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Impacts of multispecies parasitism on juvenile coho salmon (*Oncorhynchus kisutch*) in Oregon

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ABSTRACT

We are studying the impacts of parasites on threatened stocks of Oregon coastal coho salmon (*Oncorhynchus kisutch*). In our previous studies, we have found high infections of digeneans and myxozoans in coho salmon parr from the lower main stem of West Fork Smith River (WFSR), Oregon. In contrast parr from tributaries of this river, and outmigrating smolts, harbor considerably less parasites. Thus, we have hypothesized that heavy parasite burdens in parr from this river are associated with poor overwintering survival. The objective of the current study was to ascertain the possible effects these parasites have on smolt fitness. We captured parr from the lower main stem and tributaries of WFSR and held them in the laboratory to evaluate performance endpoints of smolts with varying degrees of infection by three digeneans (*Nanophyetus salmincola*, *Apophallus* sp., and neascus) and one myxozoan (*Myxobolus insidiosus*). The parameters we assessed were weight, fork length, growth, swimming stamina, and gill Na^+,K^+-ATPase activity. We repeated our study on the subsequent year class and with hatchery reared coho salmon experimentally infected with *N. salmincola*. The most significant associations between parasites and these performance or fitness endpoints were observed in the heavily infected groups from both years. We found that all parasite species, except neascus, were negatively associated with fish fitness. This was corroborated for *N. salmincola* causing reduced growth with our experimental infection study. Parasites were most negatively associated with growth and size, and these parameters likely influenced the secondary findings with swimming stamina and ATPase activity levels.

1. Introduction

Parasite associated mortality has been documented in many wild populations of animals, including fishes (Lester, 1984). We are studying the impacts of parasites on Oregon coastal coho salmon (*Oncorhynchus kisutch*), which have been listed as threatened under the Endangered Species Act (ESA) (NRC, 1996). The general consensus is that habitat loss plays the most important role in their decline (NRC, 1996).

For juvenile coho salmon, the overwintering period is recognized as a time of high mortality (Ebersole et al., 2006; Ebersole et al., 2009). Ebersole et al. (2006) used habitat quality models to predict overwinter survival of coho salmon parr from the West Fork Smith River (WFSR) in Oregon, and compared model results to survival data observed from tagged fish. They found that parr from the lower main stem of WFSR had reduced overwinter survival compared to parr from the upper reaches of this river, which was opposite to the model predictions. As a follow up to their study, we have found extremely high digenean and myxozoan parasite burdens in coho salmon parr from the lower main stem of WFSR, compared to parr rearing in the upper portion of this river (Rodnick et al., 2008). Furthermore, outmigrating smolts in this basin consistently harbor up to 95% fewer parasites than lower main stem parr of the same cohort when sampled earlier in the year (Ferguson et al., in press). Therefore, we have proposed that these heavily infected parr are either subjected to high overwinter mortality, or fail to migrate due to poor smoltification and subsequently die.

Coho salmon are infected by numerous parasite species (Hoffman, 1999; Love and Moser, 1983; McDonald and Margolis, 1995), and some studies have indicated that certain parasites can be linked to host mortality. Particularly pertinent to our study, Jacobson et al. (2008) demonstrated that a common digenean, *Nanophyetus salmincola*, is associated with early ocean mortality of coho salmon. However, similar

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studies have not been conducted on the coho salmon smolts, nor have they evaluated effects of multiple parasite infections.

There are some inherent limitations with studying effects of parasitism on wild fishes, as moribund or dead fish are likely removed from the system by predators, and sub-lethal effects of disease are hard to measure (Bakke and Harris, 1998). However, laboratory studies, conducted on either experimentally or naturally infected fish, can directly examine mechanisms that lead to mortalities in the wild. Examples of such studies with salmonids include the following: *N. salmincola* (Digenea) reducing burst swimming speed (Butler and Milleman, 1971), and immune response (Jacobson et al., 2003); *Crepidostomum farionis* (Digenea) reducing hemoglobin and hematocrit (Klein et al., 1969); *Sanguinicola klamathensis* (Digenea) reducing growth (Evans, 1974); *Gyrodactylus* spp. (Monogenea) inducing cortisol (Stoltze and Buchmann, 2001); *Eubothrium salvelini* (Cestoda) reducing swimming stamina, growth, and survival (Boyce, 1979), saltwater adaptation (Boyce and Clarke, 1983), and altering migration orientation (Garnick and Margolis, 1990); *Myxobolus arcticus* (Myxosporea) reducing swimming stamina (Moles and Heifetz, 1998); *Parvicapsula minibicornis* (Myxosporea) reducing swimming recovery rates (Wagner et al., 2005); and sea lice, *Lepeophtheirus salmonis* (Copepoda) reducing osmoregulation (Birkeland and Jakobsen, 1997), and swimming and cardiovascular performance (Wagner et al., 2003).

Here we present results of our laboratory studies using 1) coho salmon from two year classes captured in the wild harboring multiple parasite infections; and 2) coho salmon experimentally infected with *N. salmincola*. The responses we assessed in these fish were size, growth, swimming stamina, and gill Na^+,K^+-ATPase activity. These represented fitness endpoints, as many of these are either performance metrics or other parameters that are indirectly linked to fitness. Fish size influences freshwater juvenile overwinter survival (Quinn and Peterson, 1996) and smolt success (Holtby et al., 1990). Reduced swimming performance can affect fish survival by decreasing predator avoidance (Taylor and McPhail, 1985). Osmoregulation is an important factor for smolt survival (Moser et al., 1991) and gill Na^+, K^+-ATPase activity is a major component of this process (reviewed in McCormick, 2001). We performed analyses to determine if lightly infected fish had lower fitness responses than more heavily infected fish, and if parasitism was negatively associated with these responses.

2. Materials and methods

2.1. Sampling wild fish

As we are particularly interested in overwinter mortality, we captured wild parr from WFSR in September to hold and monitor in the laboratory until the typical time of smoltification. Fish were provided by the Oregon Department of Fish and Wildlife (ODFW) in conjunction with monitoring activities for their Life Cycle Monitoring Project. In September 2007, parr were gathered from the lower main stem near Crane Creek (Ck) at River kilometer (Rkm) 4.8 ($n=46$) and Rkm 2.0 of the tributary Moore Ck (enters main stem at Rkm 8.6; $n=53$) of WFSR by beach seine. Similarly, in October 2008 parr were gathered from the same area of the tributary Moore Ck ($n=88$), the upper main stem near Gold Ck (Rkm 17; $n=10$), the lower main stem near Crane Ck (Rkm 4.8; $n=61$), and the lower main stem of the WFSR near the ODFW smolt trap (Rkm 1.6; $n=11$).

