United States Department of the Interior

FISH AND WILDLIFE SERVICE

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Technical Memorandum

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SUBJECT: Response to Request for Technical Assistance – Prevalence of *C. shasta* Infections in Juvenile and Adult Salmonids

DATE: September 20, 2016

Purpose. The Arcata Fish and Wildlife Office (AFWO) Fisheries Program is working with its scientific co-investigators to develop a series of four technical memorandums that summarize recent findings of studies that contribute to our current understanding of *Ceratanova shasta* (syn *Ceratomyxa shasta)* infections in the Klamath River, in response to requests for technical assistance from the Yurok and Karuk tribes. Each of the topics addressed in the four technical memorandums: 1) geomorphic channel conditions and flow, 2) polychaete distribution and infections, 3) actinospore and myxospore concentrations, and 4) prevalence of *C. shasta* infections in juvenile and adult salmonids, were identified in a conceptual model diagram (Figure 1) taken from Foott et al. (2011), and as discussed with the requesting tribes. The intent of the technical memorandums is to provide managers with a contemporary understanding of the state of the science with regard to the *C. shasta* in the Klamath River, and to provide a scientific basis to inform and support resource management decisions. In this technical memorandum, we summarize the state of the science regarding the infection and mortality experience of salmonids exposed to *C. shasta* in the Klamath River.

Background. High infection rates by the myxozoan parasite *C. shasta* have been documented in emigrating juvenile salmon populations during spring and early summer in the Klamath River (Foott et al. 1999; Nichols and Foott 2006; True et al. 2016; among others), which have been linked to population declines in fall Chinook Salmon (Fujiwara et al. 2011, True et al. 2013). While native salmonids exposed to low doses of the parasite exhibit some degree of resistance (Ching and Munday 1984; Bartholomew et al. 2001), they can become overwhelmed by high infectious doses that result in a diseased state and cause mortality (Ratliff 1981; Ching and Munday 1984; Bartholomew 1998; Stone et al. 2008). Fish that display clinical signs of *C. shasta* infection are also likely to be more prone to mortality because of increased susceptibility to other pathogens such as *Parvicapsula minibicornis* (Figure 2*)*, to predation, and as a result of a compromised osmoregulatory system that is essential for successful ocean entry (S. Foott personal communication).
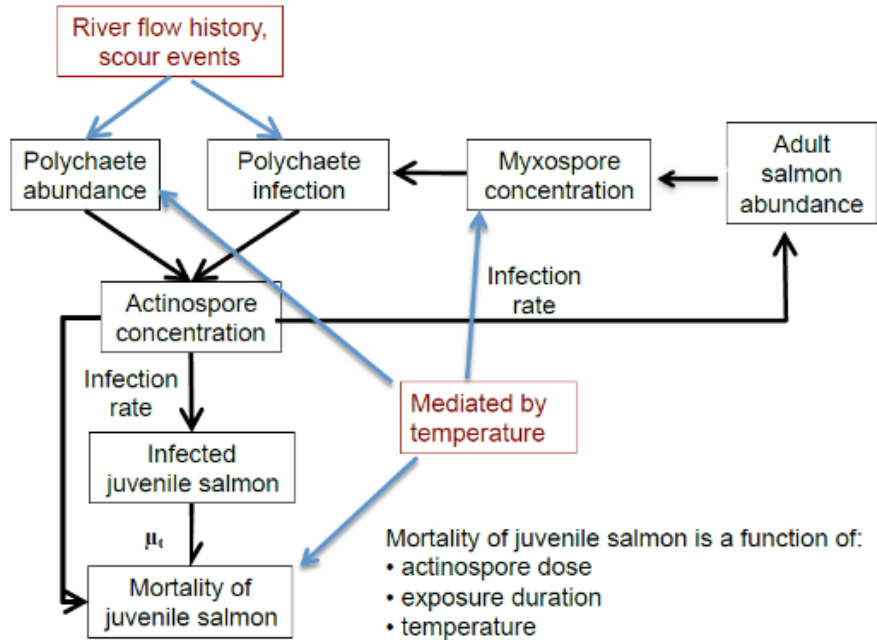
Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon. with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).
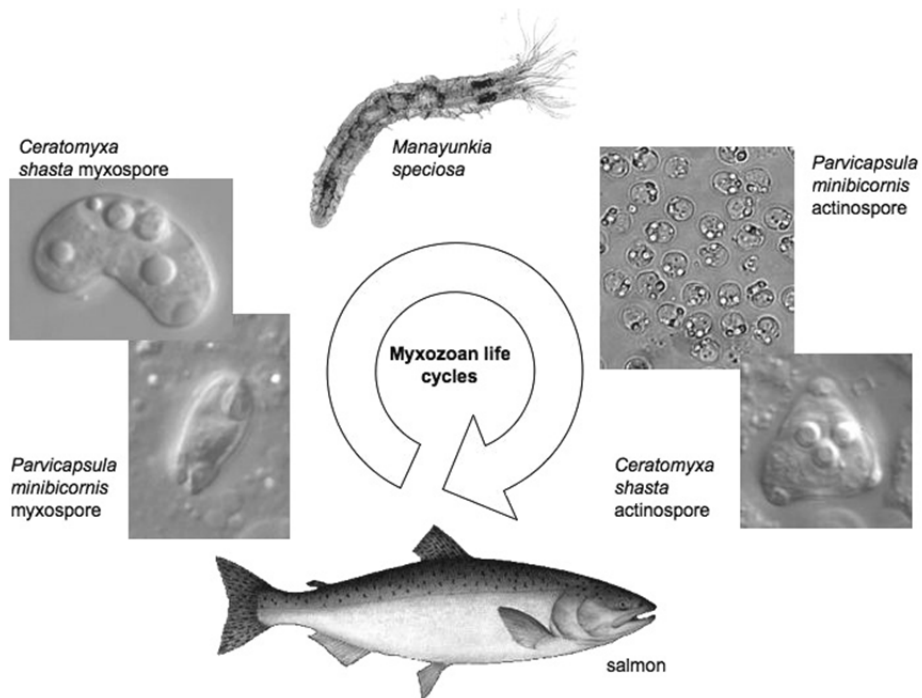

