

Sea lice and salmon population dynamics: effects of exposure time for migratory fish

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The ecological impact of parasite transmission from fish farms is probably mediated by the migration of wild fishes, which determines the period of exposure to parasites. For Pacific salmon and the parasitic sea louse, *Lepeophtheirus salmonis*, analysis of the exposure period may resolve conflicting observations of epizootic mortality in field studies and parasite rejection in experiments. This is because exposure periods can differ by 2–3 orders of magnitude, ranging from months in the field to hours in experiments. We developed a mathematical model of salmon–louse population dynamics, parametrized by a study that monitored naturally infected juvenile salmon held in ocean enclosures. Analysis of replicated trials indicates that lice suffer high mortality, particularly during pre-adult stages. The model suggests louse populations rapidly decline following brief exposure of juvenile salmon, similar to laboratory study designs and data. However, when the exposure period lasts for several weeks, as occurs when juvenile salmon migrate past salmon farms, the model predicts that lice accumulate to abundances that can elevate salmon mortality and depress salmon populations. The duration of parasite exposure is probably critical to salmon–louse population dynamics, and should therefore be accommodated in coastal planning and management where fish farms are situated on wild fish migration routes.

Keywords: salmon; sea lice; aquaculture; conservation; reservoir host; host–parasite dynamics

1. INTRODUCTION

The shifting global supply of seafood from fisheries to aquaculture (Pauly *et al.* 2003; Duarte *et al.* 2007; FAO 2007) has a corresponding shift in coastal ecosystems, where domesticated fish populations can now dwarf wild fish populations (Heuch & Mo 2001; Krkošek *in press*). Such changes in animal populations are associated with emerging infectious diseases (Daszak *et al.* 2000), which have become a threat to both aquaculture and wild fishes (Gaughan 2001; Murray & Peeler 2005; Costello 2006; Krkošek *et al.* 2007a). Understanding the mechanisms and risks of parasite transmission from aquaculture to wild fishes has depended critically on ecological processes, such as the migration of wild fish populations (Krkošek *et al.* 2006, 2007b). In this paper, we develop and parametrize a mathematical model to show how the connection between an ecological process (migration of juvenile fishes) and an epidemiological process (exposure period to parasites) is key for understanding the threat of parasitic sea lice (*Lepeophtheirus salmonis*) spreading from farmed to wild salmon.

Salmon aquaculture can increase the exposure of wild juvenile salmon to lice (Costello 2006; Krkošek *in press*), probably contributing to declines of some wild salmon stocks (Krkošek *et al.* 2006; Ford & Myers 2008). To industry, lice cost nearly \$5 billion annually in reduced

productivity and control efforts (Costello 2009). Lice are native ectoparasitic copepods, common on both wild and farmed adult salmon, which feed on host surface tissues causing morbidity and mortality (Pike & Wadsworth 2000; Costello 2006). In Pacific North America, lice are normally rare on wild juvenile salmon because salmon enter the sea uninfected and then enjoy a period of allopatry with infected adult subpopulations (Krkošek *et al.* 2007b). Although field studies of juvenile salmon migrating past salmon farms report epizootics (Morton & Williams 2003; Morton *et al.* 2004, 2008; Krkošek *et al.* 2006), experimental studies report rejection of parasites by juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon (Morton & Routledge 2005; Jones *et al.* 2006, 2007, 2008).

The disagreement among field and experimental studies, which has challenged scientific understanding and policy development, may arise because inference has not yet been extrapolated correctly. The exposure time in experimental studies, usually several hours (Jones *et al.* 2006), corresponds poorly with the two to three month migration of juvenile salmon past multiple salmon farms (Krkošek *et al.* 2006). This is a difference of two to three orders of magnitude in a fundamental epidemiological factor. In terms of population dynamics, sea lice infections in field conditions occur as an immigration and death process (continual establishment of new infections and subsequent parasite mortality), whereas experimental studies capture only parasite mortality whether direct or through mortality of the host.

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We investigated the role of exposure time in linking field and experimental studies of sea lice by developing and parametrizing a model of salmon–louse population dynamics. The model begins with an age and stage structured version of the McKendrick–von Foerster equation (Kot 2001) that was fit to replicated trials, monitoring over 7500 naturally infected juvenile salmon in ocean enclosures. We then expanded the model to account for survival and mortality of juvenile salmon, thereby estimating rates of parasite-induced host mortality associated with louse developmental stages. By combining the model with a standard fisheries model for salmon population dynamics, we obtain a mathematical framework for evaluating the sensitivity of salmon populations to varying epidemiological factors such as exposure time. The results show that modifying the duration of exposure can reproduce both experimental and field patterns of sea lice and salmon population dynamics, meaning that repeated exposure of migratory juvenile salmon to salmon farms should be considered in coastal management and planning.

(a) *Louse life cycle*

The salmon louse is a directly transmitted parasite, meaning that it has a parasitic phase and a free-living phase in its life cycle but no obligate intermediate host (Pike & Wadsworth 2000). The copepodid louse is the planktonic infectious stage that attaches to a host fish where it is distinguishable for a few days before it moults through a series of chalimus and then motile stages (Johnson & Albright 1991). The motile stages include sexually reproductive adults and gravid females from which non-feeding nauplii hatch into the water column. The nauplii can disperse for several days before developing into the infectious copepodid (Johnson & Albright 1991). The developmental progression during the parasitic phase of the life cycle from copepodid to chalimus to motile louse corresponds to changes in parasite size, mobility and feeding behaviour. The parasites grow substantially in size from the chalimus to the motile stage and also transition from being sedentary (attached by a frontal filament) to being freely mobile on the host and motile among host fish.

2. MATERIAL AND METHODS

(a) *Data collection*

The studies occurred in the Broughton Archipelago, a salmon farming region of British Columbia, Canada, where sea lice have recurrently infested wild juvenile pink and chum salmon (Morton & Williams 2003; Morton *et al.* 2004; Krkošek *et al.* 2006). We collected naturally infected juvenile pink and chum salmon and reared them in ocean enclosures for several weeks to document the subsequent louse population dynamics and fish survival. There were two replicate trials for juvenile chum salmon in April–June of 2006 and four replicate trials for juvenile pink salmon in April–June 2007.

Each trial began by collecting approximately 2500 juvenile salmon in a single beach seine catch. The seine net was 45 m long and 3 m deep with 0.5 cm knotless mesh. We transferred the fish into 25 l buckets using 15 cm × 15 cm dipnets and transported them (10–25 min) to a field laboratory consisting of a floathouse, floating docks and ocean enclosures. Once at the laboratory, we haphazardly distributed the fish into fibreglass flow-through ocean enclosures. We introduced the

same number of fish into each enclosure, either 140 fish or 160 fish, depending on the number of fish collected and the number of enclosures available at the start of the trial (between 10 and 14). During transportation and stocking, we maintained the fish in the buckets by using aerators and by exchanging fresh seawater.

The ocean enclosures were each 1.5 m square by 0.6 m deep and constructed of dark yellow fibreglass with windows approximately 0.3 × 0.5 m cut in each side covered with 0.3 mm mesh. The windows provided a continual free exchange of seawater with the outside environment. There was a 10 cm mesh screen that covered the top of each enclosure to exclude predators such as herons or raccoons. We tied buoys around each enclosure for flotation and tied each enclosure alongside one of two floating docks each of which were sheltered by a series of logs that formed a breakwater around the floating laboratory.

