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RELATIONSHIP BETWEEN TEMPERATURE AND *CERATOMYXA SHASTA*–INDUCED MORTALITY IN KLAMATH RIVER SALMONIDS

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ABSTRACT: Water temperature influences almost every biological and physiological process of salmon, including disease resistance. In the Klamath River (California), current thermal conditions are considered sub-optimal for juvenile salmon. In addition to borderline temperatures, these fish must contend with the myxozoan parasite *Ceratomyxa shasta*, a significant cause of juvenile salmonid mortality in this system. This paper presents 2 studies, conducted from 2007 to 2010, that examine thermal effects on *C. shasta*–induced mortality in native Klamath River Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon. In each study, fish were exposed to *C. shasta* in the Klamath River for 72 hr and then reared in the laboratory under temperature-controlled conditions. The first study analyzed data collected from a multi-year monitoring project to asses the influence of elevated temperatures on parasite-induced mortality during the spring/summer migration period. The second study compared disease progression in both species at 4 temperatures (13, 15, 18, and 21 C) representative of spring/summer migration conditions. Both studies demonstrated that elevated water temperatures consistently resulted in higher mortality and faster mean days to death. However, analysis of data from the multi-year monitoring showed that the magnitude of this effect varied among years and was more closely associated with parasite density than with temperature. Also, there was a difference in the timing of peak mortality between species; Chinook incurred high mortalities in 2008 and 2009, whereas coho was greatest in 2007 and 2008. As neither temperature nor parasite density can be easily manipulated, management strategies should focus on disrupting the overlap of this parasite and its obligate hosts to improve emigration success and survival of juvenile salmon in the Klamath River.

Environmental temperature is a critical factor that affects the function and efficiency of biological and physiological processes of poikilothermic animals. Prolonged exposure to either cold or warm temperature extremes can result in the cessation of these processes and eventually lead to death. The thermal tolerances of salmonids vary, depending on life stage and biological process, e.g., incubation, development, smoltification, or spawning. Optimal temperatures for rearing and growth of juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon are similar (12.2–20.0 C and 11.8–17.0 C, respectively), with Chinook capable of handling slightly higher temperatures (see review by Richter and Kolmes, 2005). The upper incipient lethal temperature (UILT) for both species is approximately 25 C (Brett, 1952). Although the UILT provides an accurate assessment of temperature-induced mortality, it fails to capture the compounding effects of other stressors, and thus chronic and acute temperature thresholds have been identified for juvenile salmon. Temperatures at the chronic (16 C) threshold can result in negative effects on salmon behavior and physiology (Campbell et al., 2001; Sullivan et al., 2001); at the acute (22 C) threshold, negative effects on the salmon are intensified and mortality can occur from temperature alone (Campbell et al., 2001). In the Klamath River below Iron Gate dam, Chinook and coho salmon encounter water temperatures ranging from 3 to 6 C in January to 20 to 22.5 C in July and August (Bartholow, 2005), thus exceeding both the chronic and acute thresholds.

In addition to direct effects on fish physiology, increased temperatures also decrease the ability of fish to cope with pathogens and other stressors (Fryer and Pilcher, 1974; Wedemeyer et al., 1980; Wedemeyer and McLeay, 1981). Indeed, as temperatures exceed 15 C, mortality from many salmonid pathogens increases, as does the rate at which fish succumb to these pathogens (Richter and Kolmes, 2005). In the Klamath River, there are several enzootic salmonid pathogens, including the myxozoan parasites *Parvicapsula minibicornis* and *Ceratomyxa shasta* (Nichols and Foott, 2006). Of these, *C. shasta* is considered a significant cause of juvenile salmonid mortality (Foott and Stone, 2004; Fujiwara et al., 2011). A relationship between increasing water temperature and elevated and accelerated mortality from *C. shasta* was first demonstrated by Udey et al. (1975). That study used strains of rainbow trout (*Oncorhynchus mykiss*) and coho salmon from river systems where *C. shasta* is not established and, therefore, are considered highly susceptible to *C. shasta*, with a lethal infectious dose as low as a single parasite (Bjork and Bartholomew, 2009). In contrast, as a result of evolving with the parasite, Klamath River salmonids have developed a degree of genetic resistance and, therefore, are considered less susceptible, with a lethal threshold dose approaching 75,000 actinospores for Chinook salmon (Ray et al., 2011). To examine the effect of water temperature on disease progression in these more resistant salmon strains, Foott and Stone (2004) exposed native Klamath River Chinook salmon to the parasite and then reared the fish at 16 and 20 C. Despite the increased resistance of this strain, mortality was unexpectedly high at both temperatures. Thus, while the findings of Udey et al. (1975) demonstrate a strong positive relationship between temperature and the rate of disease, the results of the second study suggest that some other factor, i.e., parasite density, once exceeded, may overwhelm any temperature-related effects.