Figure 2. The life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* (graphic provided with permission from J. Bartholomew, Oregon State University). *Manayunkia speciosa* is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites.

The parasite *C. shasta* is endemic to the Klamath Basin and is assumed to have co-evolved with the different species of salmonids it infects. Coevolution results in parasites that are in dynamic equilibrium with their hosts and low virulence, assuming continued environmental variation under which this equilibrium evolved (Toft and Aeschilimann 1991; Esch and Fernandez 1993). When environmental conditions are significantly altered, however, the change will most often favor the parasite because of its shorter generation time and greater genetic variation as compared to the host (Webster et al. 2007). In general, the parasite adapts more quickly to environmental change than the host, causing the parasite-host equilibrium to shift out of balance (Thompson 1994). This imbalance can be expressed as an elevated prevalence of host infections over naturally-occurring background or equilibrium levels, which is consistent with the abnormally high infection levels observed in juvenile salmon in the Klamath River during some years.

The life cycle of *C. shasta* is complicated and involves salmonids and a freshwater polychaete *Manayunkia speciosa* as alternate hosts, and two microscopic waterborne spore stages (Bartholomew et al. 1997, Meaders and Hendrickson 2009, Figure 2). Actinospores develop within infected polychaete worms that are later released into the water column where they may encounter and infect adult and juvenile salmonids. Clinical signs of the disease state exhibited by infected salmonids include necrosis of intestinal tissue that can be accompanied by a severe inflammatory reaction (enteronecrosis) and subsequent death (Bartholomew et al. 1989). The polychaete invertebrate host is necessary for completion of the life cycle and neither horizontal (fish to fish), or vertical (fish to egg) transmissions have been documented under laboratory conditions. Myxospores develop within infected salmonids and are released into the environment. After release, myxospores may be consumed by and infect polychaete worms, thus completing the life cycle.

The complexity of the *C. shasta* life cycle may lend itself to a variety of management approaches because actions can be tailored to target the different hosts or parasite spore stages, thus arresting the life cycle. Of particular interest, are aspects of the *C. shasta* life cycle that are susceptible to alteration via management alternatives (Figure 1). Given the nature of the parasite's life cycle, disruption of even a single element of the cycle could have profound impacts on survival of juvenile salmonids in the Klamath River.

Sentinel Trials. The infection and mortality experience of juvenile Klamath River salmonids has received substantial research and monitoring attention over the last decade. Most of the work has focused on sentinel exposure experiments, where groups of fish are caged in the river and exposed for several (typically 3) days, and then moved to laboratory tanks where they are reared for an extended period of time. Fish in these trials are exposed *in situ* to Klamath River water concentrations of actinospores and temperatures, with the subsequent laboratory holding temperatures often varied across trials to isolate the effects that temperatures may play in the infection and mortality experienced by exposed fish. Experimental variation in actinospore levels is achieved by conducting the experiments at different times of the year, and by natural variation in actinospore levels that occur across years.

