a = significantly different from 50 copepodids per fish, b = one fish jumped from a tank in each group, c = significantly different from 0 copepodids per fish.

intensity on Quinsam salmon was lower than that on Glendale salmon 12 days after exposure to 100 copepodids ($P = 0.02$) (Table 3).

The pattern of parasite development was very similar to that observed in Trials 1 and 2 (Table 4) and no mortalities were observed.

Comparisons between trials

Mean weight at the time of exposure, parasite abundance 6 days following exposure to 100 copepodids and cumulative mortality during the third sample interval following exposure to 100

Table 4 Developmental stages of *Lepeophtheirus salmonis*, following laboratory exposures of pink salmon from Quinsam and Glendale River stocks

Trial[a]	Day[b]	Stock	Stage							Total lice
			Co	I	II	III	IV	PAd	Ad	
1	6	Q	85							85
		G	92							92
	12	Q	2	49	39					90
		G	0	66	36					102
	37	Q						1	19	20
		G						3	16	19
2	6	Q	54	1						55
		G	54							54
	12	Q		4	22	2				28
		G		4	24	2				30
	36	Q						3	6	9
		G						8	5	13
3	6	Q	27							27
		G	30	1						31
	12	Q		2	7					9
		G		5	8					13
	36	Q						1	1	2
		G						2		2

Q = Quinsam, G = Glendale, Co = copepodids, I–IV = chalimus 1–4, PAd = preadult, Ad = adult.
[a]All copepodid densities combined, [b]days after exposure.

Table 5 Summary of the abundance of *Lepeophtheirus salmonis*, weight and mortality of Quinsam and Glendale River pink salmon after exposure to 100 copepodids per fish

Trial	Stock	Weight[a]	Abundance[b]	Mortality[c]
1	Quinsam	0.31 ± 0.09	4.3 ± 0.6	6/18 (33.3)
	Glendale	0.25 ± 0.07	4.9 ± 0.6	5/16 (31.2)
2	Quinsam	0.71 ± 0.20	4.1 ± 0.9	1/18 (5.6)
	Glendale	0.69 ± 0.17	3.0 ± 0.5	0/20
3	Quinsam	2.46 ± 0.65	2.4 ± 0.4	0/20
	Glendale	2.37 ± 0.39	2.8 ± 0.7	0/20

[a]At onset of trial, [b]6 days after exposure, [c]between 13 days after exposure and end of the trial. Number dead/population size at beginning of interval (%).

copepodids are summarized for each trial in Table 5. Within each trial, there were no significant differences between stocks in either louse abundance or fish mortality. The combined mortality that occurred during the third interval was lower in Trials 2 and 3 compared with Trial 1 ($P < 0.01$ in both cases). The abundance of lice in both stocks combined was lower in Trial 3 compared with Trial 1 ($P < 0.01$).

Histological assessment of salmon skin

Unless specified to the contrary, the following observations refer to the area of skin immediately adjacent to the lateral line and were not associated with attached *L. salmonis*. In Trial 1 at 6 dpe, the epidermis was three to five cells thick, the dermis was uniformly eosinophilic and fibroblasts were generally absent (Fig. 2a). The hypodermis was of variable thickness and contained a loose network of pale-staining fibres and chromatophores. By 12 dpe, fibroblast infiltration into the dermis, which lacked differentiation into strata, was observed in most specimens. At this time, however, fibroblast infiltration into the dermis of the dorsal and ventral surfaces was not evident. By 37 dpe, the epidermis was approximately nine to 15 cells thick and the dermis had differentiated into fibrous strata containing fibroblasts (Fig. 2b). In Trial 2, at 6 dpe, the structure of the skin was similar to that described for 37 dpe in Trial 1. By 12 dpe, the dermis had further differentiated and scales were observed in six out of ten samples examined (Fig. 2c). Scales were consistently observed by 36 dpe in Trial 2 and in all samples observed in Trial 3. Very few histological sections of skin with associated *L. salmonis* were observed.