Although there were multiple sampling locations, fish were considered to represent two main groups based on different river habits as described by Ebersole et al. (2006). Thus they are referred to as fish from the lower main stem (Rkm 1.6 and 4.8 of the main stem) and tributary (Rkm 17 of the main stem and the tributary Moore Ck), respectively. Captured parr were transferred to Oregon State University's (OSU) facilities, where they were held as described by Ferguson et al. (2010) until late April, the typical time of smoltification (100–130 mm fork length). Coho salmon undergo smoltification in spring (Groot and Margolis, 1991), so we chose the end of April (i.e., about 1 month after the Spring Equinox) to represent the typical time of smoltification for fish in our study.

2.2. Laboratory maintenance of fish

2.2.1. Captured brood year 2007 parr

Lower main stem and tributary fish were held separately in outside circular 0.6 m^2 diameter tanks at OSU's Fish Performance and Genetics Laboratory. Initial fish density was 0.68 g/L and 0.76 g/L for lower main stem and tributary fish, respectively. Flow-through, parasite free well water (12–13 C) was supplied. Fish were fed a mixture of commercial feed (1.5 mm size; Bio-Oregon Inc.) and freeze-dried brine shrimp and krill (Argent Labs), to satiation for 5 min once or twice daily.

2.2.2. Captured brood year 2008 parr

Fish were also held outside in 0.6 m^2 tanks at OSU's Salmon Disease Laboratory (SDL). Lower main stem and tributary fish were tagged with 12 mm Passive Integrated Transponder (PIT) tags (Biomark) and randomly mixed into six tanks, with approximately equal numbers of fish from each location in each tank. Initial fish density was about 0.64 g/L for each tank. Water supply, temperature, and feeding regime were the same as described above.

2.2.3. Experimental infections

In June of 2009, 120 hatchery-reared coho salmon parr were obtained from the ODFW's Oxbow Hatchery, Oregon. At the beginning of our study, six fish were examined for parasites, as described below, to determine if these fish were free of parasite infections. Likewise 30 fish from the negative control group were also evaluated for the presence of parasites at the end of the study. For this experiment, fish were divided into four groups (ca 30 fish/group): *N. salmincola* only, *Apophallus* sp. only, *N. salmincola* plus *Apophallus* sp., or no parasites. To obtain parasites for experimental infection, approximately 900 *Fluminicola* sp. and 600 *Juga silicula* snails were gathered from the lower river, near the smolt trap in WFSR from June to August 2009. *N. salmincola* utilizes only *J. silicula* as a first intermediate host (Bennington and Pratt, 1960); snails of *Fluminicola* spp. are the first intermediate hosts for *Apophallus* species in Oregon (Niemi and Macy, 1974; Villeneuve et al., 2005). However, heterophyids are fairly plastic in their affinity to intermediate snail hosts and other *Apophallus* species have been shown to use *Juga* snails (Malek, 1980). Snails were transported to the SDL where they were held in flow through tanks at 20 C under a 12 h photoperiod produced by 19 Watt aquaria lamps placed approximately 15 cm from the water surface. Here, they were screened in 12 well plates (3–4 snails per well) for cercarial shedding between 0800–1100 and 1800–2000 for several days under a dissecting microscope at ×50 magnification. Pools containing infected snails were removed and placed in flow through tanks with uninfected hatchery fish. Quantification of cercariae exposure to fish was not performed because cercarial shedding from snails was highly variable. Instead, fish were periodically evaluated for estimates of infections. Approximately 20–30 snails were used per tank and an estimated prevalence of infection in the snails, based on our screening technique, was 7% for *N. salmincola* in *J. silicula*, 2% for *Apophallus* sp. in *Fluminicola* sp., and <1% for *Apophallus* sp. in *J. silicula*. *J. silicula* were fed organic lettuce, and *Fluminicola* sp. were fed algae that were gathered from WFSR and maintained in the laboratory. Fish were exposed for 4 months (July–November 2009), and then individually PIT-tagged and separated into two 1.9 m^2 tanks outside at the SDL for a growth study, as described above for the captive fish from brood year 2008. Initial fish density was about 1.3 g/L for tanks in this study. The water source and temperature were the same as described above for the captive fish. These fish were fed to satiation daily with only the commercial feed. There was a single mortality from the "*Apophallus* sp. only" group.

2.3. Parasite evaluation

For each study, fish were euthanized at the end of April with an overdose of MS-222 (Argent Chemical Labs), carcasses were weighed, measured, subjectively assessed for degree of silver color, and macroscopically examined for neascus (black spot trematode) metacercariae. Skeletal muscle was fileted from the left side of each fish and frozen for later enumeration of parasites by microscopy as described by Ferguson et al. (2010). The posterior kidney of each fish was similarly sampled and evaluated for the presence of *N. salmincola* metacercariae. Standard histopathological techniques were used to evaluate the presence of any other parasites or pathogens in the experimentally infected and control fish. Mortalities that occurred throughout the study were noted and carcasses were processed for parasite evaluation. Prevalence (number of infected animals/total animals), intensity (number of parasites/infected animals), and density (number of parasites/infected tissue sample) of infections are reported in accordance with the definitions provided by Bush et al. (1997).

2.4. Size and growth

All fish were assessed for size at the end of April. Size was recorded as fork length (mm) and weight, to the nearest 0.01 g. Parr size (fork length and weight) was also recorded at the beginning of the study for wild fish from brood year 2008 and the hatchery fish.

Growth of individual PIT-tagged fish that were intermixed with fish from different parasitized groups was followed for individual fish by weighing fish at 6 week intervals in water throughout the experiment (four intervals total).

2.5. Swimming stamina

Fish captured from the WFSR from both years were subjected to swimming stamina tests. At the end of April, fish were evaluated for swimming stamina by determining exhaustion time of swimming at a constant water velocity. This was accomplished by acclimating individual fish in a clear polyvinyl chloride tube (7.6 cm diameter × 1.5 m long) at 10 cm/s for 15 min and then swimming them at 65 cm/s for fish from brood year 2007 and 82 cm/s for those from brood year 2008. The increased velocity for the second year was to decrease the amount of time required to run the swimming test. Water velocity was manipulated using a 1200 Watt rheostat (Staco Inc.) and a 575 Watt submersible centrifugal pump (Simer Pump Co.), which was recirculated with occasional fresh cold water added to maintain temperature. Plastic mesh was attached to both ends of the tubes to prevent fish from leaving and plastic straws were inserted at the outflow end to maintain uniform flow in the tube. When fish were exhausted, as indicated by their movement towards the outflow end of the tube and/or lack of swimming, tapping on the tube was applied to motivate them to continue swimming. If swimming was not continued after a series of three tapping cycles, of three taps each, then the time (to the nearest second) was recorded from a stopwatch, and the fish was removed.

