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Development of the salmon louse *Lepeophtheirus salmonis* and its effects on juvenile sockeye salmon *Oncorhynchus nerka*

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ABSTRACT: Responses of sockeye salmon *Oncorhynchus nerka* during infection with *Lepeophtheirus salmonis* were assessed in controlled laboratory trials. Juvenile salmon were exposed to 100 copepodids fish^{-1} (Trials 1 and 2) or 300 copepodids fish^{-1} (Trial 3) at mean weights of approximately 40, 80 and 135 g, respectively. Infections occurred on all salmon in all trials, and mean abundances (infection densities) ranged between 3.3 and 19.4 lice fish^{-1} (0.08 and 0.44 lice g^{-1} fish) in Trial 1, between 7.2 and 18.3 (0.09 and 0.22) in Trial 2 and between 19.5 and 60.7 (0.15 and 0.46) in Trial 3. A cumulative mortality of 24.4% occurred in Trial 3. At attachment sites on gills, we observed hyperplasia of basal epithelial cells and fusion of secondary lamellae occasionally associated with a cellular infiltrate. At attachment sites on fins, partial to complete skin erosion occurred, with limited evidence of hyperplasia or inflammation. Scale loss and abrasions coincided with pre-adult lice around 20 d post infection (dpi). Plasma osmolality was significantly elevated in exposed fish in Trials 1 (21 dpi), 2 (15 and 36 dpi) and 3 (20 dpi), whereas haematocrit was significantly depressed in exposed fish in Trials 1 (21 and 28 dpi) and 3 (20 dpi). Plasma cortisol was significantly elevated in exposed fish at 20 dpi (Trial 3). Physiological changes and mortality were related to the intensity of infection and became most prominent with pre-adult stages, suggesting patterns of infection and response in sockeye salmon similar to those reported for Atlantic and Chinook salmon.

KEY WORDS: Sockeye salmon · Salmon lice · Physiology · Histopathology · Host response · Susceptibility

INTRODUCTION

The salmon louse *Lepeophtheirus salmonis* is an ectoparasitic copepod that infects salmonids in the marine environment throughout the Northern Hemisphere (Kabata 1979, Pike & Wadsworth 1999). Its direct life cycle consists of 2 free-swimming nauplii, 1 free-swimming infective copepodid, 2 chalimus which are attached by a frontal filament, 2 pre-adults and 1 adult, the latter 3 stages being unattached and mobile on the host. Four chalimus were previously recognised: stages 1 and 2 are now considered chalimus 1, whereas stages 3 and 4 are now considered chalimus 2 (Hamre et al. 2013). When the infections occur on farmed Atlantic salmon *Salmo salar*, they cause a significant economic burden resulting from reduced growth performance, direct mortality, mortality due to secondary infections and the costs of treatment (Costello 2009, Torrissen et al. 2013). In addition, salmon louse infections on farmed salmon are associated with increased infection pressure on adjacent wild salmon, and there is particular concern that juvenile wild salmon most recently migrated into the marine environment are at greatest risk of adverse effects of salmon louse infection (Bjørn & Finstad 2002, Morton et al. 2004, Marty et al. 2010).

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Laboratory studies have shown that the juvenile stages of several salmon species differ in their susceptibility to infection with *Lepeophtheirus salmonis* and in their mechanisms of response. For example, a well-developed tissue response occurs at the site of louse attachment on coho salmon *Oncorhynchus kisutch* 1 d post infection, whereas the reaction is markedly reduced or absent in Chinook salmon *O. tshawytscha* and Atlantic salmon (Johnson & Albright 1992). Similarly, the intensity of *L. salmonis* was significantly lower on coho salmon when compared to Atlantic salmon and rainbow trout *O. mykiss* (Fast et al. 2002), and pink salmon *O. gorbuscha* rejected *L. salmonis* more rapidly than chum salmon *O. keta* during laboratory challenges (Jones et al. 2006, 2007). The rejection of lice observed in the latter study is associated with an earlier and quantitatively higher expression of proinflammatory genes in pink salmon (Jones et al. 2007). This natural resistance to *L. salmonis* first develops in pink salmon shortly after they enter seawater at a mean weight of less than 1 g and coincides with the development of dermal scales and with changes in the expression of genes associated with cell motility and tissue remodeling (Jones et al. 2008, Jones & Hargreaves 2009, Sutherland et al. 2011). While these data indicate that innate immunity to *L. salmonis* is enhanced in juvenile coho and pink salmon compared with Chinook, chum and Atlantic salmon, the susceptibility of juvenile sockeye salmon *O. nerka* to *L. salmonis* has not been examined.

In British Columbia, Canada, natural infections with salmon lice have been reported from adult as well as juvenile sockeye salmon. Providing a rare example of disease in a wild salmon population, Johnson et al. (1996) reported heavy infections of *Lepeophtheirus salmonis* on adult sockeye salmon whose migration into freshwater was delayed. These infections were associated with skin lesions ranging from discolouration to large open ulcers with exposed musculature and with elevated morbidity and mortality. In other studies, mixed infections with *L. salmonis* and another parasitic copepod, *Caligus clemensi*, were reported on juvenile sockeye salmon collected east of Vancouver Island, BC (Morton et al. 2008, Price et al. 2011). No evidence of pathological changes was reported in the latter cases. Thus, information about *L. salmonis* infection on juvenile sockeye salmon is relatively scarce and has been limited to field investigations. The aim of this study was to evaluate the physiological and histological responses of juvenile sockeye salmon following laboratory exposure to *L. salmonis* copepodids.

MATERIALS AND METHODS

Salmon lice

Ovigerous *Lepeophtheirus salmonis* were collected from adult Atlantic salmon during harvest from farm net pens (Trial 1 and 2) and from adult chum salmon caught during test fisheries off Vancouver Island (Trial 3). The copepods were transported in chilled aerated seawater to the laboratory, where egg strings were dissected and incubated in groups of 150 to 200 in 1600 ml of filtered (1 µm) and UV-irradiated seawater at 10°C in 2 l glass beakers. Three 5 ml samples were taken daily to determine hatch success and larval development by direct microscopic examination. An inoculum with a known number of copepodids was prepared about 7 d post incubation, when the ratio of copepodids to nauplii was greatest.