Temperature also affects the developmental rate and timing of release of the actinospore stage of *C. shasta* from its obligate invertebrate polychaete host, *Manayunkia speciosa* (Bartholomew et al., 1997). In the Klamath River, *C. shasta* infections were observed in fish held in the river beginning in April and increased through July. Although infections declined in late summer and fall, fish became infected as late as December, at water temperatures between 7 and 22 C (Hendrickson et al., 1989; Stocking et al., 2006), indicating the presence of the actinospore stage in the water column. Direct measurement of *C. shasta* in water using a quantitative molecular assay (Hallett and Bartholomew, 2006) showed a similar trend, with actinospore stages in the upper Klamath Basin detected between 10 and 22 C, and peak production occurring at approximately 17 C (Hurst et al., 2011).

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These studies illustrate a strong seasonal relationship between parasite density and salmonid infection.

The present study explores the relationship between temperature, parasite density, and *C. shasta*–induced mortality in Klamath River salmonids. First, we analyzed data from sentinel fish exposures conducted from 2007 to 2010 to determine the relative influence of temperature on parasite-related mortality between years. Second, we conducted a temperature experiment to determine the relationship between temperature and *C. shasta*–induced mortality for native Klamath River Chinook and coho. The findings of this study will facilitate better predictions of disease-related mortality and provide directions for research and management efforts to reduce the effects of this parasite on out-migrating juvenile salmonids in the Klamath River system.

MATERIALS AND METHODS

Fish exposures and handling

Juvenile (0+ age class) Chinook and coho salmon were obtained from the California Department of Fish and Game, Iron Gate Hatchery. Both species varied in size between study months and years, but coho were consistently smaller than Chinook. Chinook averaged approximately 2.0 g in May and 4.5 g in June, and the coho averaged 1.0 g in May and 3.5 g in June. Exposures were conducted in May and June 2007–2010, in the main stem Klamath River in California approximately 1 river km (Rkm) up-river from the confluence with Beaver Creek (259.1 Rkm from the Pacific Ocean). Fish were transported to the study site in aerated coolers containing approximately 38 L of specific-pathogen–free (SPF) well water. Holding cages were anchored to the river's edge using cables, then submerged approximately 1 m from the bank in about 1 m deep water. Infection by *C. shasta* was accomplished by holding fish in the river for 72 hr, after which the exposure groups were transferred to separate aerated coolers and transported to the Oregon State University–John L. Fryer Salmon Disease Laboratory (SDL), Corvallis, Oregon. At the SDL exposure groups were divided approximately in half and placed in 25-L aquaria with SPF water at appropriate experimental temperatures (see following sections). Preventative treatments for bacterial infections and external parasites were administered (Stocking et al., 2006). Fish were fed and observed twice daily. Moribund fish were removed and killed with an overdose (20 ml/L) of tricane methansulfonate (MS-222) and either immediately examined for infection or frozen for future examination. Surviving fish were killed 90 days post-exposure (DPE) with an overdose of MS-222, and 10 fish from each tank were immediately examined for infection. All moribund and a subset of up to 25 surviving fish were microscopically examined for the myxospore stage of *C. shasta* by collecting material from the posterior intestine with a sterilized inoculating loop and smearing the material in a drop of water on a microscope slide. The material was examined at 200× magnification for 3 min or until the myxospore stage was identified (Bartholomew, 2002). For each of the experiments described below, 20 control fish of each species were not exposed but were otherwise handled the same as the experimental fish.

Multi-year monitoring

Chinook and coho salmon were exposed to *C. shasta* in the Klamath River in May and June from 2007 to 2010, during the period when hatchery fish are released and migrating. After exposure fish were reared at 2 temperatures to assess the effects of temperature at different parasite densities. Due to differences in fish size between May and June, different sized cages were used (May: 67.3 cm by 17.8 cm covered with 0.3 cm mesh; June: 61.0 cm by 38.1 cm covered in 0.6 cm mesh). In May 2007 to 2009, June 2007, and June 2009, 80 each of Chinook and coho salmon were exposed as described above. In June 2008, data from the duplicate groups of fish held at 13 and 18 C in the temperature study were combined for this data point. In May and June 2010, 80 Chinook were used, but due to poor adult returns the previous year, only 60 coho were available. Following exposure, all fish were transported to the SDL and equally partitioned into 25-L aquaria at either ambient laboratory conditions (13 C) or elevated temperature, which varied between 16 and 20 C to best represent river conditions during the exposures. In May 2010 in-river conditions were 13 C; however, to continue with the thermal comparison, a group was reared at 16 C.

