Measures to Reduce *Ceratanova shasta* Infection of Klamath River Salmonids: A Guidance Document

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Introduction and Purpose of Document

In recent years, high infection rates of the parasite *Ceratonova shasta* (*C. shasta)* have been linked to population declines in Klamath River salmonids. *C. shasta* is a metazoan parasite with a complex life cycle, and it infects both salmonids and the freshwater water polychaete worm *Manayunkia speciosa* (*M. speciosa*). Infected *M. speciosa* produce actinospores which infect salmonids. Infected salmonids produce myxospores which in turn infect *M. speciosa*. 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).

In the 2013 Biological Opinion (BiOp) regarding Klamath Project (KP) operations, National Marine Fisheries Service (NMFS) determined that KP activities exacerbated the impacts of *C. shasta* on listed Coho Salmon. NMFS subsequently developed an incidental take statement that established an infection threshold of 49% of sampled juvenile Chinook Salmon. Because Coho are in such decline, not enough individuals could be captured to directly determine Coho infection rates and thus Chinook served as a surrogate to determine rate of Coho infection.

Observed infection rates of Chinook Salmon sampled between the Shasta River confluence and the Trinity River confluence in May through July of 2014 and 2015 were 81% and 91% respectively – considerably exceeding the take threshold established in the Biological Opinion (True et al. 2016, NMFS and USFWS 2013). In order to propose guidance for science-based measures intended to mitigate the effects of *C. shasta* disease infection rates in coho and Chinook salmon below Iron Gate Dam, the Disease Technical Advisory Team (DTAT) was formed, and existing information was summarized into a series of four technical memoranda by the Arcata Fish and Wildlife Office (AFWO) of the United States Fish and Wildlife Service (USFWS).

The four memoranda are:

1. Sediment Mobilization and Flow History in Klamath River below Iron Gate Dam (Conor et al 2016-Geomorphic Memo);

2. Polychaete Distribution and Infections (Som et al 2016-Polychaete Memo);
3. *Ceratonova shasta* Waterborne Spore Stages (Som and Hetrick 2016-Spore Memo);
4. Prevalence of *C. shasta* Infections in Juvenile and Adult Salmonids (Som et al 2016-Fish Infection Memo).

Research described in these four technical memoranda (summarized in Appendix A and attached in full in Appendices B-E), indicate that disease rates are largely a function of flow regimes, water temperature, adult salmonid carcass densities, sediment regimes, and are potentially exacerbated by hatchery production goals and fish release strategies. The operation of the Bureau of Reclamation's (BOR's) KP affects the total volume of flow in the Klamath River, the hydrograph, and generally alters the geomorphological features of the Klamath River (NMFS and USFWS 2013, Geomorphic Memo). A consequence of the impaired natural flow is the elevated rate of *C. shasta* infection of the Southern Oregon Northern California Coho (SONCC) Salmon which is listed as threatened on the federal endangered species list (NMFS and USFWS 2013).

The purpose of this document is to synthesize information contained in these memoranda and other information sources and to provide management guidance intended to lower the incidence of *C. shasta* in the Klamath River mainstem for both Chinook and Coho Salmon. Management guidance means identifying conditions or actions below Iron Gate Dam that will help alleviate disease conditions.

The DTAT considered actions that might contribute to lessening the prevalence and/or severity of *C. shasta* in juvenile salmonids, including both flow and non-flow related measures. The DTAT considered physical possibility, safety, and a reasonable timeline for implementation in its review of possible guidance actions. In other words, guidance provided by the DTAT must be physically possible and achievable. Furthermore, the guidance provided in this document is intended for the period between now through 2023, which is the duration of the 2013 BiOp or dam removal, whichever comes first[1]. Measures described below are intended to be implemented <u>in addition</u> to the measures and management actions described in the 2013 BiOp. It is acknowledged that implementation of these measures is complex and will involve policy considerations that are not examined in this document.

[1] Dam removal is expected to change the physical nature of the river bed and it is assumed that this issue will be re-examined at that point in light of new conditions.

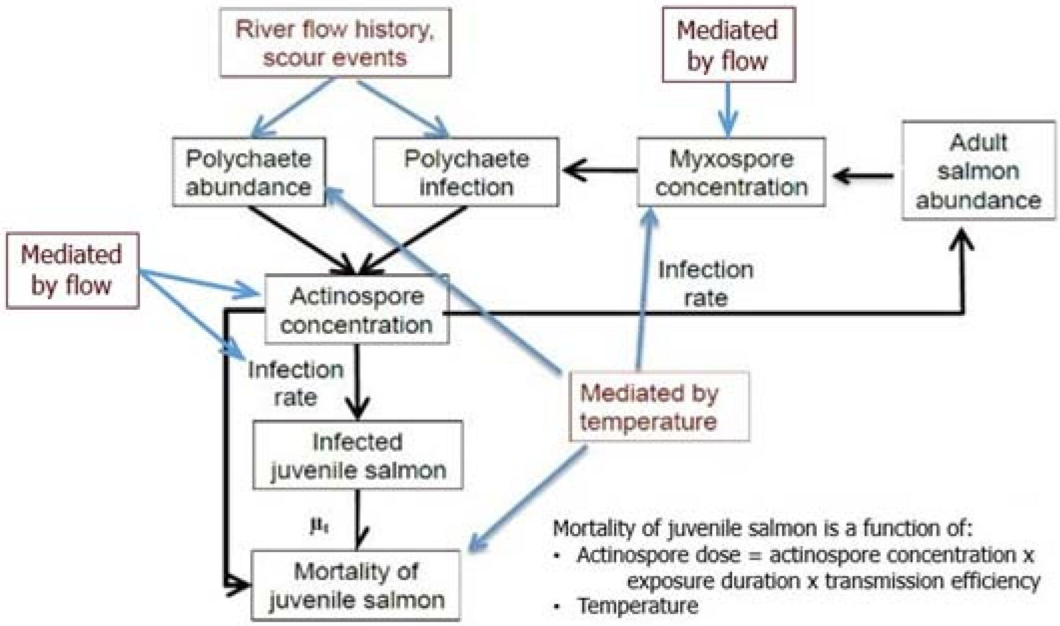

Figure 1: Conceptual model for variables that influence infection and mortality of juvenile Chinook salmon with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. Taken from Foot et al. (2011) and as cited in each of the four technical memoranda discussed below. (Figure from USFWS Memoranda). NOTE: Added "Mediated by flow" box to the conceptual model as supported by information contained in all four Technical Memoranda.

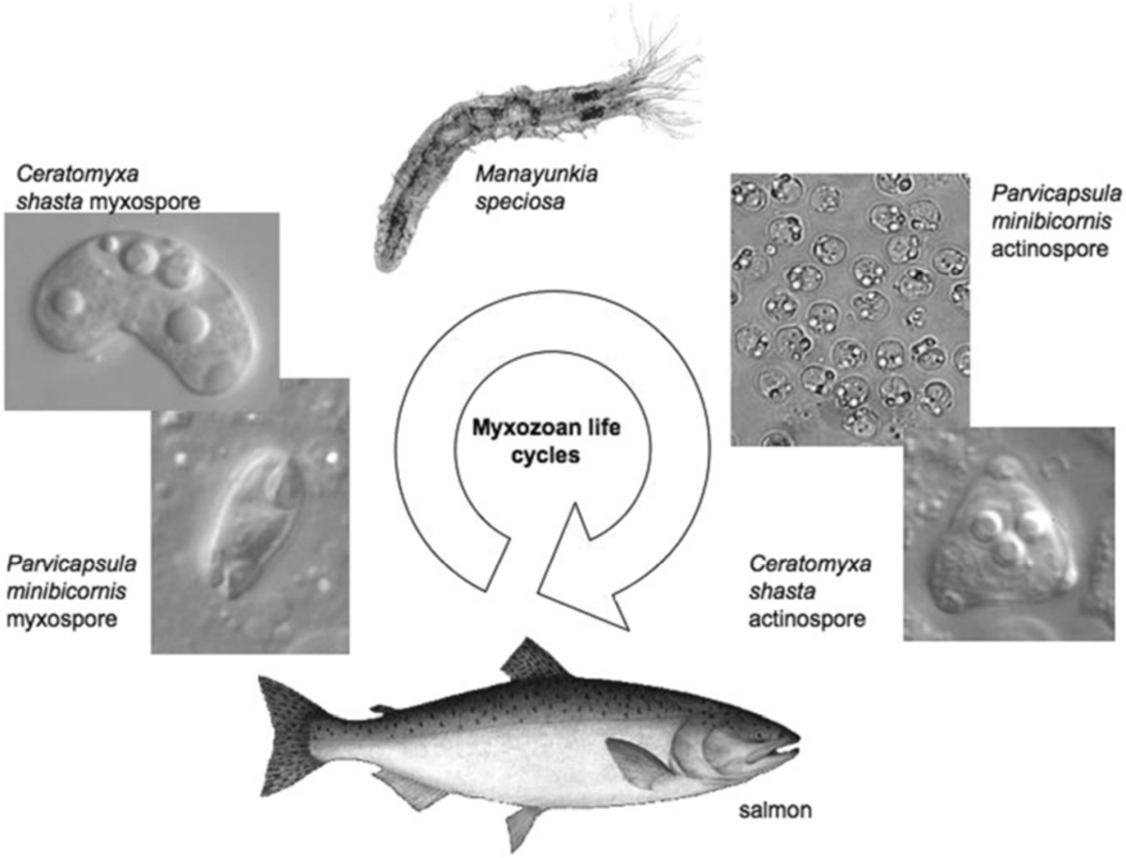

Figure 2 The life cycle of Ceratanova shasta and Parvicapsula minibicornis. Manayunkia speciosa is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites. (Figure from USFWS)

Summary of Information Available to the DTAT

As discussed above, the USFWS in cooperation with other investigators and subject to peer-review, produced a series of four technical memoranda related to the issue of *C. shasta* in the Klamath River. <u>Although the memoranda are an excellent summary of existing information on the subject, they do not contain guidance on specific management decisions to control the prevalence and severity of *C. shasta*</u>.

The four memoranda are:

1. Sediment Mobilization and Flow History in Klamath River below Iron Gate Dam (Geomorphic Memo);
2. Polychaete Distribution and Infections (Polychaete Memo);
3. *Ceratonova shasta* Waterborne Spore Stages (Spore Memo);
4. Prevalence of *C. shasta* Infections in Juvenile and Adult Salmonids (Fish Infection Memo).

Each memorandum has a summary page of important facts presented in bullet-list form. These are presented in Appendix A.

Additional relevant scientific literature was reviewed and utilized.

Members of the DTAT also had personal communication with Scott Foott, Sascha Hallett and Julie Alexander, all of whom have relevant expertise with regard to *C. shasta*.

Adaptive Management Considerations

The DTAT recognizes the importance of applying a formalized Adaptive Management component to the implementation of any management action. The lifecycle of *C. shasta* is complex and the hydrology of the Klamath River is highly dynamic. Thus, to the extent practicable, any management actions should be carried out in such a manner as to allow for scientific observations to determine if predicted outcomes of the action are realized. These observations will in turn inform future management decisions.

We recommend that the Bureau assemble a technical group from fish management agencies and Tribes to ensure that studies are developed and appropriately funded such that field observations can be made before and after management actions are implemented, and technical memoranda published to inform future management decisions. In this manner we can constantly test our conceptual model and better understand the flow thresholds necessary to disrupt the various stages of the *C. shasta* lifecycle.

Overview of Disease Control Measures

A control measure is an action or condition that leads to the objective of reducing the prevalence and severity of *C. shasta* infections in the mainstem Klamath. The DTAT discussed a number of control measures, and considered all reasonable options, not just flow related options, to reduce the incidence and severity of *C. shasta* infections. However, some were considered physically impossible, ineffective, costly, or a combination of these factors.

Short Term Disease Control Measures

The team found several types of actions that could be implemented immediately that would have a high likelihood of reducing the infection prevalence and severity of *C. shasta* in the Klamath River. If this occurred, these actions would mitigate the disease impacts associated with the operations of the KP. These fall under the following broad categories each of which will be discussed more fully in the Management Guidance Sections.

1. <u>Flow management</u>
    a. Sediment flushing and/or geomorphic flows to control infected polychaete populations and promote a more functional hydrologic and geomorphic regime in the Klamath River;
    b. Spore dilution and disruption flows during juvenile outmigration in the spring;
    c. Pulse flows to redistribute and strand myxospores and possibly carcasses in late fall/winter.
2. <u>Hatchery management</u>
    a. Alter hatchery management practices to reduce abundance of juvenile fish during times of peak actinospore concentrations.

Longer Term Control Measures

These are measures which may yield long-term benefits to the *C. shasta* disease situation on the Klamath, but will not likely yield measureable results in the shorter term.

1. <u>Large-scale nutrient reduction and eutrophication control</u>. Excessive nutrient inputs to the Klamath River results in impaired water quality, but also contributes fine organic sediments

through algal production. These fine organic sediments are hypothesized to be a major food source for polychaete worms. High concentrations of nutrients alter the food chain of the river, which could be a factor in producing high numbers of polychaete worms.

2. Removal of Iron Gate, Copco I, Copco II, and J.C. Boyle Dams. The removal of the lower four Klamath River dams has been thoroughly evaluated in an Environmental Impact Statement on Facilities Removal (USDOI and CDFG 2012) and a Secretarial Determination Overview Report (USDOI and USDOC 2013). Both of these evaluations conclude that dam removal would provide opportunities to reduce juvenile salmon disease. Dam removal would reduce *C. shasta* disease rates by creating a more natural fish spawning distribution, a more natural carcass redistribution, improving water quality, and restoring some of the natural hydrological and geomorphic functions in and below the hydroelectric project reach. Substrate mobility would be significantly increased, which would reduce polychaete densities (Malakauskas et al. 2013). Dam removal has been proposed to the Federal Energy Regulatory Commission for consideration.
3. Integration of *C. shasta* control measures and application of the principles of Adaptive Management. Control of the *C. shasta* disease situation in the Klamath River will take many individual actions. Care should be taken such that the actions will be integrated together into a coherent disease control plan and that sufficient monitoring is conducted to gage progress. Even after dam removal, it is important to track how the ecology and disease processes of the river adapt to the new situation on the river.

Control Measures Considered but Eliminated from Further Consideration

1. Dewatering. The team considered dewatering as a means to control polychaete populations. This would involve reducing the flow in the Klamath River at Iron Gate Dam to a point where a significant proportion of the polychaete population are killed through desiccation. This idea was rejected for the following reasons: 1) the flow would have to be reduced by a very large amount (i.e. to less than 300 cfs) in order to affect a large number of polychaete worms, which would cause damage to other important ecological functions of the river, such as macroinvertebrate populations which are an important food source for juvenile salmonids, 2) recent work (see polychaete memorandum) indicates that polychaete worms infected with *C. shasta* are found in deeper portions of the river (Polychaete Memo Figure 4, page 8).
2. Manual carcass removal: The team considered the removal of adult salmon carcasses as a means to limit myxospore production, thus interrupting the life cycle of *C. shasta*. However, this idea was rejected because of logistic, ecological and safety concerns. Recent publication by Foote et al (2011) also suggests that carcass removal is neither technically feasible nor an effective strategy (Spore Memo page 11). This is not to be confused with carcass redistribution, which we recommend below.
3. Direct sediment introduction: Although the Geomorphic Memo demonstrates the importance of sediment movement in controlling the polychaetes as well as the effects that dam construction has had on the sediment budget of the upper Klamath River, the imminent

prospect of dam removal precludes this option on more than an experimental, small-scale basis[2].

4. Channel restoration – Process based channel rehabilitation would provide an improved channel planform that may result in dynamics that reduce *C. shasta* prevalence. However, with the imminence of dam removal and associated expected changes in river function and the significant investment and time required to alter the channel form through mechanical channel restoration, this was removed from further consideration.

Overview of Specific Guidance for *C. shasta* Disease Control Measures

The information that describes the *C. shasta* lifecycle (Spore, Polychaete, and Fish Infection Memos) indicates that: increased river flows at certain times of years can initiate the physical, geomorphic, and ecological changes that result in less disease in juvenile salmonids including Coho Salmon. However, flows are not just a function of irrigation diversions and BOR management, but of highly variable hydrologic conditions from year to year.

Based on the science summarized in the technical memos, the following measures can be reasonably hypothesized to aid in the control of *C. shasta*:

1. Initiation of surface flushing flows
2. Initiation of deep flushing flows and armor layer disturbance
3. Initiation of geomorphically effective flood flows
4. Fall flow variability to disrupt transmission of myxospores from salmon carcasses to polychaete[3] worms.
5. Spring dilution flows to address high spore concentrations.
6. Changes in Iron Gate Hatchery practices to reduce infection rates.

The data summarized in the four technical memoranda shows that the loss of dynamic high flow events have led to river channel conditions that favor the proliferation of *M. speciosa*, *C. shasta*, and high rates of fish disease. As stated in the Geomorphic Memo:

> *"Development of flow releases from Iron Gate Dam that are intended to adversely impact the C. shasta life cycle by targeting the disruption of the obligate invertebrate host as suggested by Alexander et al. (2016) should identify specific physical objectives. The specification should identify the desired form of bed modifications (e.g., sand mobilization or gravel mobilization) and the extent of the mobilization (e.g., from riffles, from channel margins, from pools, etc.). The frequency and seasonal timing of environmental flows should also be specified. Seasonal timing should be based on biological objectives and constraints. Seasonal timing might also be based on physical objectives such as sequencing flows to occur simultaneously or following unregulated tributary peak flows.*

The first three Management Guidance Actions are designed to mobilize sediments to reduce polychaete densities and abundance, thus lowering spore production, and the last three are intended to address

[2] If it becomes apparent that dam removal will not occur, or if it becomes significantly delayed, this option should be re-examined.

[3] "Polychaete worms" or "polychaetes" refers to the species *Manayunkia speciosa* throughout this document.

acute spore concentrations and hatchery practices that are thought to lead to *C. shasta* proliferation. Implementation of the geomorphically oriented management guidance actions may significantly reduce the need for the other management guidance actions such as spring dilution, fall variability, and hatchery changes.

Management Guidance 1: Provide Surface Flushing Flows to the Mainstem Klamath River Below Iron Gate Dam

Objective: The objective of this management guidance measure is to induce the movement of fine sediments below Iron Gate Dam in order to reduce the populations of the polychaete host of *C. shasta*, thus reducing the incidence and severity of *C. shasta* in the future.

Description: Implement flows sufficient to move surface sediments as described in the Geomorphic Memo in Table 3 during the winter period (Nov 1-April 30). Specifically, provide a flow of at least 6,030 cfs from Iron Gate Dam for a 72 hour period. Given the recent scarcity of these types of flows, this action should be implemented every winter until dam removal or the end of the 2013 BiOp. Existing guidelines contained in the 2013 BiOp should be followed with regard to downramping rate unless modified by the Technical Team or FASTA as necessary and supported by scientific information. As explained in the support and uncertainties section, the descending limb is important for the river to sort and distribute fine sediments.

Support: Although the Geomorphic Memo specifies a range of flows for the mobilization of surface sediment (5,000-8,700 cfs), 6,030 cfs is recommended because as indicated in the Geomorphic Memo, that magnitude of flow would mobilize fine sediment. . This management action would increase the number of days that the Klamath River below Iron Gate Dam is in a mobile surface substrate state and therefore increase frequency, magnitude and duration of fine sediment moving flows compared to 2014 and 2015, which had high disease incidence. The Polychaete Memo describes how populations of polychaetes can be kept in check by mobilizing sediments, and the Geomorphic Memo describes what flows are needed to accomplish various geomorphic objectives.

With regard to the magnitude of discharge necessary for a surface flushing flow, the Geomorphic Memo notes different estimates from three different studies in the Klamath River. However, the Geomorphic Memo uses the flow thresholds as described in Holmquist-Johnson and Milhous (2010) in describing the thresholds for later analysis (Geomorphic Memo Tables 3 and 4, Figure 4, 5). The recommended flow of 6,030 cfs falls within the range of flows for mobilization of surface sediments.

With regard to frequency of the discharge necessary for a surface flushing flow, the guidance provides for this flow to occur every year because low winter flow conditions since 2000 (Geomorphic Memo Figure 3) have resulted in overly stable river bed conditions which in turn have caused very high mortality rates from *C. shasta*. Therefore, the guidance is for this flow to occur each year for the duration of the BiOp. Additionally, the annual occurrence of in-reservoir phytoplankton blooms in PacifiCorp Project Reservoirs, primarily in Copco I and Iron Gate, results in a large amount of dead algae that is then released into the Klamath River (NCRWQCB 2010). Dead and decaying algal materials released down the Klamath River, including from cyanobacteria species, settle out and become a potential food source to support high-density polychaete colonies. The extent of this settling out of fine organic sediments in the bed of the Klamath River is elevated during drought conditions due to the extended retention time and turn-over rate of water in PacifiCorp Project reservoirs, warmer water temperatures, and a corresponding increase in algal blooms in the PacifiCorp reservoirs (U.S. DOI and NMFS 2013).

The reduction in surface flushing flows experienced since the year 2000, relative to the 35 years prior, has resulted in fine sediment and suspended sediments accumulating on the bed of the river. This is a concern because high densities of *M. speciosa* have been observed in such deposits (Polychaete Memo). These sediments may provide prime feeding grounds for *M. speciosa*, given that in addition to being sessile suspension feeders they likely also have the flexibility to feed on organic matter in deposited sediments (Polychaete Memo).

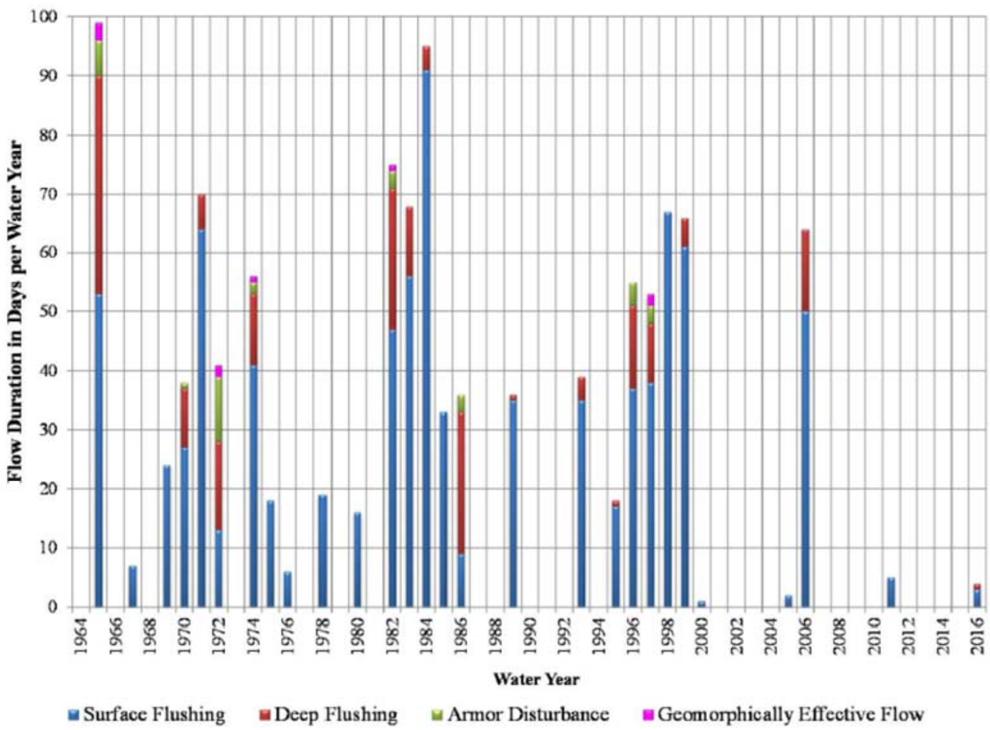

Figure 3: (Figure 5 in Geomorphic Memo), Duration of sediment mobilization flows in days per Water Year in the Klamath River below Iron Gate Dam for Water Years 1964-2016.

Uncertainties: The Geomorphic Memo provides a range of flows (5,000 cfs to 8,700 cfs) that will accomplish surface sediment mobilization (Geomorphic Memo Table 4). The estimated two year recurrence interval (Geomorphic Memo page 6) of 6,030 cfs was selected. It is uncertain exactly which sediments from which habitats will be mobilized from this magnitude and duration of flow, but indications are that at least some surface sediment will move.

In general, a longer duration event will accomplish more of the objective than a shorter duration, because more of the suspended sediments flush out of the river system, rather than being re-deposited further downstream. It is also preferable to have a gradual descending limb to the hydrograph, so that sediments can be sorted as they are deposited on the river bed. Thus it is recommended to follow the existing guidelines for downramping as contained in the 2013 BiOp unless modified by the technical team or FASTA as necessary and supported by scientific information.

The response of the polychaete worm populations to surface flushing flows is also not known with complete certainty. However, there is strong evidence to suggest that frequent surface flushing flows minimizes available habitat for polychaetes (Polychaete Memo page 4, 5).

Adaptive Management Considerations and Monitoring Guidance: The central hypothesis behind this control measure is that surface flushing flows will induce sediment movement that will decrease polychaete densities to the point where spore production and therefore disease incidence will be less. Thus appropriate monitoring of all of these factors should be done within the availability of funding and personnel resources. The ongoing work on predictive modeling of polychaete presence, polychaete infection rates and distribution should continue to be funded because it allows understanding of the effectiveness of this control measure. In addition, appropriate geomorphic monitoring should be initiated to confirm that the flows provided achieved the sediment movement objectives identified.

Management Guidance 2: Provide Deep Flushing Flows and Armor Disturbing Flows to the Mainstem Klamath River Below Iron Gate Dam

Objective: Move sediment from deeper layers of the armored bed layer to remove polychaete worms attached to the armored bed layer in order to reduce polychaete density and reduce spore production of *C. shasta*.

Description: Provide a daily average flow of at least 11,250 cfs as measured in a 24 hour period in the Klamath River at Iron Gate Dam. This should be done at least once during the period of February 15 to May 31 in 2017 and thereafter at least every other year unless precluded by drought conditions. This action should be timed with peak releases from other tributaries such as the Scott River. This flow should occur after Feb 15th if possible to avoid scouring salmon redds and disrupting incubating salmon eggs because some level of gravel movement is expected.

Downramping rates as described in the 2013 BiOp should be adhered to in order to minimize stranding and facilitate the depositional sorting of mobilized bedload unless modified by a Technical Team or FASTA team and supported by scientific information.

This management action may need to occur during times of high accretion between Link River Dam and Iron Gate Dam due to outflow limitations at Link River Dam.

NOTE: if the deep flushing event as described in Management Guidance 2 results in flows above 6,030 cfs for at least 72 hours, it will fulfill the surface sediment management guidance action (#1). In this circumstance, there would be no need for separate implementation of Management Guidance 1.

Support: In addition to the surface flushing flows described above, the Klamath River has also experienced a significant reduction in deep flushing flows and armor disturbing flows (Geomorphic Memo, see also Figure 3 this document). Deep flushing flows are described as having a magnitude of 8,700 cfs to 11,250 cfs, and armor disturbing flows have a magnitude of 11,250 cfs to 15,000 cfs (Geomorphic Memo Table 4). The management guidance here is to provide flows at the top end of the range for deep flushing and the beginning of the armor disturbing flow threshold.

Deep flushing events have become rare over the course of the past 16 years (2000 to 2016) (Figure 3). Only twice in the last 10 years (2006 and 2016) were deep flushing flows observed with only one year, 2006, having a significant number of days (>10days)where the river was in a deep flushing state. While the event in 2016 briefly exceeded the deep flushing threshold in magnitude, it had a short duration substantially less than 24 hours. Armor disturbing flows have experienced an even more significant decline.

Despite the short duration of the March 2016 deep flushing flow event (peak discharge of 11,100 cfs) this event appeared to have a significant effect on the density of *M. speciosa* (Polychaete Memo), therefore the management guidance provided herein calls for a repeat of that flow magnitude based on past success. Of all the management action prescriptions, this appears to have the most demonstrated success at significantly reducing polychaete density.

Given the extremely high levels of C. shasta experienced in recent years, as well as the lack of high flows in recent years, it is recommended that this guidance occur in the winter of 2016-2017.

Uncertainties: The provision of flows of this magnitude entails a great deal of uncertainty primarily having to do with infrastructure capacity, safety, and hydrologic support. What is far more certain is that flows of this magnitude will have a significant effect on *M. speciosa* densities and abundance. While the implementation uncertainty is high, the uncertainty regarding the effect to polychaetes is very low. In other words, if this action is implemented, past monitoring has showed that there will be a negative effect on polychaete density.

The prescribed amount (11,250 cfs) is at the upper end of the deep flushing flow criterion (Geomorphic Memo Table 4), but at the bottom of the Armor Disturbance criterion. This prescription is intended to address both needs.

With regard to ecosystem effects of this guidance action, re-establishment of a more natural flow regime that includes high flows such as called for in this management guidance is expected to restore flows that are closer to the unimpaired natural flow regime. Please see discussions on page 2 of Geomorphic Memorandum regarding importance of high flows to riverine systems and also page 3 of the Fish Infection Memo regarding parasite-host imbalances resulting from ecosystem alteration.

Adaptive Management Considerations and Monitoring Guidance: Sediment movement as well as polychaete density and abundance should be monitored as well as monitoring in support of USFWS' and OSU's polychaete predictive habitat model.

Management Guidance 3: Provide Geomorphically Effective Flows on an Opportunistic Basis.

Objective: Provide high magnitude flows that move significant quantities of gravel and large-scale disruption of polychaete colonies in a wide variety of habitats.

Description: The Bureau of Reclamation, in cooperation with PacifiCorp and other fisheries co-managers including the Tribes, and taking public safety into account, shall look for opportunities to provide safe, short-term peak flows of higher magnitudes than 11,250 cfs whenever possible. Because of the inherent unpredictability and public safety factors inherent in this recommendation, no specific flow magnitude or duration is named.

Support: The Geomorphic Memo describes in detail how flows above 11,250 cfs have declined in frequency and duration. The Polychaete Memo infers how these stable flow conditions have led to a proliferation of *M. speciosa*, and additionally show that unstable bed conditions (i.e. mobile sediment) dislodge polychaete worms (Polychaete Memo pg. 4). Provision of these flows will likely reduce the abundance and density of these worms, which will presumably lead to a reduction in spore production and a decrease in fish infection rates and severity.

Uncertainties: While there is little doubt about the effectiveness of high flows if the magnitude described above to reduce polychaete abundance, implementation of these types of flows has considerable uncertainty with regard to infrastructure capacity, public safety, future weather conditions, PacifiCorp facility operations, and other factors.

Adaptive Management Considerations and Monitoring Guidance: Ideally, it would be desirable to monitor sediment movement as well as polychaete density and abundance. In particular, it would be desirable to conduct monitoring in support of USFWS' and OSU's polychaete predictive habitat model.

Management Guidance 4: Emergency provision of flow in the spring to dilute spore concentrations of *C. shasta*.

Objective: Reduction of spore density and disease transmission through the provision of flows in the spring period (April 1 through June 15 or when 80%of the juvenile Chinook Salmon outmigration is estimated to have occurred).

Description: Hold in reserve 50 thousand acre feet (TAF) of water for the purposes of implementing spring disease dilution and disruption flows if the following thresholds are met:

1. Spore concentrations exceed 5 spores (non-specified genotype) per liter for the preceding sample based on qPCR from water filtration samples at any sampling station;

OR

2. The prevalence of infection (POI) of all captured fish (both wild and hatchery) exceeds 20% in aggregate for the preceding week at the Kinsman Rotary Screw Trap.

This guidance action should not be implemented if the juvenile catch at the Kinsman rotary screw trap is estimated to have exceeded 80% of the expected wild run. This can be based on the cumulative catch at Kinsman, Shasta, and Scott Rivers, historic averages, temperature units, and/or any other predictive tools developed in the upper Basin.

