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Title: Evaluating the Effects of a Parasitic Copepod (Salmincola californiensis) on the Performance of Juvenile Chinook Salmon (Oncorhynchus tshawytscha)

Abstract approved:

______________________________________________________

James T. Peterson

Pacific salmon (Oncorhynchus spp.) rearing in lakes and reservoirs have been known to become heavily infected with an ectoparasitic copepod (Salmincola californiensis). Little is known about the factors that affect the parasite infection prevalence and intensity. However, previous research suggests that the parasite may negatively affect the fitness and survival of the host fish. I determined the effect of water temperature, stress, and the density of the free-swimming infectious stage of S. californiensis, the copepodid, by experimentally exposing juvenile Chinook salmon (O. tshawytscha). I also evaluated the potential for cross infection by placing naïve and infected fish in the same tank for 10 weeks. The ability to osmoregulate and transition to seawater was investigated by placing unexposed and exposed fish in 34 ppt seawater and monitoring for 24 hours. I achieved infection rates observed in wild populations under certain treatment conditions: warm water (15-16°C) and high copepodid densities (150-300/L). I also observed mortality rates of 4-5% associated with copepod infection intensity during the infection experiment. Cross-infection was
achieved but at much lower infection rates. Juvenile Chinook salmon exposed to copepodid parasites experienced high mortality rates (26.7-54.5%) compared to control fish (0-8%) and could not regulate plasma Na+ concentrations back down to normal when transitioned to seawater. Our findings suggest that performance, survival, and seawater transition of juvenile Chinook salmon is negatively affected by exposure to *S. californiensis*. Recovery efforts for juvenile Pacific salmon rearing in reservoirs could be hampered by the presence of *S. californiensis*. 
Evaluating the Effects of a Parasitic Copepod (*Salmincola californiensis*) on the Performance of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*)

by

William Travis Neal

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

__________________________________________
William Travis Neal, Author
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I want to express my deepest gratitude to my advisor, Dr. James Peterson, for giving me the opportunity to research this fascinating parasite and for his incredible guidance, ability to teach, and patience as I learned to think like a scientist. Thank you.

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DEDICATION

To the memory of my father. You were the best teacher, fishing buddy, mentor and friend I could have every wished for. I love you and I miss you every day.
INTRODUCTION

Pacific salmon have disappeared from approximately 40% of their historic ranges in the Pacific Northwest (NRC 1996). These declines in salmon populations have been attributed to hydropower projects, harvest, predation by other fishes, birds, and marine mammals, reduction in freshwater habitat, ocean conditions, and interactions with hatchery origin fishes (Ruckelshaus et al. 2002). Additional threats to salmon populations come in the form of infections by pathogens, with estimates of juvenile coho salmon (Oncorhynchus kisutch) mortality as high as 68% from Ceratonova shasta (Som et al. 2019) and the high pre-spawn mortality rates (up to 83.3%) of spring Chinook salmon observed on the spawning grounds (O. tshawytscha) (Keefer et al. 2020).

Pathogens have the potential to complicate recovery efforts for Pacific salmon. For example, Monzyk et al. (2015) reported juvenile salmon parasite infection rates in most Willamette basin reservoirs by a parasitic copepod, Salmincola californiensis averaged 78-97% with an infection intensity of 6.6 adult female copepods per age-1 fish. Herron et al. (2018) showed that lightly infected fish had severely reduced swimming ability and observed gill damage in fish exposed to copepods even when no adult copepods were present. Fish infected with S. californiensis are known to avoid saltwater when given the option and die when infected fish are placed into saltwater over long periods of time (Pawaputanon 1980). Infected fish also reportedly lose weight, cease to feed and die (Roberts et al. 2004). Thus, severe copepod infections may prevent recovery of depressed salmon populations.
*S. californiensis* is a freshwater ectoparasitic copepod and its known hosts include fishes in the genus *Oncorhynchus* as well as mountain whitefish (*Prosopium williamsoni*) (Kabata 1969; Hoffmann 1999). Widespread in its distribution, *S. californiensis* is found in freshwater environments throughout the North Pacific rim from Japan to western North America (Nagasawa and Urawa 2002; Bennett et al. 2011; Bowker et al. 2012; Monzyk et al. 2015). Additionally, severe *S. californiensis* infections have been observed on transplanted *Oncorhynchus* spp., Kokanee salmon (*O. nerka*) in Colorado and Steelhead trout (*O. mykiss*) in Lake Ontario (Hargis et al. 2014; Mullin and Reyda 2020). However, almost nothing is known about population level effects of this parasite on its host.

Although previous studies have identified some potential problems *S. californiensis* pose for individual Chinook salmon, there is much that is unknown that could affect the implementation of successful salmon recovery actions. The ultimate goal of this project was to identify the factors affecting *S. californiensis* infection and its effect on juvenile salmon performance. To accomplish this, my research focused on addressing four objectives. The first objective was to experimentally determine the effect of movement space, water temperature, and copepodid density on infection prevalence and intensity. The second was to determine the effect of copepod infection intensity on juvenile salmon survival. The third was to determine whether fish can become infected via cross-infection or auto-infection. Auto-infection in parasitology refers to infection of the same individual with the next generation of a particular parasite, usually without leaving the host. In the present case, the definition is includes the infectious copepodid infecting the same host, even if it leaves the gill.
chamber for its short period of development. The fourth and final objective was to determine the ability of infected juvenile Chinook salmon to transition into seawater.

METHODS

Experimental fish- Juvenile spring Chinook salmon of South Santiam stock were reared from eggs at Oregon State University’s (OSU) Fish Performance and Genetics Laboratory (FPGL) in Corvallis, Oregon. Feeding consisted of a 2.5 mm low-lipid Bio-Oregon® pellet (Longview, WA) and fish were fed approximately 1.2% body weight each day. Fish measured 153.9 (± 27.06) mm and weighed 43.49 (± 19.7) g at the start of the experiment and were randomly placed one week before the infection experiment to acclimate in eight 91.5 cm diameter fiberglass tanks containing 348.3 liters of water and eight 61 cm diameter fiberglass tanks containing 116.1 L of water at the FPGL one week before the infection process to allow for acclimation. Tanks were located inside and supplied with flow-through well water at 12-13°C. Inflow to the 348.3 L tanks was eight liters per minute and the 116.1 L tanks received 2.67 liters per minute. These flows allowed for the same retention time and tank conditions between the different sized tanks.