Fish

Sockeye salmon were obtained on 22 June 2010 as fry from the Inch Creek hatchery (Fisheries and Oceans Canada Dewdney, BC), transported to the Pacific Biological Station, Nanaimo, BC, and maintained in dechlorinated freshwater in a 2000 l stock tank. In April 2011, fish were gradually adapted to an equal mix of freshwater and sand-filtered seawater over 4 d and subsequently maintained in this flowing brackish water at about 15 ppt. Fish were held at a natural photoperiod, fed and monitored daily for mortality or morbidity.

Salmon from this stock population were exposed to *Lepeophtheirus salmonis* copepodids in 3 trials between May and October 2011. One week prior to exposure, the salmon were acclimated in 370 l fibreglass tanks containing sand-filtered and UV-treated seawater at a mean salinity of 30 ppt.

Exposure to *Lepeophtheirus salmonis*

The water flow was stopped, tank volume was reduced to approximately 90 l, aeration was maintained, and the water was supplemented with 0.2 mg l^{-1} metomidate hydrochloride (Syndel Laboratories) as a sedative (Jones et al. 2006). Copepodids were added to each tank as described below. The exposure was conducted in darkness, and water flow resumed after 2 h. Control fish were subjected to the same conditions without the addition of copepodids. Fish were held at a daily photoperiod of 12 h light and 12 h darkness, fed and monitored daily for mortality or morbidity.

Experimental design

In Trial 1, salmon (n = 128; mean weight ~40 g) were equally distributed among four 370 l tanks. The fish in 2 tanks were exposed to 100 copepodids fish^{-1}, and those in the remaining 2 tanks served as non-exposed controls. Fish were sampled at 7 (n = 10), 14 (n = 10), 21 (n = 12), 28 (n = 16) and 34 (n = 16) d post infection (dpi). The mean temperature throughout the challenge was 11.0°C. In Trial 2, salmon (n = 150; mean weight ~80 g) were equally distributed among six 370 l tanks. Those in 3 tanks were exposed to 100 copepodids fish^{-1} and the remainder served as controls. Five fish per tank were sampled at 8, 15, 22, 29 and 36 dpi. The mean temperature throughout the challenge was 11.0°C. In Trial 3, salmon (n = 90; mean weight ~135 g) were equally distributed among six 370 l tanks. Those in 3 tanks were exposed to 300 copepodids fish^{-1} and the remainder served as controls. Five fish per tank were sampled at 10, 20 and 30 dpi. The mean temperature throughout the challenge was 10.5°C.

Examination of fish

Salmon were sedated with 0.2 mg l^{-1} metomidate hydrochloride, and individually euthanized in 200 mg l^{-1} tricaine methanesulphonate (Syndel Laboratories) in 10 l buckets. For all fish, total length and weight were measured, and salmon lice, including those in the anaesthetic bucket, were counted and staged during examination of the fish under a dissecting microscope. The developmental stages were identified by using established criteria (see Hamre et al. 2013)

Haematocrit

Immediately after salmon lice examination, blood from the severed caudal peduncle was collected into heparinized Caraway (Fisher Scientific) and haematocrit tubes and stored on ice for no longer than 2 h. Duplicate haematocrit tubes per fish were centrifuged for 10 min at 3000 × *g* at room temperature and haematocrit was measured directly. The plasma was collected and used to measure osmolality.

Histology

Right pectoral and pelvic fins and the 2 outer right gill arches from control and exposed salmon were collected immediately following blood sampling and fixed in 10% neutral buffered formalin. Preserved tissues were processed for histological examination. Serial sections of 3 µm were stained in haematoxylin and eosin and examined using a compound microscope (Carl Zeiss Canada).

Osmolality

Plasma samples (10 µl) were assayed in duplicate utilizing a vapour pressure osmometer (Vapro 5520, Wescor) following the manufacturer's instructions. The instrument was calibrated with standards of 100, 290 and 1000 mmol kg^{-1} according to manufacturer's specifications.

Cortisol

Heparinated blood was transferred from Caraway tubes into 1.5 ml Eppendorf tubes, centrifuged for 15 min at 3000 × *g* at 4°C and plasma stored at −80°C. The cortisol level was measured using an enzyme-linked immunosorbent assay (Neogen) following the manufacturer's instructions. Duplicate assays were run in a 96-well format: 50 µl of standard (0.04–10 ng ml^{-1}) or plasma, diluted 1:30 in extraction buffer, were mixed with an equal volume of enzyme conjugate per well. A control plasma sample was run in quadruplicate for every plate to account for potential inter- and intra-assay variation. The plate was incubated for 1 h at room temperature, followed by 3 washing steps and incubation with 150 µl of stabilized 3,3′,5,5′-tetramethylbenzidine in H_2O_2 per well for 30 min at room temperature. Reactions were stopped with 1 N HCl (50 µl well^{-1}) and read in a microplate reader (Dynatec Microplate Reader MR 5000) at 450 nm.

Statistical analysis

Prevalence, intensity, abundance and density of infection, as used in this paper, are as defined by Bush et al. (1997). Statistical analyses were performed using SigmaStat Version 3.5. One-way analysis of variance (ANOVA) showed no significant differences in fish size or mean parasite abundance between replicate tanks, and data were pooled into 1 control and 1 exposed group for each sample event. The statistical significance of differences in mean fish weight, length and condition factor (weight/length^3)

between control and exposed fish were tested using 2-sample *t*-tests. Pairwise multiple comparison tests (Dunn's or Holm-Sidak for nonparametric or parametric data, respectively) were used to test differences in mean abundance and infection density. One-way ANOVA was used to test differences in mean haematocrit between exposed and control fish followed by the Holm-Sidak multiple comparisons test to test for changes over time. A Kruskal-Wallis ANOVA was used to test differences in plasma osmolality and plasma cortisol between control and exposed fish followed by Dunn's pairwise comparison test. In all cases, $p < 0.05$ was considered statistically significant. Values for fish length, weight, condition factor, haematocrit, plasma osmolality and plasma cortisol are shown as mean ± SEM. Cumulative mortality was calculated as a percentage.