The most comprehensive summary of Klamath River sentinel exposures to-date was conducted by Ray et al. (2014), who analyzed the *C. shasta* mortality experience of juvenile Chinook and Coho salmon in trials conducted between 2006 and 2010. They found that increasing parasite (species-specific) concentrations and water temperatures were positively associated with the proportion of individuals succumbing to disease and to the rate at which mortality occurred. A positive relationship between discharge and mortality was also estimated, but the authors noted

several key caveats. First, the effects of temperature and spore concentration were estimated to be much stronger influences on mortality than the discharge variable, and consistent among both Chinook and Coho salmon. Second, discharge was measured at a nearby gauge and was applied as a coarse-level proxy for water velocity (and hence, potential dose) experienced by fish held in fixed-position cages, which are a weak proxy for cage-specific water velocities. Cage placement also prevented sentinel fish from seeking velocity shelter, a behavior commonly observed in the wild. Further, a separate study aimed to investigate the transmission dynamics of actinospores found that increasing velocities decreased parasite transmission and that transmission stopped above 0.2 – 0.3 m/s (Ray and Bartholomew 2013).

In addition to studying how environmental factors relate to the proportion and rate of fish mortalities, True et al. (2012) expanded on these sentinel experiments to track the progression of disease among exposed individuals. After 3-day river exposures, juvenile Chinook and Coho salmon were held at 18 °C water and groups were tested for parasite DNA levels daily for 35 days. Riverine exposure temperatures averaged 16.8 °C, and the water concentration of spores averaged 147 spores/L over the 3 days. Onset of enteronecrosis (syn. with ceratomyxosis; the disease caused by *C. shasta)* occurred at a range of 10-15 days post exposure for both species. The mean day to death was slightly (3 days) shorter for Chinook Salmon than for Coho Salmon, but a higher percentage of Coho Salmon succumbed to disease. We note that the spore concentration data were not processed for genotypic differences, which may help explain some of the small differences in the observed mortality experiences between Chinook (87%) and Coho (96%) Salmon in this study.

True et al. (2012) also noted the development of myxospores in both Chinook and Coho salmon by 15-16 days post exposure, and hypothesized that juvenile salmonids in the Klamath River could contribute spores to the system and may contribute to polychaete infections in years with high disease severity. The development and release of myxospores in juveniles was further studied by Benson (2014), who found releases from fish occurring generally at or soon (within several weeks) after mortality. Benson (2014) does note the potential for pre-spore stages (i.e., not fully developed myxospores) to be detected. The DNA assay method does not distinguish parasite developmental stage and could hinder precise timing and quantity of myxospore release. Bension (2014) also suggests the potential for hatchery Chinook Salmon to contribute more myxospores to the system than spawning adults, but notes the timing and spatial overlap of myxospore release from adults likely better aligns with the distribution of the polychaete hosts.

Extended Sentinel Trials. Building on the foundational knowledge laid down by the sentinel exposure experiments, several recent studies have attempted to address the hypothesis that 3-day exposures may underestimate the disease experience of natural and hatchery fish in the wild. These fish populations are continuously exposed to riverine conditions, and during periods of high actinospore concentrations, these extended duration exposures may lead to higher levels of infection and mortality than previously reported in the literature.

The first examination of extended exposures includes the analyses of Russell et al. (in prep). This work utilized the known exposure duration of hatchery-released fish to examine the effects of continuous, and potentially changing, temperatures and actinospore concentration levels on the prevalence of infection (POI) of juvenile Chinook Salmon. The analysis included 252 hatchery fish collected between the years of 2007 and 2011 in the "infectious zone" (Shasta River to

Salmon River confluences), and included a broad range of spore concentrations (range = 0.14 – 227 spores/L), water temperatures (range = 14.6 – 22.5°C), and exposure durations (range = 1 – 32 days). This work again demonstrated the positive association between increasing

temperatures and spore concentrations on the probability of fish becoming infected, but also captured an interaction among the physical variables (Figure 3). At higher temperatures, the model estimates a slower rate of increasing POI with increasing spore concentrations, and likely reflects the known degradation of actinospores at higher temperatures (Hurst et al. 2012). As the assay to detect spore concentrations relies on DNA content, it cannot distinguish among viable actinospores, degraded actinospores, or even myxospores. The results of this work could be applied to predict the infection prevalence given time series of spore levels and water temperatures over any specified duration.

