Experimental infections with *Lepeophtheirus salmonis* (Krøyer) on threespine sticklebacks, *Gasterosteus aculeatus* L., and juvenile Pacific salmon, *Oncorhynchus* spp.

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Abstract

Experimental infections with *Lepeophtheirus salmonis* (Krøyer) were established on threespine sticklebacks, *Gasterosteus aculeatus* L., juvenile pink, *Oncorhynchus gorbuscha* (Walbaum), and chum, *Oncorhynchus keta* (Walbaum), salmon. The prevalence and abundance of infections were initially higher on sticklebacks than on either salmon species. The initial prevalence and intensity of infections on chum salmon were higher than those on pink salmon, and declined on both species during louse development. The rate of parasite development to adult stages was similar on all species although development beyond the preadult stage was not observed on sticklebacks. These results confirm previous field observations on the occurrence and development of *L. salmonis* on threespine sticklebacks.

Keywords: abundance, *Gasterosteus aculeatus*, laboratory infections, *Lepeophtheirus salmonis*, *Oncorhynchus gorbuscha*, *Oncorhynchus keta*.

Introduction

The salmon louse, *Lepeophtheirus salmonis* (Krøyer), (Copepoda: Caligidae) occurs on anadromous salmonids in the North Atlantic and Pacific oceans. The parasite is found on adult Pacific salmon, *Oncorhynchus* spp., collected in the mid-Pacific Ocean (Nagasawa, Ishida, Ogura, Tadokoro &

Hiramatsu 1993; Nagasawa 2001) and in the coastal waters of British Columbia (BC) (Beamish, Neville, Sweeting & Ambers 2005). *Lepeophtheirus salmonis* has recently been reported on pink, *Oncorhynchus gorbuscha* (Walbaum), and chum, *Oncorhynchus keta* (Walbaum), juveniles shortly after the fish enter nearshore waters (Morton, Routledge, Peet & Ladwig 2004). The parasite is considered specific to salmonids although it has been reported on white sturgeon, *Acipenser transmontanus* Richardson, sand lance, *Ammodytes hexapterus* Pallas, and saithe, *Pollachius virens* (L.) (Kabata 1973; Bruno & Stone 1990; Lyndon & Toovey 2001). The biology of this parasite was thoroughly reviewed by Pike & Wadsworth (1999).

Lepeophtheirus salmonis was recently found on 84% of over 1300 threespine sticklebacks, *Gasterosteus aculeatus* L., from coastal BC (Jones, Prosperi-Porta, Kim, Callow & Hargreaves 2006), with mean abundance as high as 73.2 lice per fish. Nearly 20 000 specimens of *Lepeophtheirus* sp. were collected from the sticklebacks and these were primarily copepodid (40% of all stages observed) and chalimus I (23%) stages. The few adult (0.02%) stages were identified as *L. salmonis*. In addition, identities of the chalimus stages were confirmed as *L. salmonis* by sequencing the 18S rRNA gene (Jones *et al.* 2006). The rare occurrence of adult stages indicated that *L. salmonis* often failed to complete their development on the stickleback. Despite this, the high prevalence and abundance raised questions concerning the role of this host species in the epizootiology of the salmon louse. Particularly important is the need to document and compare the development of *L. salmonis* on its

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various hosts. Here we report the results of controlled laboratory experiments to test the hypothesis that *L. salmonis* parasitises but fails to complete development on the threespine stickleback.

Materials and methods

Sources and laboratory maintenance of fish

Pink and chum salmon were obtained as swim-up fry from the Quinsam River and Nanaimo River Hatcheries, respectively, on Vancouver Island, BC. They were reared in approximately equal parts of dechlorinated city water and sand-filtered sea water at the Pacific Biological Station (PBS), Nanaimo. Salinity and temperature were monitored daily. The salmon were fed pelleted commercial salmon ration at a daily rate of approximately 1.5% body weight.

