Temperature effects on hatching and viability of Juvenile Gill Lice, *Salmincola californiensis*

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Abstract

Salmonids of the genus *Oncorhynchus*, distributed throughout the Pacific Rim, can be infected by the gill lice species *Salmincola californiensis* (Dana, 1852), which makes them one of the most broadly distributed gill lice species. Despite their broad distribution and valuable obligate salmonid hosts, relatively little is known about *S. californiensis*. We evaluated effects of temperature on timing of *S. californiensis* hatching and survival of copepodids, and provide information on brood size and variability. Our results suggest that temperature was a primary driver of timing of *S. californiensis* hatching and post-hatching survival. Prior to this study, the free-swimming stage of *S. californiensis* was reported to survive approximately 2 days without a suitable host. We observed active copepodids 13 days after hatch with some individuals from most (>90%) viable egg sacs at all temperature treatments surviving ≥5 days. Our findings indicate that warmer temperatures could increase development rates of gill lice at certain life stages, potentially increasing fecundity. This information coupled with predictions that warmer water temperatures could intensify crowding of coldwater fishes, stress, and parasite transmission suggests that climate change could exacerbate negative effects of *S. californiensis*

on ecologically and economically important salmonids.

Introduction

Gill lice *Salmincola* spp. are parasitic copepods that commonly infect salmonids (Kabata 1969; Conley & Curtis 1993). Gill lice mainly attach on the gills of infected fish; however, they are also found on fins, opercula and the mouth cavity (Kabata & Cousens 1977; Gunn *et al.* 2012; Hargis *et al.* 2014). Gill lice have been associated with deleterious effects on salmonids including growth inhibition, decreased fecundity, limiting oxygen uptake, delaying sexual maturation, lowering resistance to high temperatures and increasing mortality (Gall, Mcclendon & Schafer 1972; Chigbu 2001; Gunn *et al.* 2012). Salmonids of the genus *Oncorhynchus* (distributed throughout the Pacific Rim) are infected by the gill lice species *Salmincola californiensis* (Dana, 1852) which makes them one of the most broadly distributed gill lice species (Kabata 1969). A host of *S. californiensis*, rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), is arguably one of the most widely stocked sport fish in the world, historically stocked in over 80 countries on six continents (Halverson 2010), and gill lice have been spread through stocking, moving water and other anthropogenic activities associated with fish introductions (Sutherland & Whittrock 1985). The negative effects of *S. californiensis*

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threaten native fish and hatchery stocks of fish (Modin & Veek 2011; Gunn *et al.* 2012; Hargis *et al.* 2014). Improved understanding of this species' life history may provide insight into managing popular sport fish and native salmonids in the presence of gill lice.

The only confirmed species of gill lice in Colorado (USA) to date is *S. californiensis*. Gill lice were first observed in Colorado in 1908 (Wilson 1909) on a non-native Chinook salmon *Oncorhynchus tshawytscha* (Walbaum, 1792) host. Since that time, gill lice have been found on snake river fine-spotted cutthroat trout *Oncorhynchus clarkii behnkei* (Montgomery, 1995), rainbow trout, cutthroat trout *Oncorhynchus clarkii* (Richardson, 1836) hybridized with rainbow trout and kokanee salmon *Oncorhynchus nerka* (Walbaum, 1792). Adverse effects from gill lice in hatcheries were observed in the 1990s in Colorado (Gunn *et al.* 2012). In 2007, the kokanee salmon population declined significantly in Elevenmile Reservoir (Park County, Colorado) concurrent with gill lice infestation (J. Spohn personal communication, 2014). This decline led to the cessation of the spawning operation in place to sustain the kokanee salmon population in Elevenmile Reservoir. Similar situations (lack of reproductive kokanee salmon migrating concurrent with gill lice infestation) occurred in Cheesman Reservoir (Douglas and Jefferson counties, Colorado) and Clear Creek Reservoir (Chaffee County, Colorado). These gill lice infestations occurred under poor water conditions (low levels and warm temperatures; G. Policky and J. Spohn personal communication, 2014) that have been associated with heavy infestations elsewhere (Horton & Staigmiller 2005). These populations decline, and increasing reports of gill lice infestations over the past decade in Colorado have raised questions about the interactions of gill lice and the genus *Oncorhynchus*.

Parasite interactions with hosts depend on many factors that are related to climate. These factors include host health and the health of standing stocks of parasites following winter months (Marcogliese 2001). Warmer conditions intensify crowding of cool and coldwater fishes in the hypolimnions of stratified lakes and reservoirs which could increase disease transmission (Marcogliese 2001). These factors coupled with the potential for individual fish immune systems to become compromised from other stressors could increase the susceptibility of fish to a variety of parasites (e.g. whirling disease, *Myxobolus cerebralis* (Hofer, 1903) in Colorado; Ficke, Myrick & Hansen 2007).