Copepod sourcing and processing- Adult female S. californiensis with egg-sacs were collected off dead, spawned hatchery Oncorhynchus spp. from Oregon Department of Fish and Wildlife’s South Santiam Fish Hatchery (Sweet Home, Oregon) and Rock Creek Hatchery (Idleyld Park, Oregon). Approximately 4,633 adult females were collected from the South Santiam Hatchery and 500 were collected at Rock Creek Hatchery. Female copepods were removed with forceps
from the mouth and gill tissue of the carcasses and placed in 120 mL jars, sealed with a lid and placed on ice for transport back to OSU for processing. In the laboratory, egg cases were separated from the female copepod with forceps and placed in a 100mm petri dish until a maximum of 150 egg cases were reached. Each petri dish was filled half-way with water from the fish tanks at the FPGL and 8 µL of 0.01% methylene blue, which acts as an anti-fungicidal agent. Petri dishes were placed on an orbital mixer set at 60 rpm inside an 8-10°C refrigerator for incubation. Water and methylene blue changes were conducted daily to minimize any fungal growth and to remove debris and any rotting egg cases.

Copepodids were observed in small numbers as soon as one day after being collected and incubated in the petri dishes. Hatched copepodids were collected by pouring off the petri dish water containing copepodids into a gridded petri dish. Photographs of the copepodids in the gridded petri dish were taken to facilitate estimation of the number of copepodids hatched. After the photograph, the copepodids were then be poured into a 1 L plastic beaker. Water from OSU’s FPGL and 8 µL of 0.01% methylene blue were added back to the petri dish so that ½ of it was filled. This procedure was then repeated until all copepodids were removed and photographed from remaining petri dishes. The number of copepodids on a petri dish was estimated by counting each copepodid in the photographs with imaging software tpsDig 2.0 (Rohlf 2006). This process was repeated daily until all egg-sacs hatched.

Infection Experiment

Experimental Design- I hypothesize that several factors were responsible for the relatively high copepod infection prevalence and intensity seen in wild Pacific
salmon. My first hypothesis was that infection rate increased with decreased movement space. Chinook salmon reared in smaller tanks have less space to move around less than fish held in larger tanks. To test this hypothesis, I tested two tank size treatments. Fish were stocked at a density of 1.6 g/L in four small (116.1 L) tanks and four large (348.3 L). This density corresponded to 22 fish per 116.1 L tank and 66 fish per 348.3 L tank.

My second hypothesis was that infection rate increased with copepodid density. To test this hypothesis, juvenile Chinook salmon were exposed to two levels of copepodid densities: low (35-75 copepodids/L) and high (150-300 copepodids/L). These copepodid densities are the daily exposure for the volume of water in a full tank. Because the larger 348.3 L diameter tanks have greater volume, more copepodids were added to obtain the same density as smaller tanks.

My third hypothesis was that warmer water increases the prevalence and intensity of S. californiensis infection. To test this hypothesis, two temperature treatments were compared. The “warm” water was 15-16°C and the “cool” water was 12-13°C (typical temperature of water at FPGL). These temperatures correspond to those typically encountered by wild juvenile Chinook salmon that rear in upper Willamette Valley reservoirs during summer months (personal communication; Jeremy Romer, ODFW).

I implemented these three treatments (tank volume/movement space, water temperature and copepodid density) with a fully factorial design with two replicate tanks and no controls for a total of 16 tanks. This allowed me to determine main effects, all two-way interactions and the three-way interaction. I did not include an
uninfected control because it is known that the copepods cause *S. californiensis* infections.

Our fourth hypothesis was that the high infection rates observed in reservoir rearing *Oncorhynchus* spp. could be due to cross- and auto-infection. Cross-infection being that an infected fish transmits the infection to an uninfected fish, while auto-infection being that an already infected fish is more infected. Juvenile Chinook salmon rearing in the reservoirs school and this close proximity to each other might be why they become heavily infected. To test this hypothesis, I placed naïve and previously infected fish in 116.1 L tanks and allowed a generation of copepods to mature, hatch copepodids and find a host.

*Infection Protocol*- To minimize the duration of the infection experiment and the different copepods stages, copepodids were not used to infect fish until more than 50% of the petri dishes contained hatched copepodids. I began infecting juvenile salmon approximately two weeks after collecting the egg sacs from adults. Copepodid treatment doses were created daily by dividing the total number of copepodids available with a Folsom plankton splitter (Motodo 1959) to distribute the fractions of copepodids delivered into each tank in low and high doses. This work was completed inside a cooler (set at 4°C) to prevent the copepodids from overheating. After splitting the water containing the hatched copepodids, each of the 16 copepodid doses was placed in an individually marked 120 mL specimen jars, placed on ice and immediately transported to the FPGL for exposure to the juvenile Chinook salmon.

At the FPGL, the specimen jars containing copepodids were placed sealed in their respective tanks for five minutes to start temperature acclimating the
copepodids. While acclimating, an air stone was added to each tank, the inflow to the tanks was turned off, and the center stand pipe for each tank was replaced with a modified one that reduced the 348.3 L tank water volume to 116.1 L and the 116.1 L tank water volume to 38.7 L. Once the water stopped draining from the tanks, the copepodids were released from the specimen jars into each tank containing the juvenile Chinook salmon. The tanks were left in this condition for one hour and saturated oxygen levels were monitored to ensure that the water was adequately oxygenated for the salmon. When one hour had passed, flowing water was returned to the tanks, the center stand pipes replaced with the original one, and the air stones were removed. This was repeated daily (13 times) over a two-week period.

Tanks were checked twice a day during weekdays and once a day on weekends. All moribund fish from the start of the infection to the end of the experiment were euthanized with a lethal dose of Tricaine methansulfonate (hereafter referred to as MS-222), placed on ice, and brought back to OSU for necropsy. After approximately 8 weeks from last infection date, approximately 20 random fish from each tank were euthanized using a lethal dose of MS-222 and transported on ice back to OSU where a necropsy was performed to identify and count copepods and evaluate gill damage. The remaining fish were used in the first saltwater challenge experiment described below.