When disease levels begin to climb to detectable levels, initiate a state of readiness to prepare for higher releases through coordination of FASTA and PacifiCorp and Bureau of Reclamation personnel. If critical disease and fish presence thresholds described above are met, release water from Iron Gate Dam to achieve 3,000 cfs immediately at Iron Gate Dam.

Maintain flows at 3,000 cfs until spore or POI at Kinsman Trap sampling indicates a decrease in disease levels. If disease levels are still elevated, increase flows to 4,000 cfs after 7 days of 3,000 cfs until sampling indicates a change in disease levels. If disease levels become reduced below critical thresholds, reduce flows slowly and monitor changes in disease levels to ensure flows result in disease measurements below critical thresholds. Maintain elevated flows at the level required to abate disease below critical thresholds, as determined through monitoring and adaptive management, until 80% of wild juvenile fish are predicted to have out migrated past the Kinsman trap. After initiating this action, evaluate the efficacy of specific flow targets and durations and adjust flow thresholds and durations for subsequent years as directed by the Disease Management Team to account for learning and specific within year environmental conditions.

Support: Altered Klamath River flow regimes have enhanced the reproductive success of *C. shasta* at every stage of its lifecycle. Reduced spring flows exacerbate the transmission of actinospores to rearing and migrating juvenile fish, contributing to significant population level impacts. Flow increases can play an important role in mitigating the impacts of *C. shasta*, with greater water volumes and velocities resulting in reduced disease transmission (Bjork et al. 2010; Hallett et al. 2012; Ray and Bartholomew 2013). The Spore Memo provides some insights into how resource managers can use spring flow increases to dilute actinospore concentrations and disrupt infection in juvenile salmonids.

The concentration of actinospores in the Klamath River is a function of the total number of spores and the volume of water into which they are discharged (Spore Memo page 6-8). The effective dilution capacity of the river increases with increasing flows. Infection of juvenile salmonids is a function of water temperature, exposure, duration, and spore concentration (modified with volume and velocity).Therefore, simply diluting spore concentration by increasing flows can reduce infection. This is consistent with the observation of reduced disease infection rates and mortality estimates in wetter years with higher flows as opposed to drier years and lower flows although water temperature plays a role also. Increased flows also increase water velocity and have been shown to reduce transmission rates (Fish Infection Memo).

The Fish Infection and Spore Memos discuss the efficacy of high spring releases in reducing spore density. These memos concluded that previously planned discharges for spore dilution objectives (e.g. 1900 cfs) were likely too small to be biologically effective, with larger events likely needed to effectively reduce spore concentrations. Flows in low disease years like 2012, which were approximately 3000 cfs and 4000 cfs during the critical spring periods, provide some evidence of potential magnitudes required to reduce spore concentrations meaningfully.

Despite limited examples of higher flows in the spring that coincide with high disease periods due to altered flow management, information from 2005 and 2014 provide the best insight into how dilution of actinospores through sudden flow increases can provide a means to influence infection rates. In 2005, an uncontrolled spill event of 6,000 cfs was coincident with a decrease in infected fish and non-detection of spores in water samples (Spore Memo). The 2005 event lasted several weeks. In 2014, a planned flow increase to 1,900 cfs that lasted less than a day resulted in a modest decrease in spore concentrations (Spore Memo). Spore concentrations of 5 spores per liter (Hallett et al., 2012) and prevalence of infection (POI) of 20% (True et al. 2016) are strong indicators of impending and realized high disease rates. For example Hallett et. al (2012) found that a lethality threshold of 40% mortality was reached with 10 spores per liter for Chinook and 5 spores[4] liter for Coho Salmon. These high levels of mortalities support using 5 spores per liter as a threshold to initiate this Management Guidance Action.

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.

For emergency flow releases to be effective, dilution flows need to occur as disease is worsening but before lethal doses are occurring in juvenile fish. Actinospores are generally released when temperatures are above 10°C and remain viable (able to infect salmon) at temperatures ranging from 11 to 18ºC (Foott et al. 2011). Given the lag time for analysis of samples of at least a week and trajectories of disease progression in past years, this recommendation was thought to be a prudent precautionary approach to avoid excessive mortality rates. Reclamation in the 2013 BiOp proposed flow increases for the Klamath River downstream of IGD to dilute actinospore concentrations within 24 hours of receiving information that disease thresholds have been met and used 5 spores per liter of Type II as a threshold (NMFS and USFWS 2013 page 41) so while this was only implemented once and with meager flows, this is a previously contemplated action. The unspecified genotyping referenced in the memo is in part to allow for much more rapid turnaround time for results (reduction of 2-4 days of process time), which would allow for more effective implementation for fish and water conservation. It is not disputed that there is sufficient information to assess C. Shasta through genotyping and that data would still be collected. Chinook salmon are the disease surrogate for coho in the Incidental Take Statement[5] (ITS) and incredibly important to tribal fisheries so they should be protected during these actions along with ESA listed Coho Salmon.

Uncertainties: As discussed briefly above, spring flows have been provided (whether deliberately or due to hydrologic conditions), but a direct cause and effect link is hard to establish. Part of the uncertainty is due to the effect of temperature. Low temperatures inhibit transmission of *C. shasta* (Fish Infection Memo), and low temperatures often coincide with higher flows. There is also inherent uncertainty regarding when 80% of the juvenile outmigration has been completed.

Adaptive Management Considerations and Monitoring Guidance: The implementation of this measure requires an investment in real-time data on spore concentrations and POI of fish at the Kinsman Trap. At present time, water samples are collected from various locations on the Klamath River ranging from Beaver Creek to Tully Creek once per week. *C. shasta* spores are filtered from the water and express shipped to OSU for DNA processing. Due to shipping time and logistics, the fastest turnaround time is approximately 7 days. Although it may not be possible to compress this timeline any further, it is recommended that water samples be filtered two times per week instead of one, and an alternate DNA analysis facility be employed if necessary. This will yield results twice per week instead of once. Degree days or Accumulated Thermal Unit analysis and other techniques provide promise for use in helping predict disease prevalence and outmigration timing. Tools of this type should be utilized to help maximize the efficacy of this action.

[4] Chinook and Coho are infected by different genotypes of C. shasta. The thresholds described are for type 1 genotype for Chinook Salmon and type 2 genotype for Coho Salmon.
[5] An incidental take statement (ITS) is a statement defining the allowable amount of take of an endangered species as a result of implementation of a proposed action.

Management Guidance 5: Provide flow in the late fall and early winter to redistribute salmon carcasses and myxospores.

Objective: Provide variable fall flows to redistribute salmon carcasses and move myxospores downstream.

Description: Provide a fall/early winter flushing flow of between 1500 and 2500 cfs for a period of 3-5 days between November 15th and January 15th to redistribute salmon carcasses and myxospores. Timing should coincide with tributary elevated flows during storm events when possible. Existing ramping requirements as described in the BiOp should be followed unless a Technical Team (i.e. FASTA, or DTAT) decides otherwise.

Support: The decaying carcasses of a small percentage of adult salmon post-spawn can emit large numbers of myxospores. The reduced frequency of late fall flow increases allows these carcasses to concentrate in slow velocity pools and reside within the active river channel for an extended period of time where they then release myxospores into the water column. Fall flow increases can redistribute carcasses downstream.

Modeling of polychaete habitat suggests that low velocity/ moderately (2-3m depth) deep pools have the highest concentration of infected polychaetes (Polychaete Memo). These types of slow pool habitats are presumed areas where carcasses, myxospores, and polychaetes will be in close contact resulting in higher infection rates of polychaetes, especially during times of low/stable flows (Spore Memo).

Uncertainties: While not much is known about the actual mechanisms involved in infection of polychaetes by myxospores, most experts agree that distributing carcasses further downstream or removing them from the stream channel would reduce infection rates of *M. speciosa*. QPCR methods do not distinguish between myxospores and actinospores present in the water sample, but water sampling should also occur during the time period before and after or ideally all year.

Adaptive Management Considerations and Monitoring Guidance: Real-time monitoring of the run and spawn timing and carcass counts would inform the timing of this fall pulse flow. Research and monitoring should be conducted to determine a peak flow target necessary to achieve carcass movement.

Carcass surveys already occur within the Iron Gate to Shasta Reach, but additional monitoring work load for those crews may or may not be feasible depending on year to year carcass abundances. Additional work may include more time marking carcasses, walking river banks looking for carcasses, snorkeling deep pools and taking photos. There may be a need for an additional monitoring crew in the event the carcass crews are not available.

Monitoring carcass movements could be achieved by attaching highly visible flagging and uniquely identifiable jaw tags to carcasses in variable locations (deep pools, riffles, and margins) prior to the pulse flow event. After the event, make an equal effort to re-encounter previously marked carcasses to determine new locations, travel distance, carcass condition and infection status.

In addition, gut samples from salmon carcasses should be collected to determine infection status. A sub sample of carcasses would be sampled to determine proportion of infected vs non-infected, specifically carcasses removed from the river to estimate the pulse flow effect on myxospore load. It is unclear if level of infection on individual carcasses can be measured.

Management Guidance 6: Iron Gate Hatchery Release Strategy

Objective: Minimize:

1) the perpetuation of *C. Shasta* in the Klamath River that may be caused by infected hatchery juveniles carcasses
2) the infection, and subsequent mortality, of IGH Chinook from *C. Shasta*
3) negative interactions between hatchery/natural fish in the Klamath River, while meeting the two objectives listed above

Description: Implement release strategies based on real-time conditions for Iron Gate Hatchery fall Chinook that are timed to minimize overlap with peak *C. Shasta* infection levels in the Klamath River, while minimizing negative interactions between hatchery and natural fish in the Klamath River. Such strategies could include a combination of the following:

1. Release more yearlings (typically in mid-November) and fewer fingerlings in the spring
   a. May need to increasing rearing capacity for yearlings by installing circular tanks at the Fall Creek facility or making infrastructure improvements at IGH.
2. Continue to increase fingerling releases during May, rather than during June, as has happened since 2013, to minimize overlap with high disease prevalence in the river.
   a. May require heating of water for incubation/early life stage rearing at the hatchery so more fingerlings reach minimum size requirements for release during May, especially for eggs taken late in the spawning season.
   b. This should be balanced with consideration to minimize competition with natural rearing fish.
3. Consider reduction of fingerling releases relative to natural run size, especially during times that disease rates are expected to be high (e.g. drought conditions).

Support: Iron Gate Hatchery (IGH) has a production goal to release 6,000,000 juvenile Chinook salmon annually. This production goal includes the release of 5.1 million fingerlings at 90 fish per pound (fpp) between May 1 and June 15 and the release of 900,000 yearlings at 10 fpp between October 15 and November 20 (HSRG 2012). Fish are released when two of the following criteria are met (HSRG 2012):

- Achieve target release size (fingerlings at 90 fpp, or yearlings at 10 fpp)
- Release period (May 1 to June 15 (fingerlings). October 15 to November 20 (yearlings)
- River temperatures less than 65°F

Prior to 2012, fingerling releases occurred during the month of June (personal communication and data from Keith Pomeroy, IGH Manager), occasionally not until the third week of June. Since 2013, the hatchery began releasing fingerlings during May, annually averaging a release of 65% of the fingerlings during May. The remaining annual average 35% of fingerling releases has extended through mid-June. Table 1 shows the proportion of fish released during May vs. June since 2013.

Release Year	% Fingerlings May	% Fingerlings June
2016	46%	54%
2015	68%	32%
2014	62%	38%
2013	82%	18%
% of Total Release	65%	35%

There is considerable inter-annual variation in the timing of the peak POI rate of juvenile Chinook salmon in the reach of the Klamath River from the Shasta to Scott River (Fish Infection Memo, Figure 5). However, as noted in the Fish Infection Memo, the release period for fingerlings from IGH is substantially later than when the majority of natural origin Chinook fingerlings have emigrated from the upper river, and generally aligns with the highest weekly POI estimates for each year. Summaries of the weekly POI samples over the hatchery outmigration period suggest that a high proportion of the IG hatchery stock may become infected with *C. shasta*.

It is worth noting that relatively low levels of infection by *C. Shasta* have been detected in IGH fall Chinook fingerlings prior to release. Estimated infection rates ranged from 0% to 18% from 2011 – 2015 (average = 6.5%).

Substantial mortality of IGH fingerlings from *C. shasta* is a concern in regard to the loss of mitigation production for the benefit of fisheries. However, another substantial concern is that IGH fingerlings that die from *C. shasta* may perpetuate the life cycle of *C. shasta* in the Klamath River. Just as adult carcasses infected with *C. shasta* release myxospores that infect polychaetes, juvenile carcasses also release myxospores that can infect polychaetes. These infected polychaetes may then release actinospores that infect adult Chinook while migrating up the Klamath River en-route to spawning grounds. The actinospores within the adult salmon may then develop into myxospores, thereby increasing the magnitude of myxospores released by rotting adult salmon carcasses on the spawning grounds.

As summarized in the Fish Infection Memo, True et al. (2012) noted the development of myxospores in both Chinook and Coho salmon at 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) also suggests the potential for juvenile 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.

Uncertainties:

- The timing in regard to thermal units and the infection of adults by actinospores initiated by the release of myxospores from juvenile carcasses has not been assessed (but could potentially be done with existing data).
- The number of hatchery fish that can be released per time period during May without having excessive impacts upon natural rearing fish is unclear.

Adaptive Management and Monitoring Considerations: The hypothesis inherent to this management guidance action is that disease prevalence and severity can be affected by altering release strategies from Iron Gate Hatchery. The percentage and health of hatchery fish should continue to be monitored as implementation of alternative release strategies occurs.

CONCLUSION

In 2014 and 2015 extremely high rates of *C. shasta* infection exceeded the thresholds established in the NMFS incidental take statement associated with the BiOp on the Bureau of Reclamation plan for operating the Klamath Project. These high rates of infection play a major role in the population declines of Klamath River salmonids, including ESA listed SONC Coho Salmon.

A fruitful collaboration between USFWS and disease researchers yielded a series of technical memoranda that collectively describe the current understanding of the life cycle of the fish disease causing metazoan *C. shasta* and its response to changes in river flows at various times of year. From these memoranda, we have developed this guidance document to inform the federal agencies responsible for water management and ESA compliance in the Klamath Basin on what specific measures should be taken to reduce *C. shasta* infection rates.

Although much of this effort has been couched as necessary to meet agency obligations pursuant to the Endangered Species Act, we note here that Agencies' have a further obligation as trustees of Tribal resources. This Tribal Trust obligation extends beyond ESA listed species such as coho salmon to fall and spring run Chinook salmon as well as steelhead trout, all of which suffer from *C. shasta* infections.

The technical memoranda developed by the DTAT clearly demonstrate that disease rates can best be controlled by disrupting the habitat of *M. speciosa* and diluting *C. shasta* spores with increased flows. Perfecting the magnitude, duration, and intervals of these increased flow releases will be achieved over time through adaptive management practices; however, Klamath fisheries are in dire need of measures to alleviate high disease rates immediately.

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Appendix A. – Summary of Summary of Important Facts From USFWS *C. shasta* Technical memos published in October 2016.

This is summarized information from each of the four technical memoranda prepared in support of identifying management guidance actions to address high disease levels of *C. shasta* in the mainstem Klamath River below Iron Gate Dam. Each memo had a bulleted list of important conclusions which are presented here for convenience.

Sediment Mobilization and Flow History in Klamath River below Iron Gate Dam (Geomorphic Memo)

- Environmental flows are developed by river managers to mitigate the detrimental impacts of dams and water diversions on river form and ecological functions. Environmental flow regimes designed to induce geomorphic changes are broadly divided into two categories, sediment maintenance or "flushing flows" used to modify substrate composition and channel maintenance flows intended to maintain channel form and floodplains.
- In developing environmental flow regimes, it is important to recognize conflicts in objectives and constraints on flow releases.
- There are three contemporary studies that estimated sediment transport thresholds in the Klamath River below Iron Gate Dam
- The sediment entrainment threshold estimates reported in the three studies varied due to differences in study methods employed and the dates when channel substrate and channel conditions were evaluated.
- Ayres Associates (1999) concluded that floods of approximately 10-year return period magnitude rejuvenate the Klamath River channel by reworking gravels on riffles, eroding channel banks, re widening the channel, and removing substantial amounts of aquatic vegetation in the reach between Iron Gate Dam and the Scott River.
- The 1.5-, 2-, and 10-year return period for the Klamath River below Iron Gate Dam was estimated by Reclamation (2011) to be 4,389, 6,030, and 15,610 cfs, respectively.
- The 10-year return period of 15,610 cfs reported by Reclamation (2011) is consistent with the findings of Holmquist-Johnson and Milhous (2010) who estimated the threshold for general Armor Layer Movement to be 15,000 cfs and that of Ayres Associates (1999) who estimated the gravel mobilization on riffles to occur between 9,800 and 16,500 cfs for the reach between Iron Gate Dam and Seiad Valley.
- We classify discharges that exceed 15,000 cfs in the Klamath River below Iron Gate Dam as geomorphically effective flows, which are occasional high flows required to maintain channel form and reduce riparian encroachment.
- Other important considerations in flow include ramping rates, timing, duration, and monitoring and calibration.
- From 1964 to 1999, the average cumulative duration of Surface Flushing flows exceeded 22 days per water year. From 2000 to 2016, the average cumulative duration of Surface Flushing flow exceeded five days in only one water year and no sediment mobilization flows occurred in 12 of the 17 water years.

- At flow releases less than 2,500 cfs below Iron Gate Dam, Immobile Bed conditions exist that allow suspended sediments to settle and accumulate on the bed, which are not resuspended until flows that generate Surface Flushing occur.
- Growth of fine sediment deposits on the bed and channel margins is a concern because high densities of M. speciosa have been observed in such deposits. In addition, riparian and aquatic vegetation can colonize fine sediments, further narrowing the channel and degrading fish habitat conditions such as what has been documented on the Trinity River.

Polychaete Distribution and Infection (Polychaete Memo)

- The polychaete M. speciosa has been documented as the obligate intermediate host for the parasite *C. shasta*.
- Polychaetes in the Klamath River have been documented to be sessile suspension feeders and may also have flexibility to feed on organic matter in deposited sediments.
- Polychaete reproduction typically peaks in spring to early summer, coinciding with increasing water temperatures.
- Studies indicate that dislodgement of M. speciosa increases with increasing water velocities and decreasing substrate stability.
- Results of 2-D hydrodynamic model runs predict decreasing WUA (weighted usable area) of suitable polychaete habitat with increasing discharge.
- Results of repeat samples at specific locations in the Klamath River indicate a decrease in M. speciosa following the March 2016 peak discharge event of 11,200 cfs.
- Evidence suggests that the prevalence of *C. shasta* infection in polychaetes is negatively correlated with the peak flow regime.
- Preliminary results indicate that infected polychaetes are more likely to occur within a smaller range of peak-flow depths and velocities than the general population, with infected polychaetes more associated with deeper and lower-velocity depositional habitats.

Ceratonova shasta Waterborne Spore Stages (Spore Memo)

- A DNA assay allows for the quantification of spore concentrations in water samples.
- Spore genotypes have been shown to associate with salmonid species-specific mortality.
- Myxospores are hardier than actinospores, and likely survive for a longer period after release from their host organism.
- The majority of myxospore load to the system is likely via adult salmon carcasses in the fall.
- Generally, actinospore spore concentrations increase with increasing water temperatures in the spring and then decrease as water temperatures further increase during summer.
- The location of peak actinospore concentrations varies among years, but most frequently occurs near the confluence of Beaver Creek.
- It is not uncommon for actinospore concentrations to peak as far downriver as the Tully Creek confluence. However, the magnitude of the difference between the peak spore concentrations downriver and at Beaver Creek was much higher in 2016 than for any other year since water monitoring began.
- Annual peak actinospore concentrations vary by several orders of magnitude.
- Actinospore development within polychaetes is likely a function of accumulating thermal units, and likely takes between 100 and 115 days.

- Though managed discharge events have not produced dramatic reductions in spore concentrations, the planned discharge increases were likely too small to be biologically effective. An unplanned discharge increase in 2005 likely demonstrates the potential for larger discharges to effectively reduce spore concentrations.

Prevalence of *C. Shasta* Infections in Juvenile and Adult Salmonids (Fish Infection Memo)

- 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.

Appendices B, C, D, E, F:

B. Geomorphic Memo
C. Polychaete Memo
D. Spore Memo
E. Fish Infection Memo
F. Comments and response to comments.

United States Department of the Interior

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In Reply Refer To:
AFWO

Technical Memorandum

TO: Dave Hillemeier, Yurok Tribal Fisheries, and
Craig Tucker, Karuk Department of Natural Resources

FROM: Conor Shea, Nicholas J. Hetrick, and Nicholas A. Som, Arcata Fish and Wildlife Office

SUBJECT: Response to Request for Technical Assistance – Sediment Mobilization and Flow History in Klamath River below Iron Gate Dam

DATE: September 29, 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) sediment mobilization review and streamflow history for the Klamath River below Iron Gate Dam, 2) polychaete distribution and infections, 3) actinospore and myxospore concentrations, and 4) prevalence of *C. shasta* infections in juvenile and adult salmonids, are 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. The focus of this technical memorandum is to summarize the state of knowledge regarding environmental flow releases from the Iron Gate Dam to achieve specific objectives for channel form and ecological function. Other memorandums in this series will address how achieving these objectives will potentially influence various aspects of the *C. shasta* life cycle and population.

In this technical memorandum, we first summarize the state of knowledge regarding environmental flows to achieve specific objectives for channel form and ecological function. Then, the memorandum reviews estimates of flows necessary for achieving several channel substrate movement states in the Klamath River below Iron Gate Dam that were developed by three different research teams. The final section of the memorandum examines the frequency, magnitude, and duration of sediment mobilization flows for the Klamath River below Iron Gate Dam that have occurred since dam construction.

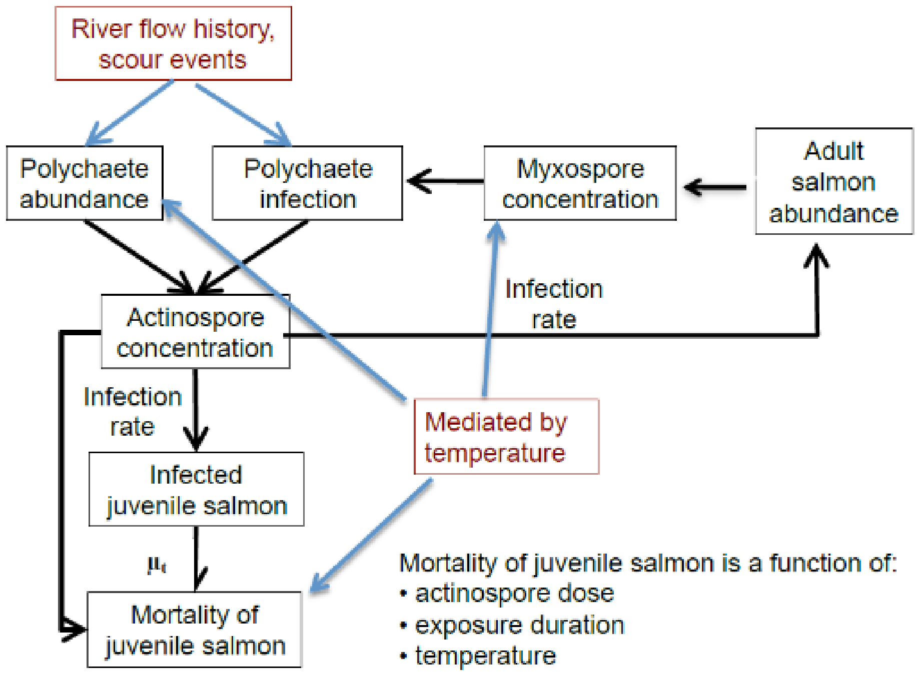

Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon, with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).

Environmental Flows. The physical and ecological responses of a river to construction of a dam or diversion of flow have been recognized for many years (Rathburn et al. 2009). A river's planform and cross section are formed in response to the flow that the river receives, the character and rate of sediment supplied to and transported by the river, and the characteristics of the vegetation, sediment, and substrate comprising the channel through which the river flows (Leopold, 1994). Similarly, a river's ecosystem is regulated and maintained by the temporal distribution, duration, and magnitude of floods and low flows (Karr 1991). The construction of dams and/or the creation of water diversions alter the natural hydrologic and geomorphic processes that maintain river form and habitat for aquatic and riparian species. Physical responses to dam construction or diversions can include downstream channel erosion and coarsening of bed substrate due to reduced sediment supplies, deposition of sediment on the bed due to reduced transport capacities, decreases in channel width and depth, and floodplain disconnection due to reduced magnitude and frequency of high flows (Kondolf and Wilcock, 1996, Williams and Wolman, 1984). Alterations to the flow regime likewise change the timing and movement of sediment, biological materials, and energy within rivers and between rivers and their floodplains, which disrupts the life cycles of riparian and aquatic species adapted to an undisturbed regime (Poff et al. 1997).

Definition. Environmental flows are developed by river managers to mitigate the detrimental impacts of dams and water diversions on river form and ecological functions. The term *environmental flow* as used in this memorandum is defined as the water regime in a river implemented to maintain geomorphic form, riparian and aquatic ecosystems, and their related benefits where flows are regulated. Environmental flows mimic components of natural flow variability including the magnitude, frequency, duration, timing, and sequencing of both high and low flow events (Arthington et al. 2006). Environmental flow regimes can include diverse components designed to meet specific physical objectives such as maintaining aquatic habitat; removal of accumulated fine sediments; maintaining sediment balance, remobilization of gravels and formation of bars, scouring of vegetation, overtopping riverbanks with flow and sediment to augment floodplain development (Whiting 2002).

Environmental flow regimes designed to induce geomorphic changes can be broadly divided into two categories: (1) *sediment maintenance flows* (also commonly called *flushing flows*) that are made with the objective of removing sediment from a channel or otherwise modifying substrate composition; and (2) *channel maintenance flows* which are flow regimes intended to maintain channel form and floodplains (Kondolf and Wilcock 1996).

Identifying Physical Outcomes. River restoration activities often have poorly defined goals and fail to specify desired project outcomes (Bernhardt et al. 2005). Similarly, environmental flow releases are often made without a clear statement of desired physical outcomes and with insufficient consideration of the physical changes that a particular environmental flow regime will create (Kondolf and Wilcock 1996). To be effective, environmental flow objectives need to be specified in terms of desired physical responses. Table 1 presents a list of common geomorphic goals with corresponding physical objectives and required environmental flow parameters.

Development of flow releases from Iron Gate Dam that are intended to adversely impact the *C. shasta* life cycle by targeting the disruption of the obligate invertebrate host as suggested by Alexander et al. (2016) should identify specific physical objectives. The specification should identify the desired form of bed modifications (e.g., sand mobilization or gravel mobilization) and the extent of the mobilization (e.g., from riffles, from channel margins, from pools, etc.). The frequency and seasonal timing of environmental flows should also be specified. Seasonal timing should be based on biological objectives and constraints. Seasonal timing might also be based on physical objectives such as sequencing flows to occur simultaneously or following unregulated tributary peak flows.

In developing environmental flow regimes, it is important to recognize conflicts in objectives and constraints on flow releases. Wilcock et al. (1996b) describes the conflict in developing flushing flow recommendations for the Trinity River below the Lewiston Dam. Optimizing the removal of fine sediment from channel would result in loss of supply limited gravel. Flushing flows were set to balance competing objectives. Constraints on flow releases may involve limits in the water available for flow due to drought or competing uses, concerns over lost power generation, and undesirable flooding or channel adjustments for downstream landowners.

Table 1. Flushing flow and channel maintenance flow goals, objectives, and requirements (adapted from Kondolf and Wilcock 1996).

Flow Regime	***Management Goal***	***Specific Objective***	***Flow Requirement***
Flushing Flow	Restore riffle habitat	Remove surficial fine sediment[1] from riffles	Generate shear stress (τ_0) sufficient to transport sand particles on riffles
		Remove interstitial fine sediment	Generate shear stress (τ_0) sufficient to entrain surface gravels
	Improve spawning gravels	Increase gravel porosity (i.e. loosen gravel)	Generate shear stress (τ_0) sufficient to entrain surface gravels
	Improve pool habitat	Scour accumulated fine sediments	Transport net sand out of pools
Channel Maintenance Flow	Maintain/Restore Channel Width, Depth and Topographic Diversity	Mobilize surface gravel layer throughout cross section	New projects [2]: Release flow equivalent to the pre-project effective (channel forming) discharge
			Old projects: Generate shear stress (τ_0) sufficient to entrain on bar surfaces
	Reduce riparian encroachment	Uproot seedlings on bar surfaces	Generate shear stress (τ_0) sufficient to entrain gravel on bar surfaces
		Remove established vegetation	May require large flow on order of 10-20 year return period
	Create/build floodplain habitat	Create vertical accretion on floodplains	Produce muddy over-bank flow (requires source of suspended sediment)
	Create diverse multiage riparian habitat	Induce channel migration and create diverse geomorphic surfaces	Flow sufficient to erode banks, deposit point bars, and create overbank deposits

Notes:
(1) Fine sediment refers to sediment where the particle diameter along the intermediate axis is less than or equal to 2 mm. Coarse sediment refers to sediment where the particle diameter along the intermediate axis is greater than 2 mm.
(2) The term new projects refers to new dams or diversions where the river retains its original form. The term old projects refers to locations where the river has undergone long-term adjustments in form in response to a dam or diversion.

Analysis Methods. Kondolf and Wilcock (1996) characterize three methods for estimating flushing flows and channel maintenance flows:

- Self-adjusted channel methods employ the assumption that the flushing flows should mimic the pre-project effective discharge (e.g., Andrews and Nankervis, 1995). Use of self-adjusted channel methods requires the assumption that the river was previously in an equilibrium condition.

- Sediment entrainment methods employ sediment transport relationships to estimate the thresholds for sand and gravel entrainment. Local observations of stream sediment and hydraulic properties are used to develop estimates. Use of these methods does not require the assumption that the river is in an equilibrium condition.

- Direct calibration methods require extensive monitoring during pilot environmental flow releases. Observations are made of flow velocity, total discharge, bed movement and sediment transport for flow events that mobilize sediment. Direct calibration methods allow for estimates of volume of sand and gravel that are mobilized (Wilcock et al. 1996a), which are critical to developing a balanced sediment regime (Wohl et al. 2015; Schmidt and Wilcock 2008).

The Role of Adaptive Management. Environmental flows should be implemented within an adaptive management framework (e.g., see Williams and Brown 2012). Projecting the responses of environmental systems to management actions often involves uncertainties. Developing environmental flows targeted at disrupting M. speciosa will involve uncertainties in the biological response, sediment transport relationships, and meteorological and channel conditions prior to releases. Development of environmental flows should be seen as an iterative process of developing flow regimes, implementing and monitoring the environmental flow, followed by assessment of sediment transport, biological response, and sediment storage on a reach by reach basis in downstream areas.

Klamath River Sediment Entrainment Analyses. There are three recent studies that developed estimates of sediment transport thresholds in the Klamath River below Iron Gate Dam (Ayres Associates, 1999; Holmquist-Johnson and Milhous, 2010; and Reclamation, 2011). These studies employ uncalibrated sediment transport relationships to develop estimates of discharge required to initiate various stages of sediment mobilization. Local observations of stream sediment and hydraulic properties are used to develop the estimates.

Although terminology and methods differs slightly between the three studies, each of the studies characterizes the channel substrate as consisting of several sediment layers:

- a mobile surface layer of fine sediment (sand, silt, and clay sized particles having median grain diameters less than 2 mm);
- an armor layer consisting of sorted coarse sediment (gravel, cobbles and boulders having median grain diameters greater than 2 mm) 1-2 grain diameters in thickness; and
- an underlying substrate layer, less coarse than the armor layer, and containing a mixture of coarse and fine sediment.