The second examination of extended exposures is a multi-year effort jointly conducted by AFWO-fisheries, the OSU Aquatic Animal Health Laboratory, and USGS Western Fisheries Research Center – Cook, WA in 2014, 2015, and 2016 (publication in preparation). These sentinel exposures mirrored the traditional 3-day exposure experiments with regard to the times of year and river locations of application. However, instead of only implementing 3-day caged exposures, simultaneous exposures of 1, 3, 5, and 7 days were conducted. Daily water temperatures across all trials ranged from 9.6 °C to 22.8 °C, and daily spore concentrations ranged from 4.8 to 900 spores/L. The formal analysis of these experiments is pending new model development for applications to a fish production model. However, summaries of the rates and percentages of mortality suggest that in addition to the influence that temperature and spore concentration play (i.e., described by Ray et al. 2014) additional days of exposure result in further increased levels and rates of mortality (e.g., Figure 4). Results from this study suggest that mortality predictions based on 3-day exposures may underestimate the population-level impact of disease.

Impacts of Disease on Outmigrant Juvenile Salmonids. Since 2005, the California-Nevada Fish Health Center has partnered with AFWO-Fisheries Program, Yurok Tribal Fisheries Program, and the Karuk Natural Resources Department to monitor the prevalence of *C. shasta* infections in outmigrating fish of the mainstem Klamath River. Though the number of sampling weeks per year has varied over time, sampling has generally occurred in a weekly-stratified fashion designed to align with Chinook Salmon population estimates generated over a comparable number of years. To summarize the weekly infection rates at an annual level, the weekly rates of infection are averaged over the period of sampling in each year. The well-designed and long-term nature of this monitoring program lead to its adoption as a metric of annual *C. shasta* infection severity and incidental take of federally listed Coho Salmon in the Klamath River (NOAA and USFWS 2013). More information on the methods and implementation of this monitoring program can be found in, for example, True et al. (2013, 2015, 2016). Between the years of 2005 and 2015, annual estimates of wild or unknown-origin juvenile Chinook Salmon POI range between 0% and 96% (Table 1).
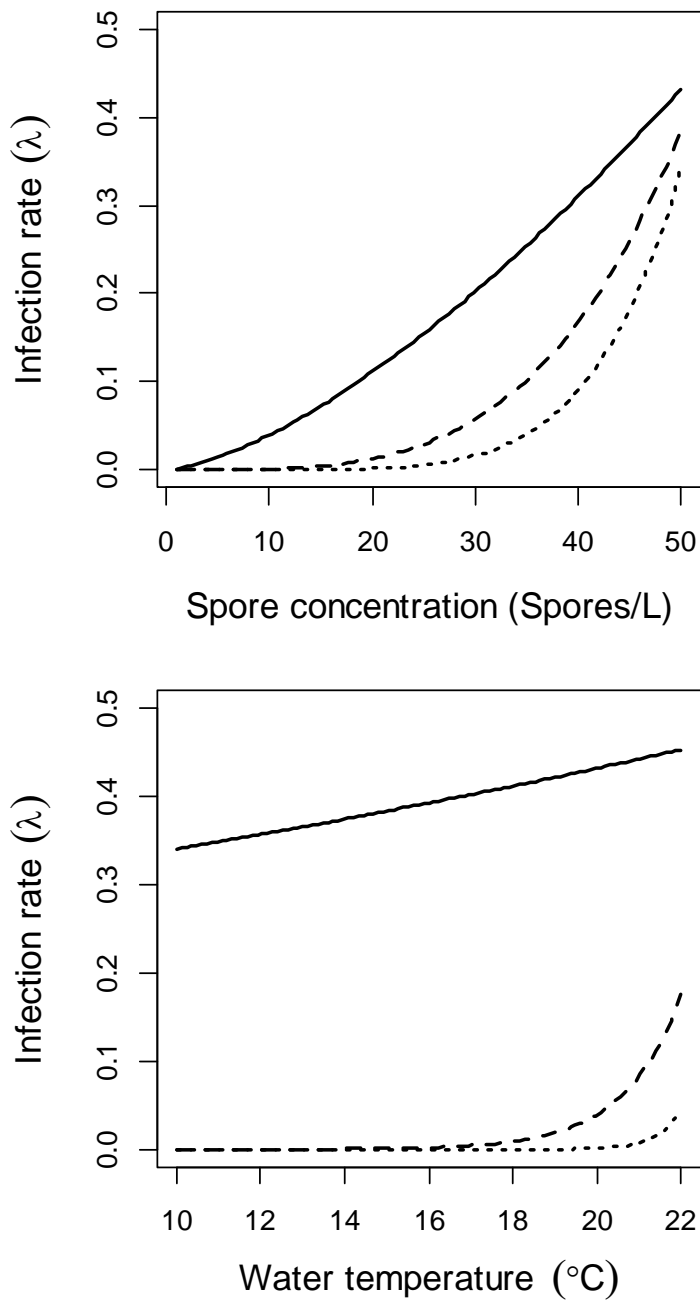
Figure 3. Effect of spore concentration and water temperature on infection rate of juvenile Fall Chinook Salmon with *C. shasta*. In the top panel, infection rates are shown as a function of spore concentration at 10 °C (dotted line), 15 °C (dashed line), and 20 °C (solid line). In the bottom panel, infection rates are show as a function of water temperature at spore concentrations of 1 spore/L (dotted line), 10 spores/L (dashed line), and 50 spores/L (solid line). Prevalence of infection is plotted using the posterior medians of the parameter values for the effect of water temperature and spore concentration on infection rate.
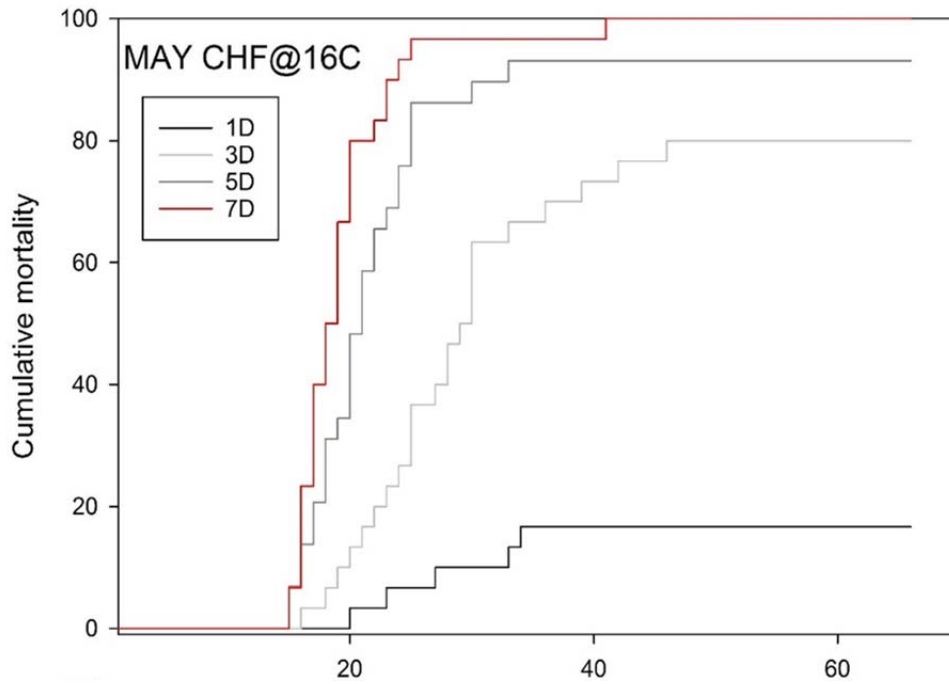
Figure 4. Cumulative percent mortality for trials of Chinook Salmon exposed for 1 day (black), 3 days (light gray), 5 days (dark gray), and 7 days (red). Each trial consisted of 30 fish exposed *in situ* to riverine conditions and then reared in disease-free laboratory water at a constant temperature for the duration of the experiment. For this set of trials, water temperatures averaged 16°C and spore concentration levels averaged 660 spores/L during the riverine exposure period. Numbers on the x-axis represent days since exposure.