2.6. Gill Na^+,K^+-ATPase activity

Fish captured from the WFSR from both brood years were evaluated for gill Na^+,K^+-ATPase activity. After each fish had undergone the swimming stamina challenge, it was euthanized with an overdose of MS-222 and gill filaments were harvested and frozen at −80 C for later analysis. Enzyme activity was measured in accordance with the protocol described by McCormick (1993).

2.7. Statistical analysis

We analyzed the data from the two different year classes of wild fish separately because of the differences in study design (i.e., different holding facility, fish densities, swimming velocities, and mixing or lack of mixing of fish subpopulations). Likewise, data from the experimentally infected fish were analyzed separately. We made between group (i.e., heavily infected vs. lightly or uninfected smolts) comparisons of results from our endpoint measurements. Additionally, we performed within group analyses to determine the relationship of parasitism to the measured fitness endpoints.

Data were assessed for normality by visual inspection of scatterplots. With the exception of weight and fork length data from all studies, and gill Na^+,K^+-ATPase activity data from the 2007 study, all fitness variables were determined to be non-normal. Therefore, we used a non-parametric bootstrap 2-sample *t*-test, replicated 100,000 times, for between group analyses.

For within group analyses, we used multiple linear regression to model the fitness responses as predicted by parasite infection. Response variables were: weight, fork length, gill Na^+,K^+-ATPase activity, swimming stamina, and growth rate. Our explanatory variables were: counts of *N. salmincola* in muscle or kidney; *Apophallus* sp.; neascus; *Myxobolus insidiosus*; and total metacercariae. The later variable was created by summing metacercariae from all digenean species (*N. salmincola* from muscle and kidney, *Apophallus*, and neascus). We also accounted for other pertinent explanatory variables, such as sex, tank, and size (fork length). Multicolinearity was assessed by visual inspection of correlation matrices and normality was assessed by visual inspection of scatterplots. We log(ln) transformed the data for all three parasite species in order to improve linearity. Many of the response variables were also transformed to meet the assumption of normality, as these data were skewed. Response variables not transformed included fork length and weight from all three studies, and ATPase activity from the 2007 study, as these data appeared to be approximately normally distributed.

Our modeling selection strategy was to compare results from full models (with predictors and interactions) to more reduced models using a backwards elimination technique. We chose final models by examining residual plots to identify outliers, non-linearity, and heterogeneous variation; R^2 values; number of variables in relation to sample size; and interpretability of model. To determine if certain trematode species were more strongly associated with decreased endpoints than the total number of trematodes, regardless of species, we evaluated two different types of parasite models separately; one with all trematode species combined (total metacercariae) plus *M. insidiosus*, and the other with individual parasite species as variables. To determine when growth may be most impacted by parasites, we considered models with two different types of responses for the growth studies; one with individual 6 week growth intervals (i.e., difference in weight between measured time interval), and the other with overall growth (i.e., difference in weight from start to end of the experiment). We also included parr fork length to account for initial size in the growth studies. Finally, we analyzed data from the swimming stamina tests both with and without accounting for fish size, because we hypothesized that parasites were correlated with size.

All statistical analyses were conducted with S-PLUS® version 8.0 software (Insightful Corp.) or R, version 2.7.2 (R Development Core Team). Significance was set at $p<0.05$ and p-values are 2-tailed. Mortalities from all studies were excluded from our analysis. There were nine mortalities from the lower main stem fish of brood year 2007, eight from this population of brood year 2008, and 11 from the tributary group of brood year 2008. Therefore, final sample sizes of wild fish were as follows: brood year 2007 fish from the lower main stem ($n=37$) and tributary ($n=56$), and brood year 2008 fish from the lower main stem ($n=64$) and tributary ($n=87$).

3. Results

Inspection of the correlation matrices provided initial insight into relationships of parasite species and host fitness responses. These correlations did not account for any other variables. However, some correlation coefficient (r) values warrant mention because they provide a simpler interpretation compared to that obtained by complex multiple linear regression modeling. For the brood year 2007 fish, *Apophallus* sp. was negatively correlated with both smolt weight and fork length (−0.44 [Fig. 1] and −0.51, respectively); *N. salmincola* in muscle was negatively correlated with smolt weight, fork length and swimming stamina (−0.48 [Fig. 1], −0.44, and −0.41, respectively); and *Apophallus* sp. and *N. salmincola* in muscle were positively correlated with each other (0.55). For the brood year 2008 fish, *Apophallus* sp. was again negatively correlated with both smolt weight and fork length (−0.54 [Fig. 1] and −0.59, respectively) and also ATPase activity and swimming stamina (−0.40 and −0.48, respectively); *N. salmincola* in muscle was consistently negatively correlated with smolt weight, fork length, and swimming stamina (−0.58 [Fig. 1], −0.57, and −0.40, respectively); and *Apophallus* sp. and *N. salmincola* were again found to be positively correlated with each other (0.63). A slight positive correlation (0.10) was found with *M. insidiosus* and ATPase activity in the brood year 2008 fish.