The actual composition of the channel substrate material varies with location relative to dams and tributaries. There are areas directly below Iron Gate Dam, which have reduced coarse grain material input due to sediment trapping behind the dam, where the armor layer has been winnowed out and a pavement layer has developed that consists of large coarse sediment (cobbles and boulders) that are several grain diameters in thickness. In other areas where fine sediment supplies are high and entrainment flows low, the armor layer has infilled with fine sediment.

The three studies develop estimates of the discharge required to achieve several sediment mobilization states. Again, terminology differs slightly between the three studies. In this memorandum, we employ the terms listed in Table 2 to describe differing degrees of sediment mobilization. The discharges ranges established by the three studies for the sediment mobilization states should be understood to be approximate values and that transitions between sediment mobilization states occur gradually, not with sudden jumps when a threshold value is exceeded.

Table 2. Sediment mobilization state definitions.

Immobile Bed	No movement of surface sediment, armor layer or substrate. Deposition of suspended sediment absorbed into voids (until full).
Stable Bed	No movement of surface sediment, armor layer or substrate. Suspended sediment in water column remains in transport.
Surface Flushing	Movement of surface fine layers on 20-30% of bed.
Depth Flushing	Removal of in-filled fine sediment from armor layer.
Armor Disturbance	Movement of individual armor layer particles.
Armor Layer Movement	Reworking of armor and substrate layers

Ayres Associates (1999). Ayres Associates prepared a geomorphic and sediment evaluation of the Klamath River from Iron Gate Dam to the ocean for the U.S. Fish and Wildlife Service (Ayres Associates, 1999). Much of the study report covers the geomorphic assessment of the river and the report provides substantive descriptions of the geomorphic controls shaping the river.

Ayres Associates (1999) conducted their field work for the report in 1997. In the period from fall 1992 to spring 1997, there were six flow events in which the daily-mean flows at Iron Gate Dam exceeded 6,030 cubic feet per second (cfs), which Reclamation (2011) estimates is the two-year return period discharge for the Klamath River below Iron Gate Dam. Daily-mean flow reached 18,500 cfs on January 1, 1997, the second highest flow since dam closure. Ayres Associates (1999) observed that neither aggradation nor channel degradation (downcutting) was apparent in the reach of the Klamath River between Iron Gate Dam and the Shasta River. They also observed that pools did not appear to be infilling with sediment and that there was minimal infilling of coarse bed substrate with fine sediment.

Ayres Associates (1999) surveyed a series of cross sections at each of six study sites. Three of the study sites were located between Iron Gate Dam and Seiad Creek. Ayres Associates used the cross sections and water surface elevation measurements to develop and calibrate 1-D hydraulic models of the study sites using Version 2.0 of the U.S. Army Corps of Engineers HEC-RAS computer program.

Ayres Associates (1999) conducted an incipient motion analysis using the hydraulic analysis to determine critical shear stress and critical discharge necessary to initiate movement of sediment from riffles and pools. The incipient motion analysis used the Shields' relationship:

$$\tau_c = \theta(\gamma_s - \gamma) D_c \quad (1)$$

where:

τ_c	is the critical shear stress required to initiate sediment transport
θ	is the Shield's parameter
D_c	is the representative sediment size
γ	is the specific weight of water
γ_s	is the specific weight of sediment

Ayres Associates (1999) set the value of D_c to the value of the median grain size (D_{50}) found on pools and riffles during site investigations. Ayres Associates (1999) set the value of the Shield's parameter to 0.047 for fine sediment and 0.035 for coarse sediment.

Ayres Associates (1999) used the U.S. Army Corp of Engineers HEC-RAS model to determine the critical discharge (Q_c) that would generate the critical shear stress calculated in equation (1) to mobilize sediment from pools and from riffles at the six study sites. Boundary shear stress was calculated using:

$$\tau_o = \frac{\rho V^2}{\left[5.75 \log\left[12.27 \frac{y_o}{k_s}\right]\right]} \quad (2)$$

where:

τ_o	is the cross section average hydraulic shear stress on the bed
ρ	is water density
V	is the cross section average flow velocity
y_o	is the cross section average flow depth
k_s	is the equivalent roughness height of the substrate

Ayres Associates (1999) set the equivalent roughness height to $3.5*D_{84}$ for coarse sediment (surface $D_{50} > 2$ mm) and to D_{84} for fine sediment (surface $D_{50} < 2$ mm). They identified the critical discharge (Q_c) required to initiate sediment motion as the mean discharge where the boundary shear stress equaled the critical shear stress ($\tau_0 = \tau_c$).

In addition to the incipient motion analysis for pools and for riffles, Ayres Associates conducted a flushing flow analysis to estimate the discharge required to flush surface sediment from pools. They assumed that the D_{84} of pool sediment was 2 mm and assumed a shear stress of twice the critical shear stress for the D_{84} would result in pool flushing. Their assumption for pool flushing flows was based on previous experience.

Results of the Ayres Associates analysis are only shown for the three most upstream sites (Sites 4 – River Mile[1] (RM) 128), 5 –RM 161, and 6 - RM 187) because the influence of Iron Gate Dam (RM 190) flows on bed conditions diminishes moving downstream from Iron Gate Dam as tributary accretions increase total flow (Table 3).

USGS – Holmquist-Johnson and Milhous (2010). The U.S. Geological Survey (USGS) conducted a study for the U.S. Fish and Wildlife Service to determine flushing flows required to improve and maintain quality spawning and rearing habitats for salmon, and to reduce the abundance of preferred habitats of *M. speciosa* (Holmquist-Johnson and Milhous, 2010). Field work for the Holmquist-Johnson and Milhous (2010) study was conducted in 2007. Just prior to data collection, three flow events occurred in 2006 where daily-mean flows at Iron Gate Dam exceeded 6,030 cfs (the two-year return period flow), but daily-mean flows in the previous six years (2000 – 2005) did not exceed 6,030 cfs.

Holmquist-Johnson and Milhous (2010) resampled sediment at the six study sites established by Ayres Associates (1999). Holmquist-Johnson and Milhous (2010) segregated samples into surface sediment, armor layer, and substrate (under the armor layer). The surface sediment was composed of silt-, clay- and sand-size sediment (i.e., fine sediment). The armor layer was composed of gravel-, cobble-, and boulder-size sediment (i.e., coarse sediment). The substrate was composed of a mix of sand-, gravel-, and cobble-size sediment.

Table 3. Ayres Associates (1999) incipient motion analysis results.

Incipient Motion Condition	Sediment Mobilization State (from Table 2)	Quantity	Site 4 RM 128	Site 5 RM 161	Site 6 RM 187
Pools Incipient Motion	Stable Bed	D_{50} – Median Grain Diameter (mm)	0.50	0.070	1.00
		τ_c – Critical Shear Stress (lbs/ft^2)	.00794	0.01100	0.0159
		Q_c – Critical Discharge (cfs)	2,300	2,600	2,500
Pool Flushing Flow	Surface Flushing	Q_c – Critical Discharge (cfs)	6,600	6,000	5,400
Riffles Incipient Motion	Armor Disturbance	D_{50} – Median Grain Diameter (mm)	86	86	86
		τ_c – Critical Shear Stress (lbs/ft^2)	1.01	1.01	1.01
		Q_c – Critical Discharge (cfs)	9,800	13,200	16,500

[1] Positions on the Klamath River are referenced by the distance in river miles measured from the river mouth and as shown on U.S. Geological Survey topographic maps.

Holmquist-Johnson and Milhous (2010) developed sediment entrainment discharge estimates using methods described by Milhous (1998). They defined four sediment mobilization states: Immobile Bed, Stable Bed, Surface Flushing, and Depth Flushing (Table 4). They estimated the sediment mobilization state for individual site conditions and discharges using the movement parameter β calculated using equation 3:

$$\beta = \frac{n^2 v^2}{1.492^2 d^{1/3}(G-1)D_{50}} = \frac{RS_e}{D_{50}(G-1)} \quad (3)$$

where:

β	is the dimensionless shear stress (movement parameter)
n	is Manning's n roughness coefficient
v	is cross section average velocity (ft/sec)
d	is cross section average depth (ft)
G	is the specific gravity of sediment (taken as 2.65)
D_{50}	is the median grain size (feet) of the bed armor later
R	is the hydraulic radius in feet
S_e	is the energy slope

The movement parameter (β) has the form of dimensionless shear stress and is analogous to the Shield's relationship. Increasing values of β imply increasing levels of shear stress applied to the river bed.

Sediment mobility states were then related to ranges of the movement parameter β (Table 4). The relationship between values of the movement parameter (β) and sediment mobility states are the same as employed in Milhous (1998). Values used to define sediment mobility states in Milhous (1998) are based on data collected at Oak Creek, Oregon in the early 1970's.

Holmquist-Johnson and Milhous (2010) employed the U.S. Army Corps of Engineers HEC-RAS hydraulic models developed by Ayres Associates (1999) and the sediment data they collected in 1997 to calculate movement parameter (β) values for discharges ranging from 1,000 to 50,000 cfs at each of the six Ayres Associates (1999) study sites. They fit a linear relation between movement parameter (β) and discharge at the Klamath River below Iron Gate Dam to estimate upper and lower discharge limits for Immobile Bed, Stable Bed, Surface Flushing, and estimate a lower discharge limit for Armor Disturbance.

Holmquist-Johnson and Milhous (2010) left a gap in their table between the upper limit of Surface Flushing (β = 0.035) and the initiation of Armor Disturbance (β = 0.045). Milhous (1998) defines Depth Flushing as the removal of fine material from within the substrate without Armor Disturbance and defines the value of the movement parameter (β) required to initiate Depth Flushing as 0.035. We adapted the Milhous (1998) study to set the range of the movement parameter (β) for Depth Flushing as 0.035 to 0.045.

Holmquist-Johnson and Milhous (2010) also computed the ratio between bed shear stress and critical shear stress required to initiate general movement of the armor layer for the range of discharges at the six study sites. They defined general movement of the armor layer as occurring when the boundary shear stress (τ_0) exceeds the critical shear stress for incipient motion of the armor layer (τ_c). Results of the Holmquist-Johnson and Milhous (2010) study found that the average critical discharge at Ayres Associates (1999) sites 4 (RM 128), 5 (RM 161), and 6 (RM

Table 4. Substrate movement state, movement parameter, and discharge limits for Klamath River below Iron Gate Dam (adapted from Holmquist-Johnson and Milhous 2010).

Substrate Movement State	Substrate Movement State Description	Movement Parameter β		Discharge (cfs)	
		Lower Limit	Upper Limit	Lower Limit	Upper Limit
Immobile Bed	No movement of surface sediment, armor layer or substrate. Deposition of suspended sediment absorbed into voids (until full).	0.000	0.009	0	2,500
Stable Bed	No movement of surface sediment, armor layer or substrate. Suspended sediment in water column remains in transport.	0.009	0.021	2,500	5,000
Surface Flushing	Movement of surface fine layers on 20-30% of bed.	0.021	0.035	5,000	8,700
Depth Flushing	Removal of in-filled fine sediment from armor layer.	0.035	0.045	8,700	11,250
Armor Disturbance	Movement of individual armor layer particles.	0.045	----	11,250	15,000
Armor Layer Movement	Reworking of armor and substrate layers ($\tau_0 > \tau_c$ of armor layer)	----	----	15,000	

187, the point at which $\tau_{0=}\tau_c$) was 15,000 cfs. Note that the average is based on ratio values of $\tau_{0/}\tau_c$ that ranged between approximately 0.65 and 1.45.

We combined the Holmquist-Johnson and Milhous (2010) estimates for discharges thresholds for Immobile Bed, Stable Bed, Surface Flushing, and Armor Disturbance with the estimates for discharge required to produce Depth Flushing (Milhous 1998), and with estimates for Armor Layer Movement to develop estimates for six sediment mobilization states for the Klamath River below Iron Gate Dam (Table 4).

Reclamation (2011). The U.S. Bureau of Reclamation evaluated sediment mobilization below Iron Gate Dam (Reclamation, 2011) as one component of numerous studies conducted to support the Secretarial determination process for the Klamath Basin Restoration Agreement and Klamath Hydroelectric Settlement Agreement (Department of Interior et al. 2013).

Reclamation (2011) developed a U.S. Army Corps of Engineers HEC-RAS model of existing conditions for the reach of the Klamath River from Iron Gate Dam (RM 190) to Happy Camp (RM 105). Model geometry was based on bathymetric surveys conducted in 2009 supplemented by LIDAR surveys conducted in 2010. Reclamation used the survey data to develop a triangulated irregular network (TIN) terrain model. Reclamation (2011) extracted 692 HEC-RAS cross sections from the TIN to develop a hydraulic model of existing conditions. Reclamation (2011) calibrated the HEC-RAS model using observed water surface elevation data and to gage data from Iron Gate Dam and at Seiad Valley (USGS 11516530, 11520500).

Reclamation (2011) analyzed stream gage records to develop flood frequency relationships for the USGS gages on the Klamath River below Iron Gate Dam (RM 190) and the Klamath River near Seiad Valley (RM 128) (Table 5).

Reclamation (2011) defined sediment mobilization states as follows:

- Under *Slight Mobilization*, there is a small, but measurable, sediment transport rate. The armor layer is only minimally disturbed and there maybe flushing of sand to a depth of the armor layer D_{90}.
- Under *Significant Mobilization,* there are many particles in motion and there is a significant sediment transport rate. Sand is mobilized from the interstitial spaces of the bed to a depth of twice the D_{90}. The armor layer is significantly disturbed.

Table 5: Flood frequency analysis for Klamath River below Iron Gate Dam (USGS Gage 11516530) and near Seiad Valley (USGS Gage 11520500 (Source: Reclamation, 2011).

Gaging Station		Klamath River below Iron Gate Dam	Klamath River near Seiad Valley
River Mile		190	128
Period of Record Used in Analysis		1961- 2009	1913 -1925 1952 -2009
Drainage Area (mi^2)		4,630	6,940
Discharge (cfs)	Median Flow	1,370	2,700
	Average Flood	7,978	28,569
	1.5 year return period	4,380	11,000
	2-year return period	6,030	17,600
	5-year return period	10,980	39,960
	10-year return period	15,610	56,540
	25-year return period	21,460	93,400
	50-year return period	26,280	131,000
	100-year return period	31,460	179,300

Reclamation (2011) developed a methodology to estimate the discharge required to generate *Slight Mobilization* and *Significant Mobilization.* Reclamation (2011) used hydraulic data from the HEC-RAS model and sediment data from their surveys to compute the Shield's parameter using equation (4), which is a re-arranged form of the Shield's relationship shown in equation (1):

$$\theta = \frac{\tau_g}{(\gamma_s - \gamma) D_{50}} \quad (4)$$

where:

τ_g is the grain shear stress

Reclamation (2011) computed the grain shear stress using results from the hydraulic modeling and methods that are detailed in Appendix J of their report.

Reclamation (2011) used the Parker Reference Transport method (Parker, 1990) to evaluate sediment mobility:

$$W^* = \frac{(s-1) g q_q}{\rho_s \left(\tau_g / \rho \right)^{1.5}} \quad (5)$$

where:

- s is the relative specific density
- q_s is the sediment transport rate
- τ_g is the grain shear stress
- ρ_s is the sediment density
- ρ is the fluid density
- W^* is the dimensionless sediment transport rate.

The Parker (1990) method replaced incipient motion with a small, but measurable transport rate, where $W^* = 0.002$. The Shield's number that yields $W^* = 0.002$ is called the reference Shield's stress (θ_r).

Reclamation (2011) characterized *Slight Mobilization* as occurring when hydraulic conditions produced a Shield's parameter equivalent to the reference Shield's stress ($\theta = \theta_r$). Reclamation characterized *Significant Mobilization* as occurring when hydraulic conditions produced a Shield's parameter equivalent to 1.3 times the reference Shield's stress ($\theta = 1.3\theta_r$).

The value of 1.3 is equivalent to the ratio between the Holmquist-Johnson and Milhous (2010) movement parameter value of 0.045 (Armor Disturbance) and 0.035 (Surface Flushing). Thus, Reclamation's (2011) slight mobilization is equivalent to the Holmquist-Johnson and Milhous (2010) Surface Flushing and Reclamation's (2011) significant mobilization is equivalent to the Holmquist-Johnson and Milhous (2010) Armor Disturbance. Reclamation (2011) notes importantly that sediment transport increases as a continuous function, not a step function. There is a continuum of sediment transport movement between conditions where $\theta=\theta_r$ and $\theta=1.3\theta_r$ rather than an abrupt change in transport states.

Combining the HEC-RAS results with measured substrate characteristics, Reclamation (2011) developed estimates of the range of discharges required to achieve *Slight Mobilization* and *Significant Mobilization* in nine reaches of the Klamath River below Iron Gate Dam. Reclamation (2011) allowed for uncertainties in the value of the reference shear stress, creating a spread in discharge estimates. The median mobilization flow estimates are shown in Table 6. Reclamation (2011) related the discharge to return period using their frequency analysis (Table 5). Figures 2 and 3 replicates Reclamation (2011) figures showing the discharge and return periods estimates with error bars for initiating *Slight Mobilization* and *Significant Mobilization.*

Reclamation (2011) flow estimates for significant and slight mobilization vary considerably between the nine reaches defined in the study. The median flow estimate of 19,100 and 20,000 cfs required to produce slight mobilization in the reaches from Shasta River to Beaver Creek far exceed the median flow estimates for significant bed material mobilization from Bogus Creek to the Shasta River. The much higher flow required to initiate sediment mobilization between Shasta River and Beaver Creek might be undesirable because they would transport gravels out of the reaches located directly below the dam that are sediment starved due to trapping of sediment upstream of Iron Gate Dam. Development of environmental flow objectives should account for differences in geomorphic controls and sediment transport capabilities between reaches.

Reclamation (2011) states that the sediment entrainment analysis is not sufficient to predict the fraction of sand remaining after an environmental flow event. Such predictions would require more information about the surface and subsurface sand fractions as well as the sand supply in the reach and would require simulation of the sand budget and bed mixing during the event. Reclamation (2011) suggests that future studies of mobilization could be done to quantify the flows necessary to accomplish a certain level of sand mobilization in the Klamath River.

Table 6. Median discharges for slight and significant mobilization for Klamath River between Bogus Creek and Indian Creek (adapted from Reclamation 2011).

Reach	Slight Bed Material Mobilization Median Flow Estimate (cfs)	Significant Bed Material Mobilization Median Flow Estimate (cfs)
Bogus Creek (RM 189.6) to Willow Creek (RM 185.0)	9,800	15,900
Willow Creek (RM 185.0) to Cottonwood Creek (RM 182.1)	10,700	17,200
Cottonwood Creek (RM 182.1) to Shasta River (RM 176.7)	8,400	13,800
Shasta River (RM 176.7) to Humbug Creek (RM 171.5)	20,000	33,900
Humbug Creek (RM 171.5) to Beaver Creek (RM 161.0)	19,100	32,900
Beaver Creek (RM 161.0) to Dona Creek (RM 152.8)	5,800	10,100
Dona Creek (RM 152.8) to Horse Creek (RM 147.3)	5,900	9,700
Horse Creek (RM 147.3) to Scott River (RM 143.0)	6,500	10,400
Scott River (RM 143.0) to Indian Creek (RM 106.8)	15,300	25,500

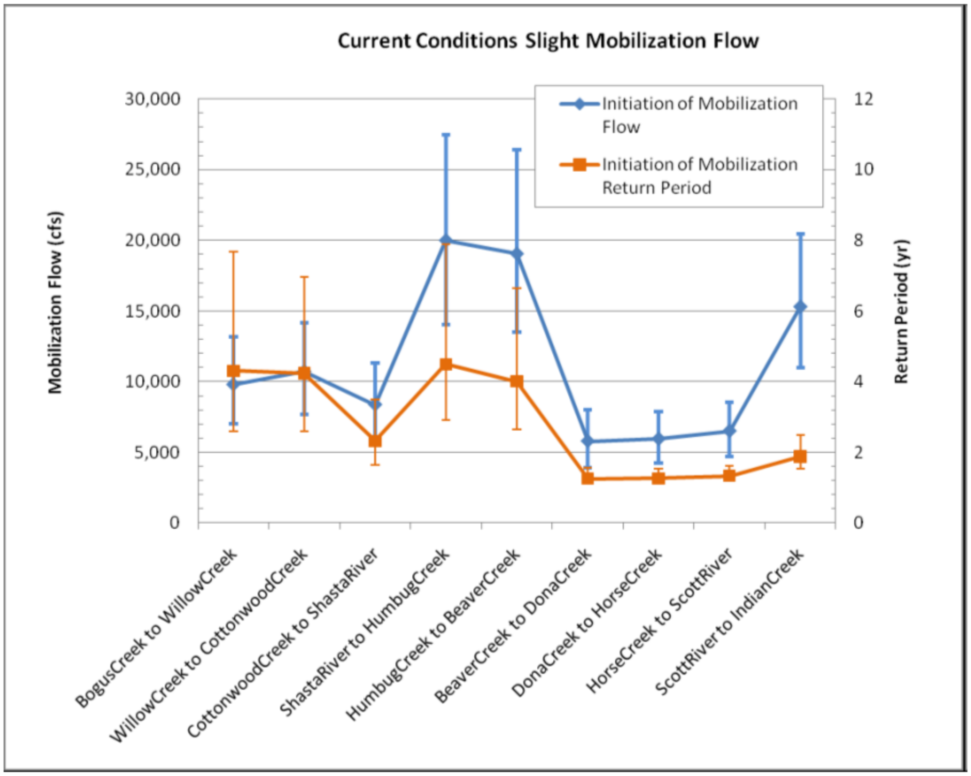

Figure 2: Slight bed material mobilization flow and return period for reaches downstream of Iron Gate Dam. (Reproduced from Figure 5-24, Reclamation, 2011).

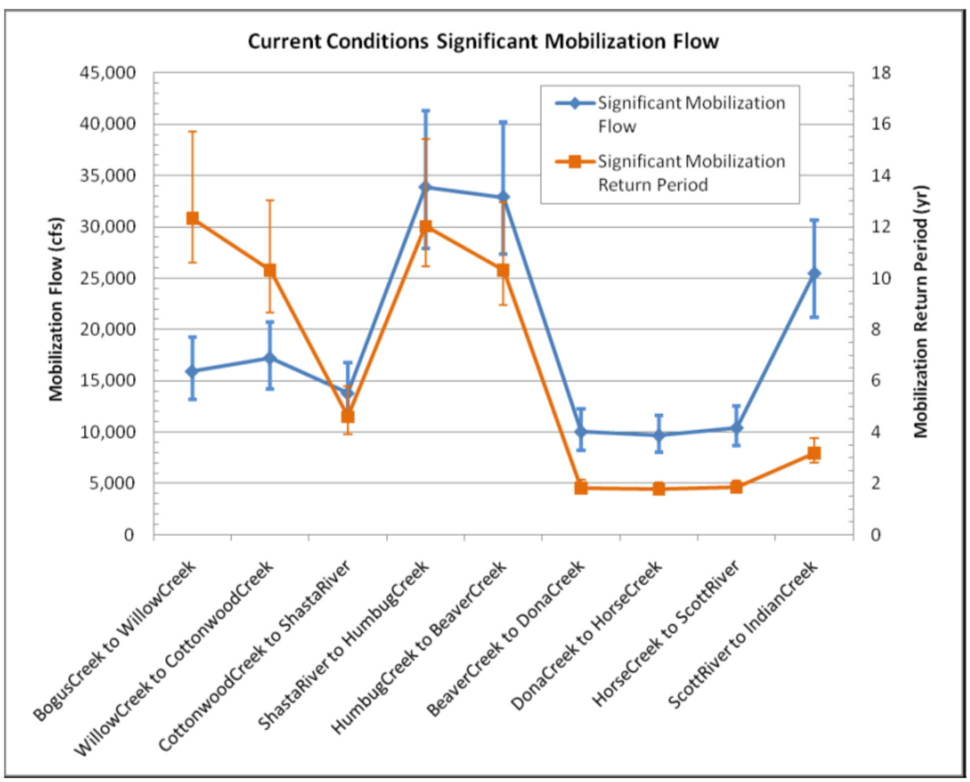

Figure 3: Significant bed material mobilization flow and return period on a reach averaged basis for reaches downstream of Iron Gate Dam. (Reproduced from Figure 5-25, Reclamation, 2011).

Comparison of Sediment Entrainment Analyses. Ayres Associates (1999), Holmquist-Johnson and Milhous (2010), and Reclamation (2011) used differing approaches to develop estimates of sediment entrainment. For purposes of comparison, we equate the Ayres Associates (1999) pool flushing flow with Holmquist-Johnson and Milhous (2010) Surface Flushing and Reclamation (2011) Slight Mobilization and we equate Ayres Associates (1999) riffle incipient motion with Holmquist-Johnson and Milhous (2010) Armor Disturbance and Reclamation (2011) Significant Mobilization (Table 7). We show the mean minimum Reclamation (2011) estimates for the Klamath River between Iron Gate Dam and the Shasta River confluence. Discharge thresholds in the Klamath River increase significantly downstream in the reach between the Shasta River and Beaver Creek reach because of its steep gradient, armor layer composed of immobile large cobbles and boulders, and occurrence of bedrock outcrops.

There is a spread in the estimates due to variances in the methods employed and the dates when channel substrate and channel conditions were evaluated, and the specific channel conditions at study locations.

Study Limitations. The three studies summarized in this technical memorandum provide useful estimates of the discharges required to mobilize bed sediment in Klamath River below Iron Gate Dam. There are some limitations resulting from the scale and scope of the studies:

- The Ayres Associates (1999) and Holmquist-Johnson and Milhous (2010) estimates are based on a hydraulic analyses that employed a limited number of stream cross sections collected at six sites that extended over 171 river miles. Only sites 4, 5, and 6 were used to evaluate conditions below Iron Gate Dam.
- All three studies developed sediment transport estimates using general sediment mobilization formulations. The estimates are not calibrated to direct observations of sediment transport in the Klamath River below Iron Gate Dam.
- All three studies used one-dimensional hydraulic models to develop estimates of hydraulic variables. The hydraulic variables extracted from the one-dimensional models are cross section averages and do not reflect the variability in flow velocities and depth across a river cross section, or across a river reach.
- The studies do not identify the mode of sediment transport (suspended or bedload).

Table 7: Comparison of sediment entrainment discharge estimates

Sediment Entrainment State	Discharge Estimate (cfs)					
	Ayres Associates (1999)		Holmquist-Johnson and Milhous, (2010)	Reclamation (2011): Bogus Creek to Shasta River		
	Low	High	Threshold Limit	Mean Minimum	Mena Median	Mean Maximum
Surface Flushing	5,400	6,600	5,000	6,900	9,600	12,900
Armor Disturbance	9,800	16,500	11,250	11,200	15,600	20,900

Additionally, the three sediment entrainment studies do not provide sufficient information to fully specify environmental flows to manage channel sediment. Wohl et al. (2015) and Schmidt and Wilcock (2008) recommend managing flow regimes below dams to produce a balanced sediment regime. Wohl et al. (2015) defines a balanced sediment regime as present when the energy of flow available to transport sediment is balanced by the sediment supply. Schmidt and Wilcock (2008) characterize a balance as occurring when the long-term transport of sediment out of a reach is equivalent to the long-term supply into the reach. Environmental flows developed to achieve a balanced sediment regime require information on sediment supply to a reach, sediment storage within a reach, and the effect of flow regimes on moving sediment out of a reach.

Additional Studies. Two additional studies bear mention because they provide the basis for further investigations into sediment mobility and developing environmental flow recommendations.

The U.S. Fish and Wildlife Service Arcata Fish and Wildlife Office (AFWO) and Oregon State University developed two-dimensional hydraulic models for three study sites in the Klamath between the Shasta and Scott Rivers (Wright et al. 2014; Alexander et al. 2016). The models are well calibrated and have been combined with statistical modeling for the purpose of analyzing distribution of *M. speciosa*. Modeling was combined with biological and physical observations made prior to and following major flows. These models could be adapted to calibrate sediment transport estimates and to tie physical outcomes of flushing flows to biological outcomes. Direct calibration of the flow required to achieve environmental flow objectives would improve the efficiency of potential water releases.

Malakauskas et al. (2013) performed flume experiments to evaluate flow requirements for dislodging *M. speciosa*. Their results identified shear velocity thresholds for dislodgement. This is another opportunity to directly relate measurable physical flow requirements to biological goals for disrupting *M. speciosa*.

Vegetation Disturbance and Geomorphically Effective Flow. Wolman and Gerson (1978) defined geomorphic effectiveness in terms of the ability of an event to alter the shape or form of the landscape. With respect to rivers, geomorphically-effective floods are described as creating a disturbance in the equilibrium river form (e.g., channel widening) that is followed by a recovery period where the channel readjusts to an equilibrium condition. Costa and O'Connor (1995) defined the energy produced by geomorphically-effective floods as a function of stream power (the product of the unit weight of water, discharge and energy slope) and flood duration for discharges above the incipient motion threshold for bed movement.

Floods are important mechanisms for maintaining channel form on rivers, including the Klamath River. During extended low-flow periods, riparian vegetation encroaches onto bar surfaces. Once riparian vegetation is established, it repeats a cycle of sediment trapping and channel narrowing and further encroachment of riparian vegetation into the channel (Ayres Associates 1999). Large, less-frequent floods of approximately a 10-year return period magnitude rejuvenate the Klamath River channel by reworking gravels on riffles, eroding channel banks and re-widening the channel, and removing substantial amounts of aquatic vegetation in the reach between Iron Gate Dam and the Scott River (Ayres Associates, 1999).

Holmquist-Johnson and Milhous (2010) estimated the threshold for general Armor Layer Movement at 15,000 cfs. Ayres Associates (1999) estimated the discharge required to rework gravel on riffles at between 9,800 and 16,500 cfs in the Klamath River between Iron Gate Dam and Seiad Valley. Ayres Associates (1999) suggested that discharges of approximately ten-year return period were required to rejuvenate the channel. Reclamation (2011) reported the ten-year return period discharge of 15,610 cfs as the approximate discharge needed to rejuvenate the river bed in the Klamath River below Iron Gate Dam.

We classify discharges that exceed 15,000 cfs in the Klamath River below Iron Gate Dam as geomorphically effective flows. This is an approximate estimate, but one which provides a general order of magnitude for a flow that will induce channel migration and create diverse geomorphic surfaces. Geomorphically effective flows remove accumulated riparian and aquatic vegetation, widen the channel where vegetation encroachment has narrowed the channel, and sort the gravel armor layer and substrate layer. The amounts of work done by geomorphically effective flows are dependent on duration and magnitude of discharges above the threshold value where Armor Disturbance occurs. After a geomorphically effective flow event, vegetation recovery, vegetation encroachment, and channel narrowing occur until the next geomorphically effective flow occurs.

Other Environmental Flow Considerations. There are several features of environmental flow releases for the Klamath River that require analysis.

Ramping Rates. Ramping rate is the rate of change in water flow released from a dam. Whiting (2002) notes than implementation of ramping rates in environmental flows are poorly addressed. Ramping rates that drop too rapidly can cause fish stranding and bank failures. Ramping rates that rise too quickly can create safety issues. Ramping rates can also be adjusted to meet other environmental flow objectives. For example, the Trinity River Restoration Program adjusts their ramping rates on environmental flow releases to encourage development of riparian vegetation.

Timing of Flows. Timing of environmental flows should consider how to minimize impacts to fish populations while identifying optimal times that flow may provide benefits, such as disrupting *M. speciosa,* in the case of the Klamath River. Timing should also consider how dam releases can interact to augment unregulated flood flows on local tributaries to cleanse fan deposits at tributary mouths to improve access by upstream migrant fish.

Duration of Flows. More analysis is required to evaluate the duration and shape of an environmental flow hydrograph. The duration should address how much sediment is available for transport and how much flow is required to cleanse the system. Specifying flow duration requires developing better information for implementing a balance sediment regime (Wohl et al. 2015) in the reaches of the Klamath River below Iron Gate Dam.