RESULTS

Fish size, condition and salmon lice infections

At the conclusion of Trial 1, the length and weight of infected fish were significantly less (16.7 ± 0.4 cm, 40.9 ± 2.5 g) than those of controls (17.9 ± 0.2 cm, 50.8 ± 1.7 g), although there was no difference in mean condition factor between the groups (0.88 ± 0.01 and 0.87 ± 0.02, respectively). By 7 dpi, the estimated parasite survival rate was 16%, intensities ranged from 7 to 30 lice fish^{-1}, and louse stages were copepodids and chalimus 1. By 34 dpi, the intensities ranged from 1 to 9, and louse stages were male and female preadults and male adults (Table 1). Infections were present on all fish throughout the trial, and infection densities ranged from 0.44 lice g^{-1} fish at 14 dpi to 0.08 at 34 dpi. Between 14 and 34 dpi, there was a statistically significant decrease both in mean abundance and density of infection (Table 1). At 21 dpi, scale loss was observed in some exposed fish, and pre-adult stages were first evident. At the same time, an apparent increase in mucus production coincided with increased jumping behaviour and loss of appetite in the exposed salmon. At 34 dpi, scale loss was evident in 100% of the exposed fish, and haemorrhage was present on the anal (13%) and caudal fins (6%). No lesions were observed on the head or body, and no control or exposed fish died during Trial 1.

At the conclusion of Trial 2, we found no statistically significant differences between infected and control mean fish lengths (20.8 ± 0.20 cm, 21.0 ± 0.28 cm), weights (83.1 ± 2.80 g, 84.3 ± 3.88 g) or condition factors (0.92 ± 0.01, 0.90 ± 0.01). An infectious dose of 100 copepodids fish^{-1} was used, and at 8 dpi, the estimated parasite survival rate was 18%. At this time, all louse stages were chalimus 1, and intensities ranged from 6 to 35 lice fish^{-1}. At 36 dpi, intensities ranged from 4 to 16, and the louse stages were female preadults and male and female adults

Table 1. *Lepeophtheirus salmonis* infecting *Oncorhynchus nerka*. Mean ± SEM abundance and density and percent of lice developmental stages on sockeye salmon following exposures to 100 (Trials 1 and 2) or 300 (Trial 3) copepodids fish^{-1}. dpi: days post infection; n: number; Co: copepodid; Ch1–Ch4: chalimus stages 1–4; PAM/PAF: pre-adult male/female; AM/AF: adult male/female

dpi	Fish (n)	Abundance (lice fish^{-1})	Density (lice g^{-1} fish)	Lice (n)	Developmental stage (%)								
					Co	Ch1	Ch2	Ch3	Ch4	PAM	PAF	AM	AF
Trial 1													
7	10	16.0 ± 2.1	0.36 ± 0.04	160	44.0	56.0	0	0	0	0	0	0	0
14	10	19.4 ± 4.2	0.44 ± 0.10	194	0.5	0.5	13.9	80.4	5.2	0	0	0	0
21	13	14.0 ± 1.3	0.36 ± 0.04	182	0	0	0	0	5.5	58.2	36.3	0	0
28	16	7.3 ± 0.9[a]	0.16 ± 0.02[a]	117	0	0	0	0	0	54.7	44.5	0.9	0
34	16	3.3 ± 0.5[a]	0.08 ± 0.01[a]	52	0	0	0	0	0	28.8	53.8	17.3	0
Trial 2													
8	15	18.1 ± 2.2	0.22 ± 0.03	272	0	100	0	0	0	0	0	0	0
15	15	18.3 ± 1.8	0.21 ± 0.02	275	0	0.7	12.7	73.5	13.1	0	0	0	0
22	15	13.8 ± 1.3	0.16 ± 0.01	207	0	0	0	0	0	44.9	55.0	0	0
29	15	10.4 ± 1.3[a]	0.12 ± 0.01[a]	156	0	0	0	0	0	34.5	50.0	11.5	3.8
36	15	7.2 ± 0.9[a]	0.09 ± 0.01[a]	108	0	0	0	0	0	0	20.4	50.0	29.6
Trial 3													
10	15	60.7 ± 3.7	0.46 ± 0.05	910	0	0	75.4	24.6	0	0	0	0	0
20	15	55.5 ± 3.4	0.44 ± 0.04	833	0	0	0	0	6.6	49.8	43.6	0	0
30	4	19.5 ± 3.1[a]	0.15 ± 0.03[a]	78	0	0	0	0	0	28.2	37.2	34.6	0

[a]$p < 0.05$ versus all earlier sample days in a given trial

(Table 1). Infections were observed on all fish throughout the trial, and infection densities ranged from 0.22 lice g^{-1} at 8 dpi to 0.09 at 36 dpi. Both mean abundance and infection density showed significant decreases at 29 and 36 dpi (Table 1). Skin abrasions and scale loss were first observed on exposed fish at 22 dpi, and pre-adult lice were first observed at this time, coincident with an apparent increase in mucus production. No control or exposed fish died during this trial.