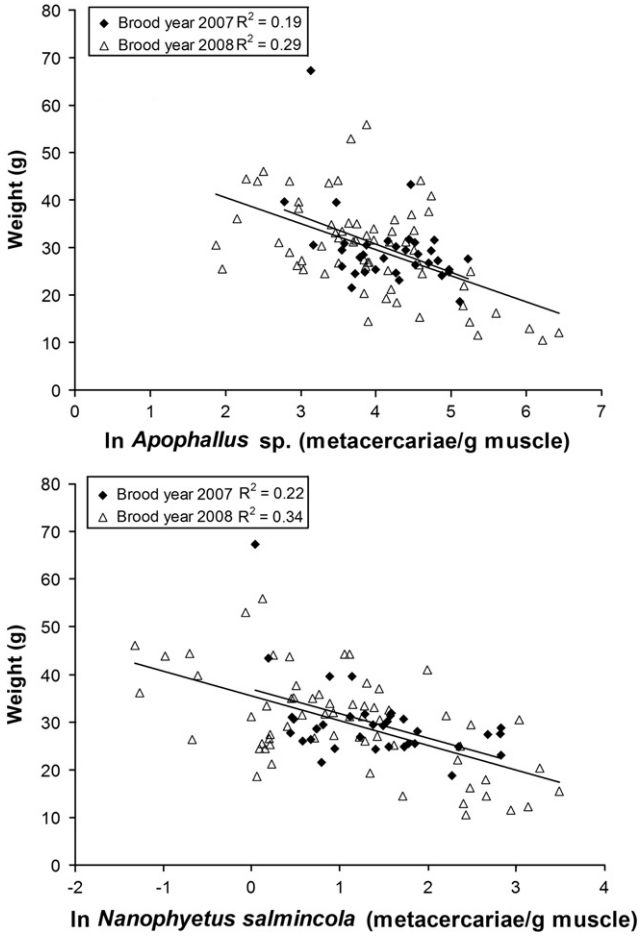

Fig. 1. Example of parasitism negatively associated with fitness endpoints of coho salmon (*Oncorhynchus kisutch*) smolts. Simple linear regressions are shown for the two parasites most negatively correlated with smolt weight for wild fish, brood years 2007 and 2008, gathered from the lower main stem of West Fork Smith River. Parasite counts were natural log transformed to improve linearity and scale to graphing. Coefficient of determination (R^2) values are shown.

3.1. Wild fish-brood year 2007

3.1.1. Parasites

Consistent with our previous studies (Ferguson et al., in press; Rodnick et al., 2008), the lower main stem fish were more heavily infected compared to tributary fish (Table 1). The most dramatic difference was in the mean density of *Apophallus* sp., with the lower main stem fish harboring approximately 80 times more metacercariae/g than fish from the tributary. The infection levels of *N. salmincola* in muscle and kidney were also significantly higher in lower main stem fish. However, *M. insidiosus* infections were not significantly different between groups ($p=0.47$; bootstrap *t*-test). There was no neascus detected in the tributary group, and the mean infection in fish from the lower main stem was 3 parasites/fish (Table 1). The prevalence of these parasites was as follows: *Apophallus* sp. was 100% and 18% in lower main stem and tributary smolts, respectively; *N. salmincola* was 100% in both smolt groups; *M. insidiosus* was 82% and 84% in lower main stem and tributary smolts, respectively; neascus was 74% in lower main stem smolts.

3.1.2. Size

Within the lower main stem group, parasitism was negatively correlated with smolt weight (Table 2; Fig. 1). Analysis incorporating multiple parasite species showed that *Apophallus* sp. and kidney *N. salmincola* were significant predictors of small fish, with almost 40% of the variation in smolt weight explained by parasites alone (Table 2). The same result occurred for smolt fork length, as *Apophallus* sp. and kidney *N. salmincola* were again significant predictors of low size values (Table 2). There was no evidence that smolt weight between the two groups differed ($p=0.34$; bootstrap *t*-test). However, there was suggestive, but inconclusive evidence for a difference in fork length ($p=0.06$; bootstrap *t*-test; Table 1).

3.1.3. Swimming stamina

Within group analysis of the lower main stem smolts showed that parasites were negatively associated with fitness. Total metacercariae was a significant predictor of lower swimming stamina, but only when not accounting for fish size (Table 2). This model explained more than 30% of the variation in swimming stamina for this group of fish (Table 2). We saw no evidence that swimming ability between the lower main stem and tributary groups differed ($p=0.20$; bootstrap *t*-test; Table 1).

3.1.4. ATPase activity

Within the lower main stem group, parasites were negatively associated with fitness when ATPase activity was used as an endpoint. *N. salmincola* in the kidney was a significant predictor of low enzyme activity, even after accounting for smolt size. This parasite contributed to explaining 27% of the variation in ATPase activity in this group of fish (Table 2). There was a difference in gill ATPase activity between the lower main stem and tributary smolts (Table 1). The fish from the lower main stem are estimated to have a mean gill ATPase activity of 1.9 μmole/mg/h (95% confidence interval: 1.3 to 2.7 μmole/mg/h) less than that of tributary fish ($p<0.01$; bootstrap *t*-test).

3.2. Wild fish-brood year 2008

3.2.1. Parasite evaluation

Consistent with the study from the previous year, fish from the lower main stem were significantly more heavily infected compared to fish from the tributary group (Table 1), with the biggest difference being in levels of *Apophallus* sp. The difference this year was even greater, as fish from the lower main stem had a mean density of about 420 times more metacercariae/g than tributary fish (Table 1). Density of *N. salmincola* in muscle was again significantly higher in lower main stem fish; however, in 2008 lower main stem fish had a lower

Table 1
Summary of parasite burden and fitness of coho salmon (*Oncorhynchus kisutch*) smolts of brood years 2007 and 2008 from the lower main stem and tributary of West Fork Smith River. Means (±95% confidence interval) are presented and were tested with a bootstrap *t*-test using 100,000 replications.

	Lower main stem, brood year 2007	Tributary, brood year 2007	Lower main stem, brood year 2008	Tributary, brood year 2008
Apophallus sp. (metacercariae/g)	77 (64.1–92.7)	1 (0.2–3.7)**	84 (62.6–117.8)	0.2 (0–0.6)**
Nanophyetus salmincola in muscle (metacercariae/g)	5 (4.0–7.2)	3 (2.4–3.6)*	5 (4.0–7.4)	3 (2.2–3.4)*
Nanophyetus salmincola in kidney (metacercariae/g)	2386 (1817.6–3149.4)	914** (754.8–1118.5)	711 (583.7–860.5)	1180** (988.3–1492.9)
Neascus (metacercariae/fish)	3 (1.6–8.7)	0	9 (8.0–11.1)	1 (0.6–1.7)**
Myxobolus insidiosus (pseudocysts/g)	51 (25.5–99.8)	36 (24.7–55.0)	47 (26.6–97.4)	5 (1.8–12.8)
Weight (g)	30 (27.8–34.8)	28 (26.1–30.3)	30 (27.6–32.4)	30 (28.9–32.9)
Fork length (cm)	14 (13.7–14.5)	13 (13.2–13.9)	14 (13.6–14.4)	14 (14.0–14.5)
Gill Na^+,K^+-ATPase activity (μmole/mg/h)	4 (3.7–5.0)	6 (4.9–7.3)**	3 (1.8–3.2)	2 (1.6–2.7)
Swimming stamina (min to exhaust)	17 (14.2–21.6)	20 (17.8–23.8)	11 (9.8–13.8)	15 (13.5–17.5)*
Growth (g/week)				
0–7week	–	–	1.2 (1.1–1.3)	1.1 (1.1–1.2)
7–12week	–	–	0.9 (0.8–1.1)	1.1 (1.0–1.2)
12–18week	–	–	0.8 (0.6–0.9)	0.9 (0.7–1.0)
18–24week	–	–	1.0 (0.8–1.1)	0.9 (0.8–1.1)
0–24 week (overall)	–	–	2.9 (2.6–3.1)	3.1 (2.9–3.3)