**Auto and Cross Infection**

To experimentally evaluate the relative roles that auto and cross infection play in copepod infection, 40 infected Chinook salmon from a pilot infection experiment were randomly selected and placed into four 116.1 L tanks described above at a
density of 10 fish per tank. An equal number of naïve (uninfected) fish from the same stock of fish were randomly selected. The adipose fin of the naïve fish were clipped to distinguish them from infected fish prior to introducing them to the tanks. The fish were maintained together for 10 weeks to allow for enough time for copepodids to hatch from the egg cases and infect an available host. The remaining pilot infection experiment fish were euthanized, placed on ice, and brought back to the lab for necropsy as described below. After 10 weeks, all 80 fish were then euthanized, placed on ice and brought back to the lab for necropsy as described below.

**Saltwater Challenge**

Three saltwater challenges were conducted to determine the ability of infected juvenile salmon to osmoregulate. The 24 h saltwater challenges were adapted from Clarke and Blackburn (1977) to determine the ability of fish to downregulate plasma Na+ concentrations when placed in saltwater. The first saltwater challenge used infected fish from the infection experiment described above and a pilot infection experiment (Supplemental information). Unexpectedly high death rate (dead and moribund fish combined were considered were tallied as “dead”) of infected fish was observed during the first challenge which warranted a repeat of the experiment. Due to the seasonal availability of copepod egg cases, number of fish available and time of year, the second and third saltwater challenges were performed approximately one year later.

For the first saltwater challenge (April), experimentally infected and uninfected control fish from the same stock were placed into 167 liter plastic containers filled with 84 liters of water from FPGL at a density of 15 fish per
container. The experimentally infected fish were infected two month prior (February), while fish from the pilot infection experiment were infected four months prior (December) to the first saltwater challenge. Instant Ocean® aquarium salt was added in the amount of 3.059kg to achieve the same salinity as seawater, 34 ppt. A refractometer was used to measure salinity and confirm that 34 ppt was achieved once all salts dissolved. One-hundred twenty fish from all 348.3 L tank treatment combinations (30 per treatment) were run in duplicate. Forty-five infected fish from the pilot experiment and 45 uninfecte control fish were run with each of those treatments run in triplicate. Each container contained 15 juvenile Chinook salmon. The containers were aerated with air stones and were placed in a larger tub with a constant flow through of 12-13°C water. All containers were periodically monitored over the 24 h period for moribund fish and water temperature. Any moribund fish were removed and euthanized in a lethal solution of MS-222 and brought to the laboratory for necropsy as described below.

The first saltwater challenge contained fish that survived the mortality associated with the initial infection and had approximately two months to develop coping ability to the parasites as well as temporally being in the period of smoltification (when they should be capable of regulating sodium). A second and third saltwater challenge experiment to test if fish osmoregulatory capacity differed due to time since infection were conducted. For the second and third saltwater challenges, juvenile Chinook salmon were exposed to S. californiensis copepodids using the protocol described above. Five hundred randomly selected juvenile Chinook salmon measuring 134.4 (± 13.94) mm and weighing 27.55 (±7.98) g were
placed in four 348.3 L tanks with 125 fish in each tank. All tanks were kept at 12-13°C, which was identical to the cool treatment. Two tanks were randomly selected and the fish exposed to copepodids during 11 one-hour periods in December at an average density of 104.4 copepods/L. Fish in the other two tanks were not exposed to copepodids but water was lowered during the same 1 hour exposure period as the infected fish to apply the same stressor. Infection events were discontinued after around two weeks as three fish in the copepodid treatment tanks died and necropsies showed very heavy infection. Fish were monitored during the growth stage of *S. californiensis* and necropsies were performed on all moribund fish. Saltwater challenges were conducted as described above. The second saltwater challenge was conducted two months after first exposure to copepodids (February) and the third saltwater challenge four months after first exposure (April). A total of 16 containers were used in each challenge with the following four treatments run in quadruplicate: exposed fish in freshwater, exposed fish in saltwater, control fish in freshwater and control fish in saltwater.

During the second and third 24 h saltwater challenges, fish were periodically monitored for signs of health and moribund fish were removed and euthanized in a lethal solution of MS-222. After 24 h, all remaining fish were euthanized. Blood was collected from each fish via severance of the caudal peduncle into ammonium-heparinized capillary tubes, plasma was collected following centrifugation and stored at -80°C. Fish were individually labeled, placed on ice, taken back to the laboratory and necropsied as described below.
Collected plasma was analyzed for Na+ levels using an i-STAT® Alinity analyzer (Abaxis, Union City, CA) and i-STAT® CG8+ cartridges with 0.1 mL of plasma into each cartridge. The CG8+ cartridges can read a range of Na+ levels of 100-180 mM/L and were intended for use with whole blood. Therefore, to validate the analysis for plasma, three juvenile Chinook salmon were euthanized and Na+ concentrations in whole blood and plasma were compared for each individual fish. There were no differences with the average Na+ concentrations for whole blood 137 (± 2.94) mM/L and the Na+ level for plasma averaging 135.7 (± 2.36) mM/L. Frozen plasma from the saltwater challenge experiments was thawed, lightly vortexed while still in the centrifuge tube and piped into the cartridge using a 5mL transfer pipette. Because all remaining fish were euthanized in freshwater, the first 5 fish from the infected group and the first 2 to 3 fish from randomly assigned control groups bled from each treatment were used in the analysis to minimize the potential concentration-altering effects of leakage of water into the gills of euthanized fish.

Necropsy

Recently euthanized fish were visually inspected to identify and enumerate all attached adult female copepods. The location of attachment also was noted. Gills were then removed from the fish using dissection scissors and gill arches separated from gill filaments using a scalpel. All eight gill filament sets per fish were wet-mounted on a glass microscope slide with a few drops of tap water and examined using a compound microscope. Using features and illustrations by Kabata and Cousens (1973), copepods attached to the gills were counted and classified into the
following developmental stages: copepodid, chalimus, immature female and mature female.