After the fish were introduced into the enclosures, we immediately enumerated sea lice on a sample of 100 juvenile salmon remaining in buckets that had been put aside for this purpose. This provided an initial estimate of sea lice abundances at the start of the survival trials. We then monitored the abundance of lice on the juvenile salmon by following a sampling schedule that had a high sampling frequency early in the trial (e.g. three samples per day) and a low sampling frequency later in the trial (e.g. one sample every third day). This yielded high-resolution data on the rapid early dynamics of copepodids and chalimi while sustaining the trial long enough to capture the transition of lice through motile stages.

For each sample, we collected approximately half the fish from an enclosure using a small seine net (3 m long, 1 m deep, 0.5 cm knotless mesh) and transferred them to buckets using seawater-filled plastic bags. Once in buckets, we enumerated sea lice and measured the fork length of every fish using the non-lethal assay described in Krkošek *et al.* (2005b). For motile stage lice, we distinguished between salmon lice (*L. salmonis*) and another occurring generalist louse, *Caligus clemensi*. We always sampled the same enclosure on consecutive sampling events to minimize the time in which the fish experienced reduced population density. The total number of enclosures in use therefore declined with time over each trial whereas fish density remained roughly constant. Once all fish in a sample were assayed, they were allowed to recover in a separate enclosure for one day before being released near the location where they were captured.

Each day we measured the temperature and salinity of the seawater at the laboratory site using a thermometer and a salinity refractometer. During each day we fed the fish commercial hatchery salmon feed every other hour during daylight hours. We inspected the enclosures for dead or moribund fish at every feeding. We removed dead fish and severely moribund fish from the enclosures and assayed the fish for sea lice and morphometrics. We endeavoured to remove severely moribund fish from enclosures before death to minimize the occurrence of sea lice leaving their host fish because it was dead. We recorded the time of death for each fish as well as the time of release for every live fish that was sampled and released.

(b) *Modelling louse population dynamics*

We formulated an age-structured model of sea lice developing through copepodid, chalimus and motile developmental stages. The model is conceptually similar to

the one used by Stien *et al.* (2005) in their analysis of data from experimental infection challenges. Most experimental studies begin with a single infection challenge and thus with lice that are all the same age. Because our data were derived from wild fish and lice, the fish may have been exposed to copepodids for days or weeks, leading to a broad age distribution of lice at the time of capture and start of the trials. To accommodate this, our model extends and departs from (Stien *et al.* 2005) so as to represent the age and stage distribution of lice preceding and over the course of the trials.

We assume the juvenile salmon entered the ocean without lice and were subsequently exposed to planktonic copepodids that attach, age and die. We track the abundance of parasitic copepodids (C), chalimi (H) and motiles (M) as well as the age distribution of lice within each stage (a_c , a_h and a_m , respectively). Ageing and mortality follow the McKendrick–von Foerster equation (McKendrick 1926; von Foerster 1959; Kot 2001) for each stage. The dynamics of copepodids are given by

$$\frac{\partial C}{\partial t} + \frac{\partial C}{\partial a_c} = \begin{cases} -\mu_c C, & \text{if } a_c \leq \tau_c \\ -\mu_c C - \nu_c C, & \text{if } a_c > \tau_c, \end{cases} \quad (2.1a)$$

$$\frac{\partial C(0, t)}{\partial t} = \beta L(t), \quad C(a_c, 0) = 0, \quad (2.1b)$$

$$L(t) = \begin{cases} L, & \text{if } 0 < t < T \\ 0, & \text{if } t \geq T, \end{cases} \quad (2.1c)$$

which says that the local density of free living copepodids to which the juvenile salmon were exposed was zero before fish entered the sea ($t < 0$) and constant at density L until the fish were captured at time T . The free-swimming copepodids attach to juvenile salmon at rate β and become age zero copepodids ($a_c = 0$), where age is measured as time since attachment. The attached copepodids then die at rate μ_c or survive to an age threshold, τ_c , when they develop into chalimus lice at rate ν_c .

The dynamics of chalimus lice are given by the McKendrick–von Foerster model

$$\frac{\partial H}{\partial t} + \frac{\partial H}{\partial a_h} = \begin{cases} -\mu_h H, & \text{if } a_h \leq \tau_h \\ -\mu_h H - \nu_h H, & \text{if } a_h > \tau_h, \end{cases} \quad (2.2a)$$

$$\frac{\partial H(0, t)}{\partial t} = \nu_c \int_{\tau_c}^{\infty} C(a_c, t) da_c, \quad H(a_h, t \leq \tau_h) = 0, \quad (2.2b)$$

which tracks the flow of copepodids exceeding age τ_c that are developing into age zero chalimus lice ($a_h = 0$), where age is measured as time since becoming a chalimus louse. The chalimi then die at rate μ_h and after an age threshold of τ_h develop into motile lice at rate ν_h . The dynamics of motile lice occur similarly

$$\frac{\partial M}{\partial t} + \frac{\partial M}{\partial a_m} = -\mu_m M, \quad (2.3a)$$

$$\frac{\partial M(0, t)}{\partial t} = \nu_h \int_{\tau_h}^{\infty} H(a_h, t) da_h, \quad M(a_m, t \leq \tau_m) = 0, \quad (2.3b)$$

which tracks the flow of chalimi exceeding age τ_h that are developing into age zero motile lice ($a_m = 0$), where age is measured as time since becoming a motile louse. The motiles then die at rate μ_m , which represents mortality from three sources: natural parasite mortality; non-parasite related host mortality; and parasite-induced host mortality. Because louse abundance was relatively low, the data are nearly Poisson distributed (figure 1), which results in μ_m being a simple linear sum of these three mortality rates (Frazer 2008).

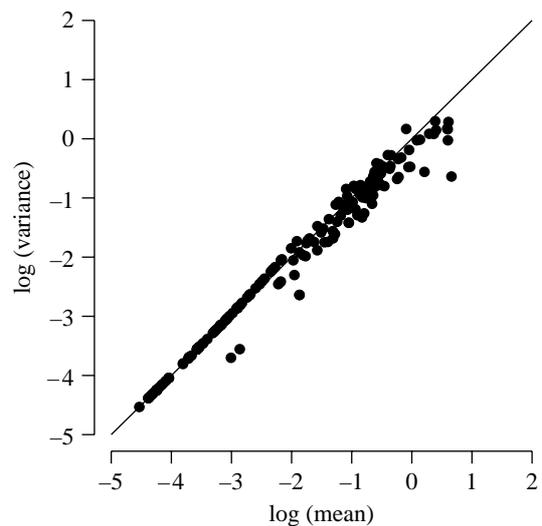


Figure 1. Log variance versus log mean for copepodids, chalimi and motiles in each sample in the study. There are approximately 80 fish per sample. Samples with zero lice are not shown. The solid line is the variance = mean line, as predicted by a Poisson distribution. Compare with fig. 5 in Shaw & Dobson (1995).