Table 1. Summaries of estimates of annual-level infection prevalence for wild and/or unknown (non-adipose fin clipped) origin Chinook Salmon passing the Kinsman rotary screw trap site. "POI" references annual summaries of weekly prevalence of infection collections aimed to monitor weekly disease rates. "PoP" references estimates for the prevalence of *C. shasta* infections in the population of juvenile Chinook Salmon. "LCL" and "UCL" reference the lower and upper confidence limits, respectively, of the infected population that account for the estimation uncertainty in abundance and weekly prevalence of infection estimates. The 2006 estimates are omitted because river discharge conditions prevented the computation of reliable weekly abundance estimates.

Year	Origin	POI	Pop. LCL	Pop. Est	Pop. UCL
2005	All	0.41	0.26	0.38	0.47
2007	All	0.28	0.07	0.1	0.15
2008	All	0.60	0.43	0.51	0.58
2009	All	0.50	0.50	0.58	0.66
2010	Wild/Unknown	0.12/0.15	0.02	0.04	0.07
2011	Wild	0.20	0.07	0.11	0.17
2012	Wild/Unknown	0.06/0.00	0.04	0.08	0.14
2013	Wild	0.18	0.03	0.06	0.09
2014	Wild	0.67	0.12	0.18	0.26
2015	Wild/Unknown	0.66/0.96	0.20	0.29	0.39