Discussion

The key finding of this study was the identification of a relationship between increased body weight, reduced parasite abundance and reduced mortality resulting from the exposure of post-emergent pink

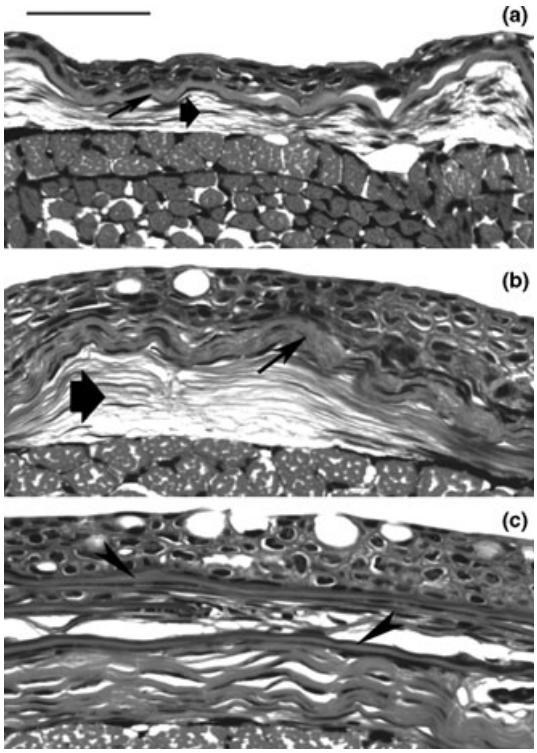

Figure 2 Skin of pink salmon. (a) Skin sample from a 0.48-g fish showing thin epidermis and poorly differentiated dermis (long arrow). Short arrow indicates hypodermis. (b) Skin sample from a 1.03-g fish showing fibroblasts within thickened dermis that is partly differentiated into strata (long arrow). Short arrow indicates hypodermis. Epidermis is well developed. (c) Skin sample from a 3.32-g fish showing scales (arrowheads) along superficial margin of dermis (H & E stain, bar = 50 μm for all images).

salmon to *L. salmonis*. Mortality associated with exposure to 100 copepodids per fish was lower in pink salmon exposed at 0.7 g compared with those exposed at 0.3 g. Similarly, the abundance of *L. salmonis* copepodids was lower in pink salmon exposed at 2.4 g compared with those exposed at 0.7 g. The trials reported here used a method of exposing salmon to copepodids that was described earlier for use on pink and chum salmon ranging from *c.* 3 to 20 g (Jones *et al.* 2006, 2007, 2008). A consistent feature of the earlier work was the absence of mortality, weight loss and reduction in haematocrit among pink salmon exposed to *L. salmonis* copepodids (Jones *et al.* 2007). Furthermore, the abundance of *L. salmonis* on the pink salmon was consistently lower than on concurrently exposed and size-matched chum salmon. Unlike the pink salmon, the exposed juvenile chum salmon weighed less, had reduced haematocrits and produced a transient spike in serum cortisol following exposure (Jones *et al.* 2007). Thus, the pattern of parasite rejection and salmon survival reported here for pink salmon in Trials 2 and 3 was similar to the earlier studies and supports the view that juvenile pink salmon possess an innate resistance to *L. salmonis* that develops in fish heavier than 0.3 g and appears to be functional by 0.7 g.

The identity of the mechanism of resistance to *L. salmonis* that develops in post-emergent pink salmon is not known. There was no evidence in the present study of the inflammatory lesions previously described at the sites of salmon lice attachment among several *Oncorhynchus* spp. (Johnson & Albright 1992a; Fast *et al.* 2002; Jones *et al.* 2007), and thought to be a mechanism of salmon lice rejection. The inability to detect similar lesions in the present study may have been because of the relatively low level of exposure used compared with the 735 copepodids per fish used earlier (Jones *et al.* 2007). The expression of proinflammatory genes in the skin and head kidney or in pools of liver–head kidney from juvenile pink salmon following exposure to *L. salmonis* copepodids (Jones *et al.* 2007, 2008), provided additional support for the inflammation hypothesis. Tissues collected in the present study will be examined for evidence of inflammatory pathways associated with the development of resistance. Resistance to *L. salmonis* may also be coupled to the development of structural complexity within the skin of post-emergent pink salmon. While the most striking feature evident in histology was the development of scales, scale formation was preceded by changes in the dermis including infiltration with fibroblasts and a differentiation into fibrous strata. There was a concurrent thickening of the epidermis. Evidence of dermal fibroblast infiltration was also observed in 2006 Quinsam pink salmon at a mean weight of 1.4 g and scales were completely formed at a mean weight of 2.7 g (W. Bennett, unpublished observations). Thus, the advent of resistance to *L. salmonis* generally coincided with the additional physical barrier afforded by scales. However, the cessation of mortality by day 26 in Trial 1 and the absence of mortality early in Trial 2 when scales were not yet evident indicated that the immature pink salmon skin elicits a mechanism of salmon louse rejection independent of the presence of recognizable scales. The enhanced resistance to *L. salmonis* observed in 0.7 g pink salmon agrees with the onset of immunocompetence reported for

this species between 0.5 and 1.0 g (Johnson, Flynn & Amend 1982).