**Definitions and statistical analysis**

**Infection Experiment**- A hurdle model was fit to test the differences in infection prevalence and intensity between infection experiment treatments Hu et al. (2011). A hurdle model is a two-part model that fits two linear models; the first a logistic linear model that estimates infection prevalence. The second part is a truncated negative binomial model that estimates infection intensity, as many parasitic infections show a negative binomial distribution (Pennycuick 1971; Peña-Rehbein and de los Ríos-Escalante 2012). The hurdle model was initially fit using all main effects (i.e., temperature, tank size and copepodid dose density) and two- and three-way interactions. I then fit a model with only statistically significant (p<0.05) parameters.

Goodness-of-fit on the full model and model with significant effects was assessed through a variety of techniques. Independence assumptions were evaluated by plotting the residuals of the all main effects model ordered by tank. The presence of overdispersion was evaluated with a rootogram, a method to graphically compare the observed and predicted values of the response (Kleiber and Zeileis 2016), and a quantile-quantile (Q-Q) plot of the residuals (Wilk and Gnanadesikan 1968). The hurdle models were fit using the hurdle function in R package pscl (Zeileis et al. 2008), rootograms were created using the rootogram function in R package countreg (Zeileis et al. 2008), and Q-Q plots created using the qnorm function (Becker et al. 1988). To aid in the interpretation of model parameters estimates, I also calculated
odds ratios (Hosmer and Lemeshow 2000) for the prevalence models, incidence rate ratios (Cameron and Trivedi 2012) for the intensity models.

Logistic regression analysis was used to examine the factors that were significantly related to death that occurred before the end of the experiment (Hosmer and Lemeshow 2000). A global model for death was fitted with all parameters that included water temperature, tank size, copepodid density, fish weight, total number of adult copepods, copepodids, and chalimus stages and two-way interactions. I then fit a model with only statistically significant (p<0.05) parameters. Goodness of fit of the all parameter and reduced was evaluated by creating Q-Q plots and by plotting residuals ordered by individual tank.

*Autoinfection experiment*- The differences in infection rate (proportion of fish infected) at the start and end of the autoinfection experiment was evaluated using a Pearson chi-square test of independence (Snedecor and Cochran 1967). Differences in infection intensity were evaluated using Welch two sample t-test (Snedecor and Cochran 1967). All analyses were conducted using R statistical software version 3.6.1 (R Core Team 2019). Comparison of infection prevalence and intensity between infected and naive fish in the cross-infection experiment were made by calculating means and 95% confidence limits.

*Saltwater challenges*- A Pearson chi-square test of independence (Snedecor and Cochran 1967) was used to test the difference in the proportions of fish with plasma Na+ concentrations that exceeded 170 mM/L. Pearson chi-square tests of independence (Snedecor and Cochran 1967) were also used to test for differences in mortalities between infected fish and control fish in the three saltwater challenges.
That threshold was based on Clarke and Blackburn (1977) who noted that fish with Na+ concentrations exceeding 170 mM/L were not ready to smolt and adapt to saltwater. Analyses were conducted using R statistical software version 1.2.5033 (R Core Team 2019).

Logistic regression analysis was used to determine which parameters explain Na+ concentrations exceeding 170 mM/L (Hosmer and Lemeshow 2000). Fish with Na+ concentrations greater than 170 mM/L and assigned a value of 1, while fish with normal Na+ values were assigned a value of 0. Variables were checked for collinearity by calculating Pearson correlation coefficients for each pair of variables. Prior to model fitting, I created binary indicator variables for fish exposure to copepods (yes=1, no=0), whether the fish was in fresh or saltwater, and the third saltwater challenge. A global model was then created using the recorded parameters saltwater exposure, copepodid exposure, third saltwater challenge, total number of copepods, and fish weight. The global model was then reduced to only include variables that were statistically significant at the p<0.05 level.

Logistic regression analysis also was used to identify the factors significantly related to death of fish during all saltwater challenges (Hosmer and Lemeshow 2000). The copepodid density and water temperatures during the pilot experiment and second and third saltwater challenges were the same as the cool and similar to the high-density treatments. Thus, I created binary indicator variables to evaluate the effect of the warm treatment and high copepodid density on death during the saltwater challenge. A global model was then created using all recorded parameters that included total number of adult copepods, copepodids, and chalimus stages
present, copepodid density high, water temperature warm, and fish length. The global model was reduced to only include variables that were statistically significant at the p<0.05 level. Goodness-of-fit of the global and reduced model was evaluated by ordering residuals by tank and container and using Q-Q plots.

**RESULTS**

**Infection Experiment**

Infection prevalence of fish held in warm water ranged from 90.2% to 97.2%, while fish held in cool water ranged from 68.2% to 73.1% (Figure 1). Results from the hurdle model indicate that copepod infection prevalence was significantly greater in the warm water treatments (Table 1). Parameter estimates indicated that infection by copepods (all stages) was on average 7.2 times more likely in fish in the warm water tanks than those with cool water (Table 1).

The total average infection intensity, including fish that died was 4.31 adult female copepods per fish (Table 2). For fish that survived until the end of the experiment, infection intensity averaged 4.45 (± 3.45) copepods. Infection intensity was significantly greater in fish infected in warmer temperatures than those infected in cooler water, with the warm and high-density treatments ranging from 4.8 to 8.31 adult female copepods per fish (Table 1; Figure 2). The attachment locations where most copepods were found included the gills, the inner operculum, and the buccal cavity (Table 2). The fish grew throughout the experiment and after approximately eight weeks, gained an average of 15.19g and 17.5mm (Table 2).
Infection intensity was significantly lower in the low density copepodids treatment and greater in the large tank and warm treatment (Table 1). The p-value for the main temperature was not significant (p=0.06) at the 95% level but was included in the model to facilitate interpretation of the significant two-way interaction between tank size and temperature. Mature female copepod infection intensity in warmer water was 2.68 times that of fish in cooler water. Fish exposed to high copepodid densities had infection intensities, on average, 1.59 times that of fish exposed to low densities. Fish held in warm water and 90 cm tanks had, on average, 1.57 times copepodids that fish in the other treatments (Table 1). Goodness-of-fit of the full and reduced hurdle models indicated that they adequately fitted the data.