Despite their widespread distribution, and relationship with valuable Pacific salmonid species, fundamental information on *S. californiensis* is unknown. Previous research focused on *S. californiensis* reported that the free-swimming copepodid stage had a life expectancy of about 2 days without finding a suitable host for attachment (Kabata & Cousens 1973). For comparison, the free-swimming copepodid stage of a similar species of gill lice *S. edwardsii* (Olsson, 1869) has been observed living for up to 17 days without finding a host (Conley & Curtis 1993). McGladdery & Johnston (1988) found the survival and development of another species of gill lice *S. salmoneus* (Linnaeus, 1758) to be directly related to water temperature. Although Kabata & Cousens (1973) reported a 2-day window for finding a host, their study was not designed to evaluate the survival of the copepodid life stage. Because of the relatively limited information available for *S. californiensis* and the disparate information for other gill lice species, we investigated several attributes of the free-swimming copepodid life stage of *S. californiensis*. We compared how long copepodids from paired egg sacs were considered viable fish parasites for three temperature treatments through time. We also compared the maximum survival time of individuals across temperature treatments. Further, we evaluated the effect of water temperature on the timing of *S. californiensis* hatching. Finally, we provide information on individual brood size and variability in gill lice egg production within (i.e. in paired egg sacs from a single louse) and across individual gill lice, and proportions of individuals hatched from viable egg sacs across temperature treatments. Quantifying these fundamental characteristics of *S. californiensis* will provide a better understanding of their transmission and ability to proliferate, and subsequent potential to produce population-level effects on important and widely distributed salmonids.

Materials and methods

Gill lice collection

On 30 October 2013, Colorado Parks and Wildlife personnel collected and killed an individual

rainbow trout from Parvin Lake, Larimer County, CO, that was infected with *S. californiensis*. This fish, and the gill lice attached to it, were transported for approximately 1 h to the laboratory while held in a cooler filled with lake water at approximately 8.2 °C. These gill lice were provided as subjects for the experiment described here. We removed individual gill lice from the host using forceps, ensuring no damage was carried out to the egg sacs. We collected gravid female gill lice ($N = 20$) with pigmented eggs for the laboratory experiment.

Egg sac partitioning

We randomly assigned 10 individual gill lice to one of two water temperature treatments: cold at 4.2 ± 0.5 °C or warm at 16.7 ± 0.2 °C. For each pair of egg sacs, we placed one sac in the randomly assigned treatment, and one in a medium temperature control at 13.9 ± 0.9 °C. Thus, we had 10 egg sacs in the cold treatment, 10 in the warm treatment and 20 in the medium control. This paired design allowed for stronger inference on temperature differences.

Incubation and enumeration

We placed each egg sac ($N = 40$) in an individual glass jar (115 mL) filled with well water and aerated with an airstone. We placed each jar in one of three incubators according to the randomly assigned temperature. We inspected individual egg sacs every 24 h and recorded the hatch date. We assessed hatching by removing the jars from their incubators and blocking light sources (by passing a hand between the jar and light source) to create a shadow. Individual gill lice responded to this stimulus by altering their activity, rapidly swimming through the water column, which was visible with the naked eye (Poulin, Curtis & Rau 1990b; *S. edwarsii*). We assumed that this response was indicative of a viable individual, capable of parasitizing a host, and we refer to this response as 'survival'. Following a post-hatch, 24-h period, individual gill lice unable to respond to the stimulus in this way were removed daily using a Pasteur pipette. Samples were preserved in vials containing 95% ethanol. We left the remaining gill lice (which we assumed were viable parasites) in the jars and placed them back in the incubators. After we removed non-responsive lice, we counted them

using a dissection microscope. When only non-responsive individuals were present in the glass jars, we noted the date and the contents were preserved in 95% ethanol and subsequently counted. We counted the number of eggs that did not develop into viable free-swimming copepodids. Two trained individuals conducted gill lice counts independently. When the counters disagreed, an average was taken; however, the observed error on average was less than three individual lice.

Statistical analyses

We compared survival of gill lice through time as a function of temperature using a log-rank test. Our null hypothesis for the log-rank test was that there was no difference in survival curves between cold and medium water temperature and warm and medium water temperature. Our alternative hypothesis was that the survival curves would differ. We compared the mean maximum survival time among temperature treatments using a Wilcoxon signed-rank test. We defined the maximum survival time as the time in which there was at least one responsive copepodid alive in each vial. We used a paired *t*-test to compare temperature treatments on days-to-hatch of paired egg sacs. We defined days-to-hatch as the number of days it took (starting when we placed egg sacs in their treatment) for one copepodid to emerge from an egg sac. We performed our analyses in R (Version 3.0.2).