This study describes the first evidence for mortality among post-emergent pink salmon juveniles following laboratory exposure to *L. salmonis* copepodids. Pink salmon below a weight threshold that occurs between 0.3 and 0.7 g were more susceptible to the parasite. Pink salmon of *c.* 3.0 g retain the ability to resist infection despite feed deprivation (Jones *et al.* 2008). The similarity in the pattern of size-associated resistance between two stocks of pink salmon further suggested that this phenomenon was species specific. The increased mortality among exposed Glendale salmon during the second interval, rather than indicating a stock-specific susceptibility, however, was probably because of the smaller size in Trial 1. In addition to mortality, exposure to 100 copepodids was associated with lower weights in Trials 1 and 2 and with increased weight in Trial 3. The pattern of mortality described here was unlike that reported earlier during laboratory exposures of Atlantic salmon or sea trout (Bjørn & Finstad 1998; Finstad *et al.* 2000; Fast *et al.* 2002). In those studies, mortality coincided with parasite development to the preadult and adult stages, and was thought to be related to more aggressive feeding behaviour of the larger parasites, and possibly to the secretion of bioactive substances by the parasite (Fast *et al.* 2007). In contrast, 81% of lice recovered from dead pink salmon in the present study had developed no further than the chalimus IV stage, suggesting that the mortality was related to factors distinct from those associated with *L. salmonis* on *Salmo* spp. It was interesting to observe that the majority of 0.3 g salmon in both stocks survived exposure to copepodids and that infections with motile lice were observed on many of the survivors. Further research is required to elucidate the mechanisms of mortality associated with immature *L. salmonis* on pink salmon.

The rationale for understanding the effects of *L. salmonis* on juvenile pink salmon stems from concerns that infections derived from farmed Atlantic salmon in British Columbia are deleterious to wild stocks of pink salmon. Laboratory research indicates that pink salmon (Jones *et al.* 2006, 2007, 2008), unlike Atlantic salmon and sea trout, are remarkably tolerant and suffer no mortality as a result of the *L. salmonis* infection. In the present study, this tolerance was shown to be present in post-emergent pink salmon by 0.7 g. In nature, pink salmon display daily growth rates between 3.5% and 7.6% following entry into the marine environment at *c.* 0.3 g (reviewed by Heard 1991). Therefore, the present study identifies a window of susceptibility, defined by temperature and the availability of forage, which in turn define growth rate, during which exposure of post-emergent pink salmon to *L. salmonis* has the potential to result in mortality. Farm-based management to minimize levels of *L. salmonis* during periods when susceptible salmon are migrating in adjacent waters is currently practiced in British Columbia (Saksida, Constantine, Karreman & Donald 2007). The present results indicate that elevated risk associated with *L. salmonis* infection among post-emergent pink salmon may occur during a relatively brief period before the fish reach 0.7 g.

Acknowledgements

The present study was funded by the Fisheries and Oceans Canada (FOC) Aquaculture Collaborative Research and Development Program in collaboration with Marine Harvest Canada. The authors thank Pieter van Will and staff (FOC) for providing fish from the Glendale River and Dave Ewart and staff (FOC) for providing fish from the Quinsam River hatchery. Thanks to Dr B. Nowak, University of Tasmania and to two anonymous reviewers for commenting critically on an earlier draft of this paper.

References

Beamish R.J., Neville C.M., Sweeting R.M. & Ambers N. (2005) Sea lice on adult Pacific salmon in the coastal waters of central British Columbia, Canada. *Fisheries Research* **76,** 198–208.

Beamish R.J., Neville C.M., Sweeting R.M., Jones S.R.M., Ambers N., Gordon E.K., Hunter K.L. & McDonald T.E. (2007) A proposed life history strategy for the salmon louse, *Lepeophtheirus salmonis* in the subarctic Pacific Ocean. *Aquaculture* **264,** 428–440.

Bjørn P.A. & Finstad B. (1997) The physiological effects of salmon lice infection on sea trout post smolts. *Nordic Journal of Freshwater Research* **73,** 60–72.

Bjørn P.A. & Finstad B. (1998) The development of salmon lice *Lepeophtheirus salmonis* on artificially infected post smolts of sea trout (*Salmo trutta*). *Canadian Journal of Zoology* **76,** 970–977.

Bowers J.M., Mustafa A., Speare D.J., Conboy G.A., Brimacombe M., Sims D.E. & Burka J.F. (2000) The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. *Journal of Fish Diseases* **23,** 165–172.

Bush A.O., Lafferty K.D., Lotz J.M. & Shostak A.W. (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83,** 575–583.

