Aquaculture 362–363 (2012) 184–192

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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

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ARTICLE INFO

*Article history:*
Received 26 October 2010
Received in revised form 28 June 2011
Accepted 1 July 2011
Available online 13 July 2011

*Keywords:*
Parasites
Mixed infections
Fitness correlates
Wild coho salmon smolts
Threatened stocks

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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doi:10.1016/j.aquaculture.2011.07.003

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.