

Effects of varying salinities on *Lepeophtheirus salmonis* survival on juvenile pink and chum salmon

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Survival of the sea louse *Lepeophtheirus salmonis* on juvenile Pacific salmon *Oncorhynchus gorbuscha* and *Oncorhynchus keta* was examined with respect to salinity (0, 7, 14, 21 and 28). Rapid mortality was observed in fresh water (0) but motile stage sea lice tolerated higher salinities (7, 14, 21 and 28) for up to 7 days. These findings suggest that salinities juvenile Pacific salmon typically encounter during early marine residence have little affect on motile sea louse survival.

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Lepeophtheirus salmonis (Krøyer), the sea louse, is an ubiquitous ectoparasitic caligid copepod on both wild and farmed salmonids in the Northern Hemisphere. As obligate marine parasites on anadromous hosts sea lice can be exposed to salinities ranging from 0 to 35. Researchers interested in salmonid behaviour, louse physiology and the use of fresh water as a delousing agent in salmon farms, have all investigated the effect of fresh water on adult sea louse survival. McLean *et al.* (1990) reported that the majority of motile sea lice on Atlantic salmon *Salmo salar* L. died within 48 h of the fish's entry into fresh water. Finstad & Bjorn (1995) found that 60% of adult sea lice remained on Arctic charr *Salvelinus alpinus* (L.) after 7 days in fresh water and Hahnenkamp & Fyhn (1985) reported that adult sea lice survived up to 7 days on Atlantic salmon in fresh water. Stone *et al.* (2002), who treated adult Atlantic salmon with a 3 h freshwater bath, found no difference in sea louse survivorship between treatment and control fish.

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There has been no investigation into the effect of intermediate salinities, that anadromous salmonids typically encounter in the nearshore marine environment, on motile *L. salmonis* survival, nor has there been any examination of the effect of these salinities on motile sea louse survival on juvenile hosts in general, or on Pacific salmon *Oncorhynchus* sp. in particular. Juvenile pink *Oncorhynchus gorbuscha* (Walbaum) and chum, *Oncorhynchus keta* (Walbaum) salmon are unique among salmonids in their precocious entry into the nearshore marine environment at 30–35 mm fork length, L_F (Groot & Margolis, 1991). Because of freshwater input, the nearshore environment, where juvenile pink and chum salmon will remain for 2–6 months before migrating to the open ocean, is more heterogeneous with respect to salinity than the comparatively homogeneous open ocean. As a result, juvenile pink and chum salmon in areas of open net cage salmon aquaculture can be exposed to sea louse infection at both a small size and range of salinities (Morton & Williams, 2003; Morton *et al.*, 2004; Krkosek *et al.*, 2005a, 2006a). The aim of the present study was to quantify how salinity affects the short-term survival of motile sea lice on both juvenile pink and chum salmon.

Juvenile pink and chum salmon ($n = 40$; mean pink $L_F \pm$ s.d. 73 ± 8 mm and mean chum L_F 66 ± 7 mm) infected with motile *L. salmonis* (mean motile sea lice fish⁻¹ \pm s.d., pink 1.78 ± 0.92 and chum 1.36 ± 0.61) were collected between 5 May and 30 May 2006, in the Broughton Archipelago, BC, Canada, following methods described in Krkosek *et al.* (2005b). Following capture, individual fishes were randomly dip netted from a holding tub, motile sea lice enumerated (Krkosek *et al.*, 2005b) and assigned to one of five aquaria (10 l) filled with water of ambient salinity (27–30) and temperature (10–15° C). This was continued until there were five fish and a minimum of six motile sea lice per aquarium (six to 11 sea lice tank⁻¹), at which point aquaria were left undisturbed for a 12 h acclimatization period. Each aquarium was then serially diluted over the course of several hours to one of four salinities (0, 7, 14 and 21) or sham diluted, by adding sea water, as a control (28). Aquaria were maintained on a flow-through table at a 16L:8D photoperiod, with water in each aquarium being replaced by two thirds every 24 h, enabling temperature and dissolved oxygen to be maintained between 10–15° C and 8–10 mg l⁻¹, respectively. Twice daily (0700 and 1900 hours) aquaria were visually assessed for moribund sea lice (*i.e.* dislodged from the host and non-responsive to tactile stimulation), which were removed and fixed in 70% ethanol. At the end of each trial (168 h) individual fishes were caught, measured (L_F), and remaining sea lice removed and fixed in 70% ethanol. This procedure was repeated four times for each species. Sea lice were identified to species and stage according to Johnson & Albright (1991).