Death during the infection experiment began shortly after the last exposure and accrued consistently through the development period of the parasite before adult females were detected (Figure 3). From the start of the infection process to the conclusion of the experiment, 68 fish (9.7%) of the fish died. Fifty-eight of the 68 mortalities (85.3%) occurred in tanks with warm water and high copepodid densities. The average prevalence of infection for mortalities in all treatments was 89.7%, while the average intensity of mature female copepods on moribund fish was 7.04 (± 5.83) per fish. Results for the logistic regression model indicate that mortality was significantly greater in tanks with warm water and significantly lower for fish in tanks with low copepodid density treatments (Table 3). Fish infected in warm water were, on average, 7.21 times more likely to die than those infected in cool water (Table 3). Fish infected with low copepodid densities were 4.11 times less likely, on average, to die than those infected at with high densities. Mortality also was positively
significantly related to total number of adult female copepods and copepodids attached to the fish. For every mature female copepod and copepodid attached a fish was 1.28 times and 4.16 times more likely to die. Fish mass was also significantly and negatively related to fish weight with a fish 1.12 times less likely to die, on average with each 1 gram increase a fish weight.

**Auto and cross-infection**

The infection prevalence and intensity of pilot infection fish were similar before and after the auto and cross-infection experiment with prevalence averaging 75% and 59% and intensity averaging 6.1 and 5.1, respectively (Figure 4). Tests comparing copepod infections in infected fish before and after the auto-infection experiment did not differ significantly for prevalence ($\chi^2 = 2.323, p=0.128$) and intensity ($t = -0.885, p=0.38$). Naïve fish infection prevalence was not significantly different than the marked (previously infected fish) at the end of the experiment and averaged 57%, indicating a similar prevalence can be achieved in the laboratory ($\chi^2 = 2.43, p=0.119$). However, infection intensity of the naïve fish was significantly lower than the previously infected fish at the end of the experiment and averaged only 1.7 adult female copepods per fish ($t = -3.5006, p=0.002$) (Figure 4).

**Saltwater Challenge**

Infected fish in the first saltwater challenge had more difficulty osmoregulating in the saltwater treatment than the control fish. During the first saltwater challenge, there was a significant difference in mortalities between fish exposed to copepodids (54.5%), and control fish (8%) in the saltwater treatment ($\chi^2 = 31.366, p<0.001$) (Figure 5). All of the warm water and high copepodid density
treatment fish were moribund within the 24-hour challenge, the warm water and low
density copepodid averaged 90% mortality and the cold water high and low
copepodid density treatments averaged 26.6% and 50% mortality, respectively.
While there were fewer mortalities in the second and third saltwater challenges, infected fish had difficulty osmoregulating when in the saltwater treatment. The second saltwater challenge had 125 fish in the saltwater treatment: 65 were in the control and 60 from the exposed group that survived the infection process. After 24 hours of saltwater exposure, there was a significant difference in mortalities between the control fish (4.6%) and from fish in the parasite exposed group (26.7%) ($\chi^2 = 10.122, p=0.001$) (Figure 5). The third saltwater challenge took place approximately two months after the second saltwater challenge in April. In the third saltwater challenge, 100 fish were used in the saltwater treatment: 46 were control fish and 54 were exposed fish. Of the 46 control fish used, none were moribund after 24 hours in saltwater, whereas 33.3% of exposed fish were moribund after 24 hours ($\chi^2 = 16.509, p<0.001$) (Figure 5). No control or parasite exposed fish in the freshwater treatments were moribund after 24 hours in either 24 h saltwater challenge.

The first three deaths from experimentally infected fish for the second and third saltwater challenges occurred 11 days after the first copepodid exposure. An examination of the fish revealed a mean infection intensity of 27.33, suggesting that the fish were sufficiently infected to warrant cessation of infection events. Mortalities before and between experiments were recorded for the fish infected for the second and third saltwater challenges (Figure 6). Of the 25 mortalities recorded, 56% were from the juvenile copepod stages and occurred within the first 10 days after the
infection period. For the first saltwater challenge, the average infection intensity was 3.56 mature female copepods and the prevalence was 78.8%. The infection prevalence for fish in the second and third saltwater challenges was 62.5% and 68.5%. The infection intensities for these two groups of fish was lower than the infection experiments and averaged 2.2 and 1.4 mature female copepods per fish, for the second and third saltwater challenge, respectively.

The high mortality rates during the first saltwater challenge prevented us from collecting sufficient plasma volume for analysis of Na+ concentrations. There were fewer moribund fish in the second and third saltwater challenges, allowing us to collect and analyze plasma from experimental fish. Uninfected control fish were by and large able to downregulate plasma Na+ concentrations to normal after 24 h, whereas even the infected fish that were able to survive the 24 h saltwater challenge had a significant number of individuals unable to downregulate plasma Na+ concentrations ($\chi^2=7.584, p=0.006$) (Table 7). Control fish exceeded the 170 mM/L plasma Na+ concentrations fewer 6.2% of the time, compared to 39.5% for fish exposed to copepodids. The logistic regression indicated that the probability of a Na+ levels exceeding 170 mM/L in juvenile Chinook salmon was significantly positively related to exposure to copepodids ($p=0.00492$) and was significantly lower for the third saltwater challenge. Juvenile Chinook salmon exposed to copepodids were 4.81 times more likely to have plasma Na+ levels exceeding 170 mM/L, whereas it was 2.65 times less likely for fish in the third challenge.

The logistic regression model of juvenile Chinook salmon mortality during the saltwater challenge indicated that mortality was significantly related to total number
of adult female copepods, exposure to copepodids during the infection period, fish length, and warm water temperature treatment (Table 4). For every single attached copepod, a juvenile Chinook salmon was, on average, 1.28 times more likely to become moribund during the saltwater challenge. For fish exposed to copepodids during the infection or held in warm water the odds of becoming moribund during the challenge increase by 5.48 and 41.15 times on average, respectively. Mortality was significantly lower for larger fish in saltwater with parameter estimates indicating that death was on average, 1.07 times less likely for every 1 mm increase in fish length.