Results

Not all of the egg sacs hatched. We only used data from which both egg sacs in a pair hatched. This resulted in six pairs in the cold and medium comparison and 10 pairs in the warm and medium comparison. Gill lice survived longer at colder temperatures (Fig. 1). The log-rank tests of the survival curves demonstrated that these relationships were statistically different in the case of the paired cold and medium temperature treatments ($P = 0$, $\chi^2(1) = 131.5$) and the paired medium and warm temperature treatments ($P = 0$, $\chi^2(1) = 55.7$). The difference between the curves was larger for the paired cold and medium temperature treatments relative to the paired medium and warm temperature treatments, as were the differences in the water temperatures of the experiments themselves.
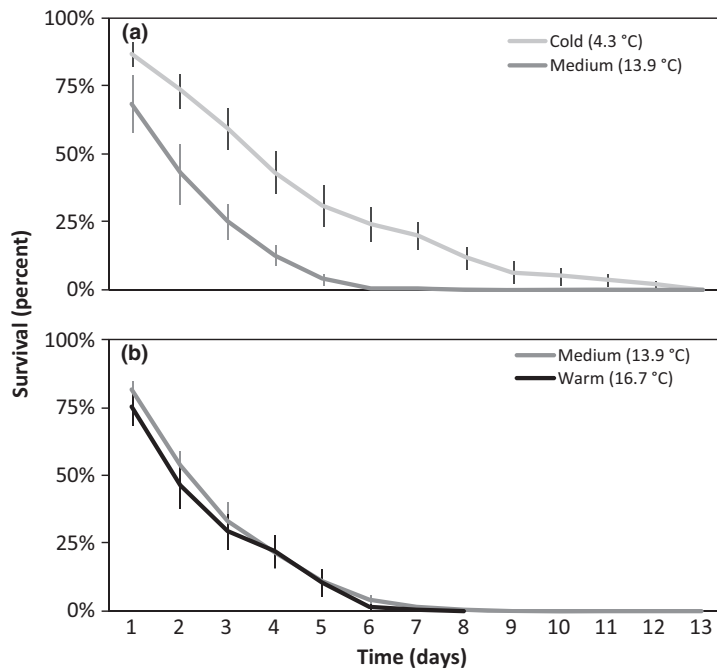
Figure 1 Survival curves of copepodids hatched from paired egg sacs as a function of temperature. Panel a illustrates the survival (per cent) of copepodids from paired egg sacs in the cold (4.3 °C; light grey line) and medium (13.9 °C; dark grey line) temperature treatments. Panel b illustrates the survival (per cent) of copepodids from paired egg sacs in the medium (13.9 °C; dark grey line) and warm (16.7 °C; black line) temperature treatments. Means and standard errors from the means of per cent survival are presented on a daily time step in both panels.

The maximum survival time of individual copepodids was longest in the cold temperature treatment (up to 13 days in two of the cold treatments; Fig. 1). The difference of this relationship (longer survival at colder temperatures) was detected at the 0.06 level with the Wilcoxon signed-rank test for the paired cold and medium temperature treatments ($P = 0.06$, $N_r = 6$, $V = 1$) and the paired medium and warm temperature treatments ($P = 0.17$, $N_r = 6$, $V = 17.5$).

The number of days that it took paired pigmented egg sacs to hatch differed by treatment. The paired t-test comparing days-to-hatch of the cold and medium temperature treatments indicated that it took longer (mean difference = 6.8 days) for egg sacs to hatch in the cold temperature treatment relative to the medium temperature treatment ($P = 0.02$, $t = -3.52$, $df = 5$). The paired t-test comparing days-to-hatch of the medium and warm temperature treatments indicated that it took longer (mean difference = 0.7 days) for egg sacs to hatch in the medium temperature treatment relative to the warm temperature treatment ($P = 0.02$, $t = 2.69$, $df = 9$).

The number of eggs produced in each egg sac by individual female *S. californiensis* ($N = 34$) varied widely (21-133 individuals with a mean of 68 and a standard deviation of 26.2). The differences in the number of eggs in the paired egg sacs from an individual female ranged from 1 to 57, with a mean difference of 24 eggs and a standard deviation of 16.5. Of the egg sacs that produced viable copepodids (i.e. excluding the four and two egg sacs that were unsuccessful in the cold and medium temperature treatments, respectively), the mean (± standard deviation) hatching percentages of eggs from the cold, medium and warm temperature treatments were 87% (±10%), 82% (±16%), and 86% (±9%), respectively.