Relationship between temperature and *C. shasta*–induced mortality

In June 2008 Chinook and coho salmon were exposed to *C. shasta* and then reared at a range of temperatures to test the relationship between temperature and ceratomyxosis-induced mortality in resistant fish. For each species, 320 fish were placed in large holding cage (123.2 cm by 40.6 cm with 0.3 mesh). After exposure, fish were transported to the SDL, and duplicate groups of 40 fish were randomly distributed into 25-L aquaria with water at 13 C. After acclimation, duplicate tanks were supplied with water at 13, 15, 18, or 21 C.

Parasite density measurement

Three 1-L samples of water were collected when exposures were initiated and concluded to approximate the density of *C. shasta* during sentinel fish exposure. In 2007 these samples were collected manually at the start and end of the exposure. In 2008–2010 an automated ISCO water sampler (Teledyne Isco, Lincoln, Nebraska) was used to collect 1 L of water every 2 hr into a 15-L composite chamber. From that composite sample, three 1-L subsamples were manually collected to estimate the average parasite density over the first and last 24 hr of exposure. Samples were processed and analyzed by qPCR using methods described by Hallett and Bartholomew (2006) with modifications described in Hurst et al. (2011). The Cq values reported from this assay are inversely proportional to the amount of parasite DNA detected in the sample. Standard samples were used to determine the Cq value of 1 and 10 actinospores/L.

Water temperature measurement

From 2008 to 2010, water temperature was recorded at the exposure site from April through August (153 days). Temperature was recorded every 15 min by a submerged HOBO temperature recorder (Onset Computer Corporation, Pocasset, Massachusetts). For each month, daily temperature averages were compared to chronic and acute temperature threshold values to determine the number of days above each threshold. Monthly temperature averages were compared between years to determine inter-annual differences.

Data analysis

DPEs to death (morbidity/moribund) were recorded for all fish that were visually *C. shasta* positive and used as a metric for the virulence and intensity of infection. The geometric mean of DPE was used to determine the mean day to death (morbidity/mortality [MDD]) for groups when more than 1 fish succumbed to infection. Cumulative mortality was measured as the total number of fish that were visually positive for *C. shasta*, divided by the total number of fish in the aquaria. Overtly morbid and moribund fish were used as a proxy for each of these metrics. Mortality attributed to factors other than *C. shasta* was minimal and was not included in our analysis.

Statistical analyses were conducted using TIBCO Spotfire S+ (TIBCO Software, Palo Alto, California). For the observational study, a 3-way ANOVA was used to analyze overall differences in parasite-induced mortality and the rate of mortality between month, year, and temperature. For the parasite-induced mortality analysis, the reported P values are based on the arcsine square root transformed data. At each individual site a χ^2 analysis was used to determine differences in mortality between temperatures within a month and between the same rearing groups within a year. For the temperature experiment, non-parametric Kaplan Meier survival curves and Cox-proportional hazard tests were used to determine differences in cumulative and rate of mortality within and between temperature groups. We report the resulting Score (log-rank) test P values from this analysis. A Wilcoxon rank-sum test was conducted to determine differences in parasite density between years and months within a year from 2007 to 2010.

RESULTS

Multi-year monitoring

Ceratomyxa shasta–induced mortality differed between years for both Chinook and coho salmon, but within a year mortality

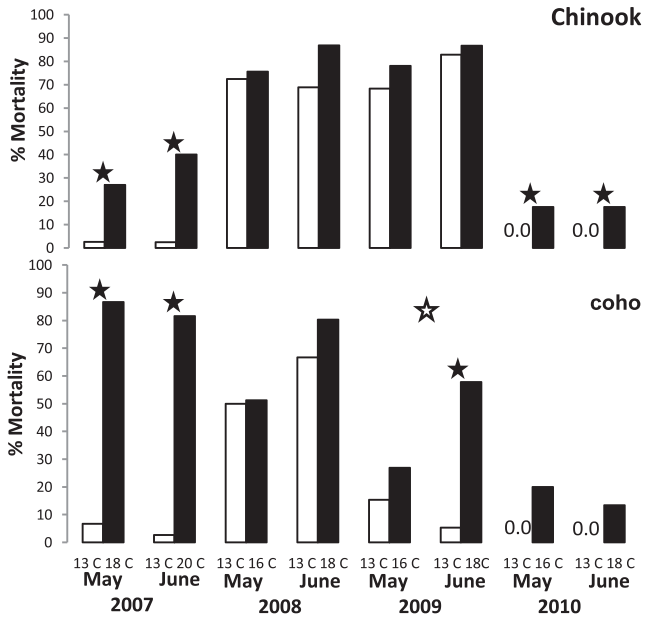

FIGURE 1. *Ceratomyxa shasta*–related mortality in Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) exposed at KBC from 2007 to 2010. Fish were reared at either ambient (13 C) or elevated temperatures (16, 18, or 20 C) in the laboratory. Statistically significant differences between ambient and elevated temperatures, within a month are indicated by H and between elevated temperatures within a year are indicated by I.