At the conclusion of Trial 3, we found no statistically significant differences between infected and control mean fish lengths (24.3 ± 0.14 cm, 24.9 ± 0.38 cm), weights (133.3 ± 3.82 g, 147.7 ± 7.91 g) or condition factors (0.94 ± 0.02, 0.94 ± 0.01). An infectious dose of 300 copepodids $fish^{-1}$ was used, and by 10 dpi, the estimated parasite survival was 20%. At 10 dpi, the louse stages were chalimus 3 or 4, and intensities ranged from 30 to 89 lice $fish^{-1}$ (Table 1). Although the higher challenge level resulted in a mean abundance at 10 dpi that was approximately 3-fold higher than that measured early in Trials 1 and 2, the initial infection density of 0.46 lice g^{-1} was comparable to Trial 1 at 14 dpi (Table 1). By 30 dpi, intensities ranged from 13 to 28 lice $fish^{-1}$, and louse stages were male and female preadults and male adults. All fish were infected throughout the trial; however, mean abundance and infection density significantly decreased at 30 dpi (Table 1). From 18 dpi, exposed fish displayed behavioural changes which included erratic and lethargic swimming behaviour at or near the surface. Erosion of the epidermis exposed underlying tissue and bone on the head in 6 of these fish. Similar lesions were observed behind the dorsal fin, and in 2 severe cases, the skeletal muscle was exposed. There was a cumulative mortality of 24.4% in exposed fish, whereas none of the control fish died. One fish died at 9 dpi, and the remaining 10 mortalities occurred between 22 and 28 dpi. The mean infection density among the 11 dead fish was 0.52 lice g^{-1} fish (range: 0.4 to 1.0), compared with a mean density of 0.15 lice g^{-1} for fish sampled at 30 dpi.

Histopathology

Histological changes were associated with the attachment of chalimus stages on fins and gill lamellae, and observations are based on samples collected on or prior to 20 dpi in Trial 3. On fins, partial to complete skin erosion was observed around chalimus attachment sites (Fig. 1a,b). The basal plate of the frontal filament was often attached to a fin ray, and despite the eroded epithelium, hyperplasia was minimal and associated with a cellular infiltrate within the adjacent epithelium consisting primarily of neutrophils. On gill lamellae, focal hyperplasia of basal epithelial cells, fusion of secondary lamellae and an occasional inflammatory infiltrate consisting of neutrophils and less often, macrophages were observed at and near to the site of louse attachment (Fig. 1c,d). The alterations to host tissue appeared more severe at attachment sites of chalimus 3 compared with chalimus 2. At sites without louse attachment on exposed fish, the tissue appeared normal and comparable to control fish.

Haematocrit

In Trial 1, haematocrit values in infected fish ranged from 13.2 to 56.3% and from 30.5 to 62.3% in controls. There was a statistically significant reduction in mean haematocrit both in infected (after 14 dpi) and control fish (after 21 dpi). However, mean haematocrits from infected fish were significantly lower than controls at 21 and 28 dpi (Fig. 2). In Trial 2, haematocrit values ranged from 30.0 to 57% in infected fish and from 22.0 to 56.0% in controls. At none of the sampling points was mean haematocrit of infected fish significantly different from controls. However, the mean values of infected fish were significantly lower at 22, 29 and 36 dpi compared to 8 dpi and at 29 dpi compared to 15 dpi. There was no statistically significant change in control values over time (Fig. 2). In Trial 3, haematocrits ranged from 29.2 to 48.7% in infected fish and from 26.3 to 54.5% in controls. Mean haematocrits were significantly lower in infected fish at 20 and 30 dpi when compared to controls (Fig. 2). There was no significant difference in control haematocrit values over time (Fig. 2).

Plasma osmolality

In Trial 1, osmolality values ranged from 304.3 to 380.8 mmol kg^{-1} in infected fish and from 314.8 to 357.3 mmol kg^{-1} in controls. At 21 dpi, mean values were significantly higher in infected fish compared to controls (Fig. 3). In Trial 2, osmolality ranged from 312.0 to 383.0 mmol kg^{-1} in infected fish and from 307.0 to 328.0 mmol kg^{-1} in controls. Compared with controls, mean values were significantly elevated in infected fish at 15 and 36 dpi (Fig. 3). In Trial 3, osmo-