(c) Fitting the louse model

The solution for the louse model can be found numerically by discretizing age and time by some small time interval Δ . The discretization transforms the model into a system of coupled matrix population models where each copepodid, chalimus and motile stage is structured by age classes organized by Δ within each stage. Each iteration updates the vector of age structured population abundances for each stage by time step Δ . The population projection matrix for each stage is a Leslie matrix with entries representing the probability of surviving from age a to age $a + \Delta$ in one time step Δ (Caswell 2001), but modified to account for the establishment of new copepodids, or the development of copepodids to chalimi and from chalimi to motiles. Specifically, the discretized model is

$$\begin{aligned} C(0, t) &= BL(t)\Delta, & \text{if } a_c = 0 \\ C(a_c, t) &= (1 - \mu_c \Delta)C(a_c - \Delta, t - \Delta), & \text{if } \Delta < a_c < \tau_c \\ C(a_c, t) &= (1 - \mu_c \Delta - \nu_c \Delta)C(a_c - \Delta, t - \Delta), & \text{if } a_c \geq \tau_c, \end{aligned} \quad (2.4a)$$

$$\begin{aligned} H(0, t) &= \nu_c \Delta \sum_{a_c \geq \tau_c} C(a_c, t - \Delta), & \text{if } a_h = 0 \\ H(a_h, t) &= (1 - \mu_h \Delta)H(a_h - \Delta, t - \Delta), & \text{if } \Delta < a_h < \tau_h \\ H(a_h, t) &= (1 - \mu_h \Delta - \nu_h \Delta)H(a_h - \Delta, t - \Delta), & \text{if } a_h \geq \tau_h, \end{aligned} \quad (2.4b)$$

$$\begin{aligned} M(0, t) &= \nu_h \Delta \sum_{a_h \geq \tau_h} H(a_h, t - \Delta), & \text{if } a_m = 0 \\ M(a_m, t) &= (1 - \mu_m \Delta)M(a_m - \Delta, t - \Delta), & \text{if } a_m > \Delta. \end{aligned} \quad (2.4c)$$

The predicted mean abundance of copepodids, chalimi and motiles at any time t is then the sum of all lice within each respective developmental stage is as follows:

$$\begin{aligned} C(t) &= \sum_{a_c} C(a_c, t) \\ H(t) &= \sum_{a_h} C(a_h, t) \\ M(t) &= \sum_{a_m} M(a_m, t). \end{aligned} \quad (2.5)$$

To fit the model to the time-series data of sea lice abundances in the ocean enclosures, we used maximum

likelihood with a Poisson error distribution. The Poisson assumption implies that lice attach as a Poisson process and subsequently survive through chalimus and motile stages as a binomial process (Krkošek *et al.* 2005a). The Poisson assumption has empirical support (figure 1) and models the abundance of copepodids, chalimi and motiles each as a Poisson random variable, Y_c , Y_h and Y_m , respectively, with a predicted mean abundance given by equations (2.5). The model was fit separately to the data from each trial using the likelihood function

$$L\{y_{s,i,n} | C, H, M\} = \prod_s \prod_i \prod_n P\{Y_n = y_{s,i,n} | \Phi\}, \quad (2.6)$$

where $y_{s,i,n}$ is the observed number of stage n lice (copepodid, chalimus and motile) on fish i in sample s ; C , H and M are the solutions to equations (2.5); and Φ is the vector of free parameters (μ_c , μ_h , μ_m , ν_c , ν_h , τ_c , τ_h , T , βL). In an attempt to generalize the parameter values, we also fit the model to data from all trials combined, but separately for pink and chum species. For this analysis, we assume that the only differing parameters among trial datasets are the time of capture, giving a likelihood function

$$L\{y_{r,s,i,n} | C, H, M\} = \prod_r \prod_s \prod_i \prod_n P\{Y_n = y_{r,s,i,n} | \Phi\}, \quad (2.7)$$

which is similar to equation (2.6) except that the function spans all trials, indexed by r . The array of parameter values is $\Phi = (\mu_c, \mu_h, \mu_m, \nu_c, \nu_h, \tau_c, \tau_h, T_1, T_2, T_3, T_4, \beta L)$ for pink salmon and $\Phi = (\mu_c, \mu_h, \mu_m, \nu_c, \nu_h, \tau_c, \tau_h, T_1, T_2, \beta L)$ for chum salmon. The T_r values are the capture times for each replicate r . These likelihood functions resulted in a 9, 12 and 10 dimensional parameter space, respectively, in which to locate the maximum-likelihood estimates. The estimation was conducted by applying optimization schemes to the negative log likelihood of the likelihood functions by starting first with a genetic algorithm (to bring the solution near the maximum-likelihood estimate) followed by a Nelder–Mead simplex to find the exact maximum-likelihood parameter values. All analyses were conducted in the statistical programming language R (www.R-project.org) using the *genalg* and *optim* packages.

(d) Modelling louse pathogenicity

To estimate the pathogenicity of salmon lice on juvenile salmon we fit and compared a series of salmon survival models that include the model solutions for sea lice abundances as well as the rate of parasite-induced host mortality. We assume that copepodids do not increase the mortality of host fish but that chalimus and motile stages cause corresponding instantaneous rates of host mortality, α_h and α_m , respectively. We also included a term for delayed mortality in the trials due to the possibility that a proportion of fish did not learn to feed. The survival function is

$$Q(t) = \begin{cases} \exp\left[-\left(\int_0^t (\alpha_h H(\tau) + \alpha_m M(\tau)) d\tau\right)\right], & \text{if } t \leq T_d \\ \exp\left[-\left(\int_0^{T_d} (\alpha_h H(\tau) + \alpha_m M(\tau)) d\tau + \int_{T_d}^t (\alpha_h H(\tau) + \alpha_m M(\tau) + \alpha_d) d\tau\right)\right], & \text{if } t > T_d \end{cases} \quad (2.8)$$

where $Q(t)$ is the probability a juvenile salmon survives to time t , T_d is the time delay between the start of the experiment and when salmon mortality begins due to emaciation of individual fish that did not learn to feed. Thus, equation (2.8) tracks juvenile salmon mortality due to three sources: parasite-induced host mortality from

chalimus lice ($\alpha_h H(\tau)$); parasite-induced host mortality from motile lice ($\alpha_m M(\tau)$); and mortality from emaciation (α_d).

From equation (2.3a) and (2.3b) the probability density function for juvenile salmon mortality events is

$$f(t) = \frac{d}{dt}(1 - Q(t)) \quad (2.9)$$

and since the data are right censored (most fish were released before death) this leads to the likelihood function for the survival model

$$L\{\tau_i | H, M, A\} = \prod_i f(\tau_i) \prod_j Q(\tau_j), \quad (2.10)$$

where H and M are the solutions to the sea lice model and A is the vector of parameters ($A = (\alpha_h, \alpha_m, \alpha_d)$). The quantities τ_i and τ_j are the mortality and release times, respectively, of each fish. We fit the survival model to the survival and mortality data from all trials combined, each separately for pink and chum salmon. The model solutions for H and M used parameter values estimated from the fits to each individual trial, not the estimates from combined trials. This is because small variation in temperature among trials would act to affect louse developmental rates more so than host mortality rates. We used maximum likelihood and the *optim* package in R to fit the models and likelihood ratio tests to test which pathogenicity parameters significantly affect juvenile salmon survival. We used likelihood profiles to find the 95% confidence intervals on α_h and α_m .

(e) Modelling pink salmon population dynamics

To evaluate how sea louse infestation of juvenile salmon affects salmon population dynamics, we begin with a Ricker model for pink salmon population dynamics. The Ricker equation (Ricker 1954) is commonly used to estimate population growth rates (Myers *et al.* 1999) and density dependence (Brook & Bradshaw 2006) from abundance time-series in fisheries and ecology. The model is commonly used to understand the standard components of productivity, over-compensatory density dependence and environmental variation that characterize fish population dynamics (Myers *et al.* 1999; Hilborn & Walters 2001), and particularly for Pacific salmon (Peterman *et al.* 2000; Ford & Myers 2008). The model has the form

$$n_i(t) = n_i(t-2)\exp[r - bn_i(t-2)], \quad (2.11)$$

where $n_i(t)$ is the abundance of population i in year t ; r is the population growth rate; and b determines density dependent mortality. The parameters in equation (2.11) have been estimated in previous work. The population growth rate is $r = 1.2$, as estimated from a meta-analysis of pink salmon stocks (Myers *et al.* 1999). The density dependent parameter is $b = 0.64$, as estimated from an analysis of pink salmon escapement data from the central coast of British Columbia (Krkošek *et al.* 2007a). These parameter estimates come from populations that were largely unexposed to salmon farms.