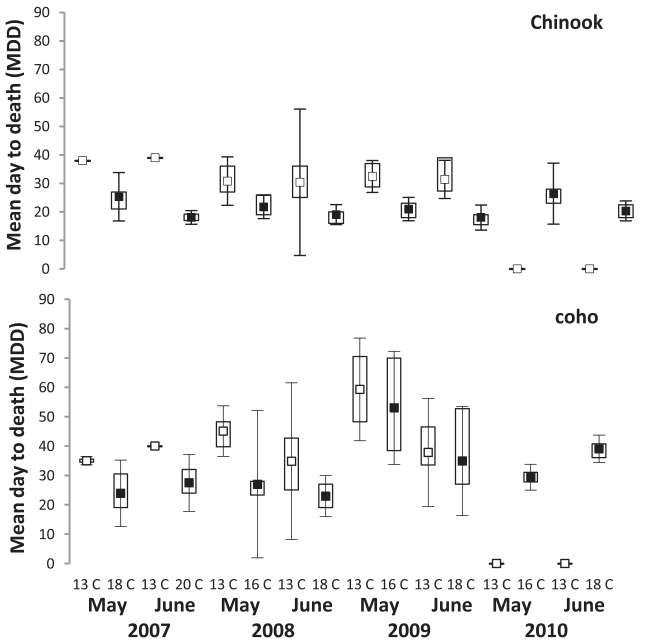

FIGURE 2. *Ceratomyxa shasta*–related mean day to death (MDD) observed for Chinook (*O. tshawytscha*) and coho (*O. kisutch*) exposed at KBC from 2007 to 2010. Fish were reared at either ambient (13 C) or elevated temperatures (16, 18, or 20 C) in the laboratory.

generally increased from May to June (Fig. 1). Chinook salmon mortality was driven by inter-annual differences (ANOVA $P = 0.002$), but within a year there was no difference in mortality for similar rearing groups (ANOVA $P = 0.995$). Differences in mortality between ambient (A) and elevated (E) treatments were significant when A mortality was low, as in May ($\chi^2 = 5.118$, d.f. $= 1$, $P = 0.024$) and June ($X^2 = 15.428$, d.f. $= 1$, $P = 0.0001$) 2007 and May ($X^2 = 4.878$, d.f. $= 1$, $P = 0.027$) and June ($X^2 = 6.806$, d.f. $= 1$, $P = 0.009$) 2010. Unlike the overall observed cumulative mortality trends, the rate at which Chinook succumbed to infection did not statistically differ between years (ANOVA $P = 0.114$), but there were differences between rearing temperature (ANOVA $P < 0.0001$) and exposure month (ANOVA $P < 0.0001$, Fig. 2). The MDD was faster in the E groups and in June than in May for each year of observations. At either rearing temperature, Chinook mortality was highest in 2008 and 2009 and lowest in 2010.

Like Chinook, there was a significant inter-annual difference in mortality of coho (ANOVA $P = 0.014$, Fig. 1). Coho mortality did not differ between months within a year for similar rearing groups, with the exception of 2009 E groups ($X^2 = 7.168$, d.f. $= 1$, $P = 0.007$). Differences in mortality between temperatures were observed in May 2007 ($X^2 = 46.484$, d.f. $= 1$, $P < 0.0001$), June 2007 ($X^2 = 48.580$, d.f. $= 1$, $P < 0.001$), and June 2009 ($X^2 = 26.062$, d.f. $= 1$, $P < 0.001$). The rate at which coho died from *C. shasta* infection was statistically different between years, months, and temperature (ANOVA $P < 0.001$, all). Faster mortality rates were observed for all the E groups compared to A, and June exposures resulted in shorter time to death in 2008 and 2009 (Fig. 2). In 2007 and 2010 the May E groups had a slightly shorter time to death than the June E groups, despite a 2 C increase in temperature. For coho, parasite-induced mortality differed greatly between years with the highest mortality occurring in 2007 and 2008.

Thus, *C. shasta*–related mortality for both species differed between years; however, the timing and severity of infection differed between species. No *C. shasta*–related mortalities were observed in the unexposed control fish for either species in any month or year.