Survival analysis was used to quantify the effect of salinity on sea louse survival because of the censored nature of the data, *i.e.* incomplete information about the survival of some individuals. A parametric survival model with salinity, host species and sea louse sex as fixed factors was fitted to the survival data. Models with exponential, Weibull and log-normal error distribution were compared using analysis of deviance to select a model with the smallest error deviance. To avoid or neutralize pseudo-replication, due to observations of more than one sea louse per tank, a frailty (random effects) term for each tank

was included in the model (Cook & Lawless, 2007). Only significant effects were included in the final model ($P < 0.05$).

Fifty-four of 321 (16%) sea lice were not recovered during the experiment. The proportion of missing sea lice to total sea louse mortalities (as calculated by subtracting the number of sea lice at the end of the trial from the number at the beginning) did not differ between salinities (Kruskal–Wallis test, $P > 0.05$) suggesting that the probability of a sea louse being missing was independent of salinity and that missing sea lice were consumed by host fishes after dying and falling off the host. Missing sea lice were assigned a survival time based on the known probability of mortality for recovered sea lice at each time interval and salinity. All analyses were performed in R version 2.3.1 (R Development Core Team, 2006).

Salinity had a significant effect on motile sea louse survival (survival regression, $n = 321$, $P < 0.001$). The best-fit model included a Weibull error distribution and salinity as a main effect (Table I). Host species and sea louse sex did not have significant effects on sea louse survival ($P > 0.05$), nor was the frailty term significant ($P > 0.05$), so all three terms were excluded from the final model. No sea lice survived in fresh water beyond 108 h and the estimated mean survival time in fresh water was 61 h (Table II). Survival in intermediate salinities, however, was considerably higher (Fig. 1 and Table II).

These results demonstrate that motile *L. salmonis* on juvenile Pacific salmon can tolerate short-term exposure to salinities below that of natural sea water (28–32) but that mortality in fresh water is rapid. It is important to note that the survival model assumes those sea lice that were not recovered during the experiments died as a result of salinity and not some other unmeasured variable. This assumption is supported by the fact that the proportion of missing sea lice to total sea louse mortalities did not differ between salinities. If this assumption was violated the model would tend to overestimate the effect of salinity on sea louse survival, though this would only be pronounced at higher salinities, where estimates of survival were less confident as a result of fewer total mortalities observed. As such the key findings of this study, that sea lice can tolerate intermediate salinities for short periods of time but that mortality in fresh water is rapid, remained unaffected.

The results of this study are consistent with those of McLean *et al.* (1990), that sea lice die quickly in fresh water, but differ from those of Hahnenkamp & Fyhn (1985) and Finstad & Bjorn (1995) who found that up to 60% of sea lice remained on host fishes after 7 days in fresh water. The findings of McLean *et al.* (1990), however, should be interpreted with caution as it has been

TABLE I. Coefficient estimates from survival regression of salinity on motile sea louse survival. Best-fit model: $\ln T = \beta_0 + \beta_1 S + \sigma e$, where T is survival time, S is salinity, σ is a scale parameter and e is an error term (*i.e.* random with a known distribution)

	Estimate \pm S.E.	P
β_0	4.120 \pm 0.072	<0.001
β_1	0.124 \pm 0.010	<0.001
σ	-0.512 \pm 0.075	<0.001

TABLE II. Predicted mean \pm S.E. survival times based on a best-fit survival regression model, for motile sea lice at five salinities

