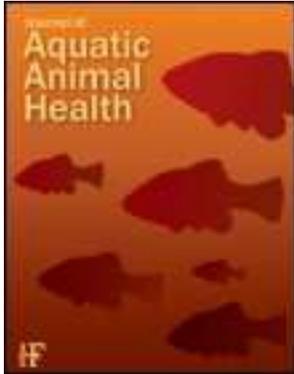


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Influence of Reduced Feed Ration on *Lepeophtheirus salmonis* Infestation and Inflammatory Gene Expression in Juvenile Pink Salmon

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Abstract.—The effect of reduced feed ration on infestation levels with the sea louse *Lepeophtheirus salmonis* and gene expression in juvenile pink salmon *Oncorhynchus gorbuscha* was tested in three laboratory trials. Body weight was significantly lower among fish on the reduced ration for 27, 34, or 65 d than fish on the full ration. Neither the prevalence nor the abundance of *L. salmonis* differed between fish on full and reduced rations at any time in any trial. In trial 2, sea louse rejection was delayed among fish on reduced rations; however, the parasite was ultimately rejected from all fish in this trial regardless of ration. Proinflammatory gene expression in salmon exposed to *L. salmonis* was modulated by reduced rations. There was a reduction in the expression of interleukin-8 in pink salmon on reduced rations 7 d after exposure but not 14 d after exposure. In contrast, the 7-d expression of interleukin-1 beta (IL-1 β) was reduced in exposed pink salmon regardless of ration. By day 14, however, expression of IL-1 β was increased in association with reduced rations among exposed salmon. Similarly, the expression of tumor necrosis factor alpha (TNF α) was increased 14 d after exposure among salmon on a reduced ration. There was no evidence that short-duration exposure of otherwise healthy juvenile pink salmon to a reduced ration affected susceptibility to *L. salmonis*. The expression data do not suggest an obvious mechanism of louse rejection; rather, they indicate that a more comprehensive suite of inflammatory pathways should be surveyed to better understand the early pink salmon response to *L. salmonis*.

Pink salmon *Oncorhynchus gorbuscha* are small (~30 mm in length and ~300 mg in weight) when they first enter the ocean (Heard 1991). First feeding coincides with the transition to seawater, and the capacity of fry to achieve daily increases in body weight of 4% to 7% is related to the availability of suitable forage (Willette 2001; Willette et al. 2001; Boldt and Haldorson 2003). Mortality among pink salmon during the first 45 d in the ocean ranges from 59% to 77% (Parker 1968; Willette et al. 2001) and their survival during this period depends on predator avoidance, as suggested by studies on size and condition factor (Boldt and Haldorson 2004), size-specific predation (Willette 2001), and the availability of alternative prey species (Cooney et al. 2001). These studies support the hypothesis that an adequate diet

among juvenile pink salmon leads to increased growth and survival. In addition to poor growth, an inadequate diet is generally associated with a reduced capacity of salmon to resist infectious disease (Waagbø 1994).

The sea louse *Lepeophtheirus salmonis* is a parasite of the skin, gills, and buccal cavity of marine salmonid fishes. The copepod develops through two planktonic (nauplii), one infective (copepodid), and seven parasitic stages (four chalimus, two preadult, and one adult) that are attached to or free-moving on the surface of the host. The parasitic stages consume host mucus, epithelial cells, and blood, and the severity of the infestation is increased in proportion to the number of parasites and their development to preadult or adult stages (Pike and Wadsworth 1999). Susceptibility to *L. salmonis* and to the resulting disease varies among its hosts. Infestations cause morbidity and mortality in Atlantic salmon *Salmo salar* and brown (sea) trout *S. trutta* whether in wild, farmed, or laboratory populations (Heuch et al. 2005; Boxaspen 2006). In contrast,

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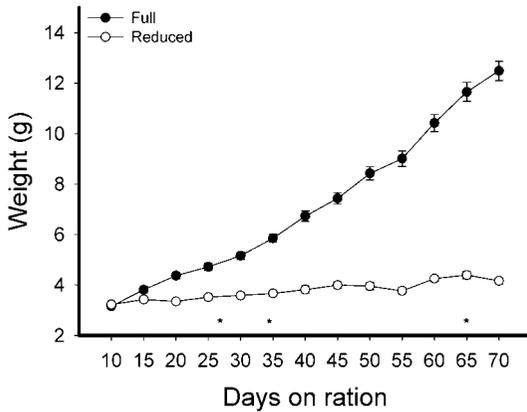