Relationship between temperature and *C. shasta*–induced mortality

Increases in rearing water temperature led to elevated and accelerated mortalities from *C. shasta* in both Chinook and coho salmon, with Chinook being more affected than coho. Cumulative mortality from *C. shasta* in Chinook increased with temperature from 68.8% at 13 C to 97.7% at 21 C (Fig. 3). The MDD was inversely correlated to temperature and decreased by almost 50% between 13 and 21 C groups (30.5 and 15.9 days, respectively). There were no differences in cumulative mortality between replicates at either 13 or 21 C (score [log-rank] test $P = 0.529$ and 0.698, respectively). However, differences were detected between the duplicates of both 15 and 18 C treatments (score [log-rank] test $P = 0.03$ and 0.01, respectively). The mortality curve of only one 15 C duplicate was statistically different from the 13 C treatment (score [log-rank] test $P = 0.005$). Each 18 C duplicate was significantly different from both the 15 C (score [log-rank] test $P < 0.0001$, for both) and 21 C groups (score [log-rank] test $P < 0.0001$, for both). Therefore, each 15 C duplicate and the combined duplicates of 18 C were graphically represented. Overall, the cumulative and rate of mortality were significantly different between all temperature groups (score [log-rank] test $P < 0.0001$). The risk of succumbing to infection increased disproportionately between adjacent temperature groups, i.e., 2.2-fold between 13 and 15 C (averaged for each 15C group), 4.7-fold between 15 and 18 C, and 6.9-fold between 18 and 21 C.

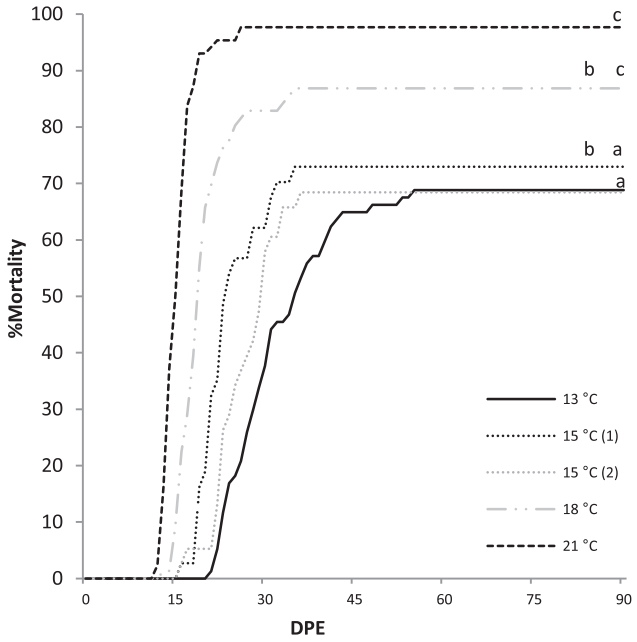

FIGURE 3. *Ceratomyxa shasta*–related mortality curves for Chinook (*O. tshawytscha*) held at 13, 15, 18, and 21 C. The 15 C groups were statistically different from each other and only 1, 15 C differed from 13 C. Both 15 C replicates were different from 18 C, which, in turn, was statistically different from 21 C; a, b, and c represent statistically different relationships. DPE = days post-exposure.

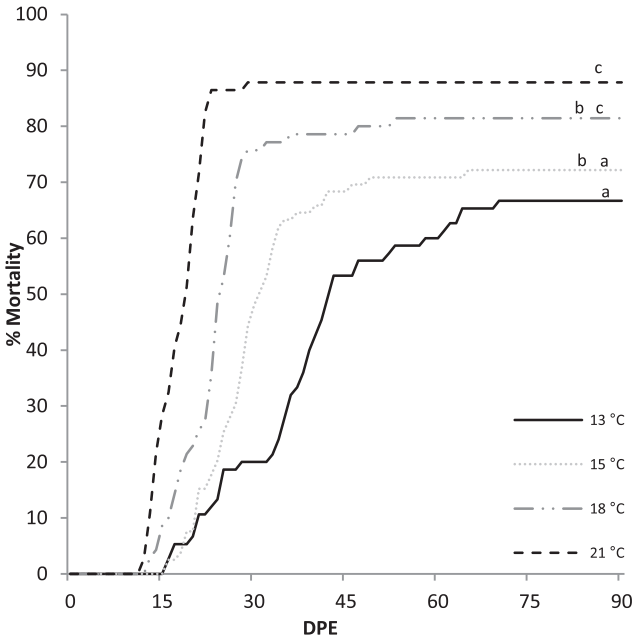

FIGURE 4. *Ceratomyxa shasta*–related mortality curves for coho (*O. kisutch*) held at 13, 15, 18, and 21 C. Mortality curves for each adjacent temperature groups (13–15 C, 15–18 C, and 18–21 C) were statistically different; a, b, and c represent statistically different relationships. DPE = days post-exposure.