Salinity	0	7	14	21	28
Mean survival time (h)	61 \pm 1	146 \pm 13	347 \pm 55	826 \pm 190	1963 \pm 595

suggested that chlorinated water used by McLean *et al.* (1990) may have accelerated the loss of sea lice (Finstad & Bjorn, 1995). There is some evidence that susceptibility to infection is species-specific with Pacific salmon being more resistant to infection than Atlantic salmon (Johnson & Albright, 1992; Fast *et al.*, 2002). This may confound the apparent differences in freshwater tolerance of sea lice between Atlantic salmon and Arctic charr hosts (Hahnenkamp & Fyhn, 1985; Finstad & Bjorn, 1995) and pink and chum salmon hosts in the present study, though the relative resistance to infection between pink and chum salmon and Atlantic salmon and Arctic charr is unknown. Host size may be another factor that influences the freshwater tolerance of sea lice. It has been suggested that adult sea lice can prolong survival in fresh water by maintaining salt balance through replenishment from mucus and becoming embedded in the host's cutaneous layer (Hahnenkamp & Fyhn, 1985). Assuming the total amount of mucus and cutaneous surface area on a host increases with host size, it could be predicted that sea lice on small hosts (*e.g.* juvenile fishes) should experience greater mortality rates in fresh water than they would on larger hosts. Hosts used in this study were orders of magnitude smaller (*c.* 3 g) than the host fishes used in other studies (Hahnenkamp & Fyhn, 1985; McLean *et al.*, 1990; Finstad & Bjorn, 1995; 350–450 mm L_F , 400–700 and 310 g, respectively), which lends support to this hypothesis. It is apparent that further

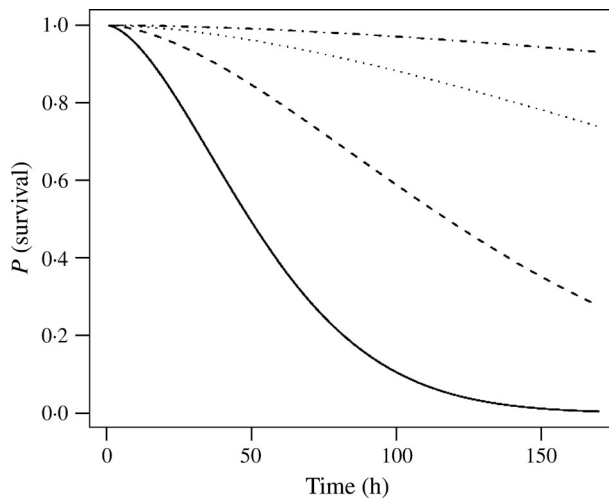


FIG. 1. Predicted survival times based on a best-fit survival regression model, for motile sea lice in four salinities [0 (—), 7 (---), 14 (.....) and 21 (-.-)].

experimental work is necessary to tease apart the influence of host species and size on the freshwater tolerance of sea lice.

In the Broughton Archipelago of BC the influence of salinity on the survival of sea lice and patterns of infection on juvenile pink and chum salmon has been debated (Brooks, 2005; Brooks & Stucchi, 2006; Jones *et al.*, 2006; Krkosek *et al.*, 2006b). During their early marine phase (March to mid-June) juvenile pink and chum salmon typically inhabit the upper 2 m of the littoral zone (Groot & Margolis, 1991; pers. obs.). Within the Broughton Archipelago salinities in the upper 2 m of the water column, during spring (March to mid-June), typically do not drop below 20 (Brooks, 2005). Although the present results can only inform on the survival of motile sea lice, and it is known that infective sea lice copepodids are sensitive to reduced salinity (Johnson & Albright, 1991; Bricknell *et al.*, 2006), they suggest that salinities juvenile pink and chum salmon typically encounter during the spring in the nearshore environment of the Broughton Archipelago (*i.e.* 20–30) are not likely to dramatically affect motile sea louse survival or their distribution among hosts.

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