Need for Calibration. Monitoring and observation of bed mobility during flow releases are required to calibrate sediment and hydraulic assessments. Direct calibration methods allow for estimates of volume of sand and gravel that are mobilized (Kondolf and Wilcock (1996). Calibration work should be combined with monitoring observations of biological responses similar to the work of Alexander et al. (2016).

Sediment Mobilization Flows at Klamath River below Iron Gate Dam. In this section, we examine the occurrence of sediment mobilization flows for the Klamath River below Iron Gate Dam since construction of the dam in 1962. We downloaded daily-mean flow records from the USGS National Water Information System for USGS gage 11516530 Klamath River below Iron

Gate Dam for the period October 1, 1964 to September 28, 2016. Data are reported on a Water Year (WY) basis, which extends from October 1 to September 30 for all calendar years.

Occurrence of Sediment Mobilization Flows. We plotted the long-term hydrograph for the Klamath River below Iron Gate Dam from WY 1964 through WY 2016 with the discharge limits for the six substrate mobilization states defined in Table 2, as shown in Figure 4. The discharge limits are based on the sediment mobilization limits developed using the Holmquist-Johnson and Milhous (2010) and Milhous (1998) studies (Table 6). Although, the discharge estimates presented in Table 6 are at the lower range of the three sets of sediment mobilization estimates previously discussed, we chose to use the Holmquist-Johnson and Milhous (2010) and Milhous (1998) set because it is the only set that establish discharge ranges for all sediment mobilization states listed in Table 6. These should be seen as a conservative estimate of the flows required to mobilize sediment.

A visual analysis of Figure 4 shows that geomorphically-effective flow events (i.e., discharge > 15,000 cfs) are rare, occurring only five times between WY 1964 and WY 1997. Armor disturbing events are slightly less rare, occurring ten times between WY 1964 and WY 1997. The Klamath River below Iron Gate Dam has not experienced a geomorphically-effective or armor-disturbing event since the January, 1997 spill event. Surface Flushing and Depth Flushing events were common prior to WY 2000. Since WY 2000, Depth Flushing events occurred only during high runoff events in winter 2006 and during a controlled spill event in March 2016 (Figure 3).

Duration of Sediment Mobilization Flows. Because the effectiveness of sediment mobilization flows are a function of both sediment mobilization capability and the duration of flows capable of mobilizing sediment (Costa and O'Connor 1995), we evaluated the duration of sediment mobilization flows over time. We plotted the number of days per Water Year that daily-mean flows in the Klamath River below Iron Gate Dam fell in the range of a substrate mobilization state that transported sediment (Figure 4). We observed that the pattern of sediment mobilization can be split into two periods: (1) the period WYs 1964 to 1999, when sediment mobilization flows were common and (2) the period WYs 2000 to 2016 when sediment mobilizations flows were rare. From WYs 1964 to 1999, the average cumulative duration of Surface Flushing was greater than 22 days per water year. From 2000 to 2016, sediment mobilization flow occurred in only five of the 17 water years; and the cumulative duration of Surface Flushing flow exceeded five days in only one water year. We conclude that the effectiveness of sediment mobilization flows in the period WYs 2000 to 2016 substantially dropped from the period WYs 1964 to 1999.

Frequency of Immobile Bed Conditions. The *Immobile Bed* sediment mobility state is estimated to occur for flow rates of 2,500 cfs or less at the Klamath River below Iron Gate Dam. Flows released from the Iron Gate Dam carry suspended materials consisting of mineral content and organic material originating from in-reservoir algal blooms (U.S. Department of Interior and California Department of Fish and Game, 2012). During Immobile Bed conditions, suspended mineral sediment and organic materials released from Iron Gate Dam can settle and accumulate on the bed and are not re-suspended until the occurrence of flushing flows. Increase in the areas of fine-grain sediment and organic material deposits on the bed and channel margins is a concern because high densities of *M. speciosa* have been commonly observed on fine sediments that are most prone to mobilization. In addition, riparian and aquatic vegetation can colonize fine sediments, further narrowing the channel and degrading fish habitat conditions (USFWS and HVT 1999).

Immobile Bed conditions persisted during WYs 2000-2016, while immobile conditions were less frequent in WYs 1964-1999 (Figure 5). For 10 of the 17 years in the period WYs 2000-2016, Immobile Bed conditions persisted for over 90% of the year. In the period WYs 1964 -1999, Immobile Bed conditions persisted over 90% of the year in only eight of the 36 years.

Sequencing of Sediment Mobilization Flows. The occurrence of Surface Flushing flows in natural rivers is a frequent event. Flows that reach or exceed the top of bank (i.e., the bankfull flow) occur on a frequency of one to two years (Leopold, 1994). Dunne and Leopold (1978) define bankfull stage as the stream level that corresponds to the discharge at which channel maintenance is most effective. Schmidt and Potyondy (2004) employed 80% of the 1.5 year return period discharge as a first approximation of the Surface Flushing flow. Robinson (2007) recommended a two-year return period discharge as a first approximation of the Surface Flushing flow for sediment supply limited streams in Oregon. Note that the two-year return period discharge for the Klamath River below Iron Gate Dam (6,030 cfs) is in the range of estimates for sediment flushing flows listed in Table 7.

Effective channel maintenance flow regimes possess flows of sufficient duration and frequency to maintain channel morphology (Schmidt and Potyondy, 2004). Lack of sufficient flows causes loss of channel capacity. The lack of sufficient duration and frequency of flows in managed systems is also detrimental to system ecology (Annear et al 2004). Poff et al. (1997) attributes flow stabilization (i.e., maintenance of a stable flow without interruption by flooding events) as a cause of overall reduction of biological diversity and increases in presence of invasive species.

In the period WYs 1964 to 1999, the duration between Surface Flushing events below Iron Gate Dam was typically one to two years (Figure 6). There were two occasions, corresponding to a drought periods in the late 1980s and early 1990s, when the duration between Surface Flushing events reached almost three years and four years. The frequency of Surface Flushing events in the period WYs 1964 to 1999 is consistent with channel maintenance needs of natural streams. Since 2000, however, there have been three occasions when the duration between flushing events was approximately five years.

Between WYs 1964 and 2000, there were five geomorphically effective events (including the December 1964 flood) for the Klamath River below Iron Gate Dam (Figure 7). Duration between events ranged between two and 14 years. As of the end of WY 2016, there has not been a geomorphically effective event since 1997, a period approaching 20 years. Geomorphically effective flow events that remove vegetation encroachment and rejuvenate the channel used to be common, but are now rare.

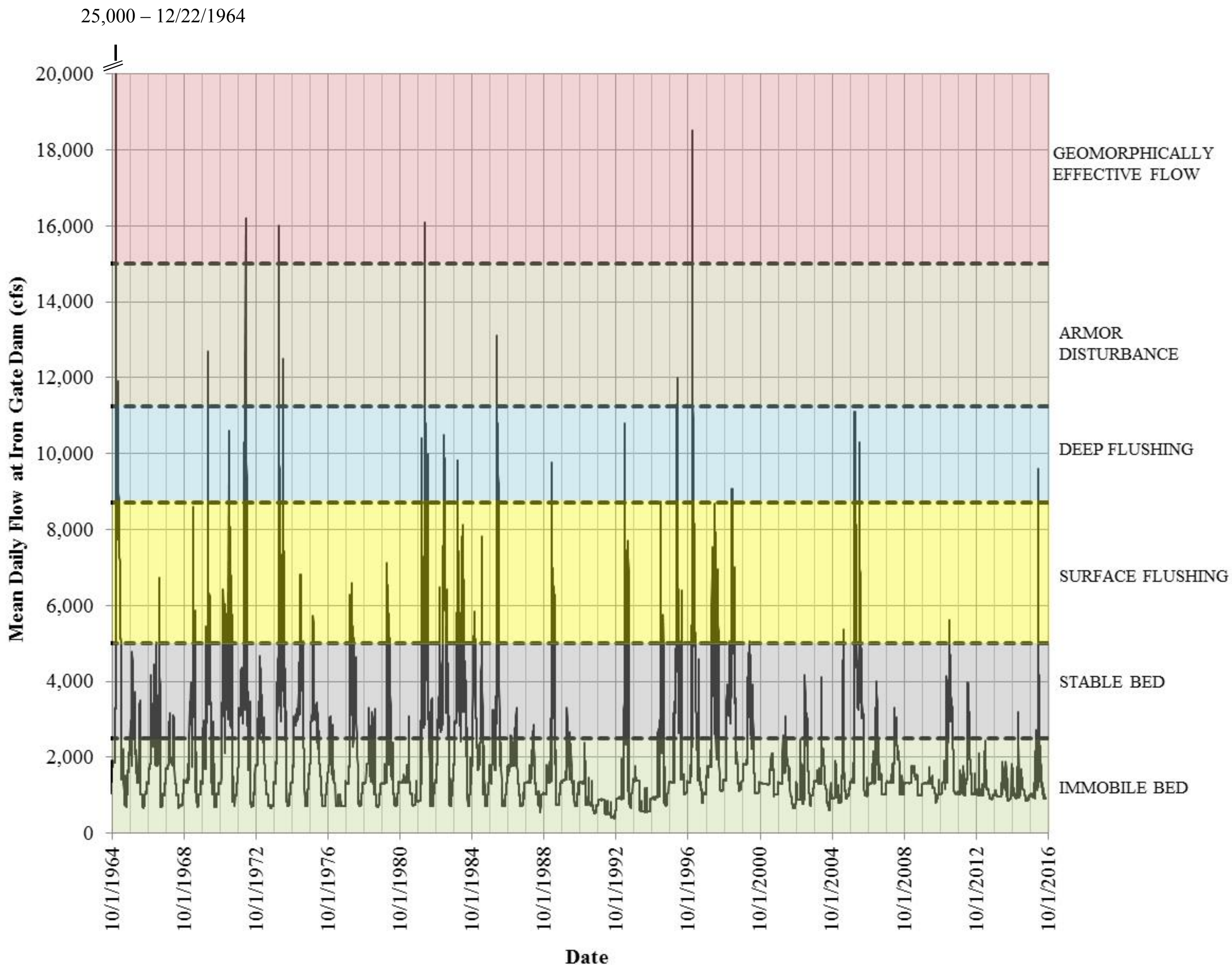

Figure 4: Daily-mean flow in cfs for Klamath River below Iron Gate Dam with substrate mobilization states. See Table 3 for definition of substrate mobilization states, Water Years 1964-2016.

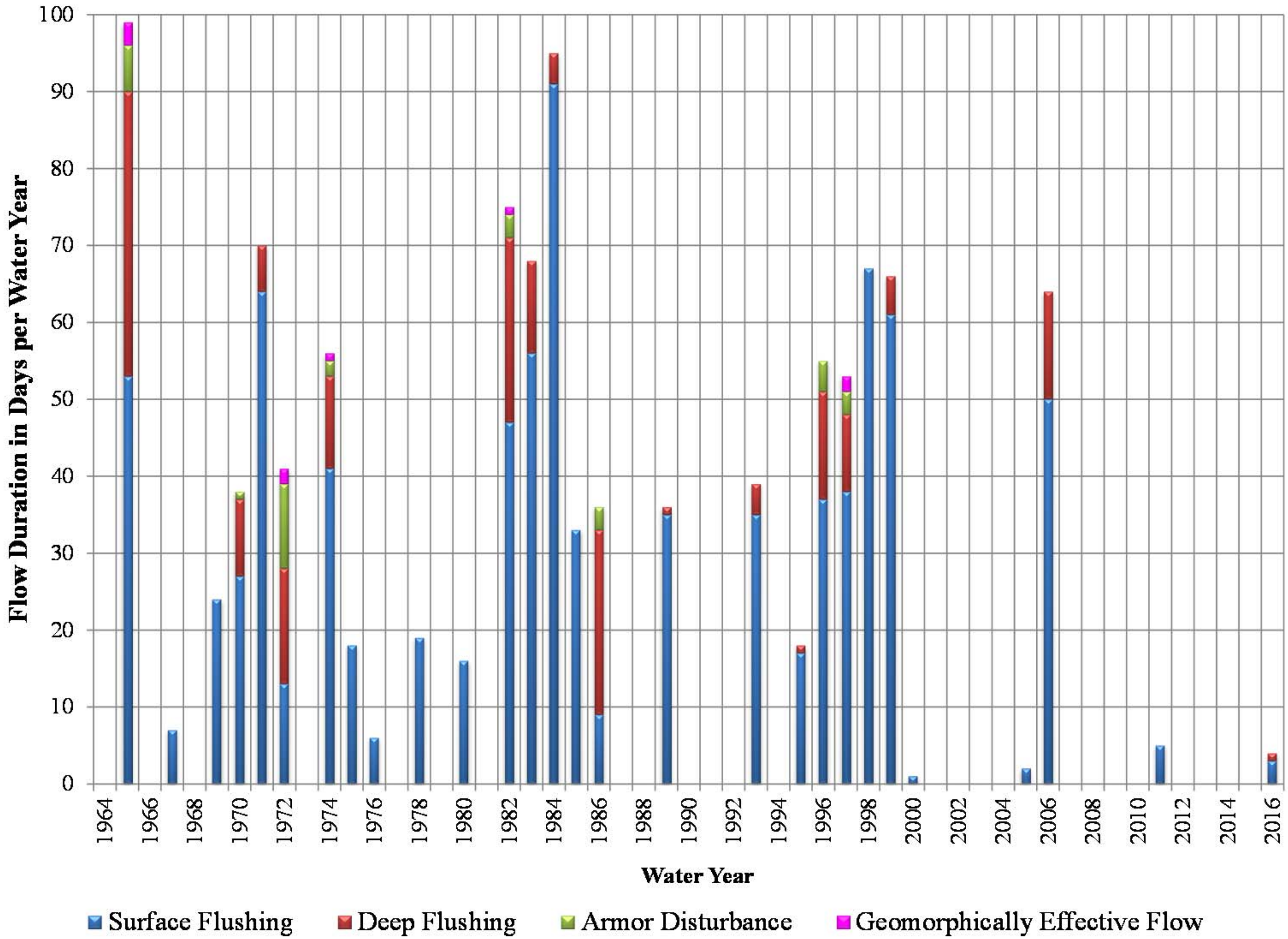

Figure 5: Duration of sediment mobilization flows in days per Water Year in the Klamath River below Iron Gate Dam Water for Water Years 1964-2016.

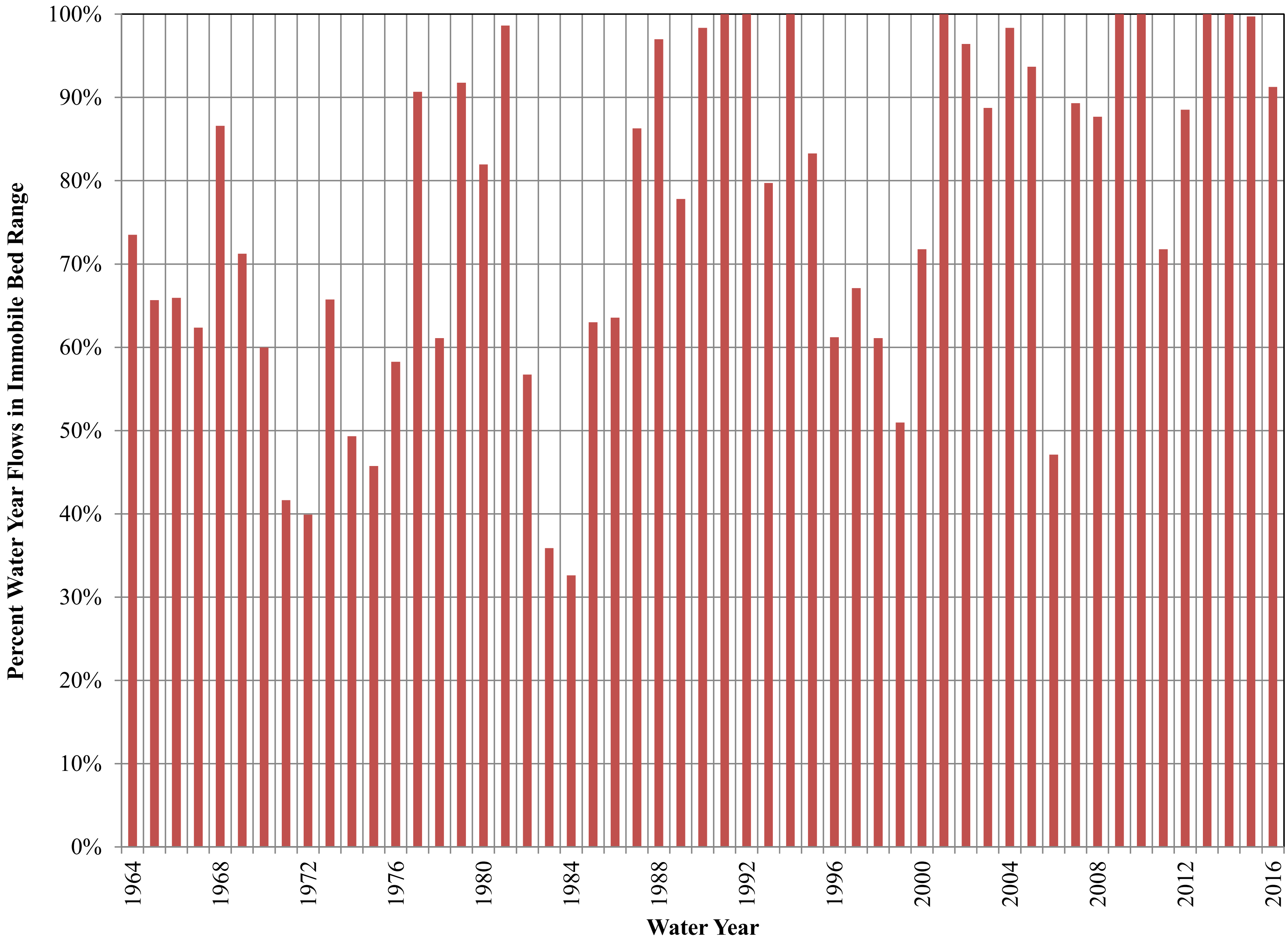

Figure 6: Percentage of Water Year Immobile Bed conditions occur in the Klamath River below Iron Gate Dam for Water Years 1964 -2016.

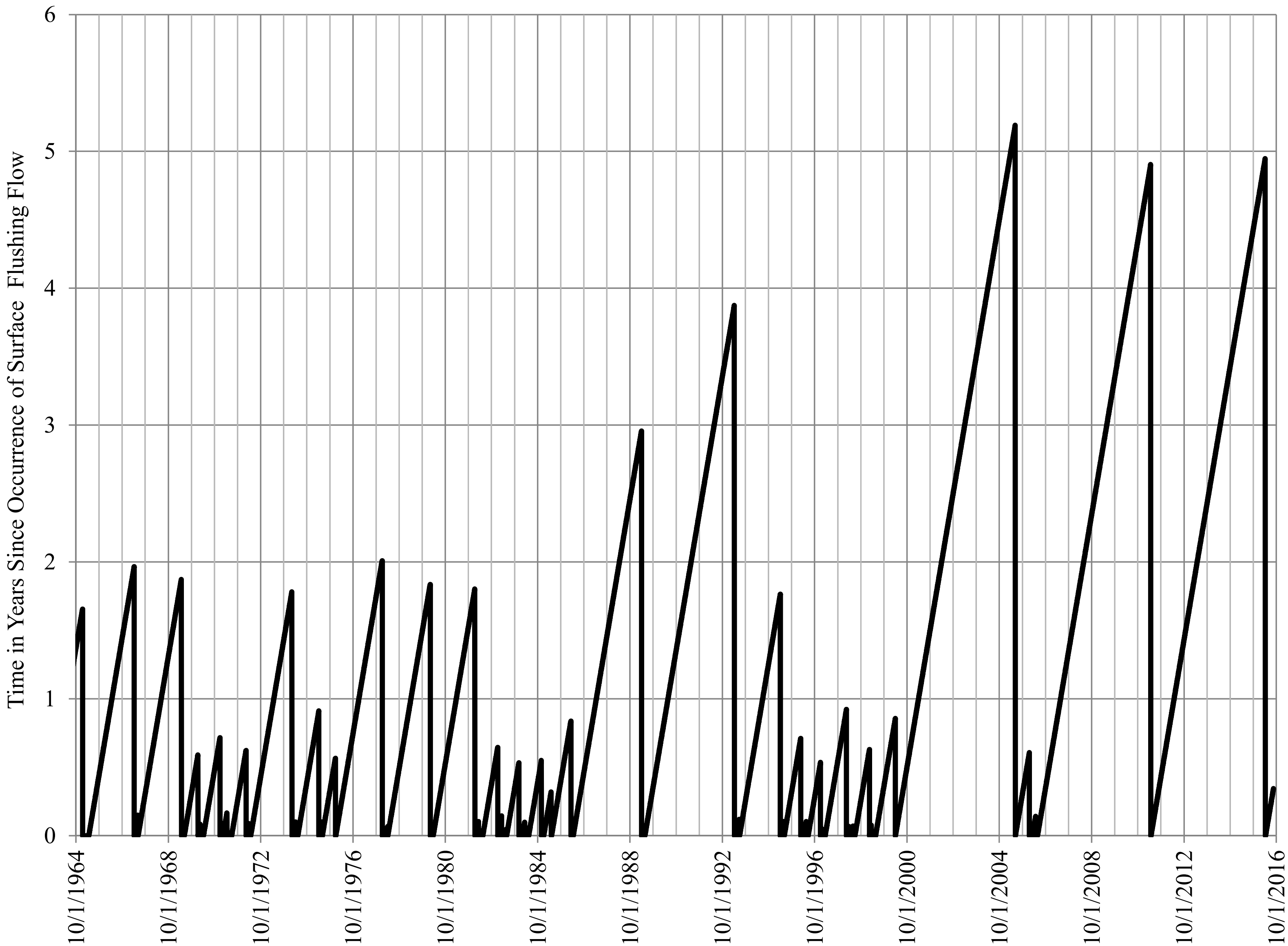

Figure 7: Time in years since occurrence of Surface Flushing flows in the Klamath River below Iron Gate Dam, Water Years 1964-2016.

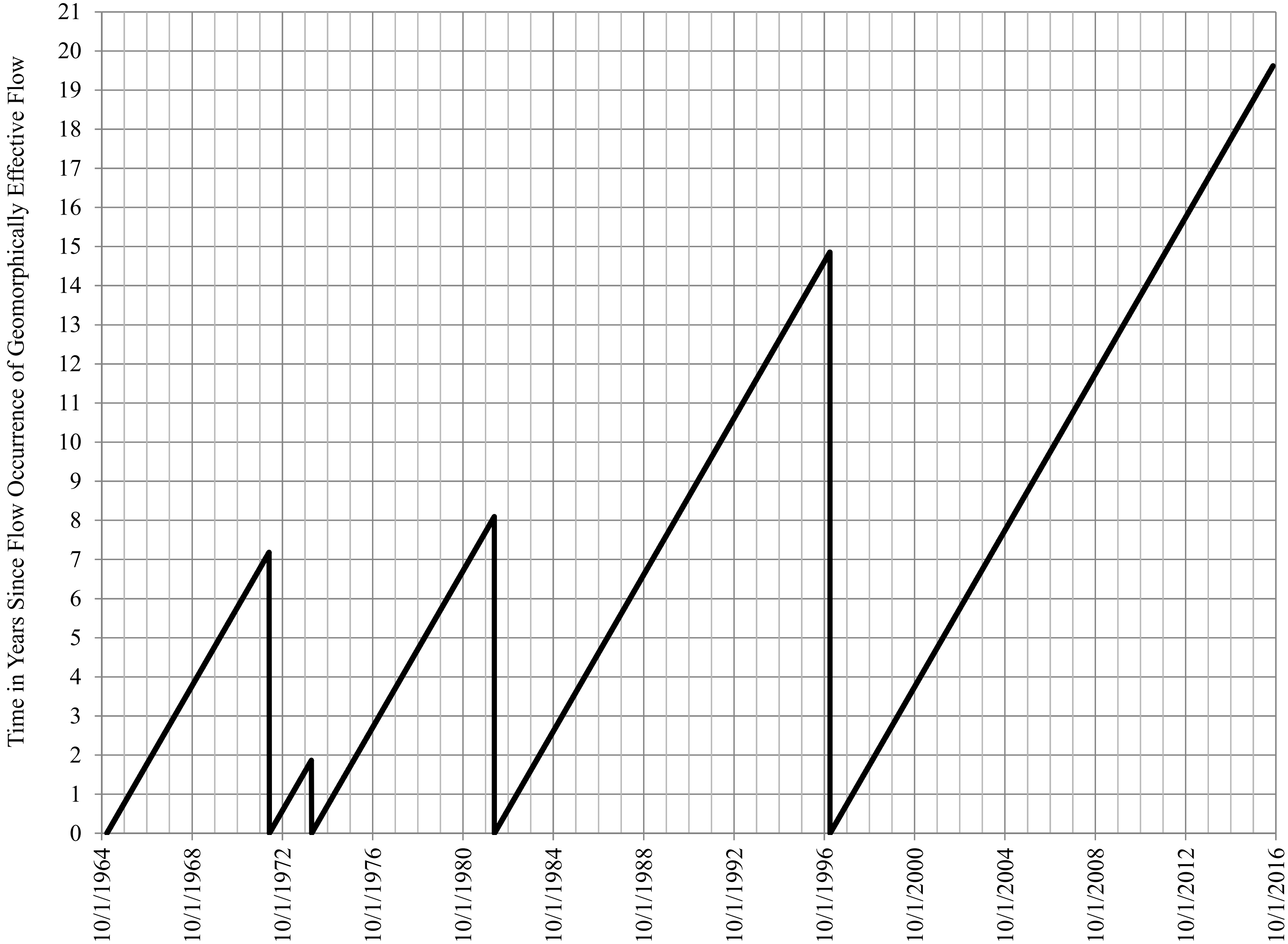

Figure 8: Time in years since occurrence of geomorphically effective flows in the Klamath River below Iron Gate Dam, Water Years 1964-2016.

Summary Guidelines.

- Environmental flows are developed by river managers to mitigate the detrimental impacts of dams and water diversions on river form and ecological functions. Environmental flow regimes designed to induce geomorphic changes are broadly divided into two categories, sediment maintenance or "flushing flows" used to modify substrate composition and channel maintenance flows intended to maintain channel form and floodplains.
- In developing environmental flow regimes, it is important to recognize conflicts in objectives and constraints on flow releases.
- There are three contemporary studies that estimated sediment transport thresholds in the Klamath River below Iron Gate Dam
- The sediment entrainment threshold estimates reported in the three studies varied due to differences in study methods employed and the dates when channel substrate and channel conditions were evaluated.
- Ayres Associates (1999) concluded that floods of approximately 10-year return period magnitude rejuvenate the Klamath River channel by reworking gravels on riffles, eroding channel banks, re widening the channel, and removing substantial amounts of aquatic vegetation in the reach between Iron Gate Dam and the Scott River.
- The 1.5-, 2-, and 10-year return period for the Klamath River below Iron Gate Dam was estimated by Reclamation (2011) to be 4,389, 6,030, and 15,610 cfs, respectively.
- The 10-year return period of 15,610 cfs reported by Reclamation (2011) is consistent with the findings of Holmquist-Johnson and Milhous (2010) who estimated the threshold for general Armor Layer Movement to be 15,000 cfs and that of Ayres Associates (1999) who estimated the gravel mobilization on riffles to occur between 9,800 and 16,500 cfs for the reach between Iron Gate Dam and Seiad Valley.
- We classify discharges that exceed 15,000 cfs in the Klamath River below Iron Gate Dam as geomorphically effective flows, which are occasional high flows required to maintain channel form and reduce riparian encroachment.
- Other important considerations in flow include ramping rates, timing, duration, and monitoring and calibration.
- From 1964 to 1999, the average cumulative duration of Surface Flushing flows exceeded 22 days per water year. From 2000 to 2016, the average cumulative duration of Surface Flushing flow exceeded five days in only one water year and no sediment mobilization flows occurred in 12 of the 17 water years.
- At flow releases less than 2,500 cfs below Iron Gate Dam, Immobile Bed conditions exist that allow suspended sediments to settle and accumulate on the bed, which are not re-suspended until flows that generate Surface Flushing occur.
- Growth of fine sediment deposits on the bed and channel margins is a concern because high densities of *M. speciosa* have been observed in such deposits. In addition, riparian and aquatic vegetation can colonize fine sediments, further narrowing the channel and degrading fish habitat conditions such as what has been documented on the Trinity River.

- From 1964 to 1999, the time between Surface Flushing events below Iron Gate Dam was typically 1-2 years, which is consistent with channel maintenance needs of natural streams reported in the literature. Since 2000, there have been three occasions when the duration between Surface Flushing events approached or exceeded 5 years.
- Between 1964 and 2000, 5 geomorphically-effective events occurred on the Klamath River below Iron Gate Dam, with the duration between events ranging between 2 to 14 years. There has not been a geomorphically-effective flow event, the events that remove vegetation encroachment and rejuvenate the channel, since 1997, in a period approaching 20 years.

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United States Department of the Interior

FISH AND WILDLIFE SERVICE

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In Reply Refer To:
AFWO

Technical Memorandum

TO: Dave Hillemeier, Yurok Tribal Fisheries, and

Craig Tucker, Karuk Department of Natural Resources

FROM: Nicholas A. Som and Nicholas J. Hetrick, Arcata Fish and Wildlife Office, and

Julie Alexander, Oregon State University

SUBJECT: Response to Request for Technical Assistance – Polychaete Distribution and Infections

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, are 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).

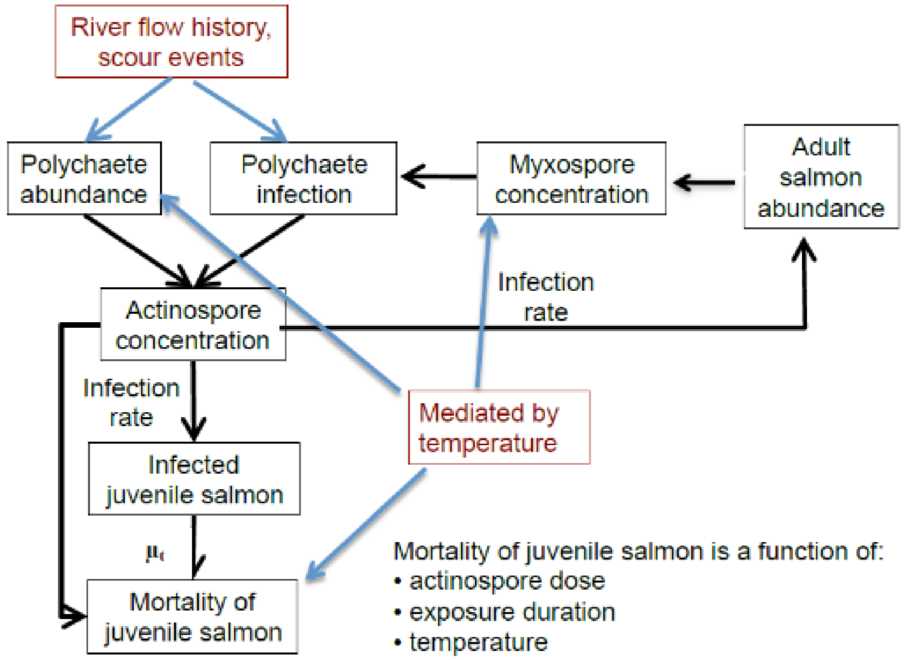

Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon. with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).