Similar trends were observed for coho salmon, although cumulative mortality was slightly lower at most temperatures (Fig. 4). As observed in Chinook, cumulative mortality increased with increased rearing temperature from 66.7% at 13 C to 87.8% at 21 C, and the MDD for coho decreased by almost 50% between 13 and 21 C (35.0 and 17.6 days, respectively). There were no significant differences in cumulative and rates of mortality between replicates at each temperature group (score [log-rank] test P = 0.116, 0.375, 0.609, and 0.176, 13, 15, 18, and 21 C, respectively). Between adjacent temperature groups, i.e., 13 and 15 C, 15 and 18 C, 18 and 21 C, there were significant differences (score [log-rank] test $P = 0.02$, < 0.01, and < 0.01, respectively). As observed in Chinook, the risk of morality increased unequally between adjacent rearing groups, i.e., 2.4-fold between 13 and 15C, 3.5-fold between 15 and 18 C, and 5.5-fold between 18 and 21 C.

Chinook cumulative mortality was higher for each temperature group, except 15 C, and was more variable, especially at the lower temperatures, than observed in coho. While there was no difference in mortality and MDD between the 2 species at either 13 or 15 C, Chinook incurred higher mortality and faster MDD than coho at 18 and 21 C (score [log-rank] test $P < 0.0001$ for both groups). The overall and rate of mortality for both Chinook and coho increased with increasing water temperatures. No *C. shasta*–related mortalities were observed in the unexposed control fish of either species.

Parasite density measurement

In 2008 and 2009, parasite densities were greatest and consistently at, or above, 10 parasites/L. Individual water sample collection, which occurred in 2007, resulted in greater variability between samples and larger standard deviations (Fig. 5). Due to this within-sample variation, 2007 was not significantly different from any of the other years. However, when similar sampling techniques (ISCO) were compared, there were differences between 2008 and 2009, 2008 and 2010, and 2009 and 2010 ($P = 0.039$, < 0.001, and < 0.001, respectively). Densities were generally higher in June than May but were significantly different only in 2008 ($P = 0.005$).

Water temperature

Temperature measurements were compared to the chronic (16 C) and acute (22 C) thresholds to distinguish differences between months and years (Fig. 6). The chronic threshold was not exceeded in April for any year; the highest average temperature during that month was in 2009 (11.4 C). The average daily temperature in May 2008 and 2009 was 15.3 and 16.2 C, respectively, and the chronic threshold was exceeded 12 days in 2008 and 16 days in 2009. In May 2010, the average daily temperature was 13.7 C, and the chronic threshold was not exceeded during this month. The chronic threshold was exceeded 100% of the days in June, July, and August for all 3 yr. Daily temperatures for July and August averaged at or above the acute thermal threshold. Of the 153 daily averages recorded for each year from 2008 to 2010, the chronic threshold was exceeded a total of 104, 108, and 88 days (respectively), and the acute was surpassed 44, 36, and 47 days (respectively). Although the temperature patterns were consistent between most of the months for all 3 yr observed, May 2010 was approximately 1.5–2 C cooler than previous years.

DISCUSSION

Temperature influences almost every aspect of the *C. shasta* life cycle, from effects on overall salmon physiology, including stress

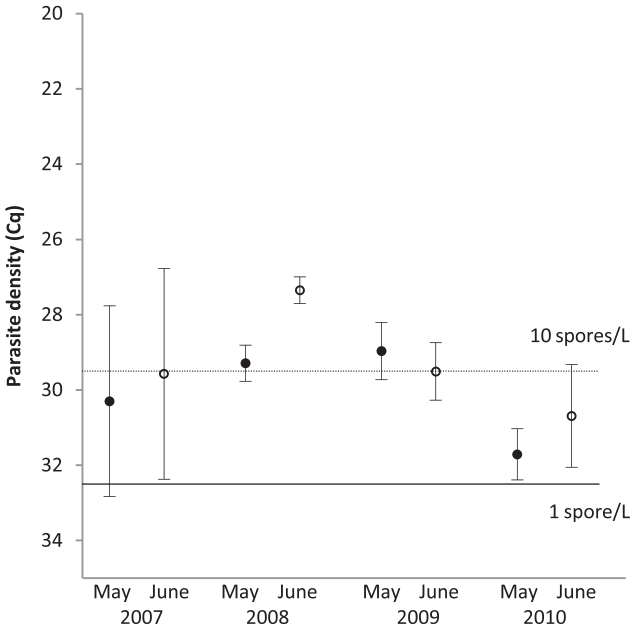

FIGURE 5. Average parasite density measurements from water samples collected at beginning and end of fish exposures. Cq values are inversely related to density. Solid and dashed lines represent standard values of 1 and 10 spores/L, respectively.