FIGURE 1.—Mean weights of stocked pink salmon on full and reduced-ration diets. The thin vertical lines represent SEs; the asterisks indicate the days on which sea lice exposure trials were initiated.

the Pacific salmon *Oncorhynchus* spp. appear to be refractory to the consequences of infestation, and morbidity and mortality tend not to be observed after laboratory exposures. Indeed, rapid rejection of *L. salmonis* by *Oncorhynchus* spp. occurs after laboratory exposure (Johnson and Albright 1992a; Fast et al. 2002; Jones et al. 2006, 2007). Inflammation elicited in coho salmon *O. kisutch* and Chinook salmon *O. tshawytscha* at the site of *L. salmonis* attachment appears to be a defense mechanism against *L. salmonis* (Johnson and Albright 1992a, 1992b). In contrast, localized inflammation was absent or limited at attachment sites on Atlantic salmon, which are relatively more susceptible to infestation (Johnson and Albright 1992a; Fast et al. 2002). Furthermore, increased expression of proinflammatory genes, such as interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF α) were associated with rejection of the parasite from juvenile pink salmon (Jones et al. 2007). Adverse conditions, however, may predispose species that are otherwise refractory to sea lice disease. Thus, coho salmon injected with hydrocortisol exhibit reduced epithelial inflammation and harbor greater parasite abundances than do coho salmon unexposed to the corticosteroid (Johnson and Albright 1992b). Furthermore, necrotic skin lesions and mortality associated with *L. salmonis* were observed on mature sockeye salmon *O. nerka* inhabiting warm, oxygen-depleted water at relatively high densities (Johnson et al. 1996). The purpose of this study was to examine the effect of ration level as a factor influencing susceptibility and responses of juvenile pink salmon to *L. salmonis*. Specifically we tested the hypothesis that reduced feed availability has no effect on levels of infestation and

expression of IL-8, interleukin-1 beta (IL-1 β) and TNF α after juvenile pink salmon exposure to *L. salmonis*.

Methods

Salmon.—Pink salmon were obtained as sac fry from the Quinsam River hatchery on Vancouver Island, British Columbia. Approximately 1,000 fish were reared in each of two 400-L stock tanks supplied with flowing water that was an equal mixture of dechlorinated freshwater and sand-filtered seawater. Temperature, salinity, and dissolved oxygen were monitored daily. Fish in the stock tanks were maintained on a daily ration of pelleted salmon diet at 1.5% biomass, as previously described (Jones et al. 2006, 2007). At a mean weight of 2.5 g, 320 fish were acclimated to seawater that was filtered to 1 μ m and irradiated with ultraviolet light (Jones et al. 2006). These fish were held in eight 33-L experimental tanks at a density of 40 fish per tank. Throughout these experiments water conditions were as follows: mean salinity of 29.3‰ (range, 29.0–29.7‰), mean temperature of 10°C (range, 9.5–10.5°C), and mean dissolved oxygen of 8.6 mg/L (range, 8.0–8.8 mg/L). Coincident with the acclimation to seawater, the fish in four tanks were maintained on the daily ration (full ration treatment) and those in the remaining four tanks were fed at 1.5% biomass, but only every third day (reduced ration treatment). Mean fish weight was calculated weekly (Figure 1).

Sea lice.—Separate batches of ovigerous *L. salmonis* were collected from wild adult sockeye salmon for each of three trials. The sea lice were transported in aerated ice-cold seawater to the laboratory where dissected egg strings were placed within porous hatching chambers and incubated in a bath of aerated and sand filtered seawater with the same salinity, temperature, and dissolved oxygen reported previously. Estimates of the numbers of cultured copepodids and nauplii were obtained from microscopic examination of triplicate subsamples obtained daily from each chamber. The contents of the hatching chambers were pooled to form a single inoculum containing a known number of copepodids.

Experimental design.—Three trials were initiated 27, 34, and 65 d after the onset of reduced ration and acclimation of fish to seawater. In each trial, 15 fish were arbitrarily allocated to each of four 33-L tanks representing the following treatments: full ration–not exposed, full ration–exposed, reduced ration–not exposed, and reduced ration–exposed. The experimental tanks were supplied with filtered and irradiated seawater as described previously. Pink salmon were exposed to copepodids as described by Jones et al.