DISCUSSION

I was able to experimentally infect juvenile Chinook salmon with *S. californiensis* in the laboratory and at levels similar to those observed in wild, reservoir dwelling *Onchorhynchus* spp. From the infection experiments, I found that infection intensity increased with temperature and copepodid density and that there was high mortality associated with the microscopic juvenile stages. The survival of juvenile Chinook salmon in the laboratory was also related to copepodid density, copepodid exposure, and copepod presence of fish. I also found that hatchery fish simply exposed to copepodids were physiologically compromised and suffered significant mortality when transitioned to seawater months later. To understand the infection dynamics of *S. californiensis* and their effect on juvenile salmonids requires knowledge of how these biotic and abiotic factors affect both the parasite and the host itself.
Fish exposed in warmer water (15-16°C) had the highest infection and mortality rates. I hypothesize this was due to multiple factors including fish respiration and parasite developmental rates. Fish respiration increases with water temperature (Beamish 1964) and thus fish in the warmer water likely passed more water volume and the free-swimming copepodids over their gills compared to fish in the cooler water treatments, leading to higher infection rates. This is consistent with previous studies that observed that increased ventilation in fish can lead to an increase in parasitism (Mikheev et al. 2014). My second hypothesis is that the higher infection rates were due to shorter development times for *S. californiensis* in warmer water. In laboratory studies, *S. californiensis* egg development in 16°C was approximately 30% shorter than at 13°C (Murphy et al. 2020). Assuming that the remaining development was similarly affected, the amount of time to complete the life cycle in the warmer treatment may have been shorter compared to development on fish held in the cooler treatment. This hypothesis is further supported by the fact that 15% of fish in warm water treatments had juvenile stages present on the gills at the end of the experiment, while juvenile stages were present on only 1% of fish in the cool water. This suggests that completion of the life cycle and production of a second generation of copepodids had occurred more frequently in the warmer water. The effect of water temperature on copepod infection and parasite reproduction have significant implications for reservoir dwelling salmonids. The optimal temperatures for growth of juvenile *Onchorhynchus* spp. range 15-20°C (Marine and Cech 2004; Yanke 2006) and juveniles actively seek out temperatures in this range in reservoirs (Swales 2006; Tiffan et al. 2009). The copepodids in stratified Willamette Valley reservoirs also
appear to concentrate at 16°C during the summer months (C. Murphy, Oregon State University, personal communication). The effect of temperature combined with the high densities of copepodid in the preferred temperature ranges may explain the infection severity in reservoirs during summer months (Hargis et al. 2014; Monzyk et al. 2015).

Brook trout (*Salvelinus fontinalis*) juveniles infected with an analogous ectoparasite, *S. edwarssii*, as *S. californiensis* had increased susceptibility to increased copepod infection when exposed again (Poulin et al. 1991). If juvenile Chinook salmon infected with *S. californiensis* exhibit similar infection dynamics as *S. edwarssii*, then my observation that previously infected fish in the auto-infection experiment did not become significantly more infected after 10 weeks, but naïve fish did is postulated because some of the existing copepods dropped off and the second generation of copepods attached. I was not able to be confirm this because the copepods present on the fish at the end of the experiment were all adult stages, making them visually indistinguishable from copepods previously attached at the beginning of the experiment, but enough time had passed for the second generation of copepodids to mature to adult stages. This suggests that wild fish previously infected by *S. californiensis* could develop heavier infections over time as observed in increasing infection intensities of reservoir dwelling juvenile Chinook salmon from June to December (Monzyk et al. 2015). This is in contrast to premunition, a common phenomenon in many parasite infections, in which pre-existing chronic infections protect the host from new infections (Bradley 1972). The infection process in the laboratory likely simplifies more complex infection processes that take place in
reservoirs. Further studies of *S. californiensis* in wild populations of *Onchorhynchus* spp. could aid in better understanding infection dynamics that take place in reservoir systems.

Juvenile Chinook salmon mortalities during the infection experiment were strongly related to the number of juvenile copepod stages (i.e., copepodid and chalimus) present on fish gills. I hypothesize that this mortality was caused by damage that occurs from these juvenile stages via implantation of copepodid attachment filaments (Figure 8) and feeding of gill tissue. I observed copepodid damage on moribund fish gills from attachment filaments and feeding, causing hyperplasia, and chopped gill filaments, reducing gill surface areas required for gaseous exchange (Figure 9). I was able to quantify copepods and identify development stages but unable to evaluate gill damage for fish in the saltwater challenge due to tissue dehydration and degradation associated with saltwater exposure. However, the strong relationship between copepodid exposure and mortality during the saltwater challenge suggests that most of the damage is caused by juvenile stages that are unobservable with the naked eye, which is similar to what (Herron et al. 2018) observed showing gill damage on the host, even when no adult copepods were present. Field observations only counting adult female copepods are likely overlooking an important contributor of mortality in wild populations. This unnoticed damage from juvenile *S. californiensis* stages is underestimated in the wild when only adult female copepods are counted. This implies that the performance of *Onchorhynchus* spp. rearing in a reservoir containing large populations of *S. californiensis* is likely compromised, even if no obvious signs of infection (adult
females) are present. In fact, the experimental fish experienced mortality even though they were kept in benign, low stress environments and fed regularly. I would expect similarly infected wild fish to experience much greater mortality.

I was surprised by the substantial mortality when transitioning fish exposed to copepodids to seawater, particularly the mortality observed in the second and third saltwater challenges where the infection rates were much lower than fish from the infection experiment. I hypothesize this mortality was caused by exposed fish not being able to effectively regulate plasma Na+ levels. One of the many functions gills provide for fish is to osmoregulate (Evans et al. 2005) and damage to this epithelial tissue can obstruct the efficiency of regulating osmotic pressure (Svobodová et al. 1993), as shown by the high number of fish exposed to copepodids with elevated Na+ levels kept in saltwater. Similar ion loading has been observed in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) with infections from the copepod Dichelesthium oblongum and thought to be from water loss due to stress or damage to the epithelial tissue (Fast et al. 2009) and also in Arctic char with salmon louse (Lepeophtherius salmonis) infections (Fjelldal et al. 2019). Water loading due to transfer and container stress that might have happened was compensated for in the 24 h saltwater challenges as shown by similar plasma Na+ concentrations in the freshwater treatments (Table 7) (Schreck and Tort 2016). Even without S. californiensis present on gills at the time of the necropsy, having been in the exposed groups and possibly infected and experiencing damage before the copepod fell off, greatly increased the odds of a fish not being able to osmoregulate. Although there were no copepods of any development stage present on the gills, there was still likely substantial damage that
occurred during the infection process from brief periods of attachment resulting in gill filament damage before the infection was lost. Again, this is similar to what Herron et al. (2018) observed with gill damage from the juvenile copepod stages while no adult stages were present. These results suggest that anadromous *Onchorhynchus* spp. rearing in reservoir environments with significant *S. californiensis* populations are more likely to sustain gill damage, even if no copepods are observed on the gill tissue, increasing their likelihood of death when transitioning to the marine environment.