– Indicates data not collected.
** $p<0.01$.
* $0.01 \geq p < 0.05$ for tests between smolts from the lower main stem and tributary within a given brood year.

infection with this parasite in kidney compared to tributary fish (Table 1). With *M. insidiosus*, again there was no difference in infection density between groups. In the 2008 study, neascus was detected in fish from the tributary group, although at a significantly lower level than that found in lower main stem fish (Table 1). The prevalence of these parasites was as follows: *Apophallus* sp. was 100% and 2% in lower main stem and tributary smolts, respectively; *N. salmincola* was 100% in both the lower main stem and tributary smolts, respectively; *M. insidiosus* was 97% and 32% in lower main stem and tributary smolts, respectively; neascus was 94% and 23% in lower main stem and tributary smolts, respectively.

3.2.2. Size and growth

As we found with the 2007 study, within the lower main stem group, parasites were negatively associated with both smolt weight (Fig. 1) and fork length. Parasites alone explained 45–50% of the variation in size, respectively (Table 2). The same parasite species from the previous year were negatively associated with size. Additionally, *M. insidiosus* also significantly predicted small values of both smolt weight and fork length (Table 2). In the 2008 study, parasites were also negatively associated with fish size in the tributary group (Table 3). Here, *N. salmincola* in both muscle and kidney were significant predictors of both reduced weight and fork length (Table 3). These associations were not as strong as those found for the lower main stem fish (Tables 2 and 3).

We found the most profound impact of parasitism on lower main stem fish with overall growth, with *Apophallus*, sp. and *M. insidiosus* being the most negatively associated species involved in explaining over 60% of the variation in total growth, after accounting for parr size (Table 2). Additionally, *N. salmincola*, infecting kidney, was also a significant predictor of reduced growth. For the tributary fish, parasitism was also negatively associated with overall growth, with *N. salmincola*, in both muscle and kidney, being the only significant species (Table 3). *N. salmincola* infections contributed to explaining almost 50% of the variation in growth of tributary fish (Table 3).

Table 1 summarizes the comparison of size and growth between the two infected groups of fish. There was no evidence that either mean fork

Table 2
Linear regression models of parasite burden and fitness endpoints for wild coho salmon (*Oncorhynchus kisutch*) smolts, originating from the lower main stem of West Fork Smith River, held in captivity from brood years 2007 and 2008.

Response variable	2007 model	R^2	2008 model	R^2
Weight (g)	−4.98 (totmeta)** + 0.44 (Myxo)	0.22	−4.44 (totmeta)** − 2.05 (Myxo)**	0.26
Weight (g)	−5.02 (Apo)* − 0.98 (M Nano) + 0.27(Myxo) − 4.16 (K Nano)*	0.39	−2.32(Apo) − 3.43 (M Nano)** − 1.34 (Myxo)*	0.44
Fork length (cm)	−0.54 (totmeta)** + 0.08 (Myxo)	0.16	−0.79 (totmeta)** − 0.36 (Myxo)**	0.30
Fork length (cm)	−0.88 (Apo)* − 0.01 (M Nano) + 0.06(Myxo) − 0.48 (K Nano)*	0.39	−0.65 (Apo)** − 0.31 (M Nano) − 0.24 (Myxo)* − 0.37 (K Nano)	0.51
ln overall growth (g/week)	–		0.24 (parr FL)** − 0.19 (totmeta)** − 0.09(Myxo)**	0.50
ln overall growth (g/week)	–		0.14 (parr FL)* − 0.15 (Apo)** − 0.06 (M Nano) − 0.07 (Myxo)** − 0.10 (K Nano)*	0.62
ln Na^+,K^+-ATPase activity (μmole/mg/h)	−0.39 (smolt FL) − 1.26 (totmeta)* − 0.01(Myxo)	0.25	0.28 (smolt FL)** − 0.05 (totmeta) + 0.20 (Myxo)*	0.22
ln Na^+,K^+-ATPase activity (μmole/mg/h)	−0.61 smolt FL − 0.74 (Apo) + 0.08 (M Nano) − 0.12 (Myxo) − 1.33 (K Nano)*	0.27	0.21 (smolt FL)* − 0.38 (Apo)* + 0.08 (M Nano) + 0.21 (Myxo)*	0.29
ln swimming stamina (min to exhaust)	−0.26 (totmeta)* + 0.08 (Myxo)	0.31	−0.31 (totmeta)** − 0.16 (Myxo)**	0.30
ln swimming stamina (min to exhaust)	NS		−0.27 (Apo)* − 0.01 (M Nano) − 0.12 (Myxo)* − 0.23 (K Nano)*	0.40

ln = natural logarithm. Density of infection (parasites/g) was used for parasite burden. Apo = *Apophallus* sp., Nano = *Nanophyetus salmincola*, M = muscle, K = kidney, Myxo = *Myxobolus insidiosus*, totmeta = total metacercariae. – Indicates data not collected. NS = not significant, indicating no associations found ($R^2<0.1$). Two models are presented for each response, one with counts of metacercariae from all digenean species pooled (totmeta) and the other with counts from each species separate. All data for parasite burdens were log (ln) transformed.
** $p<0.01$.
* $0.01 \geq p < 0.05$.

Table 3
Linear regression models of parasite burden and fitness endpoints for wild coho salmon (*Oncorhynchus kisutch*) smolts, from the tributary group of West Fork Smith River, held in captivity from brood year 2007 and 2008.