(2006). Briefly, 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 from the pooled inoculum and fish were exposed for 2 h in darkness. The fish were sedated in 0.15 mg metomidate HCl/L (Syndel, Vancouver, British Columbia) throughout the exposure. The water flow was then resumed and a photoperiod of 12 h light:12 h dark established. Nonexposed control salmon were treated in the same way without the addition of copepodids to the tanks. The exposure levels in the three trials were 222, 277, and 311 copepodids/fish, respectively, which is equivalent to 3,330, 4,155, and 4,665 copepodids/tank.

At 7-d intervals after exposure, all fish were sedated by adding 0.15 mg metomidate HCl/L to each tank. In trial 1, seven, and eight fish were killed in tricaine methanesulfonate (MS-222; Syndel) at 7 and 14 d postexposure (dpe), respectively. In trial 2, sampling was nonlethal for the first 3 weeks; all 15 sedated fish were examined at weekly intervals after exposure and allowed to recover. At 28 dpe, all fish in trial 2 were killed. In trial 3, a single lethal sample was taken at 10 dpe. In all trials, fish were examined microscopically for *L. salmonis*, and weight and length were measured. Immediately after examination, fish in trial 1 were flash-frozen in liquid nitrogen for subsequent RNA extraction.

RNA extraction.—The Trizol (Invitrogen, Burlington, Ontario) method (Fast et al. 2006a, 2006b; Jones et al. 2007) was used to extract RNA from 50-mg pools of equal parts of liver and head kidney. Total RNA was measured with the NanoDrop-1000 spectrophotometer (version 3.2.1) and samples stored in 100% formamide at -20°C for short-term storage (1–2 d) and at -80°C for longer periods (>2 d). Complementary DNA (cDNA) was produced from each RNA sample by using the Superscript III qRT-PCR kit with SYBR green (Invitrogen) as described by Fast et al. (2006a, 2006b) and Jones et al. (2007).

Real-time polymerase chain reaction.—The primers for real-time polymerase chain reaction (qPCR) amplification of the proinflammatory cytokine–chemokine genes, TNF α , IL-8, and IL-1 β , were designed from previously published rainbow trout *O. mykiss* primers and sequences conserved between rainbow trout and Atlantic salmon by means of Primer 3 software. These primers have been used and validated for pink salmon and chum salmon *O. keta* and cycling conditions followed those previously published (Fast et al. 2006a, 2006b, Jones et al. 2007). The sequencing and cloning of PCR products and the use of recombinant plasmids as PCR standards follow the earlier methods. Similarly, to ensure that genomic

DNA was not quantified in the qPCR experiments, elongation factor-1 α (EF-1A) and IL-8 primers were designed to span an intron–exon splice site and single-product amplification was confirmed through melt-curve analysis. The sensitivity of reactions and amplification of contaminant products such as primer dimers indiscriminately detected by SYBR green, were evaluated by amplifying duplicate 10-fold dilutions of the cloned DNA (between 1 and 10^{-6} ng) and by performing a blank (no cDNA) in each run. The relationship between the threshold cycle (Ct) and the $\log_{10}(\text{RNA})$ was linear ($-3.3 < \text{slope} < -3.1$) for all reactions. The gene EF-1A was used as the reference and all expression data are presented as mean \pm SE relative to the expression of EF-1A.

Statistical analysis.—The prevalence and abundance of infestation were defined according to Bush et al. (1997). The effects of ration level and exposure to *L. salmonis* on weight and the expression of proinflammatory genes (see Results) was estimated by means of two-way analysis of variance (ANOVA). The significance of differences in pairwise comparisons between expression levels was tested by means of *t*-tests. The significance of differences in the prevalence and abundance of *L. salmonis* was tested by means of chi-square and Kruskal–Wallis tests, respectively. In all cases, $P \leq 0.05$ was considered significant.

Results

Effects of Diet and Exposure to L. salmonis on Weight

Pink salmon maintained on reduced ration weighed significantly less than those on the full ration throughout all trials (Figure 1). Within each ration group, exposure to *L. salmonis* had no significant effect on weight in any of the trials (Table 1).