The stratified POI sampling described above is designed to track the progression of disease rates over the outmigration period, but is not aimed to estimate the impact of *C. shasta* disease at the population level. To estimate the annual impact of disease on the outmigrating population of juvenile Chinook Salmon, an analysis was recently completed that coupled the POI sampling data with weekly estimates of juvenile Chinook Salmon. Population estimates for fish passing the Kinsman rotary screw trap site (Gough et al. 2015) were selected because that site is the most downstream location of weekly abundance estimates, is located within the often referenced "infectious zone" of the mainstem Klamath River upstream of the Scott River confluence, and is spatially aligned with POI sampling (K4 reach between the Shasta and Scott Rivers, True et al. 2015). Using methods described in Appendix A to this memorandum, we estimated the number of infected and non-infected individuals passing by the Kinsman site for each week. We then summarized these estimates over the entire monitoring period to estimate the proportion of the monitored population infected with *C. shasta*. The methods employed to conduct this analysis allowed us to propagate all estimation uncertainty (regarding weekly infection rates and weekly abundance estimates) to the annual population impact estimates. We note that the estimates of population impact of *C. shasta* should be considered conservative as they are based on apparent survival. As such, fish succumbing to *C. shasta* before passing the Kinsman trap site are not accounted for in the weekly abundance or prevalence of infection estimates.

Estimates of the annual proportion of infected Klamath River Chinook Salmon range from 4% to 58%, and acknowledging estimation uncertainty, range between 2% and 66% (Table 1). Annual variation in the estimated percentage of infection is not only attributable to variation in weekly disease prevalence in sampled fish, but also to the temporal overlap of the migrating population and weekly disease prevalence estimates (Figure 5).

Although the progression of disease rates within a year can vary substantially in overlap with the natural population of juvenile salmon (Figure 5), the California-Nevada Fish Health Center's weekly POI samples likely do overlap well with Iron Gate (IG) hatchery-origin fish. These fish are generally released in late May and have a contracted outmigration period. The hatchery release period generally aligns with the highest weekly POI estimates of each year, and summaries of the weekly POI samples over the hatchery outmigration period suggest that a high proportion of the IG hatchery stock can become infected with *C. shasta* (Table 2). Hatchery estimates akin to those labeled as "Pop" in Table 2 cannot be generated because the Kinsman rotary screw trap is removed before the brunt of the hatchery population passes the trap site, and hatchery-specific population estimates are not available for this section of the Klamath River.
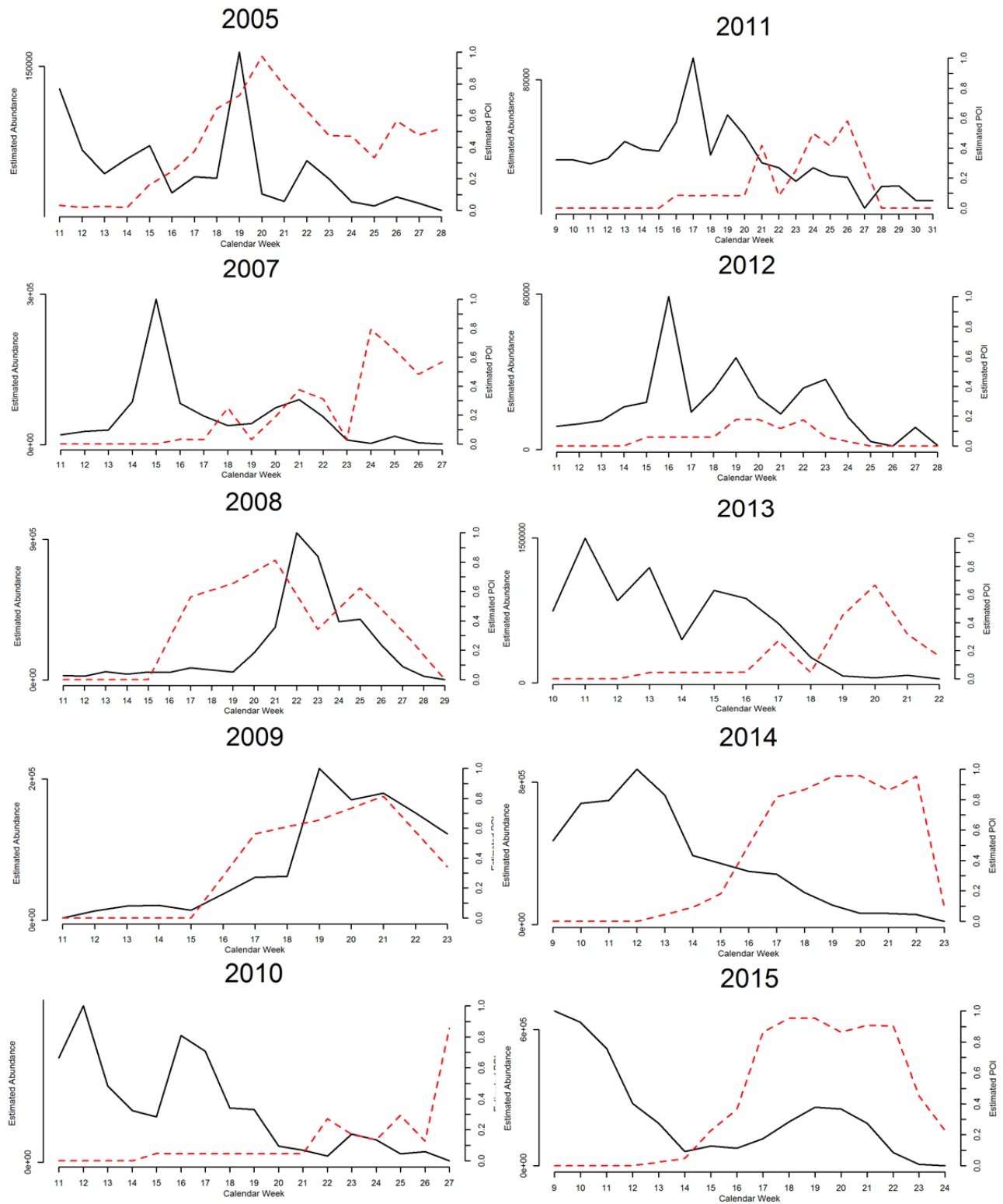
Figure 5. Weekly-stratified abundance estimates of juvenile Chinook Salmon (solid black lines) and *C. shasta* prevalence of infection (POI, dashed red line), by year. Abundance estimates reference the Kinsman rotary screw trap location (Gough et al. 2015) and prevalence of infection estimates reference the K4 reach of the mainstem Klamath River (True et al. 2016). The 2006 estimates are omitted because river discharge conditions prevented the computation of reliable weekly abundance estimates.