The effect of louse infestation on pink salmon population dynamics is a decline in salmon survival. The proportion of juvenile salmon that survive the infestation is given by

$$Q = \exp\left[-\int_0^T (\alpha_h H(\tau) + \alpha_m M(\tau)) d\tau\right], \quad (2.12)$$

where T is the duration of the juvenile salmon life cycle for which the above parametrization of louse demographic and pathogenicity rates is appropriate. We take T to be 80 days, which is the approximate time for juvenile pink and chum

Table 1. Parameter values of the louse population dynamics model (equations (2.1a)–(2.3b)) for pink salmon trials P-1 to P-4 and chum salmon trials C-1 and C-2. (Also shown are the parameter estimates from the model fit to the combined-trial data (equation (2.6)).)

parameter	P-1	P-2	P-3	P-4	P-All	C-1	C-2	C-All
μ_c	0.0000	0.0825	0.0064	0.0004	0.0210	0.0211	0.0065	0.0007
μ_h	0.0238	0.0002	0.0105	0.0257	0.0100	0.0080	0.0446	0.0029
μ_m	0.1397	0.3202	0.2367	0.3421	0.2400	0.1603	0.2417	0.2301
βL	0.3945	0.5007	0.3664	1.2523	0.7098	0.0473	0.1047	0.5381
T_1	14.7505	13.1773	16.9767	30.2599	7.5976	12.6500	57.8429	13.6500
T_2	—	—	—	—	8.1287	—	—	29.0514
T_3	—	—	—	—	9.9462	—	—	—
T_4	—	—	—	—	24.8825	—	—	—
τ_c	0.5398	0.0485	0.1500	0.4500	0.5221	0.2500	0.2365	0.4500
τ_h	14.2568	13.5890	14.0248	7.9484	8.1019	11.9489	11.1853	13.2477
ν_c	0.4528	0.4433	0.4944	1.0332	0.6637	0.5153	0.6581	0.5612
ν_h	0.1857	0.2720	0.3875	0.6169	0.1933	0.3073	0.2374	0.3297

salmon to migrate through the Broughton Archipelago (Krkošek *et al.* 2006). The lice on the juvenile salmon, *H* and *M*, can be controlled by specifying the intensity and duration of exposure to copepodids, $\beta L(t)$, in equations (2.1a)–(2.3b). For this, we used the louse survival and developmental rates estimated from the combined trial data for pink salmon (table 1).

Assuming lice act independently of other mortality factors, such as predation, the estimated direct effect of lice on pink salmon population dynamics is

$$n_i(t) = n_i(t-2)\exp[r - bn_i(t-2)]Q. \quad (2.13)$$

The effect on pink salmon productivity can be seen through the net reproductive value R_0 ,

$$R_0 = Qe^r, \quad (2.14)$$

which is the number of surviving adult offspring generated by one average adult at low population density when density dependent mortality can be ignored. As R_0 declines so too does salmon population resilience (speed of recovery following disturbance). R_0 also differentiates salmon population persistence ($R_0 > 1$) and extinction ($R_0 < 1$). We used this simple parametrized model to evaluate the effect on wild pink salmon productivity and persistence of varying the exposure time and intensity of juvenile salmon to copepodids.

3. RESULTS

The chum salmon trials lasted 35–40 days and the pink salmon trials lasted usually more than 30 days except for one trial that lasted 22 days. The juvenile salmon were 37–49 mm fork length at the start of the trials and grew approximately to 55–69 mm over the course of the trials (table 2). The salinity was 28–32 ppt and temperature was 9–11°C, except for the fourth trial for pink salmon when temperatures rose to 13–14°C. We counted a total of 47 *C. clemensi* motiles and 326 *L. salmonis* motiles during the chum salmon survival trials and 47 *C. clemensi* motiles and 375 *L. salmonis* motiles during the pink salmon survival trials. We observed no copepodids after the first few days in each trial indicating that copepodids did not enter the ocean enclosures and establish new infections.

The louse population dynamics model provided an excellent fit to the data (figures 2 and 3). In each case, there was an initial abundance of copepodid and chalimus lice that subsequently followed a developmental progression

Table 2. Average fork lengths (in mm, and associated standard deviations and sample sizes) of juvenile pink and chum salmon at the start and end of each trial.

host species	replicate	initial	final
chum salmon	1	41.6 (3.1,100)	64.5 (8.9,41)
	2	46.1 (5.9,100)	68.5 (9.2,59)
pink salmon	1	38.6 (5.0,79)	54.5 (5.8,70)
	2	37.3 (2.8,70)	55.9 (4.6,46)
	3	41.7 (3.6,70)	62.4 (6.1,70)
	4	48.6 (3.8,70)	63.3 (4.0,70)

through chalimus and motile stages. In all cases, the louse populations showed a decline to almost zero abundance after 30 days. The parameter estimates (table 1) indicated that most of the louse mortality occurred in the early motile stages. The estimated mean lifespan of a motile louse is $1/\mu_m$, which ranges from 3–6 days per trial. The parameter estimates also suggest that the copepodid stage was short lived, lasting usually less than 1 day before development to chalimus stages begins. Chalimus lice endured for 11–14 days before development into preadults began, except for the third trial for pink salmon, which occurred later in the season when water was warmer and louse developmental rates were accelerated.

There were a total of 65 and 189 mortalities in the pink and chum datasets. We removed mortalities on the first day of each trial to exclude mortality arising from stress incurred during catching and transporting the fish. This resulted in 52 and 185 mortalities remaining in the pink and chum datasets, respectively. The remaining 4964 pink salmon and 3730 chum salmon used in the trials were released alive, after they were sampled for sea lice. Most of the censored data (live releases) came from early in the trials when we sampled with high frequency to capture the copepodid to chalimus transition. Although there were few fish mortality events in the trials (figures 2 and 3), there was sufficient data to support a survival analysis. The survival model with mortality from chalimus and motile lice as well as delayed louse-independent mortality was the best-fit survival model (table 3). The mortality rate induced by chalimus lice was less than mortality induced by motile lice for chum salmon (table 4). For pink salmon, the mortality rates due to chalimus and motile lice were similar (table 4). The mortality observed later in the