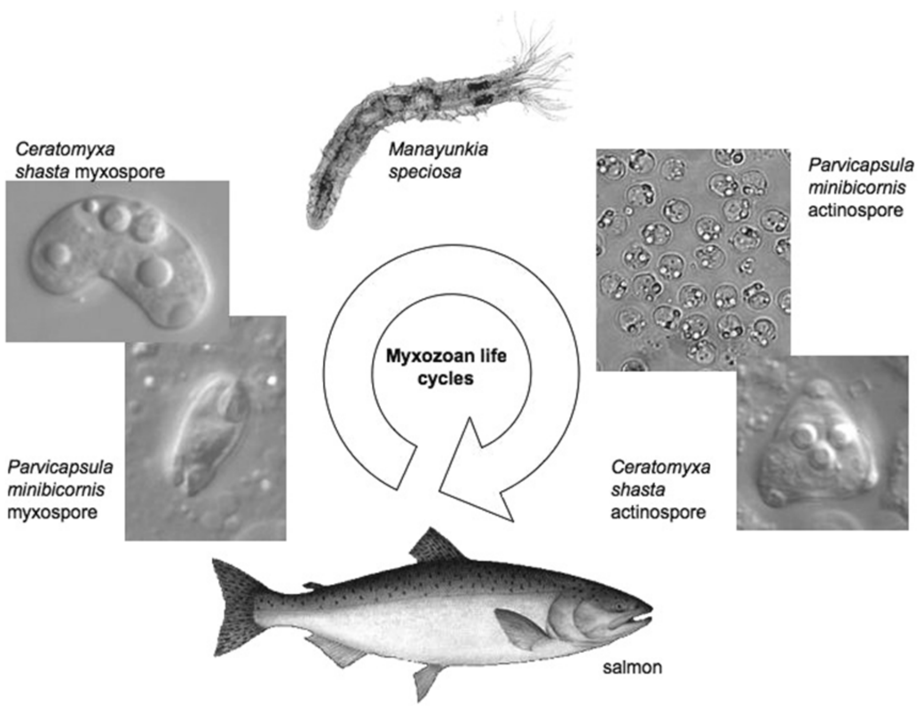

Figure 2. The life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* (graphic provided with permission from J. Bartholomew, Oregon State University). *Manayunkia speciosa* is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites.

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.

Polychaete Ecology. Morhpological (e.g., body shape, structures for attachment), physiological and behavioral adaptations enable aquatic invertebrates to feed, grow, reproduce, and maintain productive populations in a constantly moving environment (Vogel, 1996). The polychaete worm *M. speciosa* is specifically adapted to life as a semi-sessile benthic invertebrate. Morphology consists of three body regions including the anterior end composed of the branchial crown, prostomium and peristomium, the thoracic region composed of 8 serially-repeated segments, and the posterior end composed of the pygidium (Thorp and Rogers 2015). *Manayunkia speciosa* inhabit flexible tubes which they construct from mucus, sand, and silt. The tubes are attached to substrate, allowing *M. speciosa* to suspension feed in the flowing water column by extending its branchial crown structure ("tentacles") out of the tube in order to contact and ingest food particles. A series of hooks on the posterior end of the organism facilitates attachment of the worm to the inside of the tube.

Three types of feeding behavior have been reported for the genera *Manayunkia*: deposit feeding, suspension feeding, and secondary suspension feeding (Lewis 1968). *M. speciosa* has been reported in the literature as being suspension-type feeders (Stocking and Bartholomew 2007). Additionally, Stocking and Bartholomew (2007) observed populations of *M. speciosa* "freely roaming the sediment" at the mouth of the Williamson River in the Upper Klamath River Basin. This diversity in habitat use and flexibility in feeding behaviors contribute to *M. speciosa* being able survive under various environmental and nutrient availability conditions (Hendrickson et al. 2008), found in still-water depositional habitats, slow flowing habitats such as lake reservoir inflows and outflows, and lotic habitats including pools, eddies, riffles, and runs).

Knowledge of the reproduction and development of *M. speciosa* is fairly limited. The sexes are separate (dioecious) and non-feeding larvae are brooded in the maternal tube with the adult worm (Leidy 1883). Males have a dorsal sperm duct and females have a sperm storage structure located in the radiolar crown (Holmquist 1973, Rouse 1995). Eggs develop in females asynchronously in the coelem (Eckelbarger 2005) and reproduction typically peaks in spring to early summer as temperatures increase (Hendrickson et al. 2008, Willson et al. 2010, Alexander et al., personal communication). *Ceratonova shasta* infections are not commonly observed in sexually mature individuals (male or female) and infection is not thought be transmitted either vertically or horizontally among polychaetes. Progeny are reared in the maternal tube until they reach approximately 1 mm (Willson et al. 2010, Schloesser et al. 2016), which suggests they are not feeding independently (and thus not susceptible to infection by *C. shasta*) until they abandon the maternal tube (Alexander et al., personal communication).

Distribution and Habitat. Though previous work suggested that the distribution of *M. speciosa* is influenced by substrate (Stocking and Bartholomew 2007, Malakauskas and Wilzbach 2012) and a limited range of flow conditions (Jordan 2012), several recent studies have attempted to isolate how hydraulics and substrate may interact to influence the distribution of M. speciosa. Malakauskas et al. (2013) performed a series of laboratory flume experiments in which the stability and texture of substrates were varied across flume water velocities. They observed that dislodgement of *M. speciosa* increased with increasing water velocities and decreasing substrate stability, and concluded that higher flows could directly influence the distribution of polychaetes by restricting habitat use to stable substrates. They also concluded that altered flows targeting mobile substrates could effectively dislodge *M. speciosa* from readily entrained substrates, but noted that the polychaetes exhibited attachment abilities similar to taxa found in higher-gradient, rapidly-flowing environments, and that *M. speciosa* could potentially move to lower velocity sections of stable substrates (e.g., behind rock outcroppings) during high flow events. The ability of some polychaetes to persist after high flow events (Alexander et al. 2014) complicates our ability to predict the effectiveness of pulse flow events that may be targeted to scour polychaetes.

Alexander et al. (2016) implemented a designed study to assess how hydraulic conditions interact with substrate and relate to the distribution of *M. speciosa* in the Klamath River. This work coupled field sampling (measurements taken during summer base-flow periods when sampling was feasible) with the development of 2-dimensional hydrodynamic models (2DHMs, Wright et al. 2014) that allowed predictions of depth and velocity at discrete riverine locations over a range of discharges. Results of this study showed that the distribution of polychaetes is correlated with hydraulic variables occurring during the water year's winter or spring peak discharge event. Applications of the 2DHM to peak flows occurring each in 2012, 2013, 2014, and 2016 all show a consistent pattern: increasing peak discharge is associated with decreases in predicted weighted-useable area (WUA) as shown in Table 1. We note, however, that 2DHM predictions at the peak discharges of 2016 should be taken cautiously as the 2DHMs were calibrated and validated to discharges near 8,500 cfs (Wright et al. 2014). Predictions made under discharges above 12,000 cfs represent a considerable extrapolation beyond the calibration bounds of the model.

Table 1. Weighted-usable-area (WUA) for *M. speciosa* habitat at 3 sites in the Klamath River as predicted by coupling the peak discharge (Q) model of Alexander et al. (2016) with depth and velocity predictions from the 2DHM of Wright et al (2014). "T" represents the Tree of Heaven site (river kilometer (rkm) 281; 350 m in length), "B" represents the site just upstream of Beaver Creek (rkm 264; 550 m in length), and "C" represents the site near the Community Center grange (rkm 259; 850 m in length). Discharge values are in cubic feet per second, and WUA values are units of probability-weighted square-meters.

Site	Year	Peak Q (cfs)	WUA
T	2014	2154	2867
	2013	2755	2797
	2012	4520	2543
	2016	12,148	1979
B	2014	2225	6717
	2013	2825	6551
	2012	4697	5975
	2016	12,395	4552
C	2014	2331	6277
	2013	2931	6009
	2012	5015	5491
	2016	12,960	3633

The sampling design utilized by Alexander et al. (2016) also lent to the investigation of polychaete distribution dynamics by re-sampling specific geo-referenced locations in subsequent years. The main data for fitting the predictive statistical model was collected in 2012. In 2013, a largely independent (i.e., compared to the 2012 locations) data set was collected to evaluate the predictive performance of the statistical model, and so very few locations were resampled. However, many of the 2013 sample locations were resampled in 2014 and again in 2016. Of specific the locations sampled in 2013, 208 were again sampled in 2014. Of the locations sampled in 2014, 286 were again sampled in 2016. These repeated sampling locations allow us to look at changes in the density of polychaetes over time, and potentially relate changes to hydraulic conditions. Although the repeat sampling data are currently under analysis and write-up, we provide a summary of their results here. Between the 2013 and 2014 sampling period the peak discharge out of Iron Gate Dam (IGD) was 1,890 cfs, and annual peaks in 2015 and 2016 were 3,580 cfs and 11,200 cfs, respectively.

At each sampling location, a relative measure of polychaete density was assessed and recorded, with ordinal values of 0 (no polychaetes), 25, 50, 75, and 100 representing percent polychaete cover. To evaluate potential changes over each time period, we took the difference between the percent cover values recorded at the beginning and end of the period such that a larger percent cover at the end of the time period would indicate an increase. We note that our sampling was initially designed to capture the full range of hydraulic conditions at the Klamath River sites where data was collected. As such, there were a large number of samples taken and replicated at locations learned to be well outside the bounds of suitability for *M. speciosa*. Therefore, many sampling locations that were initially observed as having zero percent cover remained void of polychaete cover across the sample periods, resulting in no change in percent cover (Table 2). Between the 2013 and 2014 samples, with an IGD peak discharge of only 1,890 cfs, locations more frequently increased than decreased in percent cover across all substrate types. The opposite was observed for samples collected in 2014 and again in 2016, where decreases were more commonly observed than increases across all substrate types (Table 2), as shown visually in Figure 3. A likely mechanism for the higher frequency of observed decreases in percent cover across sampled locations is scour and bed mobility resulting from the peak discharge event of 11,200 cfs that occurred in March 2016. Notably, increases detected from 2014 to 2016 were largely limited to shallow, marginal sand habitat (sandy deposits near river banks). One explanation for this result is that polychaetes disturbed during the high discharge event of March 2016 had settled in these areas by July when sampling occurred. Polychaetes were no longer observed in these locations during subsequent sampling (August 2016, J Alexander OSU pers. comm.).

Infection Prevalence. The infection prevalence of polychaetes in the Klamath River is less understood than polychaete distribution. However, several studies and ongoing monitoring efforts (conducted by Oregon State University, Yurok Tribal Fisheries) have measured incidence of infection among sampled polychaetes. Spatial and temporal (within and among years) variation in prevalence of infection among polychaete host assemblages creates a very context dependent picture that requires further study. Variation may be explained in part by spawning adult salmon abundance, known to bring *C. shasta* to the sections of river directly downstream of Iron Gate Dam, and other unknown factors contributing to myxospore production, survival and availability for infecting polychaetes.

Table 2. Change in percent cover over two different time periods summarized across sampling sites and within each substrate type. "B/B" represents bedrock and boulder substrates, and "S/S" represents sand and silt substrates. Numbers represent, for each substrate, the proportion of locations that decreased, did not change, or increased in percent cover of polychaetes between the beginning and end of the specified time period. Peak flow between sampling periods is parenthetically noted.

Time Period	Change	Substrate			
(peak discharge cfs)		B/B	Cobble	Gravel	S/S
2013-2014					
(1,890 cfs)	Decrease	15%	6%	0%	0%
	No Change	36%	75%	78%	78%
	Increase	49%	19%	22%	22%
2014-2016					
(11,200 cfs)	Decrease	59%	20%	29%	25%
	No Change	38%	78%	71%	59%
	Increase	3%	2%	0%	16%

Figure 3. Split screen picture of an identical rock in the mainstem Klamath River taken in 2014 (left) and in 2016 (right) following the March 2016 peak release of 11,200 cfs from Iron Gate Dam.

Generally, myxozoan infection prevalence tends to be very low (0.1-2.0%) in naturally exposed invertebrate host populations (Zendt and Bergersen 2000, Ozer et al. 2002). Prevalence of *C. shasta* infection is also typically low in Klamath River polychaetes, but zones or patches of high infection prevalence have been described. For example, Stocking and Bartholomew (2007) reported 8.24% in one population sampled near the I-5 rest area, downstream from Iron Gate Dam. If factors resulting in these potential focal centers of infection could be identified, then management actions could potentially be targeted.

The sampling associated with Alexander et al. (2016) may provide some insight into the distribution of polychaetes prone to infection. To validate the relative abundance measure recorded at all sampling locations, a subset of locations were selected as validation samples, whereby all benthic material was collected and a more precise enumeration of polychaetes was conducted, as well as an assessment to diagnose the prevalence of *C. shasta* infections among the subsampled polychaetes. Though these data are still under preparation and formal analysis, graphical evidence suggests that infected polychaetes exhibit a smaller range of peak-flow discharge depths and velocities than the general population of polychaetes distributed throughout the infectious zone of the Klamath River (Figure 4). The preliminary findings of this on-going study also suggest that management actions targeted to reduce the impact of *C. shasta* on native salmonids of the Klamath River may not need to target the hydraulic habitat preferences of all polychaetes.

There is evidence from sampled polychaetes that the prevalence of infection is correlated with peak flow regime. Following the 2006 peak flow event (12,400 cfs at IGD), Alexander (2014) reported maximum prevalence of infection in polychaetes of 0.17-0.35% in populations sampled from June-September. Following moderate peak discharges (4,380 cfs in 2004; 5,700 cfs in 2011; measured at IGD), maximum infection prevalence reached 4.96% (Stocking and Bartholomew 2007) and 5.38% (Jordan 2012), respectively. Additionally, preliminary data shows that the highest polychaete prevalence of infection on record (10%, Alexander in prep.) was observed in drought years of 2014 and 2015 (peak IGD discharges of 1,890 and 3,580, respectively).

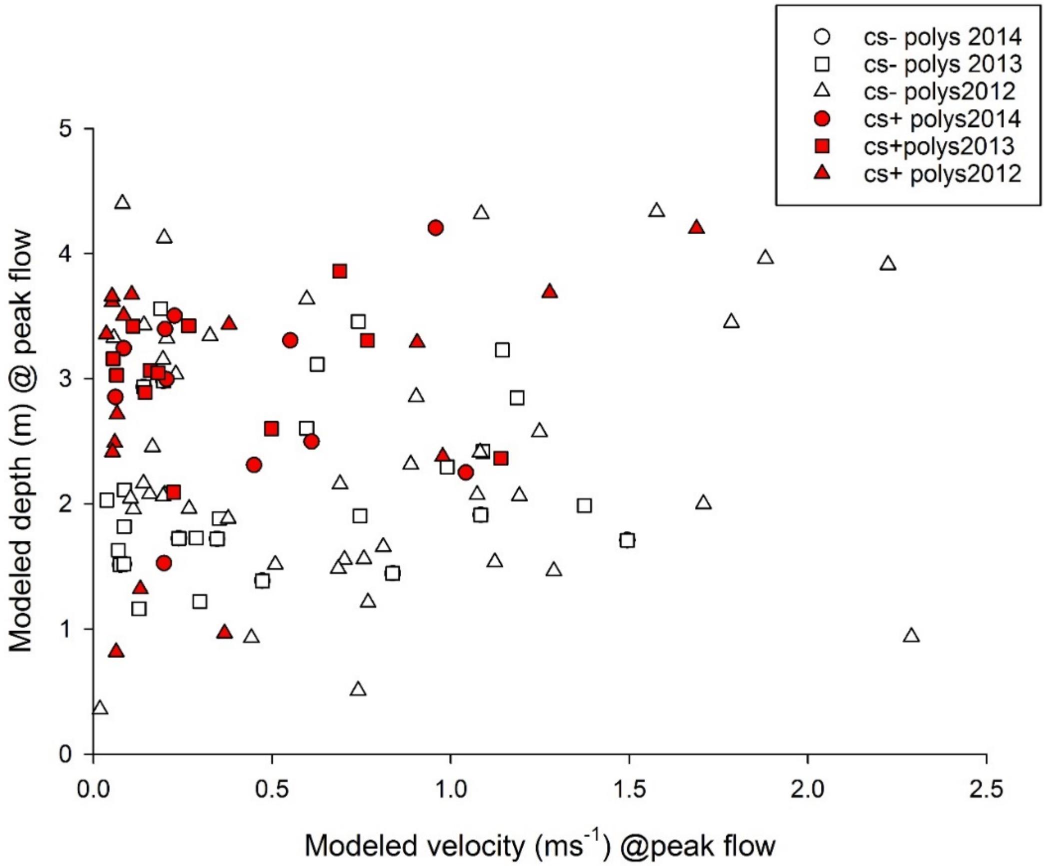

Figure 4. Infected (filled shapes) and uninfected (open shapes) polychaete host assemblages related to the peak-flow water depths and velocities as predicted by the 2-dimensional hydrodynamic model of Wright et al. (2014), and assessed under the sampling design of Alexander et al. (2016). Note that symbols in this figure reference only locations of polychaete presence and do not represent the full suite of depth and velocity combinations considered in the sampling design of Alexander et al. (2016).

Summary Guidelines.

- The polychaete *M. speciosa* has been documented as the obligate intermediate host for the parasite *C. shasta*.
- Polychaetes in the Klamath River have been documented to be sessile suspension feeders and may also have flexibility to feed on organic matter in deposited sediments.
- Polychaete reproduction typically peaks in spring to early summer, coinciding with increasing water temperatures.
- Studies indicate that dislodgement of *M. speciosa* increases with increasing water velocities and decreasing substrate stability.
- Results of 2-D hydrodynamic model runs predict decreasing WUA of suitable polychaete habitat with increasing discharge.
- Results of repeat samples at specific locations in the Klamath River indicate a decrease in *M. speciosa* following the March 2016 peak discharge event of 11,200 cfs.

- Evidence suggests that the prevalence of *C. shasta* infection in polychaetes is negatively correlated with the peak flow regime.
- Preliminary results indicate that infected polychaetes are more likely to occur within a smaller range of peak-flow depths and velocities than the general population, with infected polychaetes more associated with deeper and lower-velocity depositional habitats.

Key Questions. There are several aspects regarding the role of *M. speciosa* in the life-cycle of *C. shasta* that remain unknown, and that could help inform how the disease cycle is completed in the Klamath River. For instance, exactly when and how the infections occur, and if infection differs by parasite genotype, via suspension or deposit feeding, etc. would help inform the transmission mechanism and could help evaluate the potential efficacy and timing of pulse flow events/managed flow events. Additionally, how infection and genotype may affect the life span and survival of *M. speciosa* remains a key question.

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United States Department of the Interior

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In Reply Refer To:
AFWO

Technical Memorandum

TO: Dave Hillemeier, Yurok Tribal Fisheries, and

Craig Tucker, Karuk Department of Natural Resources

FROM: Nicholas A. Som and Nicholas J. Hetrick, Arcata Fish and Wildlife Office

SUBJECT: Response to Request for Technical Assistance – *Ceratonova shasta* Waterborne Spore Stages

DATE: September 23, 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, are identified in a conceptual model diagram (Figure 1) taken from Foott et al. (2011). 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 waterborne spore stages of the parasite and how they infect the salmonid (via actinospores) and benthic invertebrate (via myxospores) hosts 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).

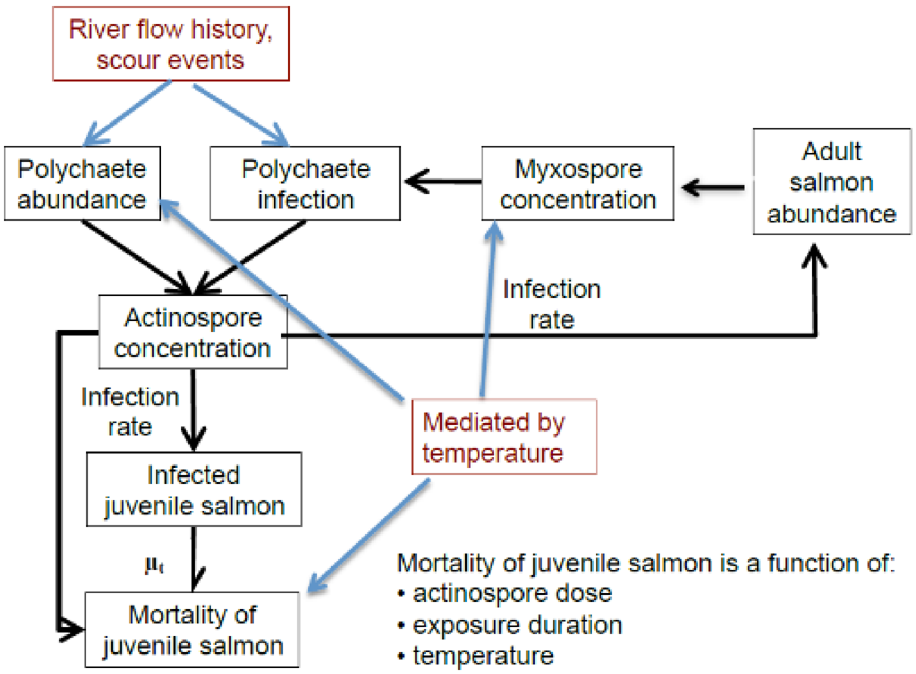

Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon. with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).

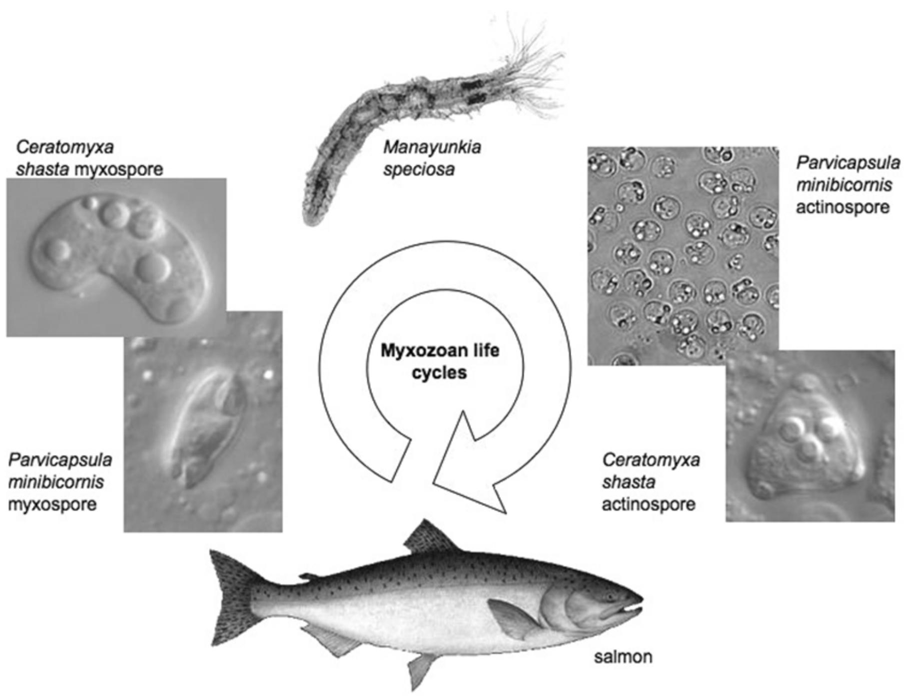

Figure 2. The life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* (graphic provided with permission from J. Bartholomew, Oregon State University). *Manayunkia speciosa* is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites.

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.

Environmental Detection. The waterborne spore stages of the parasite alternatively infect the salmonid (via actinospores) and benthic invertebrate (via myxospores) hosts. Prior to the work of Hallett and Bartholomew (2006), detection of infectious actinospores in water relied on the fates of exposed sentinel fish. Hallett and Bartholomew (2006) developed a DNA-based method for water samples that quantifies abundance of *C. shasta* by a quantitative polymerase chain reaction (qPCR) assay. This qPCR assay provides evidence of waterborne *C. shasta* spores much more quickly than exposed sentinel fish. Although this method cannot distinguish between the actinospore or myxospore stages, or between viable and non-viable (dead or partial) spores, DNA collected in water samples can be further processed to quantify the different genotypes of spores present in the Klamath River (Atkinson and Bartholomew 2010). Of most management concern in the Klamath Basin are Type I, which is associated with mortality in Chinook Salmon, and Type II, which is associated with mortality in Coho Salmon. The detection assay has been used to monitor the temporal and spatial distribution of spores in the Klamath River, and help evaluate the effectiveness of naturally occurring or prescribed experimental flows aimed to reduce the concentration of spores in water during periods of juvenile salmonid outmigration.

The J. L. Bartholomew Laboratory began monitoring spore concentrations in 2005 and added long-term monitoring index sites in 2006. In 2007, the sampling calendar was modified to begin earlier in the water year to capture the initial rise of spore concentrations associated with warming water temperatures. Over time, the sampling calendar and spatial extent of sampling have increased to address various research and monitoring needs. Currently, weekly water samples are collected and processed at five sites during the months of April through October, and year-round at two of those sites. The sampling effort at each site consists of four 1-liter samples extracted from an automatic sampler that pulls 1-liter riverine samples every 2 hours over the prior 24-hour period. The collection and filtration of water samples is coordinated with the Karuk Natural Resources Department and Yurok Tribal Fisheries Program.

Temporal and Spatial Distribution. From late winter to early summer, which encapsulates a single outmigration period, actinospore concentrations (confirmed via sentinel exposures) increase with increasing water temperatures, and then decrease forming a curvilinear pattern (Hallett et al. 2012, Hurst et al. 2012). The timing of spore release (i.e., when spore levels increase to the point of detectability in water samples) usually coincides with the descending limb of the spring hydrograph, and is likely dependent on accumulating thermal units. Meaders and Hendrickson (2009) infected polychaetes with Type II myxospores and tracked actinospore development within a laboratory. Actinospores were generated after 49 days averaging 17.3°C (approximately 850 degree days). Recent laboratory experiments suggest that Type I spores may develop more quickly (approximately 735 degree days) in polychaetes, with actinospores generated after 35 days averaging 21°C (J. Alexander, personal communication).

Actinospore and myxospore viability are both affected by water temperatures, but myxospores are more resilient to higher water temperatures (Chiaramonte 2013). At temperatures near 20°C in a controlled laboratory setting, 50% survival was observed after approximately 25 days, and the hardiest myxospores survived approximately 50 days (Chiaramonte 2013). At 15°C, 50% survival was observed as late as 100 days and the hardiest myxospores lasted 150 days. This is in stark contrast to laboratory-monitored actinospores held in 20°C water, where only 20% survival was observed after 3 days, the most robust of which lasted only 9 days (Bjork 2010). Actinospore survival also related to temperature, and at temperatures near 12°C approximately 50% survival was observed at 3 days and the hardiest spores survived 15 days.

The current hypothesis is that myxospores released from adult salmon carcasses contribute the bulk of myxospore to the system (Foott et al. 2016). The release of myxospores from adults likely occurs within several weeks of pre- or post-spawn mortality. Hence, the timing of myxospore contribution closely aligns with the timing of salmon spawning, which in recent years coincides with a stable hydraulic period below Iron Gate Dam (Figure 3). Recent laboratory studies estimate the settling rate of *C. shasta* myxospores at 0.35—0.45 m/day (Miao and Deas 2015). This rate suggests that suspended myxospores could settle in riverine locations quite distant from entry location under turbulent high-flow conditions. The settling characteristics and relative resiliency of myxospores suggests that they could be prone to redistribution and potential infection of polychaetes at spatial locations and time periods distant from their initial release time and location.

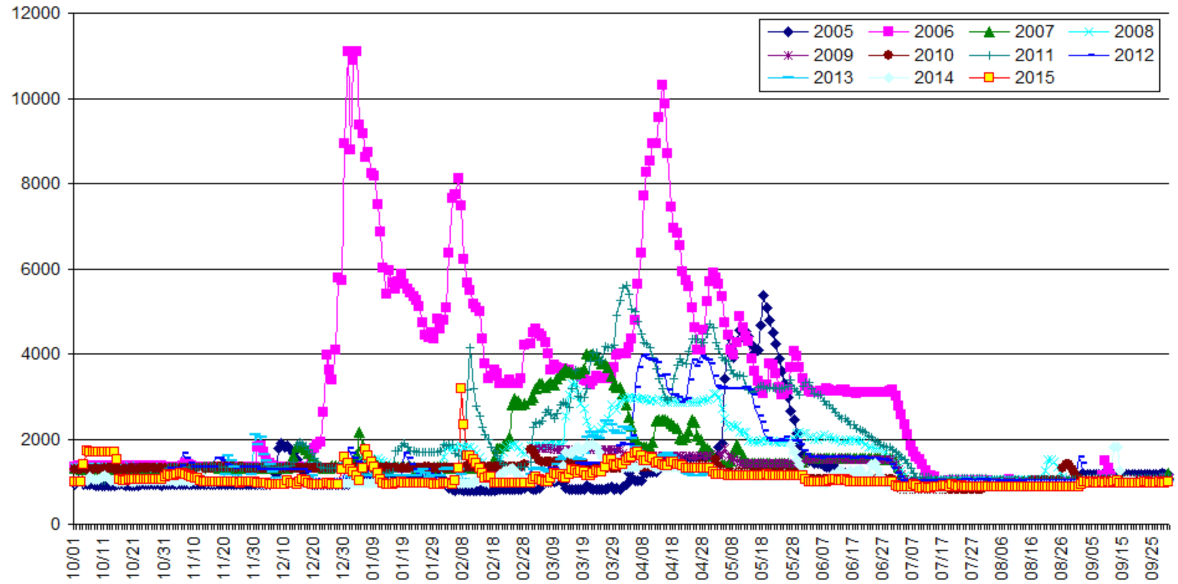

Figure 3. Daily discharge from Iron Gate Dam as measured by U.S. Geological Survey gauge 11516530 for water years 2005 – 2015. Reported discharge values are in units cubic feet per second. http://waterdata.usgs.gov/ca/nwis/uv?format=gif&period=10&site_no=11516530

The concentration of spores varies spatially within the mainstem river, with the highest spore concentrations typically detected near the confluence of Beaver Creek (Hallett et al. 2012). However, the spatial peak has occurred downstream of Beaver Creek (e.g., near confluences of Seiad Creek or Tully Creek) in some years (Bartholomew 2010, 2011, Table 1). In 2016, the index water sampling sites near Orleans and Tully Creek had much higher peak spore concentrations than any of the upstream locations, which has not previously been observed (http://microbiology.science.oregonstate.edu/content/monitoring-studies). Further, the difference between the peak spore concentrations at the Orleans index site and the upriver sites was of a much higher magnitude than observed in previous years. One hypothesis for the unique spatial pattern of spore concentrations observed in 2016 relates to the 11,200 cfs Iron Gate Dam discharge event occurring March 2016. This event could have dislodged and moved high numbers of polychaete worms downstream in the drift and these redistributed worms, if infected, may have contributed to the relatively high spore concentrations observed in the lower river (J. Alexander, pers. comm).

The annual peak concentration of actinospores varies considerably, with observed differences of several orders of magnitude among years. (Table 1). Predicting annual peak actinospore concentration levels remains elusive. The difficulty in prediction is likely attributable to the currently unavailable population-level estimates of both myxospores released by adult salmon, and abundances of infected polychaetes in each year. Further, knowledge of the exact timing or dynamics of infection and parasite transmission to polychaetes is still not well understood under riverine conditions.

Table 1. Annual maximum actinospore concentrations recorded at the Beaver Creek ("BC Peak") water monitoring index site during the Chinook Salmon outmigration period (spring and early summer). Values provided are approximations of spores per liter based on a standard curve transformation of detection assay readings, and include the totals pooling over all genotypes. In years where the system maximum value occurred at another site, the location and maximum value are indicated ("Alt. Peak"), where KTC represents Tully Creek, KSV represents Seiad Valley, and KOR represents the Orleans water monitoring index sites. Data provided by the J. L. Bartholomew Laboratory, Oregon State University.