Lepeophtheirus salmonis Infestation

Infestations were established in all three trials (Table 2). While there were no significant effects of ration on either the prevalence or the abundance of *L. salmonis* at any time in any trial, prevalence and abundance were observed to change over time. In trial 1, the abundance of *L. salmonis* in both ration groups decreased significantly between 7 and 14 d (Table 2), whereas prevalence did not change significantly during this time. In trial 2, the abundance decreased significantly between 7 and 14 dpe for pink salmon on full ration, but the decrease was not significant for those on reduced ration (Table 2). At 28 dpe there were no *L. salmonis* present on fish from either ration group. In addition there was a significant decrease in prevalence among salmon on full ration between 7 and 14 dpe. The change in prevalence during this interval was not significant for salmon on reduced ration. A total of

TABLE 1.—Effects of ration and duration of exposure to *Lepeophtheirus salmonis* on the weight of pink salmon. In trial 1, each fish was exposed to 222 copepodids after being on a reduced ration for 27 d; the corresponding values for trial 2 were 277 copepodids and 34 d, and those for trial 3 were 311 copepodids and 65 d.

Trial	Days after exposure	Ration	Exposure	Number of fish	Weight ^a (mean ± SE)
1	7	Full	Yes	7	5.5 ± 0.6
			No	7	6.6 ± 0.6
		Reduced	Yes	7	3.9 ± 0.2
	No		7	3.8 ± 0.4	
	Yes		8	5.2 ± 0.4	
	2	14	Full	No	8
Yes				7	4.1 ± 0.5
Reduced			Yes	7	4.1 ± 0.5
		No	8	3.9 ± 0.2	
		Yes	15	8.0 ± 0.6	
3		28	Full	No	15
	Yes			15	3.9 ± 0.3
	Reduced		No	14	4.3 ± 0.2
		Yes	15	10.2 ± 1.0	
		No	13	12.7 ± 1.2	
	3	10	Full	Yes	12
No				13	12.7 ± 1.2
Reduced			Yes	15	4.1 ± 0.2
		No	13	4.4 ± 0.3	

^a The results of a two-way ANOVA were as follows: Trial 1, 7 d after exposure: ration ($F = 19.5, P < 0.01$), exposure ($F = 0.9, P = 0.36$); trial 1, 14 d after exposure: ration ($F = 14.7, P < 0.01$), exposure ($F = 0.2, P = 0.70$); Trial 2: ration ($F = 70.0, P < 0.01$), exposure ($F = 1.8, P = 0.18$); Trial 3: ration ($F = 94.8, P < 0.01$), exposure ($F = 3.5, P = 0.07$).

eight fish died over the three trials. Six of these fish died within 24 h of the exposure protocol, and mortality was attributed to handling and anesthesia, whereas the other two that died were from the nonexposed groups at 5 and 7 d after the protocol.

Expression of Inflammatory Genes

The effects of ration level and exposure to *L. salmonis* on the expression of inflammatory genes were studied in samples of pooled kidney and liver tissues obtained during trial 1 (Figure 2). Analysis of variance showed significant effects of ration and exposure on the expression of IL-8 at 7 dpe ($F = 5.6, P = 0.02$) and 14 dpe ($F = 15.1, P < 0.01$), respectively, and a significant interaction between these effects at day 7 ($F = 4.0, P = 0.05$). At 7 dpe, expression of IL-8 was significantly lower among exposed pink salmon on a reduced ration than among those on a full ration (Figure 2A). At 14 dpe, expression was significantly higher in exposed fish regardless of ration group.

At 7 dpe the expression of IL-1β was lower in exposed pink salmon than in nonexposed fish in both ration groups (Figure 2B). However, this difference was only significant for salmon on full ration ($F = 12.3, P < 0.01$). At 14 dpe there was a significant interaction between ration and exposure on IL-1β expression ($F = 9.3, P < 0.01$). Among fish in the reduced-ration group, exposed salmon had significantly

TABLE 2.—Effects of ration on prevalence and abundance of *Lepeophtheirus salmonis* in exposed pink salmon. See Table 1 for further details.