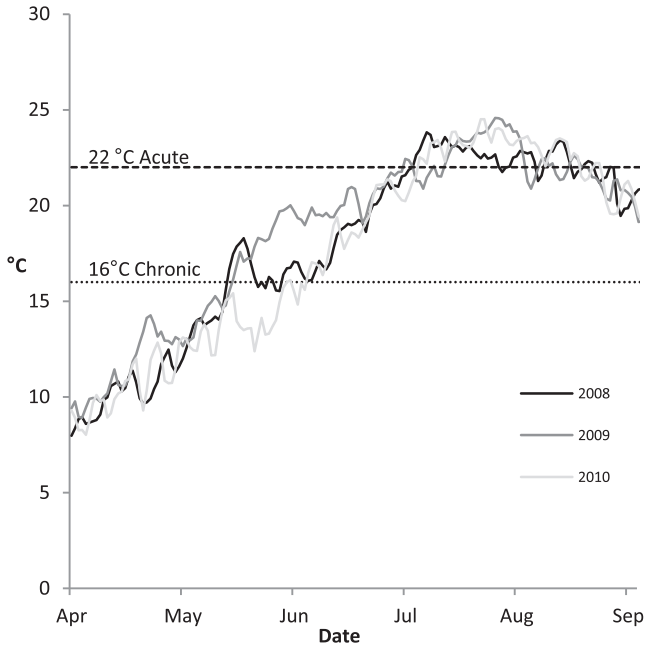

FIGURE 6. Klamath River temperature trends monitored at KBC sentinel site from March through August 2008–2010. Dotted and dashed lines represent chronic and acute thresholds for thermal tolerance of Chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon.

and disease resistance, to developmental rate and longevity of the parasite in the aquatic environment (Richter and Kolmes, 2005; Foott et al., 2007; Bjork, 2010). Both of our studies demonstrated a relationship between increasing water temperature and *C. shasta*–related mortality in native Klamath River Chinook and coho salmon. Although elevated temperatures consistently resulted in higher mortality and quicker mean day to death, the magnitude of this relationship was not consistent between months within a year or between years, and differed between species. The findings of these studies using Klamath River salmonids were consistent with the trends described by Udey et al. (1975) for the effects of temperature on *C. shasta*–related mortality of more susceptible salmonids strains. They also provided further support for the threshold of high infectivity and mortality from *C. shasta* in Klamath River salmonids when densities exceed ~10 spores/L (Hallett and Bartholomew, 2006; Ray et al., 2011). These findings also suggest a hierarchy of density, and then temperature, with respect to the relative importance of factors that affect ceratomyxosis.

The complex relationship between temperature and environmental conditions and the *C. shasta* life cycle were best exemplified by the multi-year observations. The water years 2007 to 2010 were very similar in the Klamath River Basin, with no major flooding events (USGS water data, gauging station 11516530). In May 2008–2009, water temperatures exceeded 16 C and intermittently surpassed 18 C, coinciding with the highest observed mortality for both species. In contrast, in 2010 water temperatures did not exceed 16 C until 1 June, 2 to 3 wk later than previous years. Mortalities of both species were low compared with previous years and remained low even as June temperatures increased. A strong relationship between thermal time (degree days) and growth and development has been shown for many ectothermic organisms, including invertebrates (Mullens et al., 1995; Honek et al., 1996; Trudgill et al., 2005) and for the production of the actinospore stage of *C. shasta* (Hurst et al., 2011). Therefore, it is possible that the delay of warmer water temperatures in 2010 hindered the development of the polychaete host and/or the development of the actinospore stage of *C. shasta* within this host, postponing release of the parasites. These cooler temperatures, in conjunction with the lower parasite density, may have decreased the infection prevalence and allowed the salmon adequate time to recover from infection. As there were no discernable differences in water years over the course of this study, the delayed warming in 2010 provides at least circumstantial evidence for the importance of the interaction between water temperature and the ceratomyxosis disease cycle.

Although juvenile salmonids may experience a wide range of temperatures, our experimental study focused on the effects of temperature during the peak period of salmon migration. Study temperatures overlapped the coolest temperature during this period (13 C), the chronic threshold (16 C), and approached the acute threshold (22 C) established by Campbell et al. (2001). The risk of mortality was greatest as temperatures increased from 18 to 21 C (6.9 for Chinook and 5.5 for coho), which neared the acute thermal threshold, and lowest as they increased from 13 to 15 C (2.2 for Chinook and 2.4 for coho), which does not exceed any thermal limits. This disproportionate increase in risk at temperatures near, or above, the different thermal thresholds emphasizes the compounding influences of increased thermal stress and decreased ability to cope with the infection for both species.