Year	BC Peak	Alt. Peak
2005	75	
2006	8	
2007	250	
2008	300	
2009	150	
2010	20	KTC: 100
2011	10	
2012	1	KSV: 5
2013	5	KTC: 8
2014	100	
2015	1200	
2016	50	KOR: 250

Effects of Discharge on Spore Concentrations. Due to water management concerns impacting the entire Klamath Basin, experimental discharge increases have not generally been available to assess the effects of within-year discharge changes on spore concentrations. There have been several years where elevated discharges from Iron Gate Dam have occurred between April and June, the time of year when actinospore levels are rising or at their highest. During high flows events occurring in spring 2006 (10,300 cfs) and 2011 (5,700 cfs), spore concentrations were below the detection limit prior to the increases in discharge. Hence, it is not possible to assess the dilution potential of elevating discharge in those years. Some insight into the dilution potential of elevated flows may be gained from two additional events that occurred over the last decade. The first event was an unplanned discharge increase occurring in May of 2005, and associated fish-health and water monitoring data may demonstrate an effect of discharge on spore concentrations. A planned pulse event occurred in 2014, and this event was well monitored. Both are described below.

By 2005, the impacts of *C. shasta* on salmonids in the Klamath Basin had risen to the point that fish health and water monitoring programs were being implemented throughout the basin. Weekly-stratified fish-health surveys began in March of 2005. Water monitoring assays adept at quantifying spore concentrations in water samples had just been developed (Hallett and Bartholomew 2006), and water sampling began in May of 2005.

In April, the weekly-stratified prevalence of infection estimates began to quickly rise, and eventually reached 100% of sampled fish at the beginning of May (Figure 4). Despite no water sampling occurring during this time of year in 2005, the estimated prevalence of fish infections undoubtedly demonstrates the presence of actinospores in the water. Further, given the relatively high estimated prevalence of fish infections, once could safely assume concentrations well above 10 spores/L threshold reported by Hallett et al. (2012) for infectioning Chinook Salmon.

Towards the end of April, releases from Iron Gate Dam increased slightly, but in early May discharge spiked sharply and remained elevated for nearly a month (Figure 4). Soon after the spike in discharge, weekly prevalence of infection values began to decrease. One explanation for a decrease in the weekly prevalence of infection estimates could be the addition of hatchery-released fish that were not in the river long enough to become infected. This influx of uninfected fish would dilute the weekly samples, resulting in lower prevalence of infection estimates. However, the first Iron Gate Hatchery release in 2005 occurred on May 15, several weeks after the observed decrease in weekly prevalence of infection estimates. Additionally, the first water monitoring sample was collected when discharge was elevated and resulted in a non-detection (equivalent to an estimate of ~ zero spores/L). After flows had receded to base levels, subsequent water monitoring samples revealed detectable levels of spore concentrations (Figure 4).

In 2014, water monitoring samples indicated that spore densities had reached levels of concern for Coho Salmon. To prevent or reduce a disease outbreak in juvenile fish, federal agencies and partners agreed to increase discharge as a possible solution to dilute spore concentrations or otherwise disrupt the *C. shasta* life cycle. For this event, discharge was increased to approximately 1,900 cfs on May 27, and held for 24 hours. Flows were reduced approximately 200 cfs each subsequent day until a base discharge of 1,000 cfs was reached on June 2 (Figure 5).

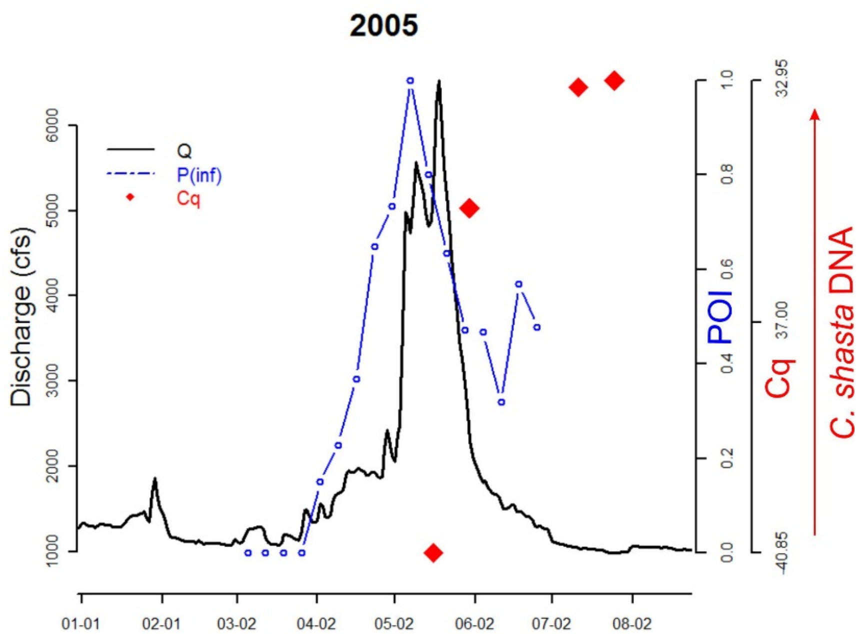

Figure 4. Daily river discharge (solid black line), weekly-stratified prevalence of *C. shasta* infection among sampled Chinook Salmon (open blue circles connected by blue lines), and Cq scores for water monitoring samples (solid red diamonds), all estimated for an area of the mainstem Klamath River between the Shasta and Scott confluences. The inset right axis represents the range of prevalence of infection values in fish, and the outset right axis represents Cq values that reflect quantities of *C. shasta* DNA; these are scaled so that increasing values correspond to increases in spore concentrations.

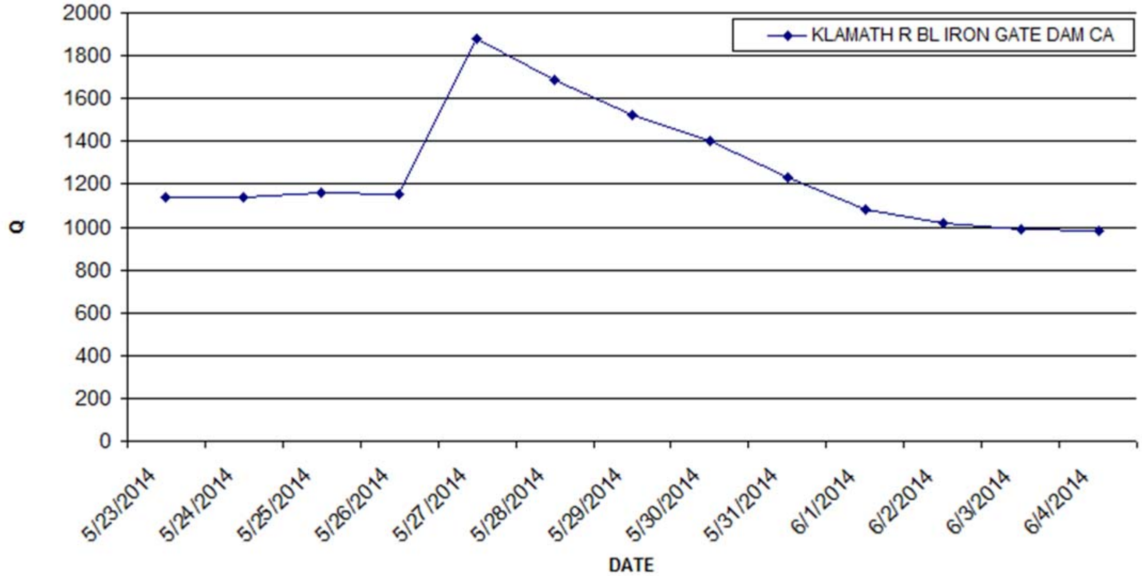

Figure 5. Daily discharge (Q) from Iron Gate Dam as measured by U.S. Geological Survey gauge 11516530 for May 23, 2005 – June 4, 2005. Reported discharge values are in units cubic feet per second.
http://waterdata.usgs.gov/ca/nwis/uv?format=gif&period=10&site_no=11516530

As a planned event, monitoring to evaluate the effectiveness of the increased discharge was coordinated. In particular, Oregon State University monitored spore concentrations at two locations. Water samples were collected daily at Beaver Creek and Seiad Valley index sites, beginning three days prior to the event and ending on May 30. Samples were collected every 2 hours using automated samplers, and pooled to make a 6-hour composite sample that was assayed using a *C. shasta*-specific qPCR. Data were more complete for Seiad Valley (Figure 6). At both sites there was a noticeable decrease in spore concentrations immediately following the increase in discharge. This reduction in spore concentrations, however, did not persist, and spore concentrations increased with decreasing discharge (Figures 5 and 6). The continued rise in spore concentrations may have been amplified by increasing temperatures.

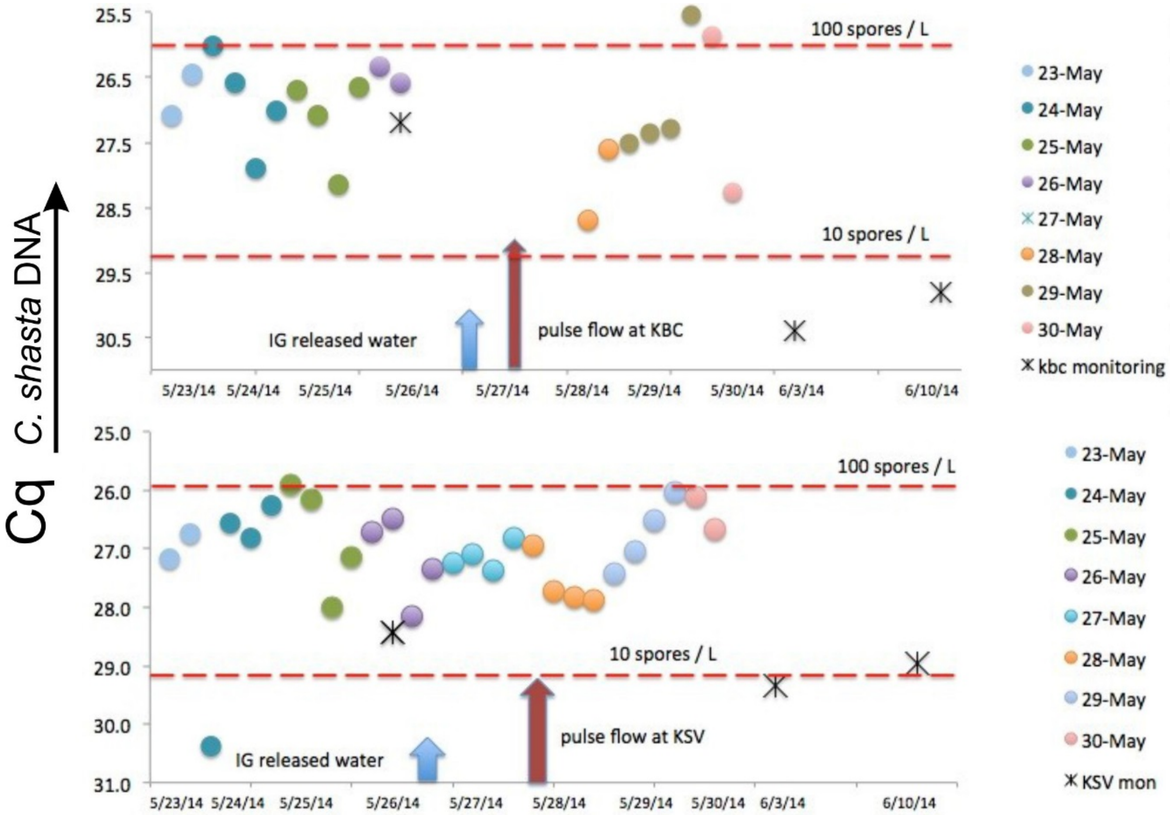

Figure 6. Quantitative PCR results, also expressed as *C. shasta* spores/L, at the Beaver Creek (top) and Seiad Valley (bottom) index water monitoring locations. Each data point is the average Cq of 3 x 1L water samples (6h composite). A lower Cq value indicates more parasite is present. Blue arrows mark the date of increasing discharge from Iron Gate Dam, and red arrows mark the arrival of the increased discharge at each index location. Figure provided by the J. L. Bartholomew Laboratory, Oregon State University.

Summary Guidelines.

- A DNA assay allows for the quantification of spore concentrations in water samples.
- Spore genotypes have been shown to associate with salmonid species-specific mortality.
- Myxospores are hardier than actinospores, and likely survive for a longer period after release from their host organism.
- The majority of myxospore load to the system is likely via adult salmon carcasses in the fall.
- Generally, actinospore spore concentrations increase with increasing water temperatures in the spring and then decrease as water temperatures further increase during summer.
- The location of peak actinospore concentrations varies among years, but most frequently occurs near the confluence of Beaver Creek.
- It is not uncommon for actinospore concentrations to peak as far downriver as the Tully Creek confluence. However, the magnitude of the difference between the peak spore

concentrations downriver and at Beaver Creek was much higher in 2016 than for any other year since water monitoring began.

- Annual peak actinospore concentrations vary by several orders of magnitude.
- Actinospore development within polychaetes is likely a function of accumulating thermal units, and likely takes between 100 and 115 days.
- Though managed discharge events have not produced dramatic reductions in spore concentrations, the planned discharge increases were likely too small to be biologically effective. An unplanned discharge increase in 2005 likely demonstrates the potential for larger discharges to effectively reduce spore concentrations.

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United States Department of the Interior

FISH AND WILDLIFE SERVICE

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In Reply Refer To:
AFWO

Technical Memorandum

TO: Dave Hillemeier, Yurok Tribal Fisheries, and

Craig Tucker, Karuk Department of Natural Resources

FROM: Nicholas A. Som and Nicholas J. Hetrick, Arcata Fish and Wildlife Office,

J. Scott Foott and Kimberly True, USFWS California-Nevada Fish Health Center

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).

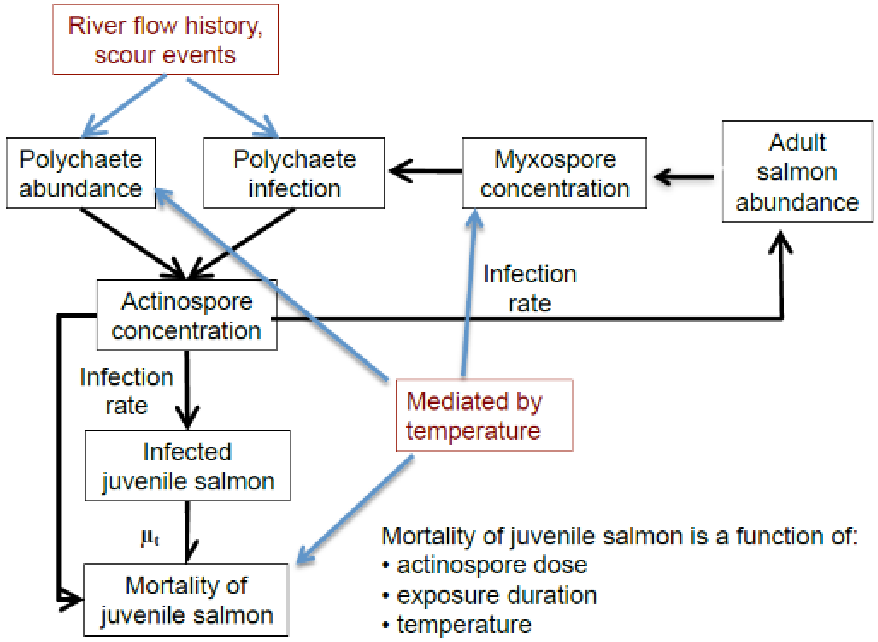

Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon. with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).

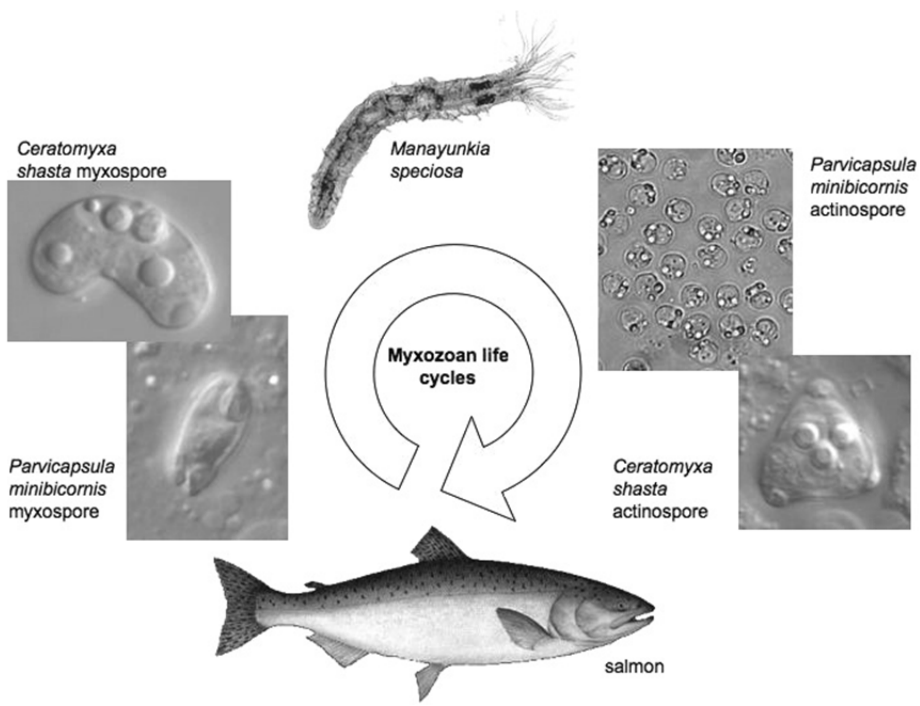

Figure 2. The life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* (graphic provided with permission from J. Bartholomew, Oregon State University). *Manayunkia speciosa* is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites.

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).

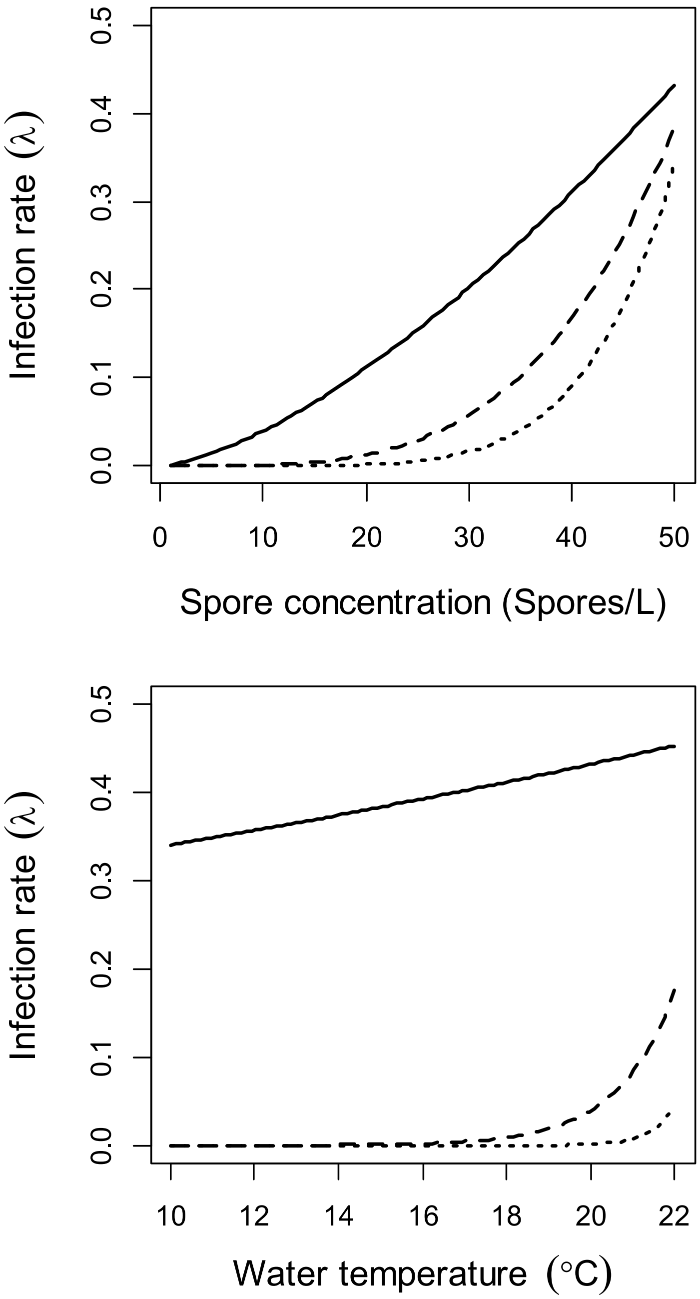

Figure 3. Effect of spore concentration and water temperature on infection rate of juvenile Fall Chinook Salmon with *C. shasta*. In the top panel, infection rates are shown as a function of spore concentration at 10 °C (dotted line), 15 °C (dashed line), and 20 °C (solid line). In the bottom panel, 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.

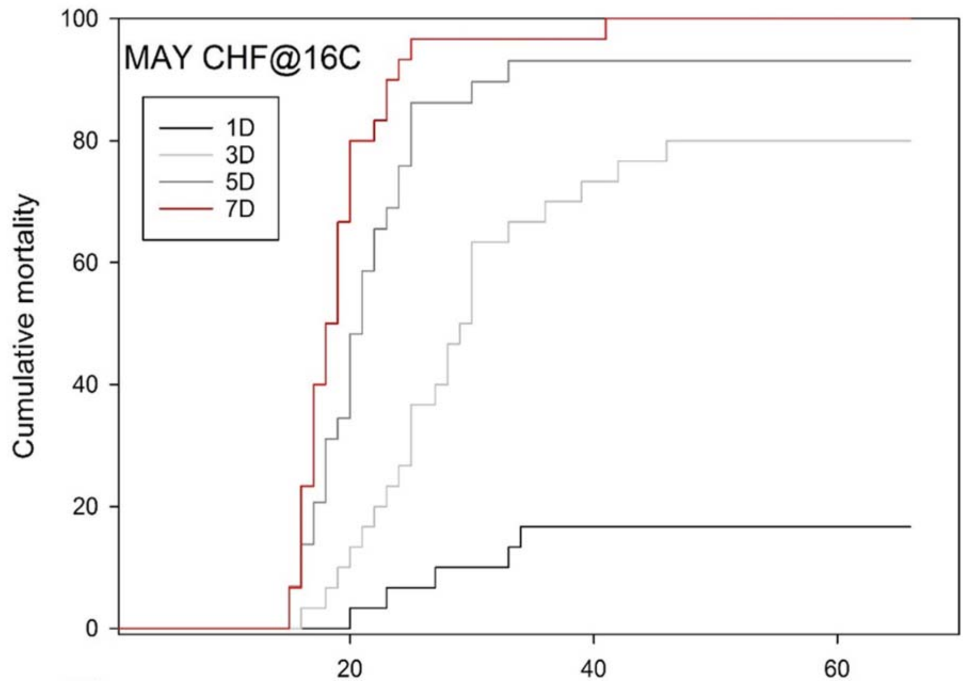

Figure 4. Cumulative percent mortality for trials of Chinook Salmon exposed for 1 day (black), 3 days (light gray), 5 days (dark gray), and 7 days (red). Each trial consisted of 30 fish exposed *in situ* to riverine conditions and then reared in disease-free laboratory water at a constant temperature 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.

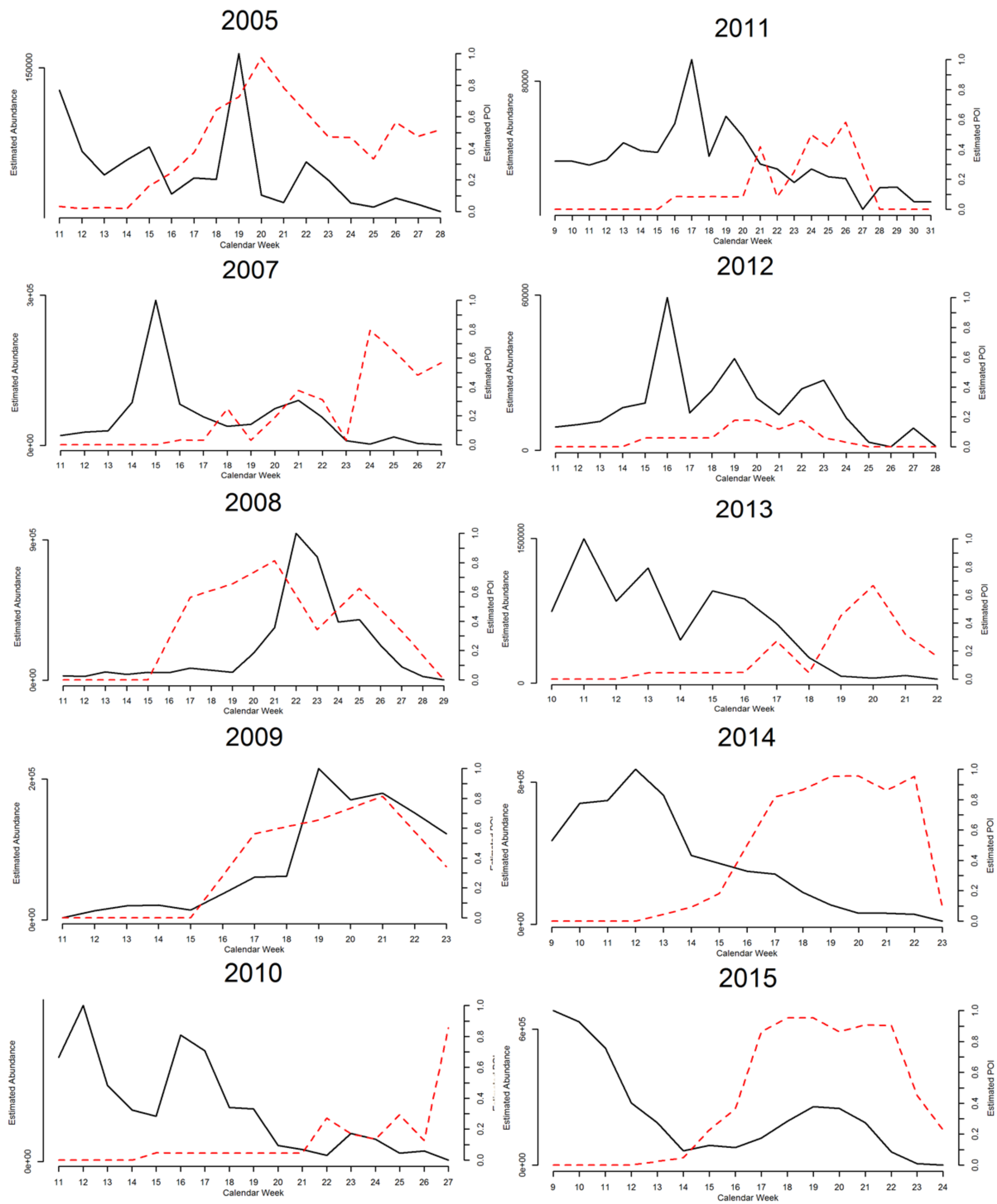

Figure 5. Weekly-stratified abundance estimates of juvenile Chinook Salmon (solid black lines) and *C. shasta* prevalence of infection (POI, dashed red line), by year. Abundance estimates reference the Kinsman rotary screw trap location (Gough et al. 2015) and prevalence of infection estimates reference 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, $\leq$13% are considered significant contributors (produce $\geq$ 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.