Trial	Day	Ration	% Prevalence (n)	Abundance (mean ± SE)
1	7	Full	100 (7)	2.0 ± 0.3 ^a
		Reduced	100 (7)	1.7 ± 0.3 ^a
	14	Full	62.5 (8)	0.8 ± 0.3
		Reduced	57.1 (7)	0.7 ± 0.3
2	7	Full	86.7 ^a (15)	1.7 ± 0.4 ^a
		Reduced	66.7 (15)	1.2 ± 0.4
	14	Full	46.7 (15)	0.7 ± 0.3
		Reduced	33.3 (15)	0.5 ± 0.2
	21	Full	6.7 (15)	0.1 ± 0
		Reduced	0 (15)	—
28	Full	0 (15)	—	
	Reduced	0 (15)	—	
	Full	33.3 (12)	0.5 ± 0.2	
3	10	Full	33.3 (12)	0.5 ± 0.2
		Reduced	13.3 (15)	0.1 ± 0.1

^a Significantly different from corresponding diet group on day 14.

higher expression of IL-1β than nonexposed salmon. Furthermore, the level of IL-1β expression in the exposed, reduced-ration group was significantly higher than that in the exposed, full-ration group (Figure 2B).

At 7 dpe, there were no significant effects of exposure or ration on TNFα expression (Figure 2C). However, at 14 dpe, there was a significant effect of exposure on the expression of TNFα ($F = 7.9, P = 0.01$) and a significant interaction between exposure and ration ($F = 9.3, P < 0.01$) (Figure 2C). At this time expression of TNFα was significantly higher among exposed pink salmon than among nonexposed fish on a reduced ration. In addition, TNFα expression was significantly lower among nonexposed salmon on a reduced ration than among both exposed and nonexposed fish on a full ration (Figure 2C).

Discussion

The present study examined the effects of ration on the susceptibility and responses of pink salmon to *L. salmonis*. Although the growth of pink salmon maintained on a reduced ration was significantly impaired, the prevalence and abundance of *L. salmonis* infestations did not differ between ration groups at any of the sample times. However, a pattern of declining prevalence and abundance was observed in both trials and characterized in trial 2, by a loss of most *L. salmonis* by 21 d without associated fish mortality. Similar declines in the abundance and prevalence of *L. salmonis* were described in earlier laboratory trials (Jones et al. 2006, 2007). In the earlier studies, infestations on juvenile pink and chum salmon underwent declines that occurred more rapidly on pink salmon and that were attributed to the differential effects of the innate defense mechanisms in these

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species (Jones et al. 2007). Thus, the infestations established in this study confirmed the reliability of the previously described exposure model. In trial 2 however, infestation appeared not to decline between 7 and 14 d of exposure among pink salmon on reduced ration, suggesting a compromise of the early rejection mechanism. Despite this, the absence of *L. salmonis* by 28 dpe in both ration groups further indicated that the compromise was transitory. However, the sample sizes were small due to the limited availability of sufficient copepodids required to establish infestations that persist more than 7–14 d on pink salmon, as reported earlier (Jones et al. 2006, 2007). Since fewer copepodids are required to establish comparable infestations on pink salmon weighing 0.3 to 2.5 g (Jones et al., in press), future studies addressing the role of ration level may benefit from the use of smaller salmon and a corresponding increase in sample size. Despite the foregoing discussion, our initial observations suggest that a 27–65d reduction in ration had limited effect on the capacity of 3.5–5.0-g pink salmon juveniles to reject *L. salmonis*. In addition, in trial 3 there were no significant differences in prevalence or abundance of *L. salmonis* between the full- and reduced-ration groups. At the onset of trial 3, the weight of pink salmon on reduced ration for 65 d was less than half that of salmon on full ration. This result further supported our hypothesis that reduced feed availability has no effect on levels of *L. salmonis* infestation on pink salmon.

The effects of ration and exposure to *L. salmonis* on the expression of proinflammatory genes in juvenile pink salmon were examined. In earlier studies, proinflammatory genes were found to be expressed in *L. salmonis*-infested salmon, both systemically and at louse attachment sites. Expression of IL-1 β was reported to increase in head kidney of Atlantic salmon during *L. salmonis* infestation (Fast et al. 2006). Similarly, IL-1 β expression increased in the fins of chum, but not pink salmon 28 dpe, IL-8 expression was up-regulated in head kidney and fins of pink salmon at 7 dpe, and TNF- α expression increased in the head kidney of pink salmon 21 d after a low exposure and 14 d after a high exposure to *L. salmonis* (Jones et al. 2007). These data support the earlier observation that inflammation elicited at the site of infestation and systemically is a defense mechanism of *Oncorhynchus* spp. against *L. salmonis* (Johnson and Albright 1992a, 1992b) that coincides with sea louse rejection on pink and chum salmon (Jones et al. 2007). The modulating effects of ration level on the expression of proinflammatory genes in *L. salmonis*-exposed pink salmon reported here, therefore, extend those earlier observations. Specifically, the expression of IL-8, which