Marine sticklebacks were collected from Knight Inlet and the channels adjacent to Gilford Island, BC by using purse seines. They were transported in aerated sea water to the PBS and maintained on sand-filtered sea water for at least 2 weeks prior to use in studies. Following arrival at the PBS, the fish were sedated (0.15 mg L^{-1} metomidate·HCl) and examined for sea lice by using a dissecting microscope. All lice were carefully removed using fine forceps. Sticklebacks were fed larval mosquitoes and chopped annelids obtained commercially.

Sources and culture of *Lepeophtheirus salmonis*

Gravid female *L. salmonis* were collected from adult Atlantic salmon, *Salmo salar* L., or Pacific salmon, *Oncorhynchus* spp., freshly harvested from farm net-pens or wild test fisheries, respectively. The lice were transported in ice-cold sea water to the PBS where groups of 25 dissected egg strings were placed into plexiglass hatching chambers constructed at the PBS. The chambers were cylindrical (length = 4.5 cm, inside diameter = 2.6 cm) and closed at each end with 200 μm Nitex screens. Chambers were suspended in flow-through sea water at a mean salinity of 29.3‰ (range 29.0–29.7‰) that had been filtered (1 μm), ultraviolet irradiated and maintained at 10 °C. The chambers were visually monitored for evidence of hatching. Following hatching, small samples from each chamber were examined microscopically and the numbers of nauplii and copepodids determined. The proportion of copepodids to nauplii was

greatest 5–7 days following the onset of hatching, and challenge trials were initiated at this time.

Experimental design

Two sea lice challenge experiments (trials 1 and 2) were conducted using pink salmon, chum salmon and sticklebacks. All trials were conducted in 33-L fibreglass tanks supplied with sand-filtered sea water (mean salinity 29.3‰). Salmon were acclimatized to the sea water at least 1 week prior to exposure to copepodids. To initiate each challenge, the water flow to each tank was stopped, the volume reduced to 2 L and supplementary aeration provided. A known number of copepodids was added to each tank and waterflow resumed after 2 h. Throughout the 2-h exposure, all fish were sedated as described above. Exposures to copepodids were conducted in darkness and, thereafter, daily photoperiod was regulated at 12 h light to 12 h dark.

The novelty of sedating fish during exposure to copepodids required validation of the challenge method. Thus, copepodids were incubated for 2 h in 0.21 mg L^{-1} metomidate·HCl at 12 °C and their motility compared with copepodids held in sea water alone. In addition, two tanks of chum salmon (1.7 ± 0.1 g, $n = 5$) were exposed to 60 copepodids each for 2 h as described above. To one tank 0.15 mg L^{-1} metomidate·HCl was added whereas the other contained sea water alone. The number and stage of sea lice on fish in both tanks were determined 7 and 14 days after exposure.

At intervals following exposures in trials 1 and 2, fish were sedated by adding 0.15 mg L^{-1} metomidate·HCl to the tank water. The weight and fork length of immobilized fish were measured. The sedated fish were examined microscopically for developing lice and returned to the tank. The prevalence, intensity and abundance of infections were determined and sea lice developmental stages occurring on the skin and fins were identified. Copepods observed on gill filaments were not counted to minimize time out of water and to avoid gill damage. Non-exposed sticklebacks were examined for sea lice. The statistical significance of differences in prevalence was determined by chi-squared analysis. The significance of differences in abundance was determined using the Mann–Whitney test. The similarity of louse development among species was estimated by comparing the proportion of developmental stages using chi-squared analysis. The significance of differences in

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fish weights at the end of each trial was determined using two-sample *t*-tests. In all cases, differences were considered significant when $P < 0.05$.