Table 2. Summaries of estimates of annual-level infection prevalence for hatchery origin Chinook Salmon passing the Kinsman rotary screw trap site. "POI" references annual summaries of weekly prevalence of infection collections aimed to monitor weekly disease rates. Years not included in the table reflect years where either sampling didn't extend far enough into the hatchery migration period or adipose-fin clip rates were too small to effectively differentiate the hatchery population.

Year	Origin	POI
2008	Hatchery	69%
2010	Hatchery	7%
2011	Hatchery	6%
2012	Hatchery	15%
2013	Hatchery	2%
2014	Hatchery	45%
2015	Hatchery	83%

Adult Salmonids. The current hypothesis is that adult salmon carcasses, above the confluence with the Shasta River (rkm 285), contribute the bulk of myxospores that continue the parasite's life cycle within the "infectious zone." A decade of monitoring has demonstrated that myxospore transmission is sufficient to continue the *C. shasta* lifecycle to a degree that juvenile salmon are at risk of disease in the Klamath River. A recent publication (Foott et al. 2016) has summarized the findings from surveys of adult salmon carcasses in the Klamath River basin, and we note the highlights of that work in the paragraphs below.

IG hatchery (via homing returns) and IG Dam (via migration barrier) influence the current concentration of adult carcasses to the river reach above the Shasta River confluence. While the majority of spawned Klamath River salmon are infected with *C. shasta,* myxospore development occurs predominately in decomposed carcasses rather than recently post-spawned adults. There is no evidence to suggest that myxospore detection is associated with fish size (age), sex, spawn timing (death), or carcass site. This data together with logistic, ecological, and safety concerns precludes carcass removal as a viable management technique to reduce *C.shasta* infectivity in the Klamath R.

Prevalence of myxospore detection from carcasses range from 22 – 52%, however, ≤13% are considered significant contributors (produce ≥ 500,000 spores). These high myxospore carcasses contribute an average of 89% of the total estimated myxospore input to the river per spawning season. This suggests that billions of myxospores are produced annually from adult carcasses in the Klamath River, and myxospores are associated with sediments on the spawning grounds after carcass decomposition. Myxospore viability is rapidly lost in temperatures greater than 18°C and limits the transmission period to the winter and early spring, and it is likely that myxospore enter the water column over the winter. There is tremendous variation in the viability of myxospores (ranging from 7 – 100%) and this has hampered efforts to model the population impact of infection based on estimated myxosore inputs.