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DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
AFWO			General	Our understanding is that the draft "Guidance Document" was prepared by the Tribes to provide science-based actions for consideration by managers that would lower the incidence of C. shasta in juvenile Chinook and Coho salmon in the Klamath River, independent of consideration of other demands on the Basin's limited water resources and/or agency obligations. Given the intended purpose of the document, we believe the authors present recommendations that capture key information and scientific findings presented in the Service's four Technical Memos, with exceptions detailed below.	
AFWO	4	3	General	Here and throughout document: we believe the language regarding monitoring and evaluation of hypotheses prompting the flow augmentation recommendations is too weak. The monitoring and evaluation efforts should be required, not optional, and need to be well designed and incorporate an adaptive management approach.	It is beyond the perview of the Guidance Document to require any action (see Guidance Scoping document). Existing language in the document regarding Adaptive management and associated monitoring already indicates the desire that such monitoring be well-designed.
AFWO	4	6, item 1.c	Pulse flows to redistribute and strand carcasses and myxospores in late fall/winter.	The flow magnitudes specified during the fall are not sufficient to strand carcasses. This observation is compounded by the findings of Foott et al. (2010) that showed that about 5-9% of carcasses in Bogus Creek were categorized as being "high spore contributors" (>1 million spores/carcass), which account for about 90% of the total myxospore load to the river. As a result, a relatively high proportion of total carcasses would need to be removed from the channel to result in a correspondingly large decrease in "high spore contributor" carcasses and therefore, spore loads. However, spore development in carcasses in relation to disturbance events is one area of research that should be conducted to test they hypothesis that carcass stability, in relation to movement and temperature, is linked to spore production within carcasses.	"possibly" added to carcasses wording regarding stranding. An additional goal is to redistribute carcasses and that is reflected in the wording.
AFWO	7	2	Given the recent scarcity of these types of flows, this action should be implemented every winter until dam removal or the end of the 2013 BiOp.	We believe the document would benefit if the authors could provide a brief description as to why a Q of 6,030 cfs below Iron Gate Dam, the flood frequency 2-yr return period presented in the Geomorphic Technical Memo, was selected to occur for a 72-hr interval annually. We further suggest that the justification be, in part, based on the annual occurrence of in-reservoir phytoplankton blooms in PacifiCorp Project Reservoirs, primarily in Copco I and Iron Gate, that are released into the Klamath River (NCRWQCB 2010). Dead and decaying algal materials released down the Klamath River, including from cyanobacteria species, settle out and become a potential food source to support high-density polychaete colonies. The extent of this settling out of fine organic sediments in the bed of the Klamath River is elevated during drought conditions due to the extended retention time and turn-over rate of water in PacifiCorp Project reservoirs, warmer water tempertures, and a corresponding increase in algal blooms in the PacifiCorp reservoirs (U.S. DOI and NMFS 2013). Also important to note that in the period WYs 1964 to 1999, the duration between Surface Flushing events below Iron Gate Dam, as calculated on a daily-mean discharge basis, was typically one to two years (Figure 6 in Geomorphic Technical Memo). The 1-2 yr. return period for Q>6,030 below Iron Gate Date is consistent with the modelled outputs of discharge from Iron Gate Dam as presented in the 2013 Joint Biological Opinion (see attached "BiOP Model Output at IGD" sheet), with mean weekly Q >6,030 cfs occurring during 35% of the modelled years and mean weekly Q >4,000 cfs occurring in 52% of the modelled years. It is assumed that a modelled mean weekly Q >4,000 cfs would likely result in a spill event that would result or could be configured to result in a spill event equating to over 6,030 cfs for 3 of the 7 days in the mean weekly value.	Language and citations added, as follows: NCRWQCB. 2010. Final staff report for the Klamath River Total Maximum Daily Loads (TMDLs) addressing temperature, dissolved oxygen, nutrient, and Microcystin impairments in California, the proposed site-specific dissolved oxygen objectives for the Klamath River in California, and the Klamath River and Lost River implementation plans. State of California North Coast Regional Water Quality Control Board, Santa Rosa, California. http://www.swrcb.ca.gov/northcoast/water_issues/programs/tmdls/klamath_river/ (U. S. Department of Interior and U. S. Department of Commerce, National Marine Fisheries Service, 2013. Interior U. S. Department of Interior Klamath Dam Removal Overview Report for the Secretary of the Interior AN ASSESSMENT OF SCIENCE AND TECHNICAL INFORMATION Version 1.1, March 2013.
AFWO	7	3	The Polychaete Memo describes how populations of polychaetes can be kept in check by mobilizing sediments.	Technically, the Polychaete Memo does not explicitly link polychaete population size to sediment mobility. The Memo does discuss modeled relationships between the probability of polychaete presence with hydraulics and substrate, and also documents reductions in polychaete occurrence associated with larger flow events.	language added to address this issue
AFWO	9	2	Provide a flow of at least 11,250 cfs in the Klamath…May 31 for a period of at least six hours…	The 6-hour duration presented by the authors is not consistent with daily-mean discharge values presented in the Geomorphic Technical Memo.	guidance changed to average 11,250 cfs for a day.
AFWO	10	4	…should look for opportunities to provide safe, short-term peak flows of higher magnitudes than 11,250 cfs whenever possible	We suggest the author's mention the importance of synching the timing of deep flushing flows to coincide with high-flow events in tributaries. Important to also establish ramp down rates that it will minimize stranding of fishes as well as facilitate the depositional sorting of mobilized bedload. We assume that during a "geomorphically-effective flow event, both the duration and ramp rates would occur as a run-of-the-river event and therefore, outside of the control of management.	language added to address this issue

DTAT Guidance Document Comments and Responses					
Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
AFWO	10	5	The Polychaete Memo describes how these stable flow conditions have contributed to a proliferation of M. speciosa.	"Describes" is too strong of a word, as the Polychaete Memo did not present any estimates of population proliferation. Perhaps one could infer this idea from the contents of the Polychaete Memo, but the language should reflect that inference.	language added to address this issue
AFWO	11	top 1/3 of page	The 4-step logical statement	The current logic of the and/or progression would trigger MG #4's augmented flows in almost every year (for instance, 8 of the last 9 years). In addition to potential issues with the and/or progression, the 5 s/L in step 1 and 13 digress C in step 4 are likely too low.	5 spores/liter was kept, due to high mortalities observed in sentinel studies; the 13C temperature threshold was eliminated.
AFWO	13	3	Fall flow increases …carcasses downstream or entrain them on the river banks and in willows.	The flow magnitudes suggested in this Management Guidance (#5) are not large enough to entrain carcasses on banks. See response in row 5.	language added to address this issue
AFWO	15	2	Continue to increase fingerling releases during May, rather than during June, as has happened since 2013, to minimize overlap with high disease prevalence in the river.	Unclear sentence as to what "has happened since 2013". Our understanding is that fingerling releases have typically been initiated in May. As written, we are concerned that in years having cooler winter and spring water temperatures that result in a late outmigration of natural fish (see 2012 Klamath Outmigrant Monitoring Summary), the overlap between natural and hatchery fish in May could be significant. For example, in colder years such as 2012, hatchery fish were not released until June 6 and yet experienced a moderate (15%) estimated infection rate (Table 2, Fish Infection Technical Memo). As such, we suggest the authors consider recommending management triggers used to adjust the timing of fingerling hatchery releases based on real-time environmental conditions, with the goal of reducing the overlap of occupancy of hatchery and natural fish in the mainstem river while minimizing the risk of high levels of infection in hatchery fish. These real-time metrics could include the timing and pattern of the natural outmigrant catch (preferably abundance or relative abundance) at the Kinsman trap and a water temperature/developmental degree day metric.	added "based on real-time conditions" to description.
USFWS Klamath Falls Office	1	3	Observed infection rates of Chinook Salmon sampled between the Shasta River confluence and the Trinity River confluence in May through July of 2014 and 2015 were 81% and 91% respectively – considerably exceeding the take threshold established in the Biological Opinion (True et al. 2016, 2013 BiOp).	Add "May through July" into sentence	Will fact-check and do so if appropriate.
USFWS Klamath Falls Office	1	3		extra "the" in sentence	Corrected; thank you.
USFWS Klamath Falls Office	2	3	The only limitations the DTAT placed on the guidance provided herein, were physical possibility, safety, and a reasonable timeline for implementation.	change limitations to limitation	Rejected; "limitations" grammatically correct.
USFWS Klamath Falls Office	4	2	Members of the DTAT also had personal communication Scott Foote, Sascha Hallet, and Julie Alexander, all of whom have relevant expertise with regard to C. shasta.	multiple errors	Changed to "Members of the DTAT also had personal communication with Scott Foott, Sascha Hallett, and Julie Alexander, all of whom have relevant expertise with regard to C. shasta."
USFWS Klamath Falls Office	4	4		extra space	extra space removed
USFWS Klamath Falls	4	6	The team found several types of actions that could be implemented immediately that would have a high likelihood of reducing the infection prevalence and severity of *C.*	Would suggest that success in reducing infection prevalence will be dependent on monitoring. It is likely that these short term control measures will have some effect on infection rates, but "high likelihood" may be too strong given the myriad factors at play here, both from a biological and hydrological perspective.	Do not agree that success in reducing infection prevalence will be dependent on monitoring. Maybe "determination of success" in which case, see section on Adaptive Management. By "high likelihood" the DTAT interprets that to mean greater than a 50/50 chance, and we stand by that conclusion based on the information provided in the memoranda and the integration of this information as presented in this memorandum.
USFWS Klamath Falls	5	2		no space	Corrected; thank you.
USFWS Klamath Falls	5	4		wrong verb tense	Corrected; thank you.
USFWS Klamath Falls	5	6		no space	Corrected; thank you.
USFWS Klamath Falls	6	3	The primary means of controlling *C. shasta* were identified as:	These were not explicitly stated in the tech memos. Suggest a modification to language such as, "Based on the science summarized in the technical memos, the following measures can be reasonably presumed to aid in the control of C. shasta:"	Sentence changed to: "Based on the science summarized in the technical memos, the following measures can be reasonably hypothesized to aid in the control of C. shasta:"

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
USFWS Klamath Falls	6	6	The DTAT has determined, based on the weight of the evidence provided in the Technical Memos and the collective expertise of the team members that implementation of the geomorphically oriented management guidance actions ~~is expected to~~ may significantly reduce the need for the other management guidance actions such as spring dilution, fall variability, and hatchery changes.	Suggest changing "is expected to" to "may".	The DTAT believes that the scientific evidence supports the likelihood that improved geomorphic function as provided for in recommendations 1-3 will in fact result in improved conditions with regard to C. shasta and thus will in fact reduce the need for other corrective actions such as spring flows. The original wording was left intact.
USFWS Klamath Falls	7	1	Management Guidance 1: Provide Surface Flushing Flows to the Mainstem Klamath River Below Iron Gate Dam	Combining these flows essentially narrows the window to Feb 15 – April 30. This is not in the spirit of the guidances, but more importantly, it leaves us with a severely hydrologically limited system. Conducting a high flow in fall and a high flow in spring makes more sense in the hydrologic function of the system. Flexibility should be sought in the frequency of these flows rather than the annual timing, so that the system can function appropriately given the availability of water in a given year.	It is unclear what this comment is referring to. Recommendation 2 (flow of 11,250 cfs) did contain a statement where it was suggested that thobjective of proficing surface flushing flows could also be met if the provision of 11,250 cfs provided a flow of 6,030 cfs that lasted longer than 24 hours). This was not required, and thus the recommendation did not narrow the window to Feb 15-April 30.
USFWS Klamath Falls	7	2	Specifically, provide a flow of at least 6,030 cfs from Iron Gate Dam for a 72 hour period	How was this time period for elevated flows determined? Please reference the technical memos or otherwise give justification for this time period.	Please see Uncertainties in this section: "In general, a longer duration event will accomplish more of the objective than a shorter duration, because more of the suspended sediments flush out of the river system, rather than being re-deposited further downstream."
USFWS Klamath Falls	7	2	Existing guidelines contained in the 2013 BiOp should be followed with regard to downramping rate	Suggest that ramping rates should be adjusted based on the seasonal situation and review by the FASTA team, as has been done in previous flows. Flexibility in ramp rates is going to be key, especially in the fall. We should look for a mechanism to streamline this process within the FASTA.	Text changed to: Existing guidelines contained in the 2013 BiOp should be followed with regard to downramping rate unless modified by the Technical Team or FASTA as necessary and supported by scientific information. As explained in the support and uncertainties section, the descending limb is important for the river to sort and distribute fine sediments.
USFWS Klamath Falls	7	3		typo: replace "an" with "a"	Corrected; thank you.
USFWS Klamath Falls	8	1	The estimated two year recurrence interval (Geomorphic Memo page 6) of 6,030 cfs was selected	Please justify making a flow with a 50/50 chance of occurring in any given year into an annual flow. This does not seem to be supported by the technical memos, nor is it within the nature of a two year recurrence interval flow	Supporting language added to support subsection of this proposed action. "With regard to frequency of the discharge necessary for a surface flushing flow, the guidance provides for this flow to occur every year because low winter flow conditions since 2000 (Figure 3) have resulted in overly stable river bed conditions which in turn have caused very high mortality rates from C. shasta. Therefore, the guidance is for this flow to occur each year for the duration of the BiOp. Additionally, the annual occurrence of in-reservoir phytoplankton blooms in PacifiCorp Project Reservoirs, primarily in Copco I and Iron Gate, results in a large amount of dead algae that is then released into the Klamath River (NCRWQCB 2010). Dead and decaying algal materials released down the Klamath River, including from cyanobacteria species, bsettle out and become a potential food source to support high-density polychaete colonies. The extent of this settling out of fine organic sediments in the bed of the Klamath River is elevated during drought conditions due to the extended retention time and turn-over rate of water in PacifiCorp Project reservoirs, warmer water tempertures, and a corresponding increase in algal blooms in the PacifiCorp reservoirs (U.S. DOI and NMFS 2013). "
USFWS Klamath Falls	8	1	However, there is strong evidence to suggest that frequent surface flushing flows minimizes available habitat for polychaetes	Please cite either technical memos or other relevant documentation to support this statement.	Citation added (Polychaete tech memo pages 4 and 5 support this statement)
USFWS Klamath Falls	8	4	However, there is strong evidence to suggest that frequent surface flushing flows minimizes available habitat for polychaetes	Monitoring the movement of fines and tying this movement, if it can be quantified, to a reduction in polychaete density will be extremely difficult. Please consider and make some reference to the difficulty in proving that these flows are having measurable positive effects outside of increased "natural" hydrologic function in the river.	Please see recent research by Alexander et al. 2016 in which polychaete distribution is correlated with hydraulic conditions during high flows. (Alexander, J. D., J. L. Bartholomew, K. A. Wright, N. A. Som, and N. J. Hetrick. 2016. Integrating models to predict distribution of the invertebrate host of myxosporean parasites. Freshwater Science. Online Early. doi: 10.1086/688342). This analysis, when combined with laboratory observations of polychaete dislodging under unstable substrates in high velocities (Polychaete Memo page 4, 5) places this hypothesis on firm footing. Therefore, the wording will remain unchanged in Guidance Document.
USFWS Klamath Falls	8	5	Adaptive management (generalized)	As a general comment on monitoring guidance in this document, the authors need to commit to greater specificity in this guidance as the measures are developed. I understand that it is difficult at this point to lay out specifics on monitoring, but this document should address that specificity in monitoring procedures, timing, and personnel should be and will be addressed before any of these management actions are implemented	The Guidance Document expresses strong support for a robust Adaptive Management aspect including hypothesis testing, monitoring and response to new information. It is beyond the scope of the document to outline a full and complete monitoring plan for each of the identified management guidance actions. The authors of the report look forward to working with fisheries co-managers to create and implement such a plan.

DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
USFWS Klamath Falls	9	3	Description of guidance (2)	What other potential ecosystem level effects may happen with this type of regime? Please address, with appropriate citation, the science supporting a net benefit from these flows to the overall ecosystem, as an imbalanced ecosystem could create unforeseen difficulties for the very species which we are trying to protect	Text added in "uncertainties" section of described management guidance #2. "With regard to ecosystem effects of this guidance action, re-establishment of a more natural flow regime that includes high flows such as called for in this management guidance is expected to restore flows that are closer to the unimpaired natural flow regime. Please see discussions on page 2 of Geomorphic Memorandum regarding importance of high flows to riverine systems and also page 3 of the Fish Infection Memo regarding parasite-host imbalances resulting from ecosystem alteration."
USFWS Klamath Falls	9	3	This action should be timed with peak releases from other tributaries such as the Scott River or other tributaries.	Add "if possible" to end of sentence.	Paragraph already says"should" and "unless precluded by drought conditions". Addition of "if possible" would therefore be redundant, because current wording captures it already.
USFWS Klamath Falls	9	4	NOTE: if the deep flushing event as described in Management Guidance 1 does not result in flows above 6,030 cfs for at least 72 hours, it will not fulfill the fine sediment management guidance action (#1)	This note has been the source of consternation in discussions with other reviewers. I read this as saying is that we can kill two birds with one stone (i.e. guidance 1 and 2), but if the ramping rates for guidance 2 preclude a flow exceeding 6030 cfs for 72 hours, the peak flow of guidance 2 will not fulfill guidance 1. Suggest clarification of this note and the purpose for its insertion here	Commentor was correct in assertion that the meaning of the note was to clarify the "kill two birds with one stone" but an unfortunate typographical error has contributed to confusion. The wording has been changed to clarify using a positive assertion. "NOTE: if the deep flushing event as described in Management Guidance 2 does result in flows over 6,030 cfs for at least 72 hours, it will also fultill the surface flushing as described in Management Guidance 1.
USFWS Klamath Falls	10	4	Management Guidance 3 (general comment)	Who will determine when these flows are available and the timing in which they should occur? Suggest clarifying within the body of this guidance as to potential decision-making process and if further hydrological modeling will be needed to define this decision-making process. Further, suggest addressing "inherent unpredictability and public safety factors" further. Are these flows intended to manifest as flood releases in wet springs, a la 2016? If so, this should be stated. Otherwise, this guidance is very similar to #3.	The Guidance 3 description states that it is the Bureau of Reclamation and PacifiCorp that would "look for opportunities" to implement this action. Have added "and other fisheries co-managers" after PacifiCorp to clarify that they should reach out to a broader technical audience. Specifying the exact circumstances for each case where this might be implemented is (as noted in the Guidance Document) complex and should be handled on a case-by-case basis.
USFWS Klamath Falls	12		Management Guidance 4 (general comment)	This entire management guidance effectively sets a new minimum in the river for a given period each spring. It will expend a great deal of water and may or may not result in the desired outcome. There is little evidence in the tech memos to support the need or effectiveness of this action. Suggest either further clarification of this guidance, with appropriate documentation, or removal of this guidance altogether.	For emergency flow releases to be effective, dilution flows need to occur as disease is worsening but before excessive levels of lethal doses are occurring in juvenile fish. Hallet (2012) found the lethality threshold of 40% was reached at 10 spores per liter for Chinook (type 1 genotype) but only 5 spores per liter (type 2 genotype) for coho salmon. Actinospores are generally released when temperatures are above 10°C, and remain viable (able to infect salmon) at temperatures ranging from 11 to 18ºC (Foott et al. 2006). A lethal combination of spores and temperatures is occurring at the trigger threshold of 5 spores per liter and 13 degrees C and given lag times of sampling of a week and likely trajectories given an examination of past trends, this was thought to be a prudent precautionary approach to avoid excessive mortality rates. Reclamation in the 2013 BiOp proposed flow increases for the Klamath River downstream of IGD to dilute actinospore concentrations within 24 hours of receiving information that disease thresholds have been met and used 5 spores per liter of Type II as a threshold (2013 BiOp page 41) so while this was only implemented once and with meager flows, this is a previously contemplated action. The unspecified genotyping referenced in the memo is in part to allow for much more rapid turnaround time for results (reduction of 2-4 days of process time), which would allow for more effective implementation for fish and water conservation. It is not disputed that there is sufficient information to assess C. Shasta through genotyping and that data would still be collected. Chinook salmon are the disease surrogate for coho in the ITS and incredibly important to tribal fisheries so should be protected during these actions along with ESA listed coho salmon.
USFWS Klamath Falls	11	1	Hold in reserve 50 TAF…	It is highly unlikely that 50 TAF can be "held in reserve" at any time in the year, much less the period annually in which water is at its most over-allocated in this system. This effectively means that 50 TAF can be requested and must be made available if the below criteria are evident, regardless of other obligations or needs. This would coopt all inflows to the lake at a critical time. Suggest a reduction in the amount requested for "reserve," which would also require adjustment of flow schedule below	Added statement in the Introduction section explaining that the purpose of this management guidance was to identify conditions in the mainstem river that would alleviate the high mortalities experienced due to disease in juvenile salmonids. The question of "how" to make these things happen is beyond the perview and scope of the document. We acknowledge that the system is complex, and variables interact with each other in unpredictable ways. none the less, we believe it is physically possible to deliver this quantity of water in the specified time period.
USFWS Klamath Falls	11	2	Spore concentrations exceed 5 spores (non?specified genotype) per liter for the preceding sample based on qPCR from water filtration samples at any sampling station;	Non-specified genotype is worrisome, given Sascha Hallett's work on genotypic specificity of C. shasta infection in a given species of salmon. There is obvious and universal interest in protecting the tribal trust species (i.e. Chinook), but the primary focus currently is on the listed Coho. Dr. Hallett's work should not be disregarded in this document. Suggest allowing specification of genotype so that species-specific threat may be more effectively evaluated, given other factors at play in the river.	We are recommending flows when the spore count reaches 5/L of any type, on the premise that 1) 5/L is indicative of a rising trend, 2) distinguishing DNA type takes precious extra days, and (?) 3) high concentrations of type 1 can mask detection of type 2. Wording as to such was added to the support section.

DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
USFWS Klamath Falls	11	2	Spore concentrations exceed 5 spores (non?specified genotype) per liter for the preceding sample based on qPCR from water filtration samples at any sampling station;	These criteria will be met in 9/10 years, effectively creating a new minimum flow for the river for much of the spring/summer. This appears to set the bar quite low for justification of these flows. Suggest reevaluating the criteria upon which these flows are based.	These criteria are based on the conclusion in the BiOp that 5 spores/L of type 2 and 10 spores/L of type 1 cause an estimated 40% mortality in Coho and Chinook juvenile salmon respectively (BiOp at 341-342). While it is acknowledged that this is a significant amount of water, it is also apparent that mortalities from this disease are a "key factor limiting salmon recivery in the Klamath River" (BiOp at 341). Furthermore, given the restrictions placed on implementation of this measure (less than 80% of the outmigration complete), it is not at all clear that 9/10 years would require additional flows. Language added under Support section: "For example Hallett et. al found that a lethality threshold of 40% mortality was reached with 10 spores per liter for Chinook and 5 spores liter for Coho Salmon. These high levels of mortalities support using 5 spores per liter as a threshold to initiate this Guidance Action. "
USFWS Klamath Falls	11	last	The concentration of actinospores in the Klamath River is a function of the total number of spores and the volume of water into which they are discharged.	While this makes sense, this does not seem to be supported by the Spore technical memo. The conceptual diagram (figure 1) suggests that actinospore concentration is a function of polychaete prevalence and infection rate. The effect of dilution, discussed in the body of the memo, is uncertain with the current science. Suggest removing these sentences.	We believe this statement is correct and is supported by analysis in the spore memo presented on pages 6-8 of the Spore Memo (Effects of Discharge on Spore Concentrations). We believe this discussion supports an alteration of the conceptual diagram to include flows and releases from Iron Gate Dam as a significant factor that affects spore concentrations. Added citation to Spore Memo to document.
USFWS Klamath Falls	12	1	Increased flows also increase water velocity and have been shown to reduce transmission rates.	This also seems to be a tenuous connection, given the current science outlined in the tech memo. Please cite with the appropriate support or remove.	Citation added to document (Ray et al.)
USFWS Klamath Falls	12	5	low temperatures often coincide with higher flows	This is anecdotal. I've not seen hydrologic research to support this, though I'd imagine there is likely some correlation. Perhaps a study should be commissioned to demonstrate the veracity of this statement in the Klamath River?	The statement says that low temperatures "often" correlate with higher flows. This is true because the higher flows come from precipitation, snowpack, and also are associated with decreased travel time from colder high elevation sites. Language remains unaltered.
USFWS Klamath Falls	13	1	Management guidance 5	Redistributing carcasses will be very difficult, and even if redistribution is successful, Foote's work shows that a single infected carcass can introduce millions of spores into the system, effectively negating the positive effect of moving carcasses about. Further, implementing guidance 1 will accomplish this goal, obviating the need for inclusion of this measure. Suggest removal or revision of this guidance.	Added statement in the support section regarding objectives. In addition to moving carcasses to different locations, it is also hypothesized that the myxospores themselves will be advected to less advantageous locations further downstream and some may become stranded on floodplains and subsequently dessicated. Management Guidance 1 (6,030 cfs flow for 72 hours), if performed during the myxospore release season (Nov-Dec) may achieve this objective, but it is more likely that if Management Guidance 1 is implemented it will be later in the year.
USFWS Klamath Falls	14	1	Management guidance 6	I agree that this should be implemented for disease control. However, this is not within Reclamation's purview, nor should it be tied in to BiOp obligations that may ultimately open the Services up to litigation in the future.	The guidance document identifies management actions that may alleviate C. shasta conditions on the Klamath without regard to implementation. This Guidance Document does not demand that Bureau of Reclamation implement any of these actions. Text will remain unaltered.
USFWS Klamath Falls	14	2	negative interactions between hatchery/natural fish in the Klamath River, while meeting the two objectives listed above	What are these negative interactions? Are they disease related? Is that disease something other than C. shasta?	This concept has been accepted in fisheries science for quite some time. No change to the text.
USFWS Klamath Falls	16	3	Adaptive Management and Monitoring Considerations for (MG6)	Perhaps language could be added here to the effect of, "The disease management team recognizes that these measure are outside of the purview of BoR and the Services. However, given the need for implementation of these measures, we strongly suggest a concerted effort between CDFW and the Yurok, Karuk, and Hoopa Tribes; with input from Reclamation, the Services, and the Klamath Tribes; to see these measures implemented at the IGD hatchery	The guidance document identifies management actions that may alleviate C. shasta conditions on the Klamath without regard to implementation. This Guidance Document does not demand that Bureau of Reclamation implement any of these actions. Text will remain unaltered.
Bureau of Reclamation	General			While the four technical memos present a science based discussion of possible factors and results, the Guidance Document does not conprehensively summarize the conclusions in the tech memos.	Disagree. Please see appendices for comprehensive summaries of each of the four tech memos as well as the memos themselves.
Bureau of Reclamation	General			The Guidance Document should be more science-based and provide recommended disease control actions independent of Klamath Project operations.	It was not focused on Klamath Project Operations, but on measures that could be implemented to minimize disease. The Klamath project is the entity tha has an Incidental Take Statement under the Federal Endangered Species Act regarding take in the Klamath River associated with Klamath Project water diversions; this ITS was substantially violated in 2014/2015, so to the extent water management is part of measures to address disease issues, the Klmath Project may be involved.
Bureau of Reclamation	General			Additional detail needs to be provided for the scientific bases of timing, duration, and frequency intervals of events for *all* management options. For example, if a management action is recommended every year, please more closely tie the recommended timing, duration, and frequency back to the technical memoranda.	Language was added in various parts of the document bolstering scientific support for implementation of various management guidance actions.

DTAT Guidance Document Comments and Responses					
Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Bureau of Reclamation	General			The most probable disease control measures proposed in the Guidance Document would involve use of flow releases to reduce polychaete densities and possibly disperse salmon carcasses downstream of IGD. However, high flow conditions (≥ 10% exceedence flows) are needed to produce an appreciable impact on polychaete and salmon carcass densities. Whether these high flows can be consistently achieved at the frequency needed is a major uncertainty, especially during moderate and dry water years (see Hardy et al. 2006, figures 5, 7, and 9). For example, how much would the frequency of high flow events >6,000 cfs increase if irrigation water was no longer stored and diverted? Instituting operational measures that rely heavily on factors outside management control, such as runoff, will not produce the desired outcome.	We agree that flow management is the most probable proposed measure to control diesease, and associated densities of infected polychaetes. There are multiple factors that affect the ability to increase flows in the Klamath River, especially for relatively short periods of time, including: end of year lake levels, management of winter flows, managing for flood control curve of the lake, management of reservoir levels, management of flows when lake is nearing full capacity, and timing associated with hydrologic events. As you are likely aware, water is naturally stored in Upper Klamath Lake. The hydrology of the Klamath River would be substantially different if it didn't have several hundred thousand acre feet of water diverted from it. However the intent, and effect even if all control measures were fully implemented, of this docment is not to eliminate agriculture and return to the pre-development hydrograph, our intent is to manage in a manner that minimizes the extremely high disease rates the river has experienced during recent years (i.e. we are not suggesting that ag diversions be halted).
Bureau of Reclamation	General			The link between coho salmon life-history events (e.g. emigration) and timing of the recommended flow management measures is not provided in the Guidance Document. Our understanding is that most of the juvenile coho emigrating through reaches proximate to IGD will have passed through the area prior to the annual onset of high C. shasta spore concentrations. These types of relationships are important to understanding the magnitude of potential benefits of some of the proposed measures (e.g. Management Guidance 4) to coho salmon population viability.	Depending on the year, disease levels can vary substantially during April - June. The years when disease levels are elevated relatively early would likely impact juvenile coho smolts prior to their emigration to the ocean. As noted in the BiOp (page 243), coho salmon migrating downstream have been found to have infection rates as high as 50% (Bartholomew and Foote, 2010). Juvenile young of year coho that are utilizing the mainstem Klamath, whether for rearing or re-distribution, would be affect by high disease levels throughout the April - June time period.
Bureau of Reclamation	General			The four USFWS C. shasta technical memos provide a logical basis to conclude that regulation of the Klamath River, particularly storing water during winter and spring, may exacerbate C. shasta infection rates for outmigrating salmon downstream of Iron Gate Dam (IGD) during some years. These impacts would be most pronounced in the first 15-20 river miles downstream of IGD where flow conditions are principally driven by IGD water releases. However, a functional relationship between IGD discharge and disease conditions (i.e. fish infection or mortality rates) in the Lower Klamath River, which would be necessary to quantify the impact of KP operations on disease-induced fish mortality, has not been developed. Therefore, the outcome of the flow management measures proposed in the Guidance Document is highly uncertain, and these measures should be regarded as investigational.	It is likely that disease conditions in the Upper Klamath River affect disease conditions in the Lower Klamath River, whether it be from dislodged/infected polychated worms, free floating actinospores, or fish that become infected in the Upper Klamath and die to release myxospores downstream. We recognize that there is still much to learn regarding the dynamics of C. shasta in the Klamath River, which is why adaptive management is highlighted as being so important in the Synthesis Memo
Bureau of Reclamation	General			The relationship between coho salmon smolt survival and flow has been quantified (Beeman et al. 2012), but this relationship includes all sources of fish mortality, not just disease. Beeman et al. (2012) did not quantify latent effects of disease that may occur after fish reach the ocean, but water temperature is the primary determinant of disease-induced mortality, and ocean temperatures are much cooler, suggesting that survival rates of infected fish would likely increase upon ocean arrival. Though latent effects of disease infection are a concern, it was generally believed that in-river survival conditions between IGD and the ocean were adequately described by the Beeman et al. (2012) study. One interpretation of the study findings is that small operational changes should not be expected to significantly reduce juvenile coho mortality. Instead, large flow events, such as those associated with large storms, were the most influential throughout most of the Lower Klamath River. The information described in the four USFWS disease technical memos also supports the idea that large flow events are needed to meaningfully alter disease conditions and subsequent fish mortality.	Of the flow related control measures identified in the Synthesis Memo to minimize *C. shasta* levels in the Klamath River, four of five measures are focused on interrupting the life cycle of C. shasta, primarily by disrupting polychaete worms (an obligate part of C. shasta life cycle), polychaete habitat, or infection of polycaetes by myxospores released from rotting carcassess. The effectiveness of these control measures to impact polychaetes was not been assessed by the Beeman study. The control measure that consist of the provision of emergency spring dilution flows if certain criteria are met, could somewhat be related to the findings of the Beeman study. Whether increasing flows to 3,000 cfs (and then going to 4,000 cfs if necessary) is considered a "small operational change" or "large flow event" is up to interpretation, however the amount of water required to meet these levels during the April/May time period can vary substantially, depending upon hydrologic conditions. During some wetter water years, these flow levels would be met without any flow augmentation.
Bureau of Reclamation	General			Please consider assessing the potential role of increasing flows from key tributaries of the Klamath River below IGD in reducing C. shasta POI. Although outside of Reclamation's jurisdiction, we feel that increasing cool water flows from key tributaries could reduce spore production and improve upstream passage into the tributaries. Concentrating efforts on increasing flows from IGD ignores the potential role tributaries could play at reducing actinospore concentrations in the mainstem Klamath River and increasing usage of those habitats by juvenile coho salmon.	It is not clear what time of year is being recommending for increasing flows in tributaries, given the reference to fish passage, however we agree that cool water accretions from Klamath River tributaries are critical for the health of the Klamath River ecosystem and could help to ameliorate disease conditions in the Klamath River to some extent. However, the magnitude of water diverted within tributaries downstream of Iron Gate Dam is relatlively small compared to IGD discharge - while the impact of these diversions is extreme within the tributaries, the impact to the mainstem Klamath flow is much less (but still important).