Discussion

Our findings expand upon what was known previously about *S. californiensis* and corroborates previous work with other species of copepodids and their responses to different water temperatures. The three temperatures selected as treatments fall within the range of those often encountered by the salmonid host. We acknowledge that the host used for this study was an individual of a single species from a single system. We observed variability in egg sacs from individual lice, and across lice from the same temperature treatments. We acknowledge that the responses of gill lice in the medium and warm temperature treatments were more similar than the cold and medium temperature treatments, but the temperatures of the

treatments themselves were more similar, so this was expected. Although the effect size was small in the medium and warm treatments, we still believe this to be biologically significant due to the temperature effect on lice development, leading to changes in host–parasite interactions (Macnab & Barber 2011). We also acknowledge that our sample size was small, having been reduced by unsuccessful egg sacs in the cold temperature treatment. There is likely inherent variability in the metrics we used in this study associated with several sources. For example, these might include variability at the system level, host community and population levels, and the level of the individual hosts themselves. Rigorously characterizing these sources of variability was outside the scope of this study. Thus, to control for these sources of variability to assess differences within and across individual wild gill lice, we used lice from a single host in a single system. However, despite these limitations, our data still supported the hypothesis that temperature was a primary driver of the timing of hatching of *S. californiensis* and survival time without a suitable host, similar to other species of gill lice (Johnston & Dykeman 1987; Poulin, Conley & Curtis 1990a; Conley & Curtis 1993).

Free-swimming copepodids were observed actively responding to stimulus on day 13 in two of the cold treatments, with at least some individuals from most (>90%) viable egg sacs surviving ≥120 h (5 days) at all treatment temperatures. Thus, there was clear evidence to extend the survival time of free-swimming *S. californiensis* copepodids beyond 2 days before needing a host at the temperatures evaluated in this experiment. Half of the egg sacs in the cold (approximately 4.3 °C) temperature treatment were unsuccessful, while the majority of the paired egg sacs in the medium temperature treatment were successful. Thus, 4.3 °C may be near a lower threshold of suitable hatching temperature for *S. californiensis* and near their upper limit of longevity post-hatch, though this would need to be tested formally to confirm. The low sample size ($N = 6$) for comparison between the paired cold and medium temperature treatments likely precluded drawing a conclusion of non-significance.

Variability in the number of eggs produced in each egg sac was relatively high across and within individual adult female gill lice. This variation must also be considered in the context of the gill lice source, a single female rainbow trout from one location and time. Thus, the variability reported here is a conservative estimate. In general, egg counts per female appeared to be higher for *S. californiensis* (mean of 68 eggs per egg sac) relative to those reported for *S. edwardsii* (20–60 per egg sac) by Poulin, Rau & Curtis (1991).

Based on these results and the work of others, it is apparent that certain life-history stages of multiple gill lice species are accelerated or enhanced (e.g. increased growth) by warmer water temperatures. For example, *S. salmoneus* was found to respond to warmer temperatures with an increase in louse body size, number of eggs per brood, number of broods per year and egg size (Johnston & Dykeman 1987). *S. edwardsii* had faster onset of hatching, and higher hatching rate at warmer temperatures (Poulin *et al.* 1990a), while they have been reported to survive and freely swim longer at colder temperatures (Conley & Curtis 1993). Although the results described here suggest that colder water temperatures result in longer survival of *S. californiensis*, copepodid survival does not mean that successful fish parasitism would necessarily occur. Further, we noted a reduction in swimming activity (although this was not quantified in this study) of individuals held in colder temperatures which we speculate might result in gill lice having lower success locating and attaching to a suitable host.

These findings inform how the life cycle of gill lice might respond to changes in environmental conditions like temperature (i.e. climate change). Our results indicate that certain life stages of *S. californiensis* are accelerated by warmer water temperatures, potentially increasing population fecundity. These findings coupled with those of Johnston & Dykeman (1987) and Poulin *et al.* (1990a) suggest that warming climates might benefit gill lice in some cases, enabling them to grow larger, and have larger broods more often. In contrast, cooler water temperatures could result in a reduction in adult gill lice size, brood size and brood frequency, while simultaneously increasing the time of survival of copepodids. Our results suggest that the dynamics that occur between *S. californiensis* and their fish hosts will likely be influenced by changes in water temperature because they have direct impacts on gill lice demographic rates (e.g. survival, fecundity, generation time). Further, factors that could be impacted by climate change, including but not limited to,

lake or reservoir stratification patterns (Marcogliese 2001; Carmack *et al.* 2014), stream flow and temperature regime (Roberts *et al.* 2013) fish species composition and distribution (Moyle *et al.* 2013), fish behaviour (Lonnstedt *et al.* 2014) and duration of growing season (Budy & Luecke 2014) should be considered in the context of gill lice dispersal and infestation. If factors like these become increasingly favourable for gill lice and unfavourable for salmonids (e.g. cutthroat trout) with climate change (Roberts *et al.* 2013), they may exacerbate gill lice infection intensity and prevalence, as well as deleterious effects on economically and ecologically valuable salmonid populations.

Acknowledgements

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