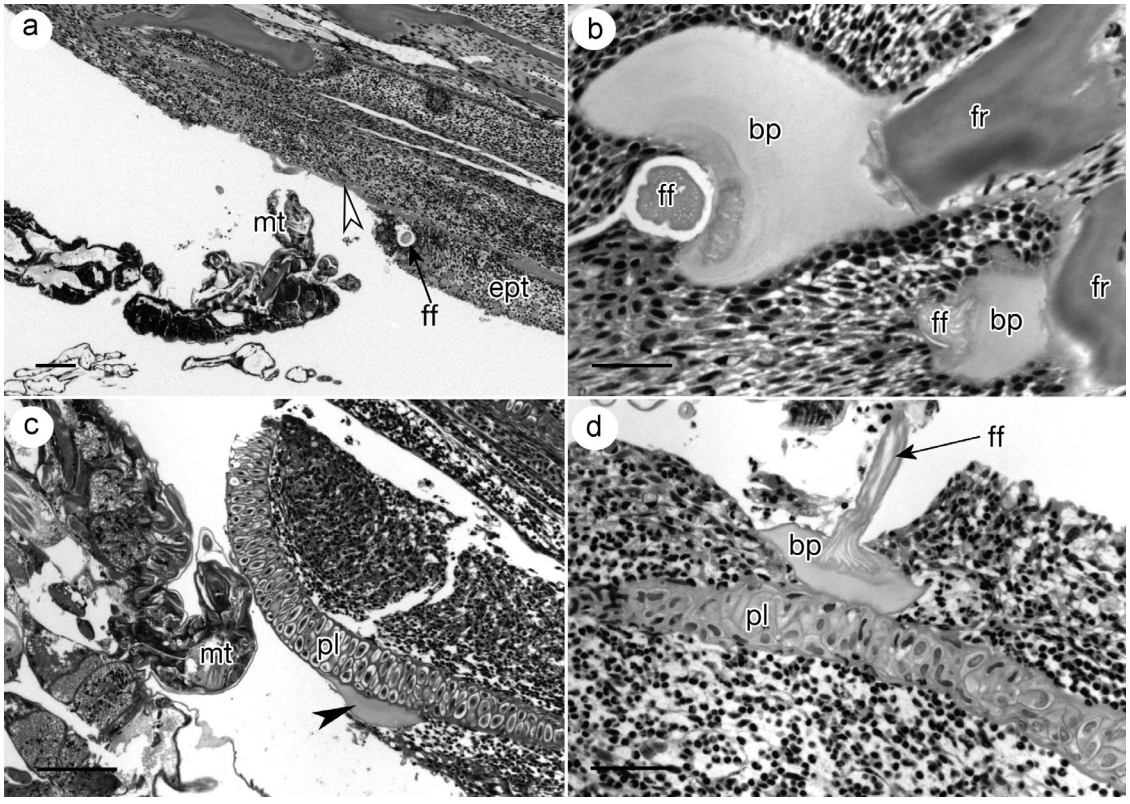

Fig. 1. *Lepeophtheirus salmonis* infecting *Oncorhynchus nerka*. Light micrographs of histological preparations of sockeye salmon tissues following infection with salmon lice copepodids. (a) Exposure of pectoral fin ray (open arrowhead) by grazing of chalimus 2 on epithelium (ept); 10 d post infection (dpi). mt: mouth tube. Note the frontal filament (ff) surrounded by a mild inflammatory infiltrate (arrow). (b) Interactions of 2 adjacent ff and basal plates (bp) with pectoral fin rays (fr); 10 dpi. (c) Erosion of respiratory tissues from cartilaginous support of primary lamellae (pl) by chalimus 3; 10 dpi. Note the bp (closed arrowhead) adjacent to cartilage and hyperplastic epithelium with inflammatory infiltrate on opposite side of lamellae. (d) ff (arrow) and bp adjacent to cartilaginous support of pl. Note cellular inflammatory infiltrate consisting principally of neutrophils; 10 dpi. Scale bars = (a,c) 100 µm and (b,d) 50 µm. Stained with haematoxylin and eosin

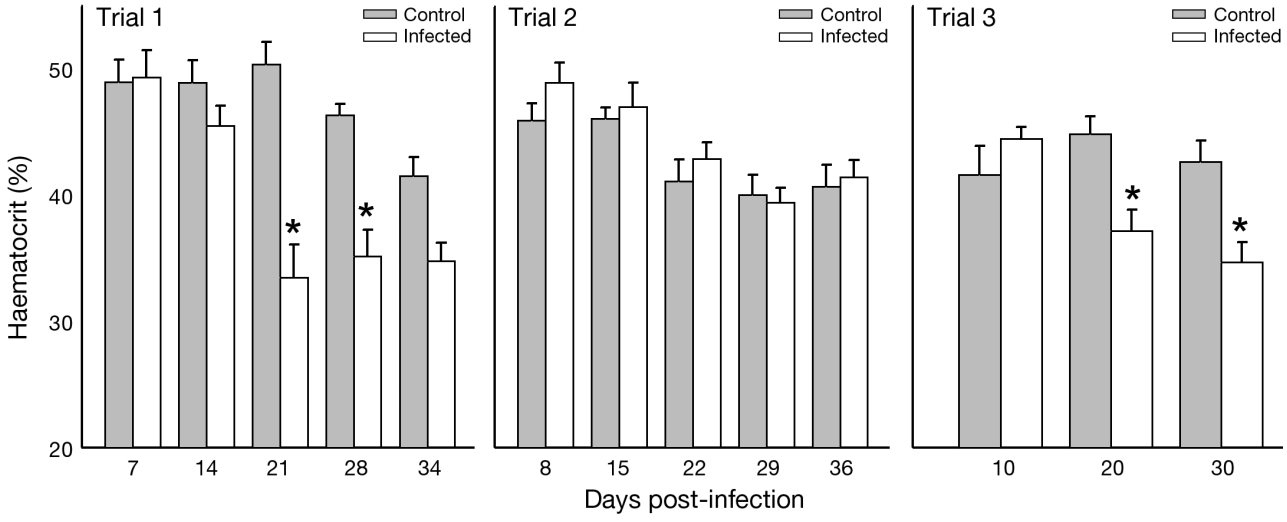

Fig. 2. *Lepeophtheirus salmonis* infecting *Oncorhynchus nerka*. Mean (±SEM) haematocrit values of control and lice-exposed sockeye salmon. *Statistically significant ($p < 0.05$) difference between control and infected fish