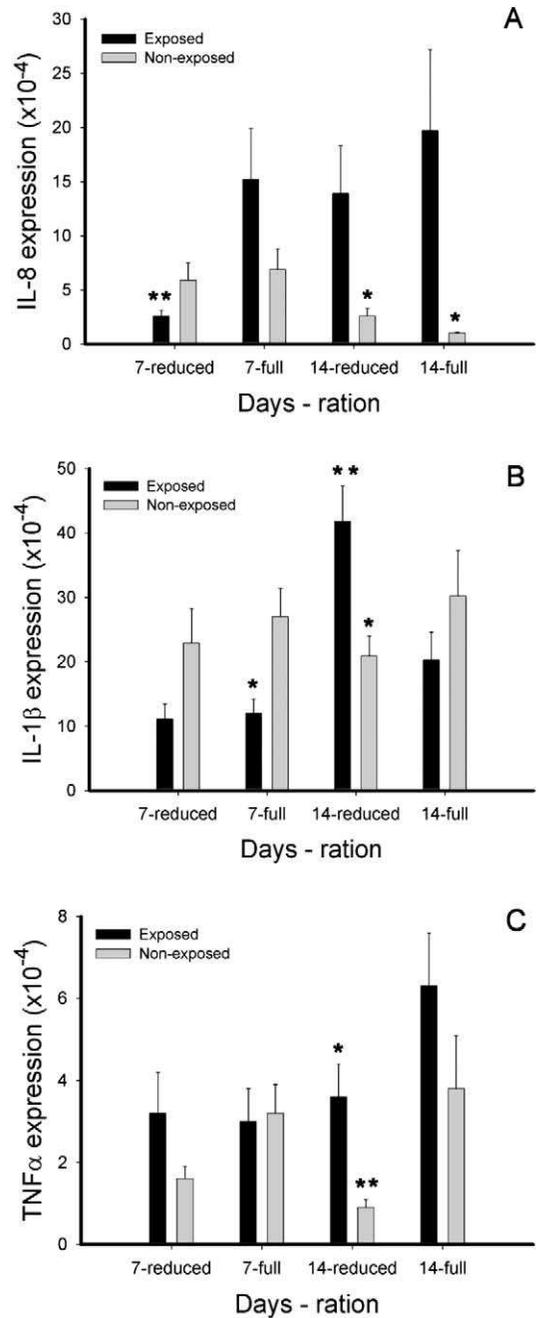


FIGURE 2.—The effects of ration and length of exposure (7 or 14 d) to *Lepeophtheirus salmonis* on the expression of three proinflammatory genes—(A) interleukin-8 (IL-8), (B) interleukin-1 beta (IL-1 β), and (C) tumor necrosis factor alpha (TNF α)—relative to elongation factor 1A in head kidney–liver pools from pink salmon. Asterisks indicate significant differences between the exposed and control groups (*t*-test), double asterisks significant differences between fish fed within exposure groups the reduced and full diets (*t*-test).

previously was shown to occur in pink salmon juveniles 7–14 d after exposure to *L. salmonis*, was transiently inhibited in fish on a reduced ration. In contrast, the expression of IL-1 β , previously not detected in pink salmon after exposure to *L. salmonis*, increased in exposed pink salmon on a reduced ration. Interestingly, the constitutive expression of TNF α in nonexposed fish was reduced among salmon on reduced ration in the 14-d samples. Previously, TNF α had not been detected until 21 d after exposure of pink salmon to *L. salmonis* (Jones et al. 2007). While the reduced expression of IL-8 among exposed salmon on reduced ration may be related to the transient inability to reject *L. salmonis*, there was little other evidence for a compromise of louse rejection associated with reduced ration. As a result, we cannot conclude that the inflammatory genes, whose expression was measured here, were solely responsible for initiating or maintaining the process of louse rejection. Rather, the data suggest a more comprehensive suite of inflammatory pathways should be surveyed, possibly through the use of microarray analysis, to better understand the early pink salmon response to *L. salmonis*.