Results

The motility of copepodids incubated for 2 h in 0.21 mg L^{-1} metomidate·HCl was not visibly impaired compared with those incubated in sea water. In addition, early developmental stages of *L. salmonis* were observed on all 10 chum salmon in the validation experiment. Although there was a trend of increased abundance on sedated fish, at neither 7 nor 14 days post-exposure (dpe) was there a significant difference in abundance (A, mean ± SEM) compared with non-sedated fish (7 dpe: $A_{sedated}$ 5.2 ± 0.9, $A_{non-sedated}$ 3.6 ± 0.5, $P = 0.17$; 14 dpe: $A_{sedated}$ 3.6 ± 0.7, $A_{non-sedated}$ 2.0 ± 0.3, $P = 0.09$). By 14 dpe most lice were third-stage chalimus and there was no significant difference in the proportion of developmental stages between sedated and non-sedated fish ($P = 1.00$). All sedated fish recovered mobility within 30 min of resuming sea water flow to the tanks.

In trial 1 (mean temperature 12.6 ± 0.1 °C; challenge level 155 copepodids per fish) at 7 dpe, the mean intensity of *L. salmonis* (±SEM) was 1.1 ± 0.1 lice per fish (range 1–2), 4.8 ± 0.6 (range 2–9) and 25.5 ± 7.7 (range 1–117) on pink salmon, chum salmon and sticklebacks, respectively. The prevalence was not significantly different among species ($P \geq 0.12$) (Table 1). The abundance, however, differed significantly among all species ($P \leq 0.01$), being highest on sticklebacks (Table 1). At 14 dpe, while there was no significant difference in louse prevalence between chum and sticklebacks ($P = 1.00$); relative to these species, lice were present on a significantly smaller percent-

age of pink salmon ($P < 0.01$). There was no significant difference in the abundance of lice on sticklebacks and chum salmon ($P = 0.29$) (Table 1). Only one louse was observed on one pink salmon at 14 dpe. At 21 and 28 dpe, the prevalence and abundance of louse infections declined but they were still detectable on chum salmon, whereas no lice were observed on pink salmon or sticklebacks. At 28 dpe, the mean weight of chum salmon (12.4 ± 0.6 g) was not significantly different from that of pink salmon (10.6 ± 0.8 g) ($P = 0.08$). The mean weight of sticklebacks was 2.4 ± 0.2 g.

In trial 2 (mean temperature, 11.7 ± 0.2 °C; challenge level, 271 copepodids per fish) at 6 dpe, the mean intensity was 2.4 ± 0.4 lice per fish (range 1–5), 2.4 ± 0.4 (range 1–5) and 17.9 ± 2.1 (range 5–37) on pink salmon, chum salmon and sticklebacks, respectively. The prevalence of lice on pink salmon was significantly less than on sticklebacks ($P = 0.03$) (Table 2), whereas differences in prevalence between chum salmon and either pink salmon or sticklebacks were not significant ($P \geq 0.24$). The abundance of lice did not differ between chum and pink salmon ($P = 0.22$), and both were significantly less than the abundance on sticklebacks ($P < 0.01$) (Table 2). At 13 dpe, louse prevalence was significantly lower on pink salmon than on either chum salmon or sticklebacks ($P < 0.01$), and was not significantly different between the latter species ($P = 1.0$). The abundance was significantly different among all fish species ($P < 0.01$) with the highest numbers of *L. salmonis* being present on sticklebacks. After 13 dpe, lice were no longer detected on sticklebacks. At 20 and 27 dpe, there were no significant differences in either prevalence ($P \geq 0.72$) or abundance ($P \geq 0.40$) of lice on pink and chum salmon. At 27 dpe, the mean weight of pink salmon

Table 1 Prevalence and abundance of *Lepeophtheirus salmonis* on juvenile pink and chum salmon and threespine sticklebacks in trial 1

Day[a]	Pink (106.2 ± 2.8 mm)[c]			Chum (111.1 ± 1.5 mm)			Stickleback (65.5 ± 2.1 mm)		
	n[b]	Prevalence (%)	Abundance	n	Prevalence (%)	Abundance	n	Prevalence (%)	Abundance
7	15	73.3	0.8 ± 0.1[d]	14	100.0	4.8 ± 0.6	15	100.0	25.5 ± 7.7
14	15	6.7	0.7 ± 0.7	14	85.7	2.2 ± 0.5	14	78.6	4.2 ± 1.2
21	15	0	0	14	50.0	0.9 ± 0.3	13	0	0
28	15	0	0	13	30.8	0.5 ± 0.3	13	0	0

The exposure level was 155 copepodids per fish and the mean water temperature was 12.6 ± 0.1 °C.
[a] Day post-exposure.
[b] Number of fish examined.
[c] Mean length at day 28.
[d] Mean ± SEM.