Fast M.D., Ross N.W., Mustafa A., Sims D.E., Johnson S.C., Conboy G.A., Speare D.J., Johnson G. & Burka J.F. (2002) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of Aquatic Organisms* **52,** 57–68.

Fast M.D., Burka J.F., Johnson S.C. & Ross N.W. (2003) Enzymes released from *Lepeophtheirus salmonis* in response to mucus from different salmonids. *Journal of Parasitology* **89,** 7–13.

Fast M.D., Ross N.W., Craft C.A., Locke S.J., MacKinnon S.L. & Johnson S.C. (2004) *Lepeophtheirus salmonis*: characterization of prostaglandin E2 in secretory products of the salmon louse by RP-HPLC and mass spectrometry. *Experimental Parasitology* **107,** 5–13.

Fast M.D., Ross N.W., Muise D.M. & Johnson S.C. (2006) Differential gene expression in Atlantic salmon infected with *Lepeophtheirus salmonis*. *Journal of Aquatic Animal Health* **18,** 116–127.

Fast M.D., Johnson S.C., Eddy T.D., Pinto D. & Ross N.W. (2007) *Lepeophtheirus salmonis* secretory/excretory products and their effects on Atlantic salmon immune gene regulation. *Parasite Immunology* **29,** 179–189.

Finstad B., Bjørn P.A., Grimnes A. & Hvidsten N.A. (2000) Laboratory and field investigations of salmon lice [*Lepeophtheirus salmonis* (Krøyer)] infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture Research* **31,** 795–803.

Grimnes A. & Jakobsen P.J. (1996) The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology* **48,** 1179–1194.

Heard W.R. (1991) Life history of pink salmon (*Oncorhynchus gorbuscha*). In: *Pacific Salmon Life Histories* (ed. by C. Groot & L. Margolis), pp. 121–230. UBC Press, Vancouver.

Johnson K.A., Flynn J.K. & Amend D.F. (1982) Onset of immunity in salmonid fry vaccinated by direct immersion in *Vibrio anguillarum* and *Yersinia ruckeri* bacterins. *Journal of Fish Diseases* **5,** 197–205.

Johnson S.C. (1993) A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naïve Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. In: *Pathogens of Wild and Farmed Fish: Sea Lice* (ed. by G.A. Boxshall & D. Defaye), pp. 68–80. Ellis Horwood, Chichester.

Johnson S.C. & Albright L.J. (1992a) Comparative susceptibility and histopathology of the response of naïve Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14,** 179–193.

Johnson S.C. & Albright L.J. (1992b) Effects of cortisol implants on the susceptibility of and the histopathology of the responses of naïve coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14,** 195–205.

Johnson S.C., Blaylock R.B., Elphink J. & Hyatt K.D. (1996) Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 2888–2897.

Johnson S.C., Treasurer J.W., Bravo S., Nagasawa K. & Kabata Z. (2004) A review of the impact of parasitic copepods on marine aquaculture. *Zoological Studies* **43,** 229–243.

Jones S.R.M. & Hargreaves N.B. (2007) The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on pink *Oncorhynchus gorbuscha* and chum *O. keta* salmon in coastal British Columbia. *Journal of Parasitology* **93,** 1324–1331.

Jones S., Kim E. & Dawe S. (2006) Experimental infections with *Lepeophtheirus salmonis* (Krøyer) on threespine sticklebacks, *Gasterosteus aculeatus* L., and juvenile Pacific salmon, *Oncorhynchus* spp. *Journal of Fish Diseases* **29,** 489–495.

Jones S.R.M., Fast M.D., Johnson S.C. & Groman D.B. (2007) Differential rejection of salmon lice by pink and chum salmon: disease consequences and expression of proinflammatory genes. *Diseases of Aquatic Organisms* **75,** 229–238.

Jones S.R.M., Fast M.D. & Johnson S.C.(2008) Influence of reduced ration on *Lepeophtheirus salmonis* infestation and expression of proinflammatory genes in juvenile pink salmon. *Journal of Aquatic Animal Health.* in press

Morton A., Routledge R., Peet C. & Ladwig A. (2004) Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **61,** 147–157.

Saksida S., Constantine J., Karreman G.A. & Donald A. (2007) Evaluation of sea lice abundance levels on farmed Atlantic salmon (*Salmo salar* L.) located in the Broughton Archipelago of British Columbia from 2003 to 2005. *Aquaculture Research* **38,** 219–231.

Wagner G.N., McKinley R.S., Bjørn P.A. & Finstad B. (2003) Physiological impact of sea lice on swimming performance of Atlantic salmon. *Journal of Fish Biology* **62,** 1000–1009.

Received: 7 September 2007
Revision received: 6 December 2007
Accepted: 7 December 2007