The importance of the diet in modulating immune function and the resistance of salmonids to infectious disease is well established (Blazer and Wolke 1984; Waagbø 1994). The purpose of this trial was to assess the effect of food availability on *L. salmonis* infestation levels and gene expression in juvenile pink salmon. Despite the metabolic cost of a reduced ration as indicated by the limited growth, the outcome of exposure to *L. salmonis* was only minimally altered. As in Alcorn et al. (2003), the defense functions of the pink salmon were apparently not compromised during a brief period (27–65 d) on a reduced ration. Alcorn et al. (2003) held juvenile Chinook salmon on three ration levels for 54 weeks. During this time, fish fed to 64% or 40% satiation were significantly smaller and had lower condition factors than controls. Among ration levels, however, no differences were observed in hematocrit, serum lysozyme, complement activity, differential leukocyte counts, nitroblue tetrazolium reduction by pronephros macrophages, and myeloperoxidase activity of pronephros neutrophils. In contrast, phagocytic activity of pronephric macrophages was enhanced in fish fed to 40% satiation. Both Alcorn et al. (2003) and Henken et al. (1987) concluded that innate and adaptive defense responses remain functional in suboptimally fed fish that retain a sufficient energy reserve. Consistent with this, resistance to coldwater vibriosis was enhanced in starved Atlantic salmon (Damsgård et al. 2004). While it is possible that the transient reduction in IL-8 expression in head kidney–liver pools from pink salmon on a reduced

ration in this study was evidence of a limited energy reserve, the elevated expression of IL-1 β and TNF α in pink salmon on reduced ration contradicts this view. The energy reserve, while poorly defined, tends to be more limited in rapidly growing juveniles relative to larger fish (Schultz and Conover 1999). Therefore, further research involving a greater duration of reduced ration is required to fully assess the effect of ration level on susceptibility to *L. salmonis* in rapidly growing juvenile pink salmon.

This is the first report of an effect of reduced ration on the expression of pink salmon genes involved in fish immunity or inflammation. Rise et al. (2006) found complement component 7 and the immunoglobulin heavy-chain variable region, among other transcripts, to be dysregulated in ration-restricted, growth hormone–transgenic coho salmon compared with the transgenic full-ration control. In other work, the expression of genes associated with the metabolism and growth of skeletal muscle and the metabolism of hepatic glucose were shown to be modulated by dietary restriction in rainbow trout (Panserat et al. 2001; Johansen and Overturf 2006). Our results indicate the potential value of quantifying gene expression as an additional measure of the effect of dietary manipulations on the immune responses of salmonids. Despite reduced size due to suboptimal ration, juvenile pink salmon retained the capacity to reject *L. salmonis* infestation after a single laboratory exposure. These results suggest that natural exposure of otherwise healthy 3-g to 12-g pink salmon to similar levels of *L. salmonis* used in this study is unlikely to result in individual or population level effects.

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References

- Alcorn, S. W., R. J. Pascho, A. L. Murray, and K. D. Shearer. 2003. Effects of ration level on immune functions in Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 217:529–545.
- Blazer, V. S., and R. E. Wolke. 1984. Effect of diet on the immune response of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 41:1244–1247.
- Boldt, J. L., and L. J. Haldorson. 2003. Seasonal and geographic variation in juvenile pink salmon diets in