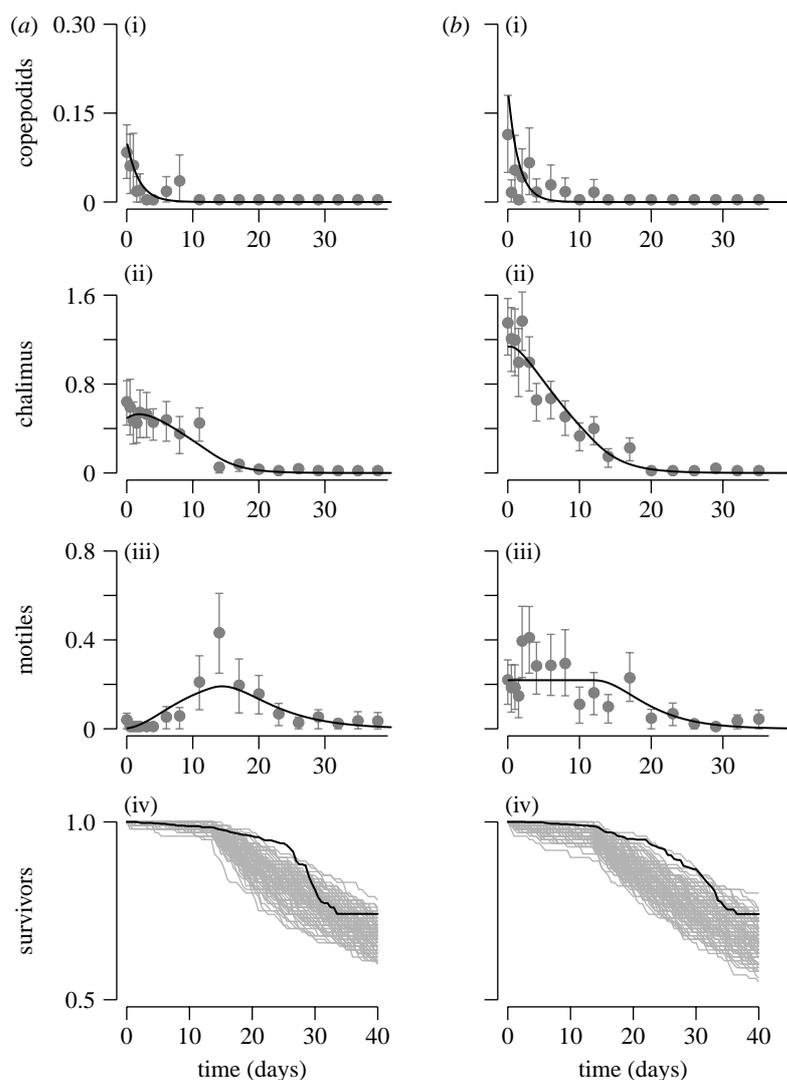


Figure 2. (i)–(iii) Salmon lice development and mortality on juvenile chum salmon and (iv) survival of juvenile chum salmon for two replicate trials (a) and (b). Shown are mean lice abundance \pm 95% bootstrap confidence intervals and the fit of the louse population dynamics model (i)–(iii) and the proportion of juvenile chum salmon surviving (black line, real data; grey lines, 1000 Monte Carlo Markov chain simulations of the best fit survival model).

trials—particularly after 25 days was associated with emaciated fish (M. Krkošek 2006 & 2007, personal observation) and near zero sea lice presence (figures 2 and 3), and few louse-associated scars indicating that some fish starved. However, overall growth rates in fish body size observed during the trials indicates that most fish learned to feed (table 2). There was a wide range in the 95% confidence intervals on the rates of parasite-induced host mortality (table 4), reflecting the fact that there were few mortality events with which to fit the survival model.

By simulating the parametrized model, we observed a large difference in sea lice population dynamics and juvenile salmon survival that depended on the duration of exposure time. For a short exposure to sea lice copepodids (e.g. 1 day), there was an initial abundance of copepodids that rapidly died off with little associated parasite-induced host mortality (figure 4). When exposure time was large (e.g. 80 days), louse numbers accumulated and reached abundances that are large enough and sustained enough to induce substantial host mortality. This effect was observed again for the productivity, resilience and persistence of salmon populations when the louse model and salmon survival model were coupled with the Ricker model

for pink salmon population dynamics (figure 5). The length of exposure time, as well as intensity, is important to distinguish between situations where salmon populations may be depressed or extirpated relative to situations where there is little effect on wild pink salmon populations. Owing to uncertainty in the estimates for the rates of parasite-induced host mortality (wide 95% confidence intervals on α_H and α_M), there was a large range in the estimated effect of lice on juvenile salmon survival and salmon population dynamics.

4. DISCUSSION

Salmon farms can increase the exposure of wild juvenile Pacific salmon to sea lice during early marine life (Krkošek *et al.* 2006) when sea lice are normally rare (Krkošek *et al.* 2007b). Our results indicate that this increased exposure may be balanced somewhat by low survival of lice on juvenile pink and chum salmon. The louse population dynamics model fit the time-series data of sea lice abundances well, showing a rapid decline in louse abundance over the initial course of each trial. This pattern of high parasite mortality on juvenile pink and

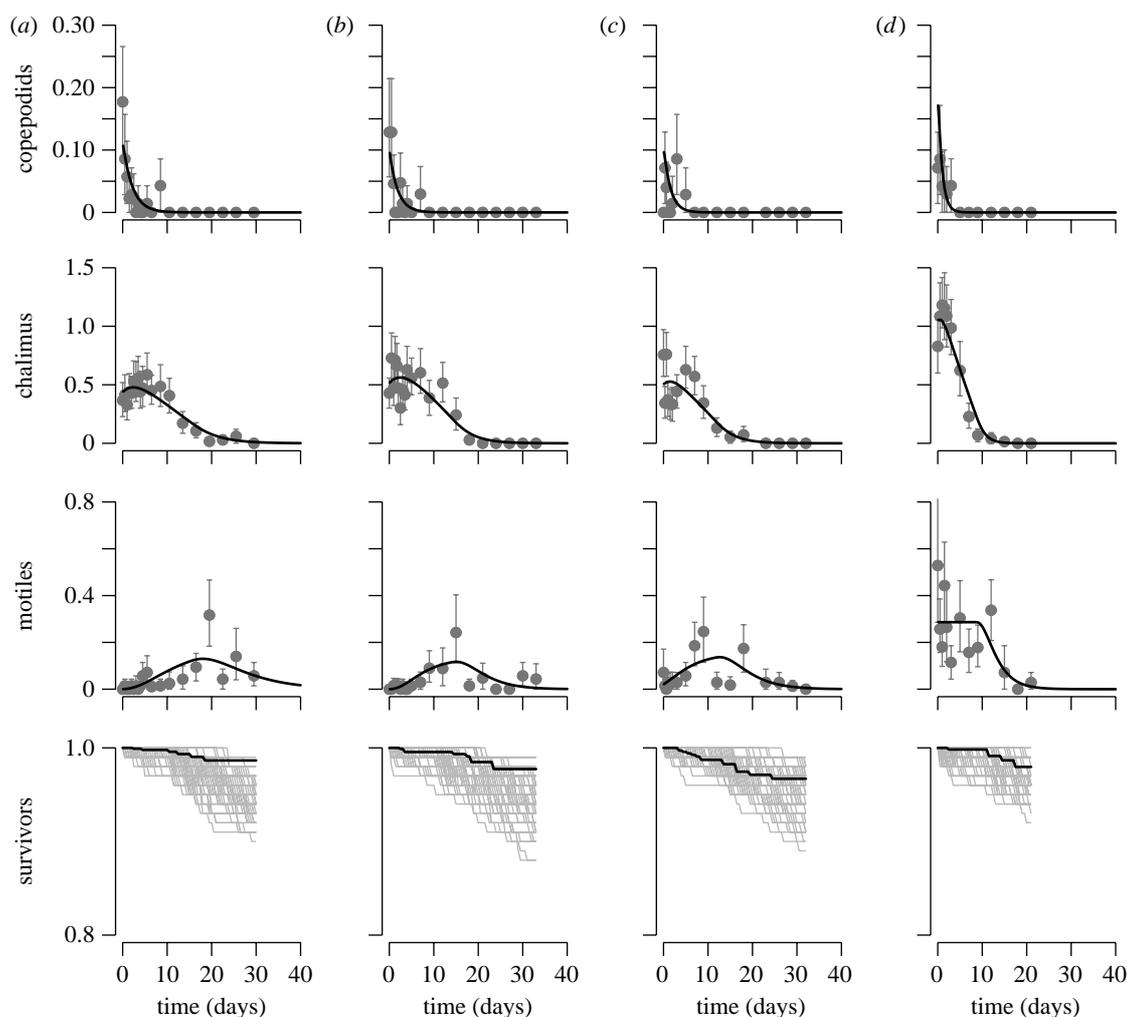


Figure 3. Same as for figure 1, except for pink salmon in four replicate trials (*a–d*) (P1–P4 in table 1).