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Table 2 Prevalence and abundance of *Lepeophtheirus salmonis* on juvenile pink and chum salmon and threespine sticklebacks in trial 2

Day[a]	n[b]	Pink (140.0 ± 1.9 mm)[c]		n	Chum (126.0 ± 2.4 mm)		n	Stickleback (65.5 ± 0.9 mm)	
		Prevalence (%)	Abundance		Prevalence (%)	Abundance		Prevalence (%)	Abundance
6	20	70.0	1.7 ± 0.4[d]	20	90.0	2.2 ± 0.4	20	100.0	17.9 ± 2.1
13	20	30.0	0.5 ± 0.2	20	95.0	3.0 ± 0.4	20	100.0	12.4 ± 1.3
20	20	25.0	0.3 ± 0.1	20	35.0	0.6 ± 0.2	20	0	0
27	20	20.0	0.3 ± 0.1	20	30.0	0.4 ± 0.2	20	0	0

The exposure level was 271 copepodids per fish and the mean water temperature was 11.7 ± 0.2 °C.
[a] Days post-exposure.
[b] Number of fish examined.
[c] Mean length at day 27.
[d] Mean ± SEM.

Table 3 Percentage of *Lepeophtheirus salmonis* developmental stages on experimentally exposed fish in trial 1

Fish	Day	Number of lice	Developmental stages (%)						
			Co	I	II	III	IV	PA	Ad
Chum	7	67	1.5	92.5	6.0				
	14	31				9.7	16.1	74.2	
	21	12						33.3	66.7
	28	7							100.0
Pink	7	12		100.0					
	14	1						100.0	
	21	0							
	28	0							
Stickleback	7	382	0.3	90.1	9.6				
	14	56[a]				3.6	7.1	33.9	55.4
	21	0							
	28	0							

See Table 1 for exposure level and temperature.
Co, copepodid; I, chalimus I; II, chalimus II; III, chalimus III; IV, chalimus IV; PA, preadult; Ad, adult.
[a] Four additional specimens were too damaged to identify stage.

(25.9 ± 1.3 g) was significantly greater than that of chum salmon (18.6 ± 1.2 g) ($P < 0.001$). The mean weight of sticklebacks at this time was 2.3 ± 0.1 g.

The abundance of *L. salmonis* was significantly lower ($P < 0.01$) on chum salmon at 6 dpe in trial 2 compared with 7 dpe in trial 1. The differences in abundance observed on pink salmon and sticklebacks at these times in trials 1 and 2 were not significant ($P = 0.21$ and 1.00, respectively).

In trial 1 at 7 dpe, most lice on all three species had developed to the first chalimus stage and by 14 dpe, the lice were predominantly preadult stages (Table 3). At neither time was there a significant difference in the proportion of louse developmental stages among host species ($P = 1.0$). At 21 and 28 dpe, lice on chum salmon were predominantly adult stages (Table 3). One chum salmon died at 21 dpe and two sticklebacks died at 13 and 18 dpe. The dead chum was infected with one preadult stage and of the dead sticklebacks, one was

uninfected and a second stage chalimus was recovered from the second.

In trial 2, no significant differences in the proportions of developmental stages were observed among species at 6 and 13 dpe ($P = 1.0$) (Table 4). Similarly, the proportion of developmental stages on pink and chum salmon was not different at 20 and 27 dpe ($P = 1.0$). No salmon or sticklebacks died during trial 2. No lice were observed on unexposed sticklebacks in either trial and no unexposed sticklebacks died.