Response variable	2007 model	R^2	2008 model	R^2
Weight (g)	NS		−4.43 (totmeta)** − 0.87 (Myxo)	0.25
Weight (g)	NS		1.43 (Apo) − 2.65 (M Nano)** − 0.65 (Myxo) − 3.78 (K Nano)**	0.34
Fork length (cm)	NS		−0.47 (totmeta)** − 0.03 (Myxo)	0.17
Fork length (cm)	NS		0.11 (Apo) − 0.33 (M Nano)* + 0.01 (Myxo) − 0.39 (K Nano)**	0.25
ln overall growth (g/week)	–		0.07 (Parr FL)* − 0.22 (totmeta)** + 0.01(Myxo)	0.38
ln overall growth (g/week)	–		0.07 (Parr FL)** + 0.06 (Apo) − 0.18 (M Nano)** − 0.36 (K Nano)** − 0.04 (Myxo)	0.47
ln Na^+,K^+-ATPase activity (μmole/mg/h)	NS		NS	
ln Na^+,K^+-ATPase activity (μmole/mg/h)	NS		NS	
ln swimming stamina (min to exhaust)	NS		NS	
ln swimming stamina (min to exhaust)	NS		NS	

Density of infection (parasites/g) was used for parasite burden. ln = natural log, Apo = *Apophallus* sp., Nano = *Nanophyetus salmincola*, M = muscle, K = kidney, Myxo = *Myxobolus insidiosus*, totmeta = total metacercariae. – Indicates no data collected. NS = not significant, indicating no associations found (R^2<0.1). Two models are presented for each response, one with counts of metacercariae from all digenean species pooled (totmeta) and the other with counts from each species separate. All data for parasite burdens were log (ln) transformed.
** $p<0.01$.
* $0.01 \geq p<0.05$.

length or weight of smolts differed between fish groups (p = 0.45 and 0.64, respectively; bootstrap *t*-test). When examining individual growth periods, there was suggestive, but inconclusive evidence that lower main stem fish grew less during the second growth interval (from 6 to 12 weeks) of the study (p = 0.06; bootstrap *t*-test). However, there was no evidence of a difference in overall growth between the two groups (p = 0.30; bootstrap *t*-test).

3.2.3. Swimming stamina

Within group analysis of the lower main stem group showed that parasites were negatively associated with swimming stamina, when not accounting for fish size (Table 2). *Apophallus* sp., *M. insidiosus,* and kidney *N. salmincola* were significant negative predictors of time to exhaust, which were involved in explaining 40% of the variation in swimming stamina (Table 2). There was evidence of a difference in swimming exhaustion time between the two groups of fish (Table 1). The mean time to exhaust swimming for the lower main stem smolts was about 3.5 min (95% back transformed confidence intervals: 1.7 to 5.8 min) less than that of tributary smolts (p = 0.02; bootstrap *t*-test).

3.2.4. ATPase activity

For the lower main stem fish, *Apophallus* sp. was negatively associated with enzyme activity, after accounting for other parasites and smolt size, and contributed to explaining about 30% of the variation of ATPase activity in this group (Table 2). *M. insidiosus* was positively associated, although the regression coefficient indicated the slope to be only slightly positive, after accounting for other variables, and that of *Apophallus* sp. to be more strongly negative. Moreover, the correlation coefficient of *M. insidiosus* was only around 0.1 (mentioned above in Results). There was no evidence of a difference in ATPase activity between the lower main stem and tributary fish for the second year study (p = 0.31; bootstrap *t*-test; Table 1).

3.3. Hatchery fish

3.3.1. Experimental infections

We experimentally infected hatchery fish to broaden the inference made from the associations in our study. We were able to successfully infect fish with moderate to high levels of *N. salmincola*, but were unable to reproduce infection levels of *Apophallus* sp. similar to those found in the field, as only about 1 worm/g or less were found in muscle from all *Apophallus* sp. exposures. Therefore, this experimental group was removed from the analysis in this study.

A few *J. silicula* snails (intermediate host for *N. salmincola*) released low numbers of *Apophallus* sp. cercariae, which correspondingly established as a low level infection in fish that cohabitated with only this species of snail. We included counts of *Apophallus* sp. in our regression models to account for the presence of this parasite. The mean densities (and range) of infections are summarized in Table 4. High infections of *N. salmincola* were established in the kidney for both treatment groups, with the *N. salmincola*-only and *N. salmincola* plus *Apophallus* sp. groups having about 11,000 and 4000 worms/g, respectively. Only low infections of *N. salmincola* were established in muscle (ca ≤ 2 worms/g) of fish from both groups (Table 4). Controls were free of the infection and histopathologic evaluation revealed no other parasites in any group.

3.3.2. Size and growth

Within the infected group, total *N. salmincola* (counts from muscle and kidney combined) was negatively associated with growth (p = 0.04; multiple linear regression). Overall growth was modeled as follows: ln overall growth = − 0.12 (ln total *N. salmincola*) − 0.02 (ln *Apophallus*) + 0.21 (parr fork length). This model explained 43% of the variation in overall growth. Because these data were from a controlled experiment, we present the following interpretation for this model: a doubling of *N. salmincola* causes a decrease in overall median growth by 8% (back transformed 95% confidence interval: 3% to 13%), after accounting for initial parr fork length and *Apophallus* sp.

Table 4 summarizes the size and growth of experimentally infected fish compared to the uninfected negative control fish. There was no evidence of a difference in the mean fork length or weight of smolts between fish groups (p = 0.14 and 0.19, respectively; bootstrap *t*-test). When looking at individual growth intervals, there was suggestive, but inconclusive evidence, in a difference in mean growth between treated and untreated fish for the second growth period (p = 0.06; bootstrap *t*-test). However, overall growth did not differ between groups (p = 0.28; bootstrap *t*-test).

4. Discussion

We consistently showed that digenean and myxozoan parasites in our study were negatively associated with size, growth, ATPase activity, and swimming stamina. In addition to the importance in their own right, these performance traits are all associated with the parr-smolt transformation process. The most dramatically negative association with parasitism and these performances occurred with fish from the lower main stem of WFSR, which are most heavily parasitized. Here, the most profound negative association with parasitism occurred with growth, and clearly size is strongly linked to swimming stamina and ATPase activity, and thus survival. Moreover, the reproducibility was remarkable for the negative association between weight and parasites for both *Apophallus* sp. and muscle infections of *N. salmincola* between study years. Indeed, even the slopes and Y intercepts were almost exactly the same, suggesting that these associations have relatively low temporal variation. In biological terms, most fish within the top 50th

Table 4
Summary of size, growth, and infection density from uninfected and experimentally infected hatchery coho salmon (*Oncorhynchus kisutch*) smolts. Mean values and ranges (in parentheses) are shown. Comparisons of mean size and growth between experimentally infected and uninfected fish were made with a bootstrap *t*-test using 100,000 replications, all of which were found to be not statistically significant ($p > 0.05$). ** = $p < 0.01$, * = $0.01 \geq p < 0.05$ for tests between smolts from the experimentally infected fish and uninfected. *n* = sample number, wk = week.