Table 3. Comparison of salmon survival models fit to pink and chum datasets. (Models are identified by their component mortality sources—motile lice (*M*), chalinus lice (*H*) and delayed louse-independent mortality (T_d).

species	model	par	$-\log(L)$	AIC	Δ AIC
pink	<i>M</i>	1	454.6	911.2	314.3
	T_d	1	398.6	799.1	202.2
	<i>M</i> , <i>H</i>	2	454.2	912.4	315.5
	<i>M</i> , <i>H</i> , T_d	4	294.5	596.9	0.0
chum	<i>M</i>	1	1369.2	2740.4	500.3
	T_d	1	1193.3	2388.6	148.5
	<i>M</i> , <i>H</i>	2	1369.2	2742.4	502.3
	<i>M</i> , <i>H</i> , T_d	4	1116.0	2240.1	0.0

chum salmon has been observed previously in laboratory and ocean enclosure settings (Morton & Routledge 2005; Jones *et al.* 2006, 2007, 2008). The model demonstrates that when exposure is brief (e.g. 1 day), the commonly observed pattern of rapid parasite loss and high salmon survival results. When exposure is sustained for several weeks, as occurs in the Broughton Archipelago where juvenile salmon migrate at approximately 1 km d^{-1} through an 80 km zone of salmon farms (Krkošek *et al.* 2006), louse abundance can accumulate to epizootic levels. Thus, the experimental studies do not disagree with field observations of epizootics, they rather point

Table 4. Estimates for parameters in the salmon survival model for pink and chum salmon. (The trial-specific parameters (table 1) were used to generate the *H* and *M* solutions in the salmon survival model *Q* (equation (2.8)), used to analyse the survival and mortality data from all trials. Shown in brackets are the 95% confidence intervals for the rates of parasite-induced host mortality calculated by likelihood profile. Shown are rates (per louse per day) of parasite-induced host mortality for chalinus and motile stages (α_h and α_m , respectively), and the rate of mortality due to emaciation (α_d) following a time delay (T_d , in days).

	α_h	α_m	α_d	T_d
pink	0.00093 (0.00028, 0.0016)	0.00060 (0, 0.0052)	0.00097	11
chum	0.00074 (0.000051, 0.0018)	0.00343 (0.00061, 0.0067)	0.00866	14

to exposure time as an important determinant of salmon–louse population dynamics.

The results indicate that a large proportion of louse mortality occurred during early motile stages. The average motile lifespan ranged only 3–6 days in the ocean enclosures, meaning that motile lice disappeared as young preadult lice and did not reach maturity. As the motile stages (two preadult stages and one adult stage) span several weeks of duration, the results indicate motile lice experienced exceptionally high mortality in our trials.

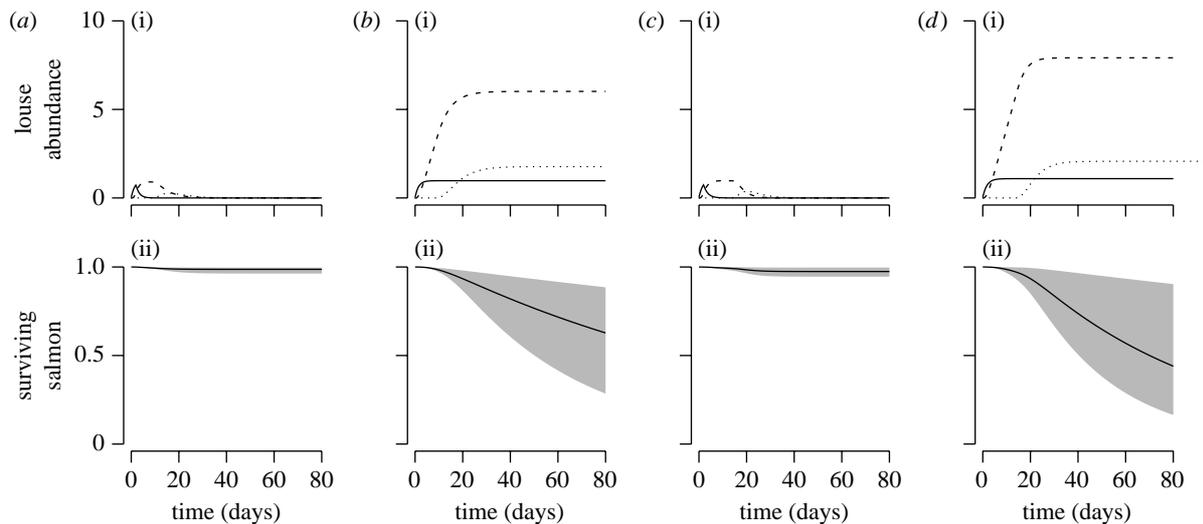


Figure 4. Salmon–louse population dynamics on (a,b) juvenile pink and (c,d) chum salmon for exposure times of (a,c) one day and (b,d) 80 days. (i) Louse dynamics are shown for copepodids (solid lines), chalimi (dashed lines) and motiles (dotted lines), as determined by the louse population dynamics model parametrized by the combined-trial data (P-All and C-All in table 1). (ii) Salmon survival was calculated for the point estimates for the rates of parasite-induced host mortality for chalimus and motile lice (solid lines) as well as the upper and lower 95% confidence intervals for these parameters (shaded region, table 4).