Juvenile Chinook salmon rearing in Willamette Valley reservoirs have been observed to have infection rates that begin to increase over the summer and peak in December (Monzyk et al. 2015). Additionally, the highest catches of outmigrating juvenile spring Chinook salmon typically occur in March to May (Kirk Schroeder et al. 2016), three to five months later, respectively. The saltwater challenge experiment in this thesis has shown that juvenile Chinook salmon infected with *S. californiensis* at a much lower infection intensity four months prior are still heavily compromised as they transition to seawater with a mortality rate of 33% after 24 hours. This suggests that wild outmigrating juvenile *Onchorhynchus* spp. rearing in reservoirs containing strong populations of *S. californiensis* are likely compromised months after infection as they migrate to the marine environment, an effect that could be particularly dangerous for fall migrating smolts and possibly yearling smolts in the spring.
Literature cited
Kabata, Z. 1969. Revision of Genus Salmincola Wilson, 1915 (Copepoda-
Pawaputanon, K. 1980. Effects of parasitic copepod, Salmincola californiensis (Dana, 1852) on juvenile sockeye salmon, Oncorhynchus nerka (Walbus). The University of British Columbia.


TABLES

Table 1. Parameter estimates, standard errors (SE), lower and upper 95% confidence limits and p-value from negative binomial hurdle model fit to the experimental copepod infection prevalence and intensity. These estimates are for fish in the infection experiment testing water temperature, copepodid density and movement space on infection rates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infection prevalence (logit link)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.900</td>
<td>0.168</td>
<td>0.572</td>
<td>1.229</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Warm water temperature (15-16°C)</td>
<td>1.975</td>
<td>0.352</td>
<td>1.284</td>
<td>2.666</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Infection intensity (log link)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.041</td>
<td>0.139</td>
<td>0.769</td>
<td>1.313</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Copepodid density low (35-75 copepodids/ L)</td>
<td>-0.462</td>
<td>0.098</td>
<td>-0.654</td>
<td>-0.271</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Warm water temperature (15-16°C)</td>
<td>0.985</td>
<td>0.158</td>
<td>0.676</td>
<td>1.295</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tank size large (348.3 L)</td>
<td>0.322</td>
<td>0.173</td>
<td>-0.016</td>
<td>0.660</td>
<td>0.0622</td>
</tr>
<tr>
<td>Warm water temperature (15-16°C) x tank size (348.3 L)</td>
<td>-0.453</td>
<td>0.208</td>
<td>-0.861</td>
<td>-0.045</td>
<td>0.0297</td>
</tr>
<tr>
<td>Negative Binomial Dispersion</td>
<td>0.827</td>
<td>0.169</td>
<td>0.495</td>
<td>1.158</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Summary of mean juvenile Chinook salmon length and mass pre infection. Summary of mean juvenile Chinook salmon length, mass, copepodid stages, chalimus stages, attachment filaments, location of adult copepods and total number of adult *S. californiensis* present.

<table>
<thead>
<tr>
<th>Start of Infection</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish mass (g)</td>
<td>43.49</td>
<td>19.76</td>
<td>7.00</td>
<td>77.60</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>153.90</td>
<td>27.06</td>
<td>91.00</td>
<td>192.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-infection</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
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<tbody>
<tr>
<td>Fish mass (g)</td>
<td>58.68</td>
<td>33.37</td>
<td>3.30</td>
<td>200.26</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>171.40</td>
<td>33.58</td>
<td>75.00</td>
<td>238.00</td>
</tr>
<tr>
<td>Copepodids</td>
<td>0.07</td>
<td>0.34</td>
<td>0.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Chalimus</td>
<td>0.08</td>
<td>0.38</td>
<td>0.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Filaments</td>
<td>0.03</td>
<td>0.26</td>
<td>0.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Adult copepods on gills</td>
<td>1.98</td>
<td>2.29</td>
<td>0.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Adult copepods on operculum</td>
<td>1.13</td>
<td>1.57</td>
<td>0.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Adult copepods on buccal cavity</td>
<td>1.04</td>
<td>1.51</td>
<td>0.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Adult copepods in mouth</td>
<td>0.07</td>
<td>0.27</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Adult copepods on fins</td>
<td>0.04</td>
<td>0.19</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Adult copepods: all other places</td>
<td>0.05</td>
<td>0.24</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Total adult copepods</td>
<td>4.31</td>
<td>4.30</td>
<td>0.00</td>
<td>22.00</td>
</tr>
</tbody>
</table>
Table 3. Parameter estimates, standard errors (SE), lower and upper 95% confidence limits and p-values from logistic regression model describing mortality during the infection experiment. These estimates are the significant parameters (p<0.05) from the reduced model for mortality that occurred during the infection experiment.

<table>
<thead>
<tr>
<th>Estimate</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.489</td>
<td>0.511</td>
<td>-0.531</td>
<td>1.494</td>
</tr>
<tr>
<td>Warm temperature (°C)</td>
<td>1.976</td>
<td>0.516</td>
<td>1.009</td>
<td>3.048</td>
</tr>
<tr>
<td>Copepodid density low (35-75 copepodids/ L)</td>
<td>-1.414</td>
<td>0.471</td>
<td>-2.382</td>
<td>-0.522</td>
</tr>
<tr>
<td>Total adult female copepods</td>
<td>0.247</td>
<td>0.06</td>
<td>0.137</td>
<td>0.371</td>
</tr>
<tr>
<td>Copepodids</td>
<td>1.424</td>
<td>0.667</td>
<td>0.323</td>
<td>2.974</td>
</tr>
<tr>
<td>Fish weight (g)</td>
<td>-0.113</td>
<td>0.017</td>
<td>-0.151</td>
<td>-0.082</td>
</tr>
</tbody>
</table>

Table 4. Parameter estimates, standard errors (SE), lower and upper 95% confidence limits and p-values from logistic regression model describing mortality from the 24-hour saltwater challenges. These estimates are the significant parameters (p<0.05) from the reduced model for mortality that occurred during the 24 h saltwater challenges.