- the northern Gulf of Alaska and Prince William Sound. Transactions of the American Fisheries Society 132:1035–1052.
- Boldt, J. L., and L. J. Haldorson. 2004. Size and condition of wild and hatchery pink salmon juveniles in Prince William Sound, Alaska. Transactions of the American Fisheries Society 133:173–184.
- Boxaspen, K. 2006. A review of the biology and genetics of sea lice. ICES Journal of Marine Science 63:1304–1316.
- Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83:575–583.
- Cooney, R. T., J. R. Allen, M. A. Bishop, D. L. Eslinger, T. Kline, B. L. Norcross, C. P. Mcroy, J. Milton, J. Olsen, V. Patrick, A. J. Paul, D. Salmon, D. Scheel, G. L. Thomas, S. L. Vaughan, and T. M. Willette. 2001. Ecosystem controls of juvenile pink salmon (*Oncorhynchus gorbuscha*) and Pacific herring (*Clupea pallasii*) populations in Prince William Sound, Alaska. Fisheries Oceanography 10(Supplement 1):1–13.
- Damsgård, B., U. Sørum, I. Ugelstad, R. A. Eliassen, and A. Mortensen. 2004. Effects of feeding regime on susceptibility of Atlantic salmon (*Salmo salar*) to coldwater vibriosis. Aquaculture 239:37–46.
- Fast, M. D., S. C. Johnson, and S. R. M. Jones. 2006a. Differential expression of the pro-inflammatory cytokines IL-1 β -1, TNF α -1, and IL-8 in vaccinated pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon juveniles. Fish and Shellfish Immunology 22:403–407.
- Fast, M. D., N. W. Ross, D. M. Muise, and S. C. Johnson. 2006b. Differential gene expression in Atlantic salmon infected with *Lepeophtheirus salmonis*. Journal of Aquatic Animal Health 18:116–127.
- Fast, M. D., N. W. Ross, A. Mustafa, D. E. Sims, S. C. Johnson, G. A. Conboy, D. J. Speare, G. Johnson, and J. F. Burka. 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar*, and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. Diseases of Aquatic Organisms 52:57–68.
- Heard, W. R. 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*). Pages 121–230 in C. Groot and L. Margolis, editors. Pacific salmon life histories. UBC Press, Vancouver.
- Henken, A. M., A. J. Tigchelaar, and W. B. van Muiswinkel. 1987. Effects of feeding level on antibody production in African catfish, *Clarias gariepinus* Burchell, after injection of *Yersinia ruckeri* O-antigen. Journal of Fish Diseases 11:85–88.
- Heuch, P. A., P. A. Bjørn, B. Finstad, J. C. Holst, L. Asplin, and F. Nilsen. 2005. A review of the Norwegian “National Action Plan Against Salmon Lice on Salmonids”: the effect on wild salmonids. Aquaculture 246:79–92.
- Johansen, K. A., and K. Overturf. 2006. Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and refeeding in rainbow trout. Comparative Biochemistry and Physiology 144B:119–127.
- Johnson, S. C., and L. J. Albright. 1992a. Comparative susceptibility and histopathology of the responses of naive coho, Atlantic, and Chinook salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms 14:179–193.
- Johnson, S. C., and L. J. Albright. 1992b. The effects of cortisol implants on the susceptibility and histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms 14:195–205.
- Johnson, S. C., R. B. Blaylock, J. Elphink, and K. D. Hyatt. 1996. Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 53:2888–2897.
- Jones, S. R. M., M. D. Fast, S. C. Johnson, and D. B. Groman. 2007. Differential rejection of salmon lice by pink and chum salmon: disease consequences and expression of proinflammatory genes. Diseases of Aquatic Organisms 75:229–238.
- Jones, S., E. Kim, and W. Bennett. In press. Early development of resistance to the salmon louse, *Lepeophtheirus salmonis* (Krøyer), in juvenile pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Journal of Fish Diseases.
- Jones, S. R. M., E. Kim, and S. Dawe. 2006. Experimental infections with *Lepeophtheirus salmonis* (Krøyer) on threespine sticklebacks, *Gasterosteus aculeatus* L. and juvenile Pacific salmon, *Oncorhynchus* spp. Journal of Fish Diseases 29:489–495.
- Panserat, S., E. Plagnes-Juan, and S. Kaushik. 2001. Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 204:2351–2360.
- Parker, R. R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. Journal of the Fisheries Research Board of Canada 25:757–794.
- Pike, A. W., and S. L. Wadsworth. 1999. Sea lice on salmonids: their biology and control. Advances in Parasitology 44:233–337.
- Rise, M. L., S. E. Douglas, D. Sakhrani, J. Williams, K. V. Ewart, M. Rise, W. S. Davidson, B. F. Koop, and R. H. Devlin. 2006. Multiple microarray platforms utilized for hepatic gene expression profiling of GH-transgenic coho salmon with and without ration restriction. Journal of Molecular Endocrinology 37:259–282.
- Schultz, E. T., and D. O. Conover. 1999. The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. Oecologia 119:474–483.
- Waagbø, R. 1994. The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar* L.: a review. Aquaculture and Fisheries Management 25:175–197.
- Willette, T. M. 2001. Foraging behavior of juvenile pink salmon (*Oncorhynchus gorbuscha*) and size-dependent predation risk. Fisheries Oceanography 10(Supplement 1):110–131.
- Willette, T. M., R. T. Cooney, V. Patrick, D. M. Mason, G. L. Thomas, and D. Scheel. 2001. Ecological processes influencing mortality of juvenile pink salmon (*Oncorhynchus gorbuscha*) in Prince William Sound, Alaska. Fisheries Oceanography 10(Supplement 1):14–41.