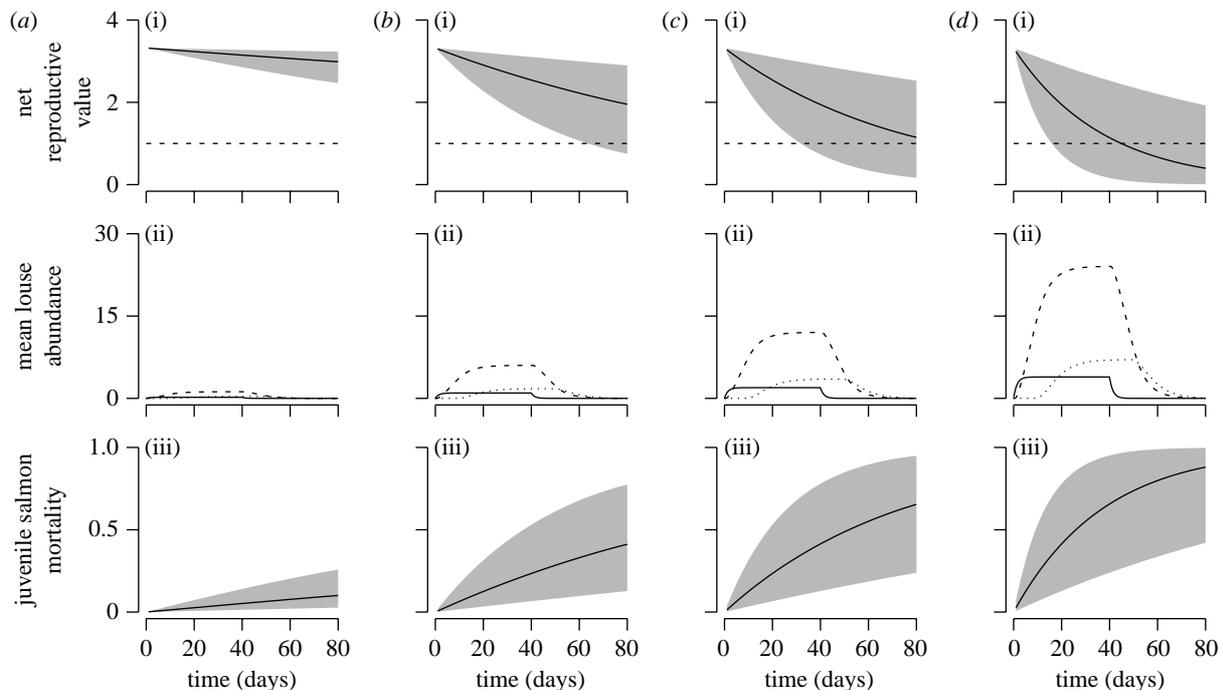


Figure 5. Relationship between pink salmon productivity and the duration of sea lice exposure for (a–d) four different infestation intensities. (i) The salmon net reproductive value, R_0 , as predicted by the point estimates for rates of parasite-induced host mortality (solid line) as well as the 95% confidence region (shaded area) as determined by the 95% confidence intervals on the rates of parasite-induced host mortality (table 4). The dashed line is $R_0 = 1$ that differentiates population persistence and collapse. To illustrate underlying louse dynamics, (ii) the mean louse abundances are plotted for copepodids (solid lines), chalimi (dashed lines) and motiles (dotted lines) when exposure time is 40 days. The infestation intensities used were (a) $\beta L = 0.01$, (b) 0.05, (c) 0.1 and (d) 0.2. (iii) The cumulative mortality of juvenile pink salmon after 80 days when being exposed to larval lice for the duration shown on the abscissa.

Motile lice can move among hosts (Ritchie 1997), presumably to find mates or to escape predation on their host (Connors *et al.* 2008). Also, the small size of juvenile pink and chum salmon may increase motile louse detachment (actively or accidentally) because there are fewer (or no) host locations for lice to seek shelter from hydrodynamic forces. The juvenile salmon were 40–60 mm fork length and a free-swimming preadult louse would be a suitable prey item. The juvenile salmon may have eaten

the preadult lice as the lice moved among the fish. We did not keep any frozen samples or analyse stomach contents, so further detailed observational study and lethal sampling is needed to evaluate if predation of juvenile salmon on lice explains the high motile louse mortality we observed. The inflammatory response that juvenile pink and chum salmon mount in response to louse infection (Jones *et al.* 2007) may contribute directly to louse mortality or indirectly by increasing the movement of motile lice. Further work is

also needed to evaluate if predation on free-swimming motile lice is a genuine ecological interaction or an artefact of the ocean enclosure setting.

There was variation among trials in parameter estimates, which may be due to environmental variation. In particular, trial 4 for pink salmon was conducted relatively late in the season, when water temperature was warmer. This may explain the drop in the estimated time lag for chalimus lice to develop to motiles, τ_h , as increased temperature is associated with increasing louse developmental rates. Variation in the time lag of copepodids becoming chalimi, τ_c , may be because the analysis does not resolve that parameter well due to the rapid transition from copepodid to chalimus as well as the mixed age and stage distribution of lice at the start of the trials. Variation in parameter estimates may also come from changes in parasite–host interactions as the juvenile salmon grow (Jones *et al.* 2008). The size of the fish at the start of the trials varied among trials and the juvenile salmon grew during the trials. This variation in host size may also affect the parameter estimates. Another possible source of variation is that the exposure of juvenile salmon to sea lice preceding the trials was not constant, as assumed by the model. Departures from this assumption would affect the age and stage distributions at the start of a trial and the subsequent course of louse development.

There were small deviations from the model predictions at certain time points. These deviations could have been the result of founder effects—the initial abundances of lice could vary from enclosure to enclosure simply due to chance when stocking the enclosures. In addition, there may have been variation among enclosures in characteristics such as sunlight, temperature or disturbance from waves or people. This could cause variation in louse developmental or survival rates as well as variation in juvenile salmon stress and behaviour, thereby leading to variation in louse abundance among enclosures. Because each enclosure was sampled twice, with the first sampling event removing half the fish and the second event removing the remainder, there was a period of reduced fish density that could further contribute variation to the results. However, we sampled fish from each enclosure on consecutive sampling events thereby minimizing the period of reduced density in each enclosure. This did mean, however, that data from consecutive sampling events from the same enclosure were not truly independent observations on the time-series of sea lice and salmon population dynamics. The two samples from each enclosure would share the factors that may cause variation in that particular enclosure and thereby group deviations from model predictions into data pairs. Despite these limitations, the model provided excellent fits to the data, indicating that these sources of variation were small relative to the overall pattern in sea lice and salmon population dynamics.

The mortality rates induced by chalimus and motile lice were lower than previous estimates of louse mortality. Krkošek *et al.* (2006) estimated the rate of host mortality induced by motile lice as $\alpha = 0.02$ (motile lice·day)^{−1}, whereas the estimates in this paper are considerably lower. It may be that we have underestimated the rate of parasite-induced host mortality. Most of the motile lice died as preadults and so did not reach their larger adult stages when they would presumably be more damaging to their host. Because there were few mortality events and high parasite mortality in this study, the survival analysis

involved data that did not capture higher louse abundances over longer periods that have been observed in the field. The fish used in this study were mostly above 50 mm fork length at the end of the trials, indicating that the development of louse resistance at this size (Jones *et al.* 2008) may have contributed to low louse survival. As juvenile salmon encounter farms at fork lengths as low as 30 mm, further study is needed to evaluate the survival rate of juvenile salmon over a broader range of body sizes, exposure intensity and exposure duration to better resolve how sea lice affect wild salmon population dynamics.

If the rates of parasite-induced host mortality we have estimated here are also correct for adult stage lice and at higher louse abundance, then the rate of parasite-induced host mortality is less than that calculated from interannual changes in average louse abundance on juvenile pink salmon and adult pink salmon abundance (Krkošek *et al.* 2007a). There are several plausible interactions that could mediate host mortality. It may be that at higher louse abundances, the rate of parasite-induced host mortality increases nonlinearly. Repeated exposures to lice may weaken host defences and improve louse survival. Ecological interactions with predators may also mediate how louse infestation affects wild salmon population dynamics, in ways that might dampen or intensify mortality. In addition, there is a substantial amount of environmental stochasticity in the pink salmon population dynamics (Krkošek *et al.* 2007a), which might act to blur the model predictions on mortality and productivity as well as mask the corresponding trends in nature. While there is more work needed to fully understand how sea lice affect salmon populations, in particular experiments to test model predictions and assumptions, these results indicate that the duration of exposure of juvenile salmon to sea lice is important to sea lice and salmon population dynamics. For policy, this means that coastal planning and management should consider minimizing the exposure time of juvenile salmon to sea lice from multiple salmon farms sited sequentially on migration routes in addition to the abundance of lice on individual farms.