Discussion

Infections with *L. salmonis* were established on laboratory populations of pink and chum salmon and on threespine sticklebacks. However, by 6 or 7 dpe to copepodids, the prevalence and/or abundance differed significantly among species, being consistently highest on sticklebacks. Despite this difference, parasite development proceeded at the

Table 4 Percentage of *Lepeophtheirus salmonis* developmental stages on experimentally exposed fish in trial 2

Fish	Day	Number of lice	Developmental stages (%)						
			Co	I	II	III	IV	PA	Ad
Chum	6	44		100.0					
	13	60		1.6	6.7	41.7	41.7	8.3	
	20	12						33.3	66.7
	27	8						25.0	75.0
Pink	6	33		100.0					
	13	10				40.0	60.0		
	20	6						50.0	50.0
	27	5						20.0	80.0
Stickleback	6	357	0.6	83.2	16.2				
	13	248		1.6	1.6	62.1	26.2	8.5	
	20	0							
	27	0							

See Table 2 for exposure level and temperature.
Co, copepodid; I, chalimus I; II, chalimus II; III, chalimus III; IV, chalimus IV; PA, preadult; Ad, adult.

same rate among all three species, and preadult stages were evident on all species by 14 dpe. Adult stages, while present on both salmon species by 20 dpe, were not observed on sticklebacks. Treatment with metomidate during exposure of salmon to *L. salmonis* copepodids has not been previously employed, although Sevatdal (2001) exposed salmon that had been anaesthetized in benzocaine and rinsed prior to exposure. In a preliminary trial, we observed a relatively high settlement rate of *L. salmonis* copepodids among juvenile pink salmon that had died because of aeration failure during exposure (S. Jones, unpublished data). Given that louse settlement is enhanced with reduced host swimming velocity (Genna, Mordue, Pike & Mordue (Luntz) 2005), we reasoned that host immobility during exposure should maximize copepodid settlement, consisted with the observations of Sevatdal (2001). The present study confirmed that neither copepodid motility nor the settlement and development of *L. salmonis* were impaired by the sedative. The results did suggest, however, differences in the infectivity of the two copepodid inocula. In trial 2, fish were exposed to approximately 75% more copepodids than in trial 1 without a corresponding increase in infection rates. The initial abundance of lice on chum salmon, but not on other species, was significantly lower than in trial 1. The difference in infectivity is not likely due to host size alone (see below). Rather, variations in the infectivity of copepodids between trials may be related to copepodid age or energy content (Tucker, Sommerville & Wootten 2000).

Previous work has related the success of *L. salmonis* settlement to host size. A positive correlation between *L. salmonis* abundance and the length of

Atlantic salmon and anadromous sea trout was noted (Glover, Skaala, Nilsen, Olsen, Teale & Taggart 2003; Glover, Hamre, Skaala & Nilsen 2004). Similarly, the absolute number of lice settling onto larger Atlantic salmon was greater than on smaller salmon; however, parasite density, measured in lice per cm^2 of host surface area, was greater on the smaller fish (Tucker, Sommerville & Wootten 2002). There was no significant difference in the weight of pink and chum salmon at the completion of trial 1, and in trial 2 the weight of pink salmon was significantly greater than that of chum salmon. Despite this, the initial abundance (and intensity in trial 1) of *L. salmonis* was consistently higher on chum compared with pink salmon. Indeed, the sticklebacks were very much smaller than the salmon in both trials yet, as described above, the intensity of lice on sticklebacks 6 or 7 days after exposure was consistently higher than on the salmon. Thus, size does not appear to explain the differences in infections observed among these host species.