<table>
<thead>
<tr>
<th>Estimate</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.387</td>
<td>0.797</td>
<td>-1.96</td>
<td>1.176</td>
</tr>
<tr>
<td>Total adult female copepods</td>
<td>0.248</td>
<td>0.061</td>
<td>0.138</td>
<td>0.376</td>
</tr>
<tr>
<td>Exposed to copepodids</td>
<td>1.710</td>
<td>0.400</td>
<td>0.957</td>
<td>2.54</td>
</tr>
<tr>
<td>Warm temperature (°C)</td>
<td>3.717</td>
<td>0.644</td>
<td>2.598</td>
<td>5.202</td>
</tr>
<tr>
<td>Fish length (mm)</td>
<td>-0.016</td>
<td>0.005</td>
<td>-0.027</td>
<td>-0.008</td>
</tr>
</tbody>
</table>
Figure 1. Infection prevalence of adult female copepods for the eight treatments in the infection experiment testing the effect of water temperature, tank size and copepodid density with associated 95% confidence limits. Warm water (15-16°C) had significantly greater infection prevalences compared to cool water (12-13°C) treatments (<0.001).
Figure 2. Infection intensities of adult female copepods for the eight treatments in the infection experiment testing the effect of water temperature, tank size and copepodid density with associated 95% confidence limits. Low copepodid density (35-75 copepodids/ L) treatments had significantly fewer adult female copepods as the high density treatments (150-300 copepodids/ L) (p<0.001) and warm water (15-16°C) temperature treatments had significantly higher intensities as the cool water (12-13°C) treatments (p<0.001).
Figure 3. Laboratory infections of Chinook Salmon with *Salmincola californiensis*. Accumulated fish mortality for all treatments during the infection process. Juvenile and adult copepod development stage transition is marked with dash-dotted line. The spike in mortality was euthanasia for sick fish. The fish were exposed to copepodids 14 times over 15 days, with 46 fish dying in the next 58 days.
Figure 4. Mean infection prevalence (top) and intensity (bottom) for naïve surrogate Chinook salmon and fish exposed to copepods during the pilot experiment with associated 95% confidence limits. The infected pre-cross infection fish were the infection rates for juvenile Chinook salmon at the start of the experiment and the infected post-cross infection were the infection rates for juvenile Chinook salmon at the end of the experiment. The naïve group, which had no infection at the start of the experiment, became lightly infected by the end of the experiment when kept with infected fish after 10 weeks and was significantly lower than the previously infected fish at the end of the experiment ($t = -3.5006$, $p = 0.002$).
Figure 5. Proportion of mortalities for each of the three saltwater challenges with associated 95% confidence limits. The exposed groups were those in tanks where copepodids were added and control tanks contained naïve fish. The first saltwater challenge (SWC 1) only had saltwater treatments (SW). The saltwater treatments and freshwater (FW) treatments were in the second saltwater challenge (SWC 2) and third saltwater challenge (SWC 3). There were no mortalities in the FW groups.
Figure 6. Accumulated mortality over time for fish infected before the second and third saltwater challenges with primary copepod development stage transition marked. Juvenile Chinook salmon (n=280) were infected in the first 11 days and 14 fish died in the 10 day period following with only juvenile S. californiensis stages found. On day 36 the first mortality was observed with adult S. californiensis stage present. Eleven fish died from days 36-86 with adult S. californiensis stages present.
Figure 7. Plasma Na+ concentrations for juvenile Chinook salmon during the second and third 24 h saltwater challenges. Each dot represents a fish in the treatment. Plasma Na+ concentrations for control fish in freshwater (Control FW) and infected fish in freshwater (Infected FW) appear to hold around the 130-140 mM/L range, with the infected freshwater treatment having two fish that were below the 120 mM/L. The infected fish in saltwater (Infected SW) had more fish hit the upper range of the plasma analyzer (180 mM/L) than uninfected fish in saltwater (Control SW) suggesting difficulty in downregulating Na+ levels.
Figure 8. Arrows pointing to *S. californiensis* copepodid attachment filaments in all three pictures. In picture B, “C” marks a copepodid with arrows pointing to the attachment filament. (Photo credit: Justin Sanders, Oregon State University)
Figure 9. Juvenile Chinook salmon gills with A) attached copepodids “C”, B) damage from the juvenile S. californiensis stages, C) chalimus stage attached and D) regeneration of gill filaments after damage from copepods. (Photo credit: Justin Sanders, Oregon State University)
SUPPLEMENTARY INFORMATION

I conducted a pilot experiment to determine whether hatchery juvenile Chinook salmon could be experimentally infected with *Salmincola californiensis* in the laboratory. Juvenile Chinook salmon were stocked into two 348.3 L tanks and two 116.1 L tanks at Oregon State University Fish Performance and Genetics Laboratory at a density of 95 fish per 348.3 L tank and 25 fish per 116.1 L tank. The densities were the same, proportional to tank volume. They were fed daily with 2.5mm Bio-Oregon® pellet (Longview, WA) at approximately 5% body weight. Fish were infected with copepodids during 20 one-hour exposures beginning in mid-November, 2018 with hatched copepodids from approximately 2,159 female copepods using the protocols detailed in the main document. Four weeks after last infection, approximately 20 fish from each tank were euthanized in a lethal dose of MS-222, placed on ice, and transferred back to OSU for a necropsy as described in the main document.

Nine deaths (3.8%) occurred before the end of the experiment. All moribund fish were infected and the average intensity was 20.56 (±8.75) copepods per fish. The juvenile Chinook salmon in the two 348.3 L tanks had infection prevalences of 91.6% and 87%. The infection intensities of copepods on fish in the 348.3 L tanks was 10.64 (±7.74) and 8.8 (±6.52). The juvenile Chinook salmon in the two 116.1 L tanks had infection prevalences of 90.0% and 95.0%. The infection intensities of fish held the 116.1 L tanks was 7.28 (±9.42) and 3.74 (±4.12). Forty of the remaining fish were used in the cross infection experiment and 45 were used in the first saltwater challenge.