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REFERENCES

- Brook, B. W. & Bradshaw, C. J. A. 2006 Strength of evidence for density dependence in abundance time series of 1198 species. *Ecology* **87**, 1445–1451. (doi:10.1890/0012-9658(2006)87[1445:SOEFDD]2.0.CO;2)
- Caswell, H. 2001 *Matrix population models*. Sunderland, MA: Sinauer.
- Connors, B., Krkošek, M. & Dill, L. M. 2008 Sea lice escape predation on their host. *Biol. Lett.* **4**, 455–457. (doi:10.1098/rsbl.2008.0276)

- Costello, M. J. 2006 Ecology of sea lice parasitic on farmed and wild fish. *Trends Parasitol.* **22**, 475–483. (doi:10.1016/j.pt.2006.08.006)
- Costello, M. J. 2009 The global economic cost of sea lice to the salmonid farming industry. *J. Fish Dis.* **32**, 115–118. (doi:10.1111/j.1365-2761.2008.01011.x)
- Daszak, P., Cunningham, A. A. & Hyatt, A. D. 2000 Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* **287**, 443–449. (doi:10.1126/science.287.5452.443)
- Duarte, C. M., Marbá, N. & Holmer, M. 2007 Rapid domestication of marine species. *Science* **316**, 382–383. (doi:10.1126/science.1138042)
- FAO 2007 *State of the world's fisheries and aquaculture*. Rome, Italy: Food Agricultural Organization of the United Nations.
- Ford, J. S. & Myers, R. A. 2008 A global assessment of salmon aquaculture impacts on wild salmonids. *PLoS Biol.* **6**, e33. (doi:10.1371/journal.pbio.0060033)
- Frazer, L. N. 2008 Sea-lice infection models for fishes. *J. Math. Biol.* **57**, 595–611. (doi:10.1007/s00285-008-0181-3)
- Gaughan, D. J. 2001 Disease-translocation across geographic boundaries must be recognized as a risk even in the absence of disease identification: the case with Australian Sardinops. *Rev. Fish Biol. Fish.* **11**, 113–123. (doi:10.1023/A:1015255900836)
- Heuch, P. A. & Mo, T. A. 2001 A model of salmon louse production in Norway: effects of increasing salmon production and public management measures. *Dis. Aquat. Organ.* **45**, 145–152. (doi:10.3354/dao045145)
- Hilborn, R. & Walters, C. J. 2001 *Quantitative fisheries stock assessment*. Norwell, MA: Kluwer.
- Johnson, S. C. & Albright, L. J. 1991 The developmental stages of *Lepeophtheirus salmonis* (Kroyer, 1837) (Copepoda, Caligidae). *Can. J. Zool.* **69**, 929–950. (doi:10.1139/z91-138)
- Jones, S., Kim, E. & Dawe, S. 2006 Experimental infections with *Lepeophtheirus salmonis* (Kroyer) on threespine sticklebacks, *Gasterosteus aculeatus* L., and juvenile Pacific salmon, *Oncorhynchus* spp. *J. Fish Dis.* **29**, 489–495. (doi:10.1111/j.1365-2761.2006.00742.x)
- Jones, S., Kim, E. & Bennett, W. 2008 Early development of resistance to the salmon louse, *Lepeophtheirus salmonis* (Kroyer), in juvenile pink salmon, *Oncorhynchus gorbuscha* (Walbaum). *J. Fish Dis.* **31**, 591–600. (doi:10.1111/j.1365-2761.2008.00933.x)
- 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. *Dis. Aquat. Organ.* **75**, 229–238. (doi:10.3354/dao075229)
- Kot, M. 2001 *Elements of mathematical ecology*. Cambridge, UK: Cambridge University Press.
- Krkošek, M. In press. Sea lice and salmon in Pacific Canada: ecology and policy. *Front. Ecol. Environ.* See <http://www.esajournals.org/toc/fron/0/0>. (doi:10.1890/080097)
- Krkošek, M., Lewis, M. A. & Volpe, J. P. 2005a Transmission dynamics of parasitic sea lice from farm to wild salmon. *Proc. R. Soc. B* **272**, 689–696. (doi:10.1098/rspb.2004.3027)
- Krkošek, M., Morton, A. & Volpe, J. P. 2005b Nonlethal assessment of juvenile pink and chum salmon for parasitic sea lice infections and fish health. *Trans. Am. Fish. Soc.* **134**, 711–716. (doi:10.1577/T04-133.1)
- Krkošek, M., Lewis, M. A., Morton, A., Frazer, L. N. & Volpe, J. P. 2006 Epizootics of wild fish induced by farm fish. *Proc. Natl Acad. Sci. USA* **103**, 15 506–15 510. (doi:10.1073/pnas.0603525103)
- Krkošek, M., Ford, J. S., Morton, A., Lele, S., Myers, R. A. & Lewis, M. A. 2007a Declining wild salmon populations in relation to parasites from farm salmon. *Science* **318**, 1772–1775. (doi:10.1126/science.1148744)
- Krkošek, M., Gottesfeld, A., Proctor, B., Rolston, D., Carr-Harris, C. & Lewis, M. A. 2007b Effects of host migration, diversity, and aquaculture on sea lice threats to Pacific salmon populations. *Proc. R. Soc. B* **274**, 3141–3149. (doi:10.1098/rspb.2007.1122)
- McKendrick, A. G. 1926 Applications of mathematics to medical problems. *Proc. Edinb. Math. Soc.* **40**, 98–130.
- Morton, A. & Routledge, R. 2005 Mortality rates for juvenile pink *Oncorhynchus gorbuscha* and chum *O. keta* salmon infested with sea lice *Lepeophtheirus salmonis* in the Broughton Archipelago. *Alaska Fish. Res. Bull.* **11**, 146–152.
- 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. *Can. J. Fish. Aquat. Sci.* **61**, 147–157. (doi:10.1139/f04-016)
- Morton, A. B. & Williams, R. 2003 First report of a sea louse, *Lepeophtheirus salmonis*, infestation on juvenile pink salmon, *Oncorhynchus gorbuscha*, in nearshore habitat. *Can. Field Nat.* **117**, 634–641.
- Morton, A. M., Routledge, R. & Krkošek, M. 2008 Sea lice infestation of wild juvenile salmon and herring associated with fish farms off the east central coast of Vancouver Island, BC. *North Am. J. Fish. Manage.* **28**, 523–532. (doi:10.1577/M07-042.1)
- Murray, A. G. & Peeler, E. J. 2005 A framework for understanding the potential for emerging diseases in aquaculture. *Prev. Vet. Med.* **67**, 223–235. (doi:10.1016/j.prevetmed.2004.10.012)
- Myers, R. A., Bowen, K. G. & Barrowman, N. J. 1999 Maximum reproductive rate of fish at low population sizes. *Can. J. Fish. Aquat. Sci.* **56**, 2404–2419. (doi:10.1139/cjfas-56-12-2404)
- Pauly, D., Alder, J., Bennett, E., Christensen, V., Tyedmers, P. & Watson, R. 2003 The future for fisheries. *Science* **302**, 1359–1361. (doi:10.1126/science.1088667)
- Peterman, R. M., Pyper, B. J. & Grout, J. A. 2000 Comparison of parameter estimation methods for detecting climate-induced changes in productivity of Pacific salmon (*Oncorhynchus* spp.). *Can. J. Fish. Aquat. Sci.* **57**, 181–191. (doi:10.1139/cjfas-57-1-181)
- Pike, A. W. & Wadsworth, S. L. 2000 Sealice on salmonids: their biology and control. *Adv. Parasitol.* **44**, 233–337. (doi:10.1016/S0065-308X(08)60233-X)
- Ricker, W. E. 1954 Stock and recruitment. *J. Fish. Res. Board Canada* **11**, 559–623.
- Ritchie, G. 1997 The host transfer ability of *Lepeophtheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* **20**, 153–157. (doi:10.1046/j.1365-2761.1997.00285.x)
- Shaw, D. J. & Dobson, A. P. 1995 Patterns of macro-parasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**, S111–S133.
- Stien, A., Bjorn, P. A., Heuch, P. A. & Elston, D. A. 2005 Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. *Mar. Ecol. Prog. Ser.* **290**, 263–275. (doi:10.3354/meps290263)
- von Foerster, H. 1959 Some remarks on changing populations. In *The kinetics of cellular proliferation* (ed. F. Stohlman), pp. 382–407. New York, NY: Grune and Stratton.