Since Udey et al.'s (1975) original experiments describing the relationship between water temperature and mortality from ceratomyxosis, there have been advances in parasite detection and our understanding of the biology of *C. shasta*. Two of the most significant were the elucidation of the parasite life cycle (Bartholomew et al., 1997) and the development of molecular

assays that could detect *C. shasta* DNA in fish (Palenzuela et al., 1999) and quantify parasite DNA in water (Hallett and Bartholomew, 2006). The latter assay allows for the estimation of parasite density in a given amount of water, which can be extrapolated to estimate the exposure dose. Variation in density between years provides an explanation for the differences in mortality observed, at similar temperatures, during the temporal study, as well as between this and previous studies. In 2007 and 2010, parasite density measured less than the 10 spores/L threshold. In these years mortality was lowest in groups held at 13 C and mortality in groups held at elevated temperatures was significantly higher, except for coho in 2010, where low fish numbers may have limited the statistical sensitivity. In 2008 and 2009, parasite density exceeded the 10 spores/L threshold, and high mortality was observed regardless of rearing temperature, except for 2009 coho, which will be further discussed below. Thus, temperature effects were most significant below 10 spores/L, supporting the lethal threshold density identified by Hallett and Bartholomew (2006).

Another recent development in our understanding of this parasite was the identification of *C. shasta* genotypes associated with different salmonid hosts. Atkinson and Bartholomew (2010a, 2010b) identified 4 unique genetic types (genotypes 0, I, II, III) of *C. shasta* in the Klamath Basin. It was observed that mortality in Chinook was consistently associated with genotype I and in coho with genotype II, even though most genotypes were simultaneously detected in the river during exposure. These specific host associations may explain some of the disparities we observed in the temporal monitoring and temperature experiment. For example, between 2007 and 2009, there was a switch in cumulative mortality between the species, with higher mortality in coho in 2007 and higher mortality in Chinook in 2009. Parasite density was similar between these years, thus differing proportion of host-specific genotype may provide an explanation for the differential mortality.

Coho are generally considered more sensitive to elevated temperatures, and this is supported in the June 2007 exposure. During this exposure, Atkinson and Bartholomew (2010a) estimated an approximate 1:1 ratio of *C. shasta* genotype I (Chinook) to genotype II (coho) in water samples. Mortality in exposure groups held at 13 C was equally low for both species, but coho held at the higher temperature died at a rate double that of Chinook. This suggests that, as temperature increases, coho became less capable of preventing the onset of ceratomyxosis. However this trend is not repeated in the temperature experiment. During this exposure, parasite densities were higher than in 2007, and mortality was significantly lower in coho at both 18 and 21 C. This suggests that during this exposure a greater proportion of the total dose was genotype I, associated with Chinook. These examples emphasize the importance of understanding how all 3 factors, e.g., temperature, density, and genotype, in combination, can affect the survival of juvenile salmonids as they migrate toward the ocean.

The magnitude of the relationship between temperature and mortality was affected by parasite density during exposure, i.e., when densities were high thermal influences were dampened. In the Klamath River, current environmental conditions are marginal for out-migrating and over-summering juvenile salmonids based solely on temperature, which is predicted to increase 0.3–0.6 C per decade (Bartholow, 2005). The compounding stress of *C. shasta* infections likely further impedes restoration of these commercially and culturally important species. Use of cool water refugia provided by tributaries and groundwater-influenced hyporheic zones by salmonids may mitigate some of the adverse effects of increasing temperature (May and Lee, 2004; Hatch et al., 2006; Sutton et al., 2007). However, these areas may provide only minimal relief for salmonids if the density of *C. shasta* remains at, or near, the densities observed over the course of our study. As this is a hatchery-driven system, a potential management action would be to release salmon earlier in the spring or later in the fall to avoid both peak parasite production and higher temperatures. To restore and stabilize the salmonid population in the Klamath River, river-wide modifications, such as dam removal and habitat restoration, have been proposed as part of the Klamath Basin Restoration Agreement (2010). Even if these projects do not alter in-river temperatures, they could disrupt the spatial or temporal overlap of hosts and parasite currently observed in the Klamath. Temperature and parasite density are difficult parameters to control; however, our findings provide avenues for research and management actions to potentially circumvent or lessen the adverse affects of *C. shasta* on the juvenile salmon population.

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