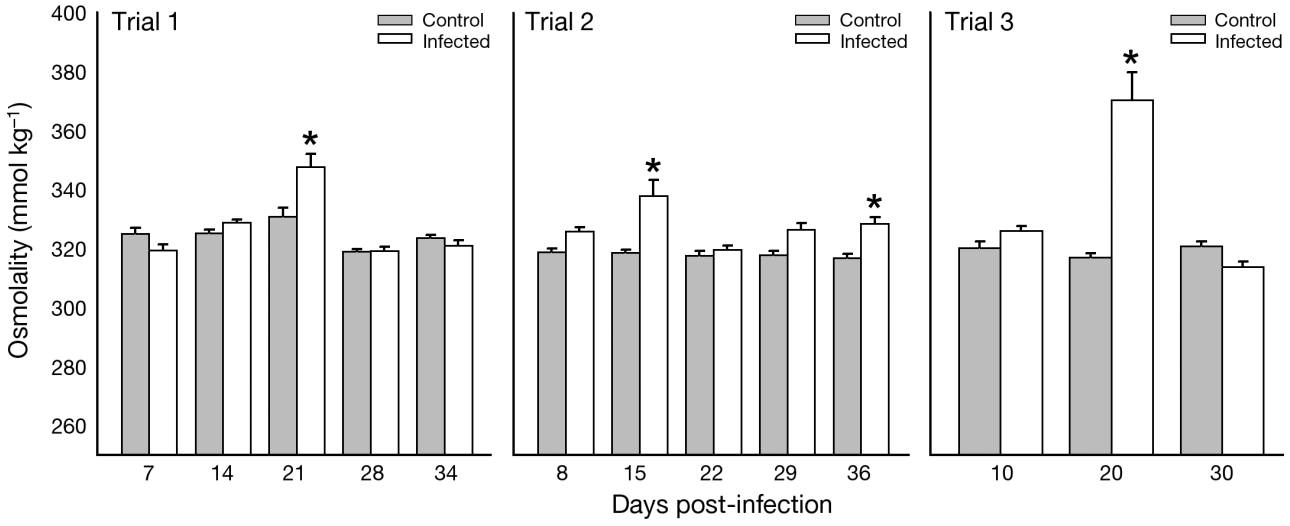

Fig. 3. *Lepeophtheirus salmonis* infecting *Oncorhynchus nerka.* Mean (±SEM) plasma osmolality of control and lice-exposed sockeye. *Statistically significant ($p < 0.05$) difference between control and infected fish

lality ranged from 310.0 to 444.0 mmol kg^{-1} in infected fish and from 310.0 to 341.0 mmol kg^{-1} in controls. Mean values were significantly higher in infected fish compared to controls at 20 dpi (Fig. 3).

Plasma cortisol

In Trial 1, cortisol values ranged from 0.9 to 66.5 ng ml^{-1} in infected fish and from 2.7 to 112.7 ng ml^{-1} in controls. Mean values were highly variable among sampling days in both treatment groups. In Trial 2, values ranged from 0.4 to 42.0 ng ml^{-1} in infected fish and from 0.4 to 40.9 ng ml^{-1} in controls. Mean values in infected fish were significantly less than in controls at 8 dpi and significantly greater than controls at 22 dpi (Fig. 4). In Trial 3, values ranged from 1.0 to 126.5 ng ml^{-1} in infected fish and from 1.6 to 29.1 ng ml^{-1} in controls. At 20 dpi, the mean cortisol value was significantly higher in infected fish compared to controls (Fig. 4).

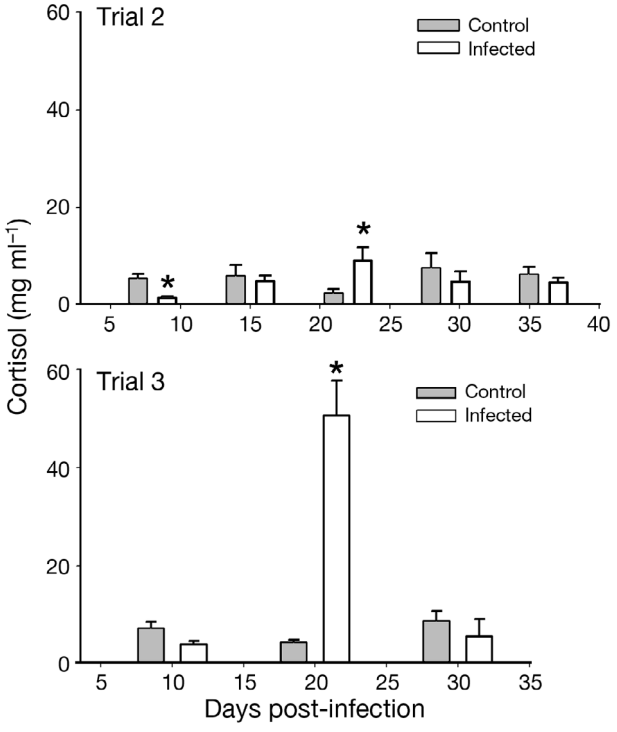

Fig. 4. *Lepeophtheirus salmonis* infecting *Oncorhynchus nerka.* Mean (± SEM) plasma cortisol levels in control and lice-infected sockeye salmon. *Statistically significant ($p < 0.05$) difference between control and infected fish

DISCUSSION

This is the first investigation of the consequences to laboratory-reared sockeye salmon of controlled exposure to *Lepeophtheirus salmonis*. In this study, a common stock of juvenile salmon was used in all exposure trials, which resulted in exposures of increasingly larger fish. Two levels of parasite challenge were adopted to investigate possible effects of host size and parasite intensity. The similar parasite abundances observed in Trials 1 and 2 following exposure to 100 copepodids^{-1} fish and the uniform composition of developmental stages observed midway through all trials indicate that parasite infectivity and rate of development was not influenced by host

size. However, a comparison with previous studies on wild sockeye suggests the morbidity and mortality associated with mean abundances ranging from of 19.5 to 60.7 lice fish^{-1} in Trial 3, following exposure to 300 copepodids fish^{-1}, and the apparent association of the parasite with reduced growth in Trial 1 may have been aggravated by the relatively small fish used in these studies. At the conclusion of Trial 3, the mean length was 24 cm in comparison to a mean length of 59 cm in adult sockeye (Beamish et al. 2005). These authors reported a mean abundance of 41.1 lice fish^{-1} among adult sockeye collected by hook and line in coastal British Columbia with only minor skin damage. Similarly, the abundances of *L. salmonis* reported from relatively healthy adult sockeye collected by seine nets from the Alberni Inlet on 5 occasions over 2 yr ranged from 7.4 to 77.1 lice fish^{-1} (Johnson et al. 1996). In the latter study, a mean abundance of 300 lice fish^{-1} was associated with death and morbidity of sockeye, suggesting that adult salmon have a greater tolerance to the pathological effects of infections at intensities capable of causing disease in the smaller salmon. Abundances of up to 1.0 *L. salmonis* fish^{-1} were reported for juvenile sockeye salmon presumably exposed to salmon aquaculture (Morton et al. 2008, Price et al. 2011). Although these values are well below those shown here to be harmful, there is a need for additional controlled exposure data from a similarly small (<12 g) size class of sockeye salmon reported in the latter studies. Few other studies have explicitly explored effects of salmon size on responses to *L. salmonis*. A series of papers reviewed by Sutherland et al. (2011) and Brauner et al. (2012) describe the immunological and physiological changes that occur in juvenile pink salmon over the first few weeks in the ocean and how these influence susceptibility to *L. salmonis*. During this time, pink salmon increase from ~0.2 to ~2.0 g and develop from newly transformed larvae lacking scales to immunocompetent juveniles with increased resistance to *L. salmonis* infection. While not explicitly explored in our study, changes of a similar magnitude are unlikely in juvenile salmon over the range of sizes used here since sockeye are already immunocompetent at 1 g (Johnson et al. 1982). Overall, the exposure trials reported here demonstrated a high degree of reproducibility in establishing *L. salmonis* infections on juvenile sockeye salmon.