Alternatively, these unequal early measures of infection may reflect differences in natural resistance to infection among the species. Johnson & Albright (1992) reported that coho and chinook salmon were less susceptible to *L. salmonis* infection than Atlantic salmon, and Fast, Ross, Mustafa, Sims, Johnson, Conboy, Speare, Johnson & Burka (2002) reported coho salmon to be less susceptible to *L. salmonis* than both rainbow trout and Atlantic salmon. Intraspecific variations in susceptibility to *L. salmonis* have also been reported. The enhanced susceptibility to *L. salmonis* observed among fresh water compared with sea-run brown trout appeared to be genetically based and related to an historical

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association between the parasite and the sea-run population (Glover, Nilsen, Skaala, Taggart & Teale 2001). In the present study, repeated sampling may have elicited a stress response in some of the fish that interfered with the host response to infection (Wendelaar Bonga 1997). Prior to handling, however, fish were sedated in metomidate which inhibits stress-related cortisol production (Mattson & Riple 1989), suggesting that host-related differences in susceptibility cannot be ruled out. Therefore, based on initial mean intensity, the stickleback appeared to possess the least natural resistance to infection, followed by the chum and pink salmon. The mechanisms of this innate immunity are poorly understood but may be associated with enhanced inflammation and epithelial cell hyperplasia at the site of infection in resistant species (Johnson & Albright 1992).

The rates of *L. salmonis* development on pink and chum salmon and on sticklebacks were similar to those reported in earlier studies of this parasite on Atlantic salmon, chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), coho salmon, *Oncorhynchus kisutch* (Walbaum), rainbow trout, *Oncorhynchus mykiss* (Walbaum), and sea trout, *Salmo trutta* L. (Johnson & Albright 1991, 1992; Johnson 1993; Grimnes & Jakobsen 1996; Finstad, Bjørn, Grimnes & Hvidsten 2000; Fast *et al.* 2002). Fast *et al.* (2002) also reported that the parasite matured more slowly on coho salmon compared with the other salmon species. The development of *L. salmonis* on sticklebacks was characterized by a high intensity of early developmental stages relative to that observed on the salmon, and a loss of parasites between the second and third week of infection. The timing of this loss coincided with, or followed shortly after the moult to the preadult stage. In some areas of coastal BC, sticklebacks were infected with up to 290 *L. salmonis*. Copepodids and chalimus stages represented approximately 97.6% of the specimens examined from the natural infections, whereas preadult stages represented 2.3% and adult stages approximately 0.02% (Jones *et al.* 2006). The proportions of *L. salmonis* developmental stages in laboratory infections therefore mirrored those in natural infections and confirmed that *L. salmonis* typically fails to complete development on the stickleback. The evident coincidence of the disappearance of *L. salmonis* on sticklebacks with louse maturation to the preadult stage suggested that parasite motility and/or an increase in parasite size were associated with the loss. Jones *et al.* (2006)

concluded that the threespine stickleback serves as a temporary host for the early development of *L. salmonis*. Bruno & Stone (1990) showed that preadult and adult *L. salmonis* actively transfer from saithe to cohabiting Atlantic salmon. The possibility of similar transfers from sticklebacks to salmon is presently under investigation in our laboratory.

No evidence generated in this study refuted the hypothesis that *L. salmonis*, although commonly referred to as the salmon louse, parasitises and subsequently develops on the threespine stickleback. The relative impacts of *L. salmonis* infection on sticklebacks or the salmon were not explored in these trials. Two of 15 sticklebacks died in trial 1 and none died in trial 2. Coincidentally, only one of 69 salmon died during the trials, despite exposure to relatively high numbers of copepodids. However, insufficient data were collected to adequately assess subclinical impacts or the extent to which the mortalities were directly linked to the sea lice infections. The virtual absence of mortality and the relatively low intensity of mature lice stages suggested little obvious adverse impact on either salmon or sticklebacks during the observation periods. Jones *et al.* (2006) reported no significant correlation between sea lice intensity and condition factor and concluded that infections among naturally infected sticklebacks were associated with minimal impact. By contrast, it is noteworthy that in an earlier study, exposure of 55.5 g Atlantic salmon to a similar number (approximately 200 per fish) of *L. salmonis* copepodids resulted in infections with an average of 178 lice per fish (Ross, Firth, Wang, Burka & Johnson 2000). All fish died by 12 dpe coinciding with the moult to the preadult stage. Ongoing studies (S. Jones, unpublished data) will characterize in more detail the responses and consequences of *L. salmonis* infections on laboratory-reared juvenile pink and chum salmon.

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