Summary Guidelines.
- Temperature and spore concentrations are positively correlated with infection and mortality of both Chinook and Coho salmon.
- Carcasses of juvenile salmon infected with *C. Shasta,* particularly hatchery-produced Chinook Salmon due to their timing of release and associated water temperatures, may be contributing significantly to the total myxospore load.
- Mortality predictions based on 3-day sentinel trial exposures likely underestimate the population-level impacts of ceratomyxosis.
- Both outmigration timing and pattern of POI levels in natural-origin juvenile Chinook Salmon can vary between years, and the more these distributions overlap, the greater the adverse effect on the population.
- Carcass removal is not a viable method for reducing myxospore levels, in addition to being contrary to natural ecological processes.
- The majority of myxospores contributed to the system are most likely released by adult carcasses during a typically stable hydraulic period of managed water years.

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Appendix A. Methods for Annual Population Impact Assessment

Our analysis goal was to estimate the proportion of the natural juvenile Chinook Salmon population infected with *C. shasta* each year that comparable data have been collected. We relied on abundance estimates generated for the Kinsman rotary screw trap location because that site sits within the so-called "infectious zone" (Shasta River to Salmon River confluences), and the trapping occurs during a period of the year aimed to capture as much of the passing natural population as possible (Steven Gough, pers. comm). Due to weather and discharge constraints, as well as natural variation in immergence timing, the Kinsman trap can miss a portion of the outmigrant run in some years. Hence, in some cases our estimates refer to a percentage of the measured population, rather than strictly referring to the entire population of natural juvenile Chinook Salmon passing the Kinsman trap site. We also note that if sections of the natural run are missed by the trapping schedule, they are more prone to miss early portions of the run that occur when C. shasta conditions in the river are always benign (both in terms of temperature and spore concentrations). Hence, we are unlikely to miss a substantial portion of the infected run of natural juvenile fish in any year. Additionally, the discharge conditions in 2006 were so extreme that they prevented sufficient implementation of the mark-recapture experiments necessary to construct reliable abundance estimates.

To estimate the annual proportion infected in each year, we started with estimates of weekly abundances and weekly prevalence of infection (POI), each obtained via Bayesian methods. Abundance estimates were generated via the methods of Bonner et al. (2009), which apply Bayesian p-splines to generate weekly-stratified abundance estimates that account for potentially missed sampling weeks. This method also accounts for the capture probability of the rotary screw trap via a series of mark-recapture experiments that occur during each trapping season. More details on the abundance estimates can be found in David et al. (2016). After burn-in and thinning, 6000 posterior distribution draws were retained for each weekly-stratified abundance. Convergence of chains was assessed via Bayesian p-values and visual assessment. In all cases Bayesian p-values were less than 1.1 and no traceplots indicated issues related to convergence.

Prevalence of infection estimates were generated by summarizing the weekly samples collected over the course of a fish health survey jointly implemented by the California-Nevada Fish Health Center, the AFWO-Fisheries Program, the Yurok Tribal Fisheries Program, and the Karuk Natural Resources Department. This survey generally calls for weekly collections of at least 20 fish in designated reaches of the Klamath River. For this analysis we focused on the section of river labeled "K4" which corresponds with the previously noted infectious zone and lies above the Kinsman trapping location. In the event, either planned or conditions permitting, that sampling was not conducted in a given week, we estimated a missed week's POI by averaging the POI for the weeks directly before and after the missed week. For all weeks where abundance estimates commenced before the onset of fish health sampling, we imputed POI values in two ways. If the number of infected individuals in the first week(s) of fish health sampling was zero, then the POI for all weeks prior to the onset of fish health sampling were all set to zero. If the number of infected individuals in the first week of fish health sampling was non-zero, then the POI estimate for the week immediately prior to the onset of fish health sampling was set to the average of zero and the first week's POI value, and all previous weeks' POI values were set to zero.

Weekly-stratified POI estimates were generated using JAGS software, implemented with the R statistical computing environment. Vague priors were specified for all parameters. After burn-in and thinning, 6000 posterior distribution draws were retained for each weekly-stratified POI sample. Convergence of chains was assessed via Bayesian p-values and visual assessment. In all cases Bayesian p-values were less than 1.1 and no traceplots indicated issues related to convergence.

By applying Bayesian methods and estimating the weekly abundance and POI values via Markov Chain Monte-Carlo methods, we were able to compute the weekly number of infected individuals as derived parameters in a broader Bayesian implementation. We next summed the number of infected and non-infected individuals annually, and propagated all estimation uncertainty in order to compute estimates and credible intervals for the annual POI in the natural (or measured natural) population of juvenile Chinook Salmon.

Appendix A References

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