Microscopic pathological lesions of the skin caused by *Lepeophtheirus salmonis* have been reported from salmon belonging to several species. Most of these reports concern infections with chalimus stages and show that louse feeding behaviour is an important contributor to skin pathology. In Atlantic salmon, lesions ranged from non-existent to mild hyperplasia, apoptosis or necrosis of epithelial cells (Jones et al. 1990, Johnson & Albright 1992, Nolan et al. 1999). In contrast, responses of the skin of Pacific salmon vary among species: well-developed hyperplasia with mixed inflammatory cell infiltrates, haemorrhage and necrosis are evident in *L. salmonis*-infected coho salmon, whereas little epithelial response is observed in Chinook salmon (Johnson & Albright 1992). Also, chalimus stages of *L. salmonis* elicit necrosis and a mixed leucocyte response in the skin of juvenile pink and chum salmon (Jones et al. 2007). The limited hyperplastic response of sockeye salmon skin epithelial cells with occasional inflammatory infiltrate is similar to the responses observed in Chinook salmon (Johnson & Albright 1992). Branchial infections with *L. salmonis*, such as those observed on the gills of sockeye salmon, are frequently reported during laboratory infections and are considered an artefact of this exposure method (Bron et al. 1991, Johnson & Albright 1991, Tucker et al. 2000, Jones et al. 2007). Severe infections of the sockeye gill were similar to those reported in other species and associated with erosion, necrosis or hyperplasia of epithelial cells and necrosis. Although comparative studies have shown that branchial infections do not occur with the same severity in juvenile pink and chum salmon (Jones et al. 2007), their occurrence mainly in laboratory trials indicates a limited value in predicting impacts during natural exposures. In addition to histopathological lesions, gross lesions on the head and body of sockeye in Trial 3 following copepod development to the pre-adult and adult stages were similar to those reported from naturally infected adult sockeye salmon (Johnson et al. 1996) and from farmed (Jónsdóttir et al. 1992) and laboratory infected Atlantic salmon (Jónsdóttir et al. 1992, Grimnes & Jakobsen 1996, Jakob et al. 2011). These lesions are induced by the feeding activities of the larger mobile parasites and may be exacerbated by secondary bacterial infections (Wootten et al. 1982, Johnson et al. 1996, Jakob et al. 2011).

Early survival of *Lepeophtheirus salmonis* following copepodid settlement on the host provides an estimate of host susceptibility. Jones et al. (2007) reviewed earlier laboratory studies and found higher survival rates on susceptible species including Atlantic salmon (3 to 75%), sea trout (58%) and three-spine stickleback (6.6 to 16.5%). In contrast 7 d survival on the more resistant juvenile pink salmon ranged from 0.4 to 3.6% (Jones et al. 2007). In the present study, louse survival at 7, 8 and 10 dpi was

16, 18 and 20%, respectively, in the 3 trials, suggesting that susceptibility of juvenile sockeye salmon to *L. salmonis* is intermediate between that of pink and Atlantic salmon. Comparative data from previous studies indicate that rejection of *L. salmonis* is host species dependent: lice are rejected more rapidly from pink and coho salmon than from chum, Chinook or Atlantic salmon or from rainbow trout, and lice tend to be rejected from pink and coho salmon during chalimus stages of development (Johnson & Albright 1992, Fast et al. 2002, Jones et al. 2007). Whereas the retention of higher numbers of more aggressive mobile parasites contributes to the pathology observed on susceptible salmon, concurrent development of epithelial pathology in coho salmon or of the expression of proinflammatory genes in skin of pink salmon (Johnson & Albright 1992, Jones et al. 2007, Braden et al. 2012) suggests that cutaneous inflammation is partly responsible for rejection of lice from less susceptible salmon. On sockeye salmon, decreases in the abundance (lice $fish^{-1}$) and density (lice g^{-1} fish) of *L. salmonis* coincided with the first appearance of adult stages. Further research is therefore required to provide a quantitative and comparative assessment of cutaneous inflammation associated with *L. salmonis* in juvenile sockeye salmon.