Group	*n*	Infection density (parasites/g)	Smolt length (cm)	Smolt weight (g)	0–8 wk growth (g/wk)	8–16 wk growth (g/wk)	Overall growth (g/wk)
Uninfected control	28	0	15.0 (13.4–17.2)	44.3 (30.6–65.5)	1.6 (0.8–2.6)	1.7 (0.5–2.9)	1.7 (1.0–2.5)
Apophallus sp. only	25	0.1 (0–0.6)	14.9 (12.9–16.8)	44.2 (27.1–71.3)	1.3 (0.1–2.1)	1.9 (0.8–3.5)	1.7 (0.6–3.1)
Nanophyetus salmincola only	29	Kidney = 10,575.3 (1,420–27,900) Muscle = 2.4 (0.5–9.6) *Apophallus* = 1.1 (0.2–4.7)	15.3 (12.8–18.4)	46.5 (25.6–83.6)	1.4 (0.7–2.6)	2.0 (0.8–4.1)	1.7 (0.7–3.3)
N. salmincola + *Apophallus* sp.	28	Kidney = 3,945.6 (290.9–9,500) Muscle = 0.4 (0–1.4) *Apophallus* = 0.1 (0–1.1)	15.6 (12.5–19)	50.4 (25.4–98.8)	1.6 (0.6–4.4)	2.3 (0.8–5.5)	1.9 (1.0–4.9)

percentile of infection densities were also in the bottom 25th percentile of size.

We also found differences in fitness between lower main stem fish and fish from the tributary group of WFSR. Although these results were not constant between study years, it is difficult to make comparisons between wild fish populations, even from the same watershed. There are many other environmental and genetic factors that influence growth, swimming performance, and smoltification. In addition, the timing of smoltification is not necessarily consistent between years for any stock, and hence sampling time could also account for discrepancies.

4.1. Size

Reduced growth and size are frequently associated with chronic parasite infections in fishes (reviewed in Barber and Wright, 2005), and these growth-related endpoints were most consistently associated with parasite burdens in our study. Indeed, *Apophallus* sp. and *N. salmincola* were most strongly associated with low values of size and growth in both brood year groups of naturally infected fish, which was corroborated for *N. salmincola* in our study with the experimentally infected fish. *M. insidiosus* was also negatively correlated with size and growth, although only in lower main stem fish.

Our findings that the parasites in our study are associated with reduced fish size may be important for understanding indirect sources of salmon mortality. For example, smaller fish have reduced survival due to increased predation risk and reduced physiological fitness (Beamish and Mahnken, 2001). Furthermore, fish size may not only influence freshwater juvenile overwinter survival (Quinn and Peterson, 1996), but also successful smolt survival (Holtby et al., 1990) and ocean migration of salmonids (Beckman et al., 1998). This is particularly evident for overwinter survival in years with more severe winter conditions (Quinn and Peterson, 1996). Effects on size and growth have been evaluated on other host–parasite systems. Cutthroat trout (*Oncorhynchus clarkii clarkii*) experimentally infected with *S. klamathensis* had significantly less growth than uninfected fish (Evans, 1974), and *E. salvelini* causes reduced growth of sockeye salmon (*Oncorhynchus nerka*), even with light infections (Boyce, 1979). Particularly pertinent to our study, Johnson and Dick (2001) found that yellow perch (*Perca flavescens*) in four Canadian lakes heavily infected with *Apophallus brevis*, had significantly lower growth in terms of total length and somatic mass than those lightly infected.

Reduced growth in parasitized fish most likely involves an excessive energy demand of infection. Parasites divert energy from their hosts to undergo growth and development, and even seemingly quiescent parasites utilize host energy for basal maintenance (Barber et al., 2000). Furthermore, the accompanying immune response to infection requires additional energy expenditure (Barber et al., 2000). To compensate for the increased energetic cost of parasitism, fish behavior may be affected, such as devoting increased time to foraging. Changes in foraging time of hosts have been well studied in three-spined sticklebacks (*Gasterosteus aculeatus*) infected with plerocercoids of *Schistocephalus solidus*. In our study, we fed fish to satiation, so the impacts of these parasites under natural conditions could be even more dramatic, as fish would need to balance foraging for limited resources with exposure to predation. Consistent with our study, Ebersole et al. (2009) also found that neascus was not associated with fish size in coho salmon from the WFSR. However, they did not examine fish for internal parasites. This stresses the importance of evaluating the role of more than one parasite species on host fitness.

4.2. Swimming performance

Salmon live in fast moving streams and rivers during their freshwater phase, and thus their swimming ability is implicitly tied to their survival. Reduced swimming performance could lead to mortality either directly by smaller fish being purged from systems during heavy winter water flows (Pearsons et al., 1992) or indirectly by decreasing predator avoidance (Taylor and McPhail, 1985). We found reduced swimming stamina was negatively associated with *N. salmincola* and *Apophallus* sp. in fish from the lower main stem of WFSR from both brood years. Boyce (1979) demonstrated that sockeye salmon experimentally infected with *E. salvelini* had significantly reduced critical swimming speeds (U_{crit}) compared to controls. Likewise, the U_{crit} of Atlantic salmon (*Salmo salar*) is reduced when challenged with sea lice, which is likely due to the accompanied decrease in cardiac output (Wagner et al., 2003). Butler and Millemann (1971) examined both burst and sustained swimming abilities of juvenile salmonids experimentally infected with *N. salmincola* and reported the biggest impairment to swimming occured shortly after initial infection. They concluded this was due to the migration of cercariae destroying tissue and that encysted metacercariae may impact swimming only when occurring in very high numbers.

We found a negative association with swimming and *M. insidiosus* in lower main stem smolts for only the 2008 year class. The difference in our results between the two study years could have been due to differences in infection, as the maximum infection in fish from the second year was about twice that of the heaviest infection in fish from the first year (data not shown). Moles and Heifetz (1998) reared wild sockeye salmon to smoltification in the laboratory, and found that fish naturally infected with *M. arcticus* had significantly lower U_{crit} than those without infections. Similarly, sockeye salmon experimentally infected with the myxozoan *P. minibicornis* have reduced swimming recovery rates (Wagner et al., 2005).