To compensate for increased fish size, a challenge level of 300 copepodids $fish^{-1}$ was used in Trial 3 to obtain a physiological outcome that was similar to that obtained in Trial 1. Also, the intensity of infection was expressed as infection density (lice g^{-1} fish) as previously reported (Bjørn & Finstad 1998, Wagner et al. 2008), to standardize for differences in host weight among trials. Although this strategy resulted in similar mean infection densities observed at 14 to 22 dpi in Trials 1 and 3, parasite abundances at these times were approximately 4-fold higher in Trial 3 compared with Trial 1. Furthermore, mortality and the highest mean plasma osmolality and plasma cortisol values occurred in Trial 3, suggesting that in juvenile sockeye salmon, very high parasite intensities induced an added physiological burden that was independent of host size and probably directly related to a loss of skin integrity (Bjørn & Finstad 1998, Bowers et al. 2000, Finstad et al. 2000). The coincidence of significant plasma cortisol responses with the first detection of mobile *Lepeophtheirus salmonis* in sockeye salmon is similar to the timing of responses measured in Atlantic and chum salmon during laboratory *L. salmonis* infections (Bowers et al. 2000, Fast et al. 2002, Jones et al. 2007). Plasma cortisol levels also increased in sea trout during infections with chalimus stages of *L. salmonis* (Bjørn & Finstad 1997). Plasma cortisol is a widely used indicator of the stress response in salmonid fishes (Wendelaar Bonga 1997); however, levels vary among species and in response to environmental conditions, intensity of infection, sampling methods, host size and host condition. Johnson & Fast (2004) corrected for some of these factors by comparing different experiments based on the lice infection densities on Atlantic salmon. Despite similarities in study design resulting in 0.16 lice g^{-1} fish at the time of moult to pre-adult, cortisol levels were 7.3 ng ml^{-1} in Fast et al. (2002) and ranged from 54.5 to 72.7 ng ml^{-1} in Bowers et al. (2000). Plasma cortisol levels in juvenile chum salmon were 80 ng ml^{-1} in exposed fish and 15 ng ml^{-1} in controls at 21 dpi, with an infection density of 0.08 lice g^{-1} fish (Jones et al. 2007). In sockeye salmon, plasma cortisol at 9.1 ng ml^{-1} was measured at 22 dpi, with a mean infection density of 0.16 lice g^{-1} fish, and 50.5 ng ml^{-1} was measured at 20 dpi when there was an infection density of 0.44 lice g^{-1} fish. These observations support an association between a stress response in sockeye salmon and the appearance of mobile stages of *L. salmonis* and show that the magnitude of the stress response increases with intensity or density of infection. Together, these observations confirm that absolute values of plasma cortisol are highly contextual and appear most informative in assessing stress among concurrent treatment groups following *L. salmonis* exposures under the same experimental conditions.

Mean haematocrits were significantly lower in infected sockeye salmon than in controls in Trials 1 and 3, and these differences only occurred following the first appearance of preadult lice. Similarly, Grimnes & Jakobsen (1996) observed reduced haematocrits in *Lepeophtheirus salmonis*-exposed juvenile Atlantic salmon coincident with the appearance of pre-adult stages at infection densities of 1.6 lice g^{-1} fish. Reduction in haematocrit has also been associated with chalimus and later stages of *L. salmonis* on sea trout and chum salmon (Bjørn & Finstad 1997, Jones et al. 2007). In addition to haematocrit, an increase in plasma osmolality preceded (Trial 2) or coincided with the first appearance of preadult stages in sockeye salmon (Trials 1 and 3). Other workers have also reported perturbations in plasma chloride levels indicative of osmoregulatory disturbance during infections with chalimus or following the first appearance of pre-adult *L. salmonis* on sea trout and Atlantic salmon (Grimnes & Jakobsen 1996, Bjørn & Finstad 1997, Finstad et al. 2000). The decrease in haematocrit reported in these studies was likely the

combined result of osmotic shrinking of erythrocytes and loss of body fluids through lesions in the skin. During more intense infections, these factors may therefore exert a greater impact on haematocrit than plasma cortisol, which has been associated with an increase in haematocrit (Mazur & Iwama 1993). Wootten et al. (1982) and Wagner et al. (2008) reported that increased feeding activity and mobility of the pre-adult and adult stages on Atlantic salmon results in a stress response, disruption of the skin and in severe cases, formation of lesions leading to osmoregulatory breakdown. Changes in haematocrit among control salmon, as described here, have also been reported (e.g. Bowers et al. 2000) and may result from handling or confinement (Mazur & Iwama 1993). The increased haematocrit reported by Bowers et al. (2000) in Atlantic salmon following exposure to *L. salmonis* may have been due to swelling of erythrocytes and increased erythropoiesis caused by a catecholamine-mediated increase in oxygen uptake capacity (Wendelaar Bonga 1997). However, more research is required to determine the effect of salmon size on the relationship between haematocrit and parasite intensity, skin lesions and plasma cortisol, since the Atlantic salmon used by Grimnes & Jakobsen (1996) were 40 g whereas those used by Bowers et al. (2000) were 680 g.

The consequences of *Lepeophtheirus salmonis* infections on salmon belonging to a variety of species led Wagner et al. (2008) to establish 3 categories of infection density: 1, up to 0.1 lice g^{-1} fish are designated subclinical without any physiological impact; 2, between 0.1 and 0.75 lice g^{-1} fish are subclinical with observed physiological impact; and 3, above 0.75 lice g^{-1} fish are clinical infections. The physiological risk categories of Wagner et al. (2008) emphasize the combined importance of fish size and intensity as factors in determining the outcome of infection and provide a conceptual framework for interpreting the present findings. Mean infection densities throughout the sockeye exposure trials ranged from 0.1 to 0.75 lice g^{-1} fish and, consistent with category 2, physiological perturbations were measured. However, salmon in Trial 3 experienced clinical disease with mortality despite a mean infection density among dead fish of 0.52 lice g^{-1} fish: an infection density greater than 0.75 lice g^{-1} fish was only measured in 3 of the 11 dead fish. The similar infection densities between Trials 1 and 3 suggests that infection density may not always be the best predictor of a physiological outcome, particularly in those circumstances in which salmon are exposed to extremely high infection pressure or in which small body weight results in high parasite intensity. For example, a threshold density of 7.5 *L. salmonis* g^{-1} fish was only useful in predicting mortality among pink salmon weighing 0.7 g or less (Jones & Hargreaves 2009). The risk categories of Wagner et al. (2008) may be more useful indicators of physiological effect under more moderate levels of infection pressure such as those in Trials 1 and 2.

In conclusion, the mild inflammatory response of juvenile sockeye salmon to infection with *Lepeophtheirus salmonis* was most similar to that of Chinook salmon, whereas the loss of lice during mobile stages of development and the evidence for osmotic perturbations were similar to those reported for Atlantic salmon, chum salmon and sea trout. Challenge level and the stage of parasite development were shown to be important determinants of the outcome of infection in juvenile sockeye salmon.

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