Size is linked to swimming performance in fish (e.g., Ojanguren and Braña, 2003; Taylor and McPhail, 1985), and thus most studies investigating swimming performance with salmonids evaluate results

compensating for size. Therefore, we were interested in the association that parasites may have had on both size/growth and swimming separately. Parasites and size/growth were both strongly associated with swimming, and thus compensating for size/growth in regards to swimming tended to negate the effects of parasitism. In other words, parasites most strongly affected swimming by their association with reduced size/growth. Ebersole et al. (2006) suggested that reduced overwinter survival in lower main stem fish in the WFSR may be associated with fish being flushed out of the system during heavy overwinter flows due to the simplified substrate at this location. However, parasites could further exacerbate this situation due to their association with a reduced swimming ability of the host.

Many studies have illustrated the importance of predation on salmon survival (e.g., Larsson, 1985; Parker, 1968, 1971; Ward and Larkin, 1964), and both size (Bams, 1967; Beamish and Mahnken, 2001; Parker, 1971; Patten, 1977) and swimming performance (Bams, 1967; Taylor and McPhail, 1985) are principal factors influencing predation. Therefore, reduction of either fish size or swimming performance would be advantageous to parasites with complex life cycles involving piscivorous definitive hosts. There are many examples of host manipulation by parasites with complex life cycles (referred to as Parasite Induced Trophic Transmission; PITT) involving fish (reviewed in Barber et al., 2000). The digenean species infecting fish in our study complete their life cycle in piscivorous avian and/or mammalian hosts. Therefore, the reduction in swimming stamina and growth of fish associated with parasites in our study is consistent with the strategy of PITT. Although myxozoan parasites do not share a similar life cycle with digeneans, spores of *Myxobolus cerebralis* can survive passing through the digestive tract of birds (El-Matbouli and Hoffmann, 1991; Taylor and Lott, 1978), which may act as phoretic hosts by transmitting parasites to alternate oligiochaete hosts in other watersheds.

4.3. ATPase activity

Another measure of fitness used in our study was Na^+,K^+-ATPase activity, a major component of osmoregulation (reviewed in McCormick, 2001). The ability for anadromous fish to osmoregulate is central to the physiological preparedness of salmon migrating to sea water, and hence is an important factor for survival (Moser et al., 1991). Aside from the direct mortality associated with poor osmoregulation, poor enzyme activity has also been linked to increased avian predation risk (Kennedy et al., 2007).

Smoltification involves changes in energy storage and is thought to be a stressful transition for salmon (Hoar, 1988), and thus the effects of infectious agents could be amplified during this sensitive stage. For example, Price and Schreck (2003) showed that Chinook salmon (*Oncorhynchus tshawytscha*) infected with *Renibacterium salmoninarum*, the bacterium that causes bacterial kidney disease, had less preference for seawater than control fish. Parasites have also been associated with decreased osmoregulation of fishes. Boyce and Clarke (1983) showed that sockeye salmon infected with *E. salvelini* had significantly higher mortality and plasma sodium levels when challenged to saltwater than controls. Interestingly, these investigators did not find fish size to be significantly correlated with parasites, suggesting that the parasite effects on osmoregulation were direct. Birkeland and Jakobsen (1997) showed that sea lice infected juvenile (post smolt) sea trout returned to the freshwater environment prematurely compared to uninfected control fish, when simultaneously released in the ocean. In our study, a decrease in ATPase activity was associated with all parasites, except for neascus. Therefore, when these fish migrate with the rest of the population in the system, they are likely to either directly die due to poor saltwater adaptation or are subjected to greater predation when lingering in the estuary awaiting maturation (Schreck et al., 2006).

4.4. Parasites

In general the total number of metacercariae, regardless of species, typically did not predict the fitness responses as well as individual trematode species. Summarizing our findings by parasite species, the negative associations of parasitism with host fitness, within heavily infected groups, demonstrated that *Apophallus* sp. had the strongest negative relationships with all performance endpoints. *N. salmincola* was negatively associated mostly with size and swimming stamina. This species was also associated with decreased growth in both the naturally and experimentally infected fish. *M. insidiosus* was an important species only in the second wild fish captive study, and was negatively associated with size, growth and swimming stamina. The weakly positive association this parasite had on ATPase likely would have been negated by the much stronger negative association of *Apophallus* sp., as suggested by the magnitude of the regression coefficients. The difference in associations of *M. insidiosus* between study years could be attributed to differences in the composition of parasite burden. For example, kidney infections of *N. salmincola* were much less in the lower main stem fish the second year. Perhaps this allowed *M. insidiosus* to have a stronger effect, which may be expected if parasite species compete. Neascus was never associated with the performance endpoints measured in our study, which could suggest that not all parasite species contribute equally to the reduction of certain aspects of host fitness.

It was difficult to separate the effects of each parasite species as the infections were correlated with each other. For example, *N. salmincola* and *Apophallus* species were positively correlated, share the same intermediate snail host (*J. silicula*) (present study; Malek, 1980), and utilize mammals and birds as definitive hosts (Niemi and Macy, 1974; Schlegel et al., 1968). Therefore it is plausible that the effects of these parasite species could be additive in fashion to decrease host fitness and thus susceptibility to predation. Indeed, our analysis is suggestive of such an effect, as more than one parasite species was significantly associated with decreased performance after accounting for the mixed infection. This stresses the importance of studying more than one parasite, as fish are commonly infected by numerous species of parasites (Hoffman, 1999).

5. Conclusion

In conclusion, we have consistently shown that the parasites examined in our study (i.e., *Apophallus* sp. and *N. salmincola*) were negatively associated with several fitness endpoints in both naturally and experimentally exposed coho salmon smolts. Reductions in growth, swimming stamina and ATPase activity are linked to overwintering and smolt mortality and thus parasites associated with critical negative effects on individual smolts could have negative effects at the population level. Therefore, the much lower observed parasite burden of up to 95% in outmigrating smolts compared to parr from the lower main stem of WFSR (Ferguson et al., in press) could indicate parasite associated mortality, which may be indirect via decrease in these performances. Understanding why certain salmon populations are heavily infected with these parasites, which likely are driven by landscape characteristics, could help in management or recovery planning, given that our data indicates that severity of these infections are associated with survival. Such understanding would help with life history based population viability models by identification of the importance of a heretofore unconsidered mortality factor.

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