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Bureau of Reclamation	1	2	General	There are inaccuracies in this paragraph. For example, in the BiOp, NMFS concluded that they "cannot quantify the magnitude of the increased disease risk to coho salmon under the proposed action" (2013 BiOp, page 376). As such, to the extent statements such as those in paragraph 2 are needed, they should be limited to factual statements. Please verify the accuracy of other statements in this paragraph.	Page 349 of the BiOp: "The proposed action is likely to adversely affect coho salmon fry by reducing habitat availability and increasing susceptibility to diseases." AND NMFS believes the high incidence of disease in certain years within the mainstem Klamath River results largely from the reduction in magnitude, frequency, and duration of mainstem flows from the natural flow regime under which coho salmon evolved. The proposed action's effects on spring flows and channel maintenance flows and their relationship to disease are discussed below. Research on the effects of C. shasta on coho salmon juveniles is applicable to coho salmon fry because the parasite targets species not life stages (Hallett et al. 2012). (page 341 of the BiOp) AND: "Therefore, when environmental conditions are conducive to actinospore release in the spring (e.g., elevated water temperature), the proposed action will likely result in hydrologic conditions in the mainstem Klamath River that contribute to high C. shasta actinospore concentrations (e.g., ≥5 spores/L actinospore genotype II), which will likely increase the percentage of disease-related mortality to coho salmon fry in the mainstem Klamath River between Trees of Heaven (RM 172) and Seiad Valley (RM 129) in May to mid-June (Foot et al. 2008, Hallett et al. 2012, Ray et al. 2012). The proposed action will also likely increase the percentage of coho salmon fry in the mainstem Klamath River between Klamathon Bridge (RM 184) and Orleans (RM 59) that will experience sublethal effects of C. shasta infections during April to mid-June. Sublethal effects include impaired growth, swimming performance, body condition, and increased stress and susceptibility to secondary infections (Hallett et al. 2012)." (page 343). It seems that all of the statements in this paragraph are accurate. Please be more specific if you don't agree.
Bureau of Reclamation	1	2	General	Please refer to the Klamath Project as the Klamath Reclamation Project or Klamath Project throughout document.	Done
Bureau of Reclamation	1	3		The third paragraph on p. 1 of the Guidance Document does not seem to be directly related to the purpose of the Guidance Document. There is no reference to the ITS in the scoping document, rather the purpose of the exercise was to provide scientific information as to possible controls of the disease that could be implemented by Reclamation. Please remove first full sentence. Additionally, revise second sentence to be consistent with Scoping Document as follows: "In order to propose guidance for science-based measures intended to mitigate the effects of Ceratanova shasta (C. shasta) disease infection rates in coho and Chinook salmon below Iron Gate Dam, ...the disease technical advisory committee......".	The first sentence of paragraph 3 gives context for the document. While the scoping document doesn't specifically refer to the ITS, it does refer to the BiOp and the fact that the Synthesis Memo (referred to as Guidance Document in the Scoping memo) is intended for consideration and implementation for the duration of the 2013 BiOp.
Bureau of Reclamation	2	1		What are the specific mechanisms and empirical evidence linking KP to C. shasta infection in fish? The reviewer is concerned that operation of the Klamath Project may be less influential than other factors like meteorological conditions. Yes, the Klamath Project changes the hydrograph, but what is the relative contribution as compared to meteorological conditions, etc? There is a large amount of uncertainty whether the types of flows needed for disease control can be reliably, artificially produced. Figure 3 in the DTAT Guidance Document shows the flow duration for various types of high flow events in the Klamath River, 1964-2016. During the last 16 years there was one very wet water year (2006) and that's the year that produced the types of flows desired for disease control. The Geomorphic Memo stated that "From 2000 to 2016, the average cumulative duration of Surface Flushing flow exceeded five days in only one water year and no sediment mobilization flows occurred in 12 of the 17 water years." Due to water storage limitations, the Klamath Project may not be able to artificially create high flow conditions during dry or moderate water years, which indicates that the quantity and duration of the requested flow management measures may not be implementable.	The comment states: "There is a large amount of uncertainty whether the types of flows needed for disease control can be reliably, artificially produced". In regard to reliability, it depends upon which flows are being referenced, and, of course, the hydrologic conditions. However, surface flushing flows could be provided in most years, and deep flushing flows in many years. In regard to "artificially produced", we would argue that in many years the lack of higher flows, especially surface flushign flows), is "artificially" prevented from happening. Your reference to the minimal amount of high flows during the past 16 years,especially relative to the prior 26 years, is what we are trying to remedy. We think that part of the solution is management of the project, lake levels, and river flows. The driest yeaers will be the most challenging, however the surface flushing flows would consume approximatley 30,000 - 35,000 acre ft. of water (depending upon details), which should be technically feasible in all water years.
Bureau of Reclamation	2	3	3	On page 2, the document states "the only limitation the DTAT placed on the guidance provided herein, were physical possibility, safety, and a reasonable timeline for implementation". The limitation criteria described on page 2 is not followed in Page 5 list of control measures considered but elimiated (ie. Dewatering and Direct Sediment Introduction). The reasons for rejecting these control measures should reflect this list, or the list should be revised to include the reasons noted for each eliminated control measure starting on page 5.	Clarifying language added on page 2.
Bureau of Reclamation	2			The discussion in the first full paragraph of page 2 regarding operation of the Klamath Project is not relevant to the task of determining possible science-based controls of disease. Also, the reference to the impaired natural flow is an oversimplification of a more detailed discussion of the BiOp. Also, there is no explanation on how the Klamath Project affects water quality that relates to the disease issue at hand.	Language re: WQ deleted, reference to 2013 BiOp and Geomorph memo added
Bureau of Reclamation	3			The underlined text on p. 3 of the Guidance Document does not seem necessary as the Tech Memos were not for the purpose of suggesting management decisions.	The tech memos were developed, at the Yurok and Karuk Tribes request, so we could draft the synthesis memo which is based upon the information from the tech memos.
Bureau of Reclamation	4	2	1	typo, should be Sascha Hallett not Sasha Hallet	Thank you

DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
Bureau of Reclamation	4	3	3	Predicted outcomes are a key element of adaptive management. As such, to be able to meaningfully carry out the adaptive management considerations described on page 4, clear definition is needed in each of the management options. Please include.	This will be a task for technical group identified in the 2nd paragraph under "Adaptive Management Considerations".
Bureau of Reclamation	4	3	General Comments	In the Adaptive Management Considerations section (page 4), the authors recommed that 'Reclamation assemble a techncial group from fish management agencies and Tribes to ensure that studies are developed and appropriately funded such that field observations can be made before and after management actions are implemented and monitored.' The FASTA team, comprised of technical experts from the Tribes, fish management agencies, and universities already exists and has been developing and carrying out adaptive management activities; hypotheses are often discussed prior to implementation of management actions, monitoring plans are coordinated, and results are conveyed. As an example, the FASTA team recently agreed to deviate from the formulaic approach to implement a pulse flow capable of displacing carcasses and limiting myxospore load. Do the authors envision the need for an additional technical group? How often would this group convene and what would they discuss. Reclamation believes the composition and forum is captured with the existing FASTA team and does not recommed the forming of an additional and largely redundant group.	The relationship between the disease technical group and the Fasta team should be discussed among Co-Managers to determine the most efficient/effective way to meet the adaptive management needs associated with measures to minimize disease issues.
Bureau of Reclamation	4	4	2	typo, should be thresholds not thresh holds	Thank you
Bureau of Reclamation	4	5		"Spore dilution" is mentioned as an objective of one of the DTAT's flow management recommendations. However, spore dilution via flow augmentation has not been shown to appreciably decrease infection rates in Klamath River salmon (2014, tech memo). Infection rates are most strongly correlated with water temperature, especially in cases where spore concentrations are high. Lab studies have also shown that temperature is the key factor for predicting mortality of infected fish, which means releasing more warm water above the tubine capacity at IGD may not appreciably (if at all), affect mortality rates.	We believe that the rationale behind the spore dilution management guidance is adequately explained and backed up with appropriate scientific literature
Bureau of Reclamation	4	6	1 & 2	The sentences noted below contradict each other, and the text in page 2, paragraph 2, sentence 2. Page 4, Paragraph 6, Sentence 1 states "high likelihood" whereas sentence 2 states "would" - a positive assurance of results while Page 2, paragraph 2, sentence 2 implies considerable uncertainty. Change "would" to "could" since the outcomes of all management guidance are not fully understood at this time.	language changed for clarification
Bureau of Reclamation	6	3	General Comments	The authors state that implemention of the geomorphically oriented management guidance actions is expected to reduce the need for other management guidance (dilution, fall carcass, and hatchery changes etc.). The observation of polychaete distribution/density and C. shasta POI in juenvile Chinook and coho after the 2016 'deep flushing flow' event supports the statement that geomporhically active flows can disrupt the C. shasta lifecycle. However, it's unclear how the authors anticipate managers will make decisions on whether or not management actions in addition to, or other than, geomorphically oriented events are also needed for disese abatement. Please provide specific instruction and/or criteria that provides for the decision making process on when and how management guidance 4-6 are implemented (or not). Do the authors anticipate that these decisions will be necessary on an annual basis. If so, what are the criteria and who will make them (FASTA?)?	The guidance document identifies management actions that may alleviate C. shasta conditions on the Klamath without regard to implementation. This Guidance Document does not demand that Bureau of Reclamation implement any of these actions. Text will remain.

DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
Bureau of Reclamation	7	2		IGD discharges of 6,030 cfs flow was noted to have a two-year recurrence interval, but the recommendation is for this to occur every year until dam removal. What is the rationale for a recommendation that exceeds the frequency of high flow events observed over the period of record? This point is especially valid given climate change projections and the already reduced frequency of these events observed over the last 16 years.	With regard to the climate change portion of the document, the BiOp says the current drought cycle is an anomaly. (source is: USBR [U.S. Bureau of Reclamation]. 2011. SECURE Water Act Section 9503(3) - Reclamation Climate Change and Water, Report to Congress. U.S. Dept. of Interior, Denver, Colorado.) "Reclamation also projects annual increases in runoff during the 2020s compared to the 1990s, based on the global climate models. The annual volume of flow in the Williamson River is expected to increase by approximately 8 percent, with increases of approximately 22 percent during December through March and decreases of approximately 3 percent during April through July (Reclamation 2011). The Klamath River below Iron Gate Dam is expected to experience an approximate 5 percent increase in annual flow volume, with increases of approximately 30 percent during December through March and decreases of approximately 7 percent during April through July (Reclamation 2011)." (BiOp at 67)(source is: USBR [U.S. Bureau of Reclamation]. (same source as cited above). Now with regard to frequency being more than historic: The first reason we have asked for 6,030 more frequently than every other year is because of the dire situation with regard to disease in the Klamath River. The frequency of flow in the Geomorphic memo is a description of how frequently a given flow occurred over the specified period of record. The flow magnitude of 6,030 was selected because it does initiate movement of fine sediments and surface layer sediment (Geomorphic Memo). The polychaete memo, combined with the spore and fish infection memo describe what are the ecological results of the lack of these flows (see Figure 5 of Geomorphic Memo on page 21).
Bureau of Reclamation	7	last	last	typo, should be Polychaete not Polycheate	Thank you
Bureau of Reclamation	7,8	6	Management Guidance 1: Provide Surface Flushing Flows	The authors recommend that Reclamation follow the 2013 BiOp ramp up and ramp down rates during the implementation of the fall surface flushing flows of 6,030 cfs in November-December. Reclamation, when implementing the fall carcass displacement in November 2016 did not need to follow the downramping rate in the BiOp because, as individuals on the FASTA noted, 'there were very few, if any juvenile salmon in the mainstem Klamath River at that time and stranding was not a significant concern'. Also, the surface flushing flow time period overlaps with Recommendation #5 (carcass displacement flow). Reclamation seeks clarification on whether the fine flushing flows and carcass displacement flow could be combined into one event. And, if combined into one event, and given the need to remove carcasses from the wetted channel, the authors should clarify whether they feel the BiOp ramping rates should be adhered to (as recommended) or whether there's more benefit than risk associated with steeper ramp up and ramp down rates.	NOTE: we don't refer to "fall surface flushign flows", but "surface flushing flows". The surface flushing flow does not necessarily overlap with the carcass displacement flow - see response to comment in row 29 below. In regard to ramping rates, this should be considered by technical experts, such as the potential Disease Management Recommendations Team mentioned by BOR in the Scoping Document.
Bureau of Reclamation	7,8	6	Management Guidance 1: Provide Surface Flushing Flows	It is unclear whether the recommended surface flushing flow of 6,030 cfs can be incorporated on the beginning or end of a larger geomorphic flow event in the spring months (Feb-April) or a carcass displacement flow in the fall (Novemeber - December). The authors should clarify whether these events can be combined into one singular flow event. If the authors recommend two discrete flow events, justification and rationale should be provided.	As noted on page 9 of the Synthesis Memo, "if the deep flushing event as described in Management Guidance 1 does not result in flows above 6,030 cfs for at least 72 horus, it will not fulfill the fine sediment managemetn guidance action (#1)." Therefore, deep flushing events and surface flushing flow events can be piggy backed on top of each, as long as flows are above 6,030 cfs for at least 72 hours. Potential concerns with piggy-backing the surface flushing flows with the fall carcass/myxospore redistribution flows include: 1) scouring of redds in the Upper Klamath, 2) effectiveness of the flows at reducing polychaete densities for the spring/early summer. Our opinion is that the spring time, especially during an ongoing hydrologic event, is likely the best time for the surface flushing flows. However, this would be a good topic for the experts to consider, such as the Disease Management Recommendation Team that is described by BOR in the Scoping Document.
Bureau of Reclamation	8	3		While there is considerable discussion to support the 6,030 cfs flow, the true target is the reduction in the host population where the scientific basis included in the Guidance Document is that there is "strong evidence to suggest". This does not provide the "predicted outcome" assumed in the adaptive management consideration section on page 4. Clear definition is needed. Please include.	Not clear what this comment is asking or recommending. The predicted outcome of the surface flushing flows is the mobilization of surface sediments, reduction in available polychaete habitat, and resultant reduction in polychate densities.

DTAT Guidance Document Comments and Responses					
Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Bureau of Reclamation	9	6	General Comment	Since there has been a demonstrated successful reduction in polychaete density at a much shorter duration than suggested in the management guidance, recommend including a component in the adaptive management strategy that provides for testing and success of various flow management alternatives that include scenarios with a magnitude/duration/frequency less than suggested in the Guidance Document that may result in similar successes.	These recommendations are intended for the period from now through 2023, when the BiOp expires and after dam removal has occurred; six years is not enogh time to explore several paramters (i.e. magnitude/duration/frequency). Furthermore, our intent is provide management actions that prevent high *C. shasta* infections, such as occurred in 2014 and 2015, between now and 2023. We fully support an adaptive management approach, however it must be implemented with an eye toward minimizing the risk of high *C. shasta* infection rates. We do not recommend exposing the the fish to additional high risk of *C. shasta* infections for the sake of science or for the sake of conserving a relatively small amount of water.
Bureau of Reclamation	9		Management Guidance 2: Provide Deep Flushing Flows and Armor Disturbing Flows	Given that Reclamation enacted a large deep flushing flow in the spring of 2016 (March) of 11,200 CFS and the recommendation is to have these events occur every other year, what is the justfication and scientific basis for requiring this flow to occur in water year 2017?	language supporting implementation in 2017 added to support section
Bureau of Reclamation	10		Management Guidance 3: Opportunistic Geomorphic Flows	This recommendation seems redundant with recommendation number 2. Reclamation and NMFS will seek to maximize the magnitude of geomorphically active flows within the inherent constraints of infrastructure and public safety.	This recommendation is for flows higher than deep flushing flows, so it is not redundant with recommendation #2. We agree that such flow events need to be done wtihin constraints of infrastructure and public safety.
Bureau of Reclamation	10	9		From the Spore memo: “Though managed discharge events have not produced dramatic reductions in spore concentrations, the planned discharge increases were likely too small to be biologically effective. An unplanned discharge increase in 2005 likely demonstrates the potential for larger discharges to effectively reduce spore concentrations.” “Actinospore development within polychaetes is likely a function of accumulating thermal units…” The Fish Infection Memo, Figure 3 shows that infection rates will be between 35% and 45% across a range of water temperatures (10-22°C) when spore concentrations reach 50 spores/L. Monitoring sites in the lower Klamath River show spore concentrations regularly exceeding 50 spore/L even following experimental flow releases. Taken together, these observations would lead to the thought that a small dilution effect associated with Guidance Measure 4 would have little effect on fish infection rates. Please clarify.	Agreed, the flows (1,900 cfs) provided in 2014 indicate that minimal flow increases (which result in minimal dilution effect) would have little effect on prevalence of infection rates. However, the natural hydrologic event that occurred in May 2005 (briefly > 6,000 cfs) indicates that prevalence of infection dropped substantially from this event. These two flow levels set the side boards, as we suspect the "sweet spot" for reducing spore levels lies somewhere in between them. We propose starting with 3,000 cfs and if that is not effective, then increasing to 4,000 cfs. The text will remain unchanged.
Bureau of Reclamation	11	1	Mangement Guidance 4: Emergency Provision Flow to dilute spores	In general, Reclamation believes the thresholds listed for detemining whether to implement a diluting flow are difficult to quantify, especially given the dynamic nature of natural systems. This has the potential to make implementation of a diluting flow incredibly difficult. For example what tool or approch is available and accepted as a means to estimate whether 80% of the expected wild run has passed the Kinsman, Scott and Shasta? It doesn't seem like this threshold is easily quantifiable, especially in real time (i.e., how do we know what 80% is until we get to the end?). Looking back at past data, water temperature and prevalance of infection are also highly dynamic, leading to the possibility of an on-again/off-again management action.	USFWS staff (operators of the Kinsman trap) have indicated that they can develop a method for estimating when 80% of the run has passed the trap site - this would be independent of real-time trapping information. Dynamic flows in the spring time would not necessarily be a bad thing. Management Guidance Action will remain the same.
Bureau of Reclamation	11		Mangement Guidance 4: Emergency Provision Flow to dilute spores	The scientific support and mechanism for the diluting flow recommendation is less robust and direct than the other recommendations. Increasing spring flows to dilute actinospores likely leads to complex biotic interactions, including altered habitat use by juvenile coho and Chinook salmon (increased time in margin habitats with slower water velocities). Given Adam Ray's dissertation, which describes the effect of water velocity on the likelihood of actinospores finding and attaching to a host, and the compounding effects of increasing flow on fish behavior and habitat use, there seems to be a lot of uncertainty surrounding the actual benefits of a diluting flow. The authors also do not provide a justification for the thresolds listed; how did the authors identify 5 spores and 20% POI as the thresholds and how are those thresholds related to population level mortality? Given the inherent uncertainty regarding the efficacy of this action based on past events and the complex interactions between flow, fish behavior, and infectivity rates, Reclamation recommends that this guidance be removed from the Guidance Document.	The flows identified in the Synthesis Memo (3,000 cfs initially, followed by 4,000 cfs if necessary) for spore dilution provide improved habitat conditions for coho fry (see 2013 BiOp, Figure 11.17) and coho juveniles (see 2013 BiOp, Figure 11.18), relative to flows below these levels. See next response (row 37) in regard to justification for the criteria. No change to Management Guidance Action.

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Bureau of Reclamation	11		Management Guidance 4: General Comment	It's clear that the authors selected conservative thresolds for implementing the diluting flow. As such, please provide the scientific basis for reduced disease management thresholds (ie. 5 spores per liter, irrespective of genotype, and 13 degrees celsius water temperature at Seiad Valley) from those identified in the BiOp. Daily average water temperature of 13 deg C at Seiad ran from about May 1 through Oct 15 in 2016 and 5 spores per liter (no genotype req's) happened by mid April in 2016 and continued through October. Furthermore, please provide the scientific basis for using unspeciified genotyping as part of the criterion. Consistent with Scoping Document, the strategies or actions are being proposed in an effort to reduce infection rates of juvenile coho and Chinook salmon, to the extent such information on Chinook salmon is relied on to address measures for coho salmon. Since there is sufficent information to assess the risk of C. Shasta infection in coho salmon through genotyping, this data should be relied on to indicate the need for implementation of this management action. Disregarding the genotyping ignores the effects specific to ESA listed coho, the subject of this exercise.	The following language was added to document: "For emergency flow releases to be effective, dilution flows need to occur as disease is worsening but before excessive levels of lethal doses are occurring in juvenile fish. Hallet (2012) found the lethality threshold of 40% was reached at 10 spores per liter for Chinook but only 5 spores per liter for coho salmon. This level of mortality is extremely high with small changes in mortality rates translating to a large difference in overall survival rates (Fujiwara 2014). Actinospores are generally released when temperatures are above 10°C, and remain viable (able to infect salmon) at temperatures ranging from 11 to 18ºC (Foott et al. 2006). A lethal combination of spores and temperatures is occurring at the trigger threshold of 5 spores per liter and 13 degrees C and given lag times of sampling of a week and likely trajectories given an examination of past trends, this was thought to be a prudent precautionary approach to avoid excessive mortality rates. Reclamation in the 2013 BiOp proposed flow increases for the Klamath River downstream of IGD to dilute actinospore concentrations within 24 hours of receiving information that disease thresholds have been met and used 5 spores per liter of Type II as a threshold (2013 BiOp page 41) so while this was only implemented once and with meager flows, this is a previously contemplated action. The unspecified genotyping referenced in the memo is in part to allow for much more rapid turnaround time for results (reduction of 2-4 days of process time), which would allow for more effective implementation for fish and water conservation. It is not disputed that there is sufficient information to assess C. Shasta through genotyping and that data would still be collected. Chinook salmon are the disease surrogate for coho in the ITS and incredibly important to tribal fisheries so should be protected during these actions along with ESA listed coho salmon.
Bureau of Reclamation	11		Mangement Guidance 4: Emergency Provision Flow to dilute spores	The thresold criteria for Kinsman rotary screw trap is specific to wild fish (to not exceed 80%), but the criteria number 2 regarding overall POI does not specify whether the POI is all fish captured, or just wild? Please clarify.	The 80% is specific to wild fish emigration, the POI level pertains to all fish sampled prior to when 80% of the wild run is estimated to have passed the Kinsman trap site. Clarifying language added to document in description of Management Guidance Action.
Bureau of Reclamation	11		Mangement Guidance 4: Emergency Provision Flow to dilute spores	Just as there are inherent infrastructure limitations with providing a 11,250 cfs geomorphic flow, there are also inherent infrastructure limitations and public safety risk limitations to the amount of storage in UKL during the spring months when UKL is at or near full pool. In other words, there are safety limitations associated with storage operations and UKL will not have the capacity to store an additional 50K AF for this purpose in many years.	Understood, we did not anticipate there would be additional water stored in UKL. No change made to document.
Bureau of Reclamation	11		Mangement Guidance 4: Emergency Provision Flow to dilute spores	The temperature benefit from increasing flows at Iron Gate Dam are uncertain and overstated - spring flows at Iron Gate Dam, especially above turbine capacity have the potential to increase water temperatures between Iron Gate and the confluence with the Shasta River.	We could not find corroborating evidence that increases in flow during the time period specified would increase water temperatures by any significant degree. Even if that occurred (which we doubt), the effects would be very limited in geographic scope, based on temperature modeling from the Sec Determintaiton report and observation. No change to text.
Bureau of Reclamation	11	1		There may be an important tradeoff between potential benefits from diluting spore concentrations and increasing water temperature. Artificial increases in spring flows from IGD have been shown to increase water temperature, particularly upstream of the Shasta River, which would cause spore concentrations and fish infection rates to increase as well. Therefore, this management measure could reduce fish survival. The authors should utilize existing predictive tools such as the S3 temperature submodel to better understand the multiple effects of increasing flows form IGD on water temperatures and fish POI.	See previous comment response.
Bureau of Reclamation	11	1		Smolt trapping data at Bogus Creek (USFWS 2002-2005) showed that coho salmon smolts migrated through the most upstream reach of the Lower Klamath River between March 1 and May 1, nearly a month prior to the increase in C. shasta spore concentrations recorded in 2005 (Spore Memo, Figure 4). If coho emigration regularly precedes spore concentration increases, dilution strategies would not be effective.	In addition to some coho smolts being in the Upper Klamath River during May, there are also young of year coho in the Upper Klamath at this time; rearing and/or re-distributing from one area to another. For example see USFWS report titled *Arcata Fisheries Data Series Report DS 2016-47 Summary of Abundance and Biological Data Collected During Juvenile Salmonid Monitoring on the Mainstem Klamath River Below Iron Gate Dam, California, 2014* (David et al, 2016) - available on USFWS Arcata web site. No change to text.
Bureau of Reclamation	11	7	1	The authors state that 'Altered Klamath River flow regimes have enhanced the reproductive sucess of C. shasta at every stage of its lifecyle'. The authors mention reduced spring flows as one aspect of the annual hydrograph that has been altered and contributed to the reproductive success of C. shasta. However, the authors should also note that reduced variability throughout the year, inclulding stable base flows during the summer months may also be contributing to C. shasta's reproductive success.	Noted
Bureau of Reclamation	12	3	5	Add (POI) after "prevalence of infection"	Thank you

DTAT Guidance Document Comments and Responses					
Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Bureau of Reclamation	13		Mangement Guidance 5: Provide flow in the late fall and early winter to redistribute salmon carcasses and myxospores	The mechanism the authors provide for disrupting the production and contribution of mxyospores is intuitive. However, it's unclear how the authors arrived at the specific magnitude, duration, and timing recommended. Also, the discussion does not indicate what amount/kind/extent of carcass redistribution is needed to meet an undefined goal. The benefit in terms of reduced infection rates is unclear. Is there also the potential to just move the myxospore load downstream, or are there fewer or less dense polychaete beds below Beaver Creek? Please justify. Monitoring conducted before/after the fall 2016 flow (and future years) may provide additional information for the range of magnitudes and durations needed to redistribute and remove carcasses from the channel. Additionally, the authors should specify whether BiOp ramp up/ramp down rates will be required?	We had little data to base this recommendation upon, however the concept appears to be supported by the scientific evidence (TechMemos) and relatively speaking, it does not require a large volume of water. We agree that the efficacy of this measure, along with the magnitude, duration, and timing of flow should be investigated through an adaptive management process. Clarifying language re: ramping rates has been added.
Bureau of Reclamation	14		Mangement Guidance 6: Iron Gate Hatchery Release Strategy	Although this management guidance is outside of Reclamation's control, authority, and discretion, Reclamation supports this management guidance and recommends that appropriate parties coordinate immediately and prior to the upcoming season to determine ways that hatchery management could be modified to reduce their contribution to the disease incidence in the Klamath River during the critical spring time period. However, as the authors note, infected and dying fingerlings released from Iron Gate Hatchery are likely contributing myxospores to the Klamath River. Given the high production goals (up to 5.1 million annually) and late releases (compared to wild fish outmigration), there is reason to believe that hatchery fish are contributing myxospores to the system and perpetuating the C. shasta lifecycle. This is especially true in years of drought or above average temperatures (e.g., 2014 and 2015) when the vast majority of hatchery fish showed clinical signs of the disesase (and likely died) within days of release. What is unknown is the relative contribution of myxospores between adult and juvenile fish and future monitoring should attempt to clarify the reletive contribution between lifestages.	See response in row 25 above.
Bureau of Reclamation	14	2		The second objective of this measure is to reduce hatchery fish exposure during peak spore concentration periods. This appears to be a logical basis for this management action. Additionally, juvenile hatchery fish could also be released in coordination with river operations to push fish through the IGD reach more quickly and potentially increase smolt survival.	There are complexities associated with release of IGH fish, such as size of fish due to incubation/rearing temperatures, infrastructure limiations, etc...., however we agree that such strategies should be considered amongst Co-managers.
Bureau of Reclamation	36	Table 7		typo, Mena should be Mean	Thank you
Bureau of Reclamation	52	3	1	typo, Morhpological should be Morphological	Thank you
Bureau of Reclamation	76	1	1	The effects of water temperature and actinospore concentration are estimated to be much stronger influences on mortality than discharge (especially when increased flows come from the hydropower reach). The authors should examine ways to increase shading, hyporheic inflows, or expanding the influence of cold water tributaries.	Actinospore concentration is related to discharge, however more data regarding this relationship would be beneficial. Ways to increase shading, hyporheic inflows, and expanding the influence of cold water tributaries are worthy of consideration, however they are better fit for the bin of "Longer Term Control Measures". No change made to text.
Klamath Water Users Association	General			Respectfully, some aspects of the Draft Guidance appear to contemplate a reversion to the failed paradigm of looking to the Klamath Irrigation Project to attempt to address and solve all issues of concern in the basin.	We have added language under the "purpose of the document" section to clarify that this Management Guidance simply describes conditions necessary below Iron Gate Dam to alleviate acute disease mortality issues that affect both Coho and Chinook salmon. We do not agree that this document points directly at the Klamath Reclamation Project.
Klamath Water Users Association	General			The Draft Guidance should be reviewed for consistency and objectivity. Various alternatives are rejected because of cost, safety considerations, or effects on certain resources (e.g., invertebrates). Yet there has been no consideration of the potential consequences of any of the retained Management Guidance actions for irrigation water supplies and related communities, national wildlife refuges, endangered suckers, or other resources that could be affected. Nor has there been sufficient consideration of legal authority to implement any or all of the actions.	We have added language under the "purpose of the document" section to clarify that this Management Guidance simply describes conditions necessary below Iron Gate Dam to alleviate acute disease mortality issues that affect both Coho and Chinook salmon. While it is acknowledged that any departure from current water management will have implications to other parts of the system including farmers, such analysis is beyond the scope of this document. Same applies to any analysis or discussion of legal authority to implement any or all of the actions.
Klamath Water Users Association	General			As noted above, the Draft Guidance, or at least portions, appears to target the Klamath Project as the only factor related to disease in the Klamath River.	Disagree with this statement. The guidance document identifies management actions that may alleviate C. shasta conditions on the Klamath without regard to implementation. This Guidance Document does not demand that Bureau of Reclamation implement any of these actions. To the extent that Klamath Project operations are identified or mentioned, it is because it is the 2013 BiOp that is at the center of this analysis.

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Klamath Water Users Association	General			In addition, KWUA had understood that various flow-related actions that might be considered would only be proposed for an interim period, with an emphasis on opportunistic approaches that would avoid adverse water supply consequences for other parties. This appears to have been abandoned.	As stated in the "Purpose of Document" section of the Guidance Document, the purpose of this document was to identify flow and/or other conditions in the river below Iron Gate Dam that would alleviated acute disease mortality currently being experienced in the Klamath River. It was, and still is, beyond the perview of the Guidance Document to discuss impacts to other users from implementation of any of the guidance actions identified.
Klamath Water Users Association	General			We do observe, however, that in some locations the Draft Guidance refers to "hydrologic support" or similar concepts. We recommend that it be made clear what this means.	noted: will add clarifying language as appropriate. In general, hydrologic support means not trying to implement large flows during extreme drought. There is much room for flexibility in this concept.
Klamath Water Users Association	General			Also, KWUA's general concerns can be mitigated if the Guidance Document makes clear, as we believe it should, that the implementation of any measure would: be accomplished through, or affect only, the Environmental Water Account (EWA) established in the Klamath Project biological assessment and opinions; or provide offsetting adjustment of fall-winter flows. This also, however, has not been made clear.	It is beyond the scope of this Guidance Document to identify specific ways in which flow conditions could be achieved. The Guidance Document does not specify any source for identified additional flows.
Klamath Water Users Association	General			The Draft Guidance appears to include multiple actions directed to the same end. It would be helpful if there were a clear prioritization.	Noted. The Management Guidance is presented in a generalized prioritized order in that the first three Guidance Actions focus on prevention, the fourth one (spring dilution flows) is an emergency response. The fifth and sixth actions are additional actions the DTAT believed would also help.
Klamath Water Users Association	General			The Draft Guidance describes the 2014 and 2015 situation as critical, and refers to an immediate dire need. We believe parties would benefit from a more complete understanding of the consequences of C. shasta for overall populations of coho or other species through time and over the range of conditions that exist in the river, and the relative role of disease and other factors affecting populations, including at the present time.	Please see Fujiwara et al. 2011, True et al. 2013 as cited in the Fish Infection Memo (attached as appendix to the Guidance Document) for a peer-reviewed fully developed analysis of population effects. Although this does not include the most recent data, it discusses population level effects to chinook and coho salmon.
Klamath Water Users Association	General			We also recommend a more fully-developed discussion of post-2015 disease rates and any significance to be assigned. Some of this information may be lacking, and KWUA would support work to improve our understanding.	Noted. In general the 2016 data was not available to our knowledge as we were drafting the Guidance Document.
Klamath Water Users Association	General			We also are interested to work with DTAT parties who are willing to engage in collaborative approaches.	We welcome your technical participation.
Klamath Water Users Association				Affected communities in the upper basin have not been included in this process. This approach is not consistent with progress in addressing Klamath basin issues that has been achieved over the past 10 years. We recommend immediate efforts to include potentially affected parties in any consideration of the Draft Guidance.	The identification of Management Guidance was a thoroughly technical exercise. The next steps, which will include discussion on whether, and if so, how to implement any of these actions is another process and will address concerns you have raised. The DTAT is not involved in that process.
Klamath Water Users Association	Page 1	Third paragraph:			
Klamath Water Users Association				The focus here falls to the operation of the Klamath Project; KWUA encourages a broader perspective. The flows affected by Klamath Project operations also supplement natural flow conditions in certain times of year. Additionally, the last sentence in the paragraph makes an assumption regarding infection rates in coho salmon. We are not aware of data on this issue and ask that it be provided if it exists and is available.	Citations added for clarity. Again, this document identifies conditions downstream of Iron Gate Dam that could help alleviate high disease mortality and does not specify management actions needed to achieve those conditions. Thus, we do not believe we targeted the Klamath Project.
Klamath Water Users Association					
Klamath Water Users Association	Page 2	First full paragraph:			
Klamath Water Users Association	Page 2	Second paragraph:		The term "management guidance" should be defined.	Noted: will add.
Klamath Water Users Association	Page 2	Second paragraph:		It has been stated by fish biologists that less water will be needed if dam removal occurs. There should be an acknowledgement that any guidance considerations or recommendations would be expected to change.	The Guidance document clearly states that the Management Guidance provided is valid until the BiOp concludes or is superceded. Presumably dam removal would trigger reconsultation on this BiOp.
Klamath Water Users Association	Page 2	Last Sentence		"Measures described below are intended to be implemented in addition to the measures and management actions described in the 2013 BiOp." This statement is not consistent with KWUA's understanding. It was explained to us that these measures were being identified but implementation of any measures is to be discussed among the affected parties (i.e. Reclamation, irrigators, Klamath Tribes, USFWS (including Refuges)) and Reclamation would decide how to proceed.	The Guidance document rationale for this statement is that the acute disease conditions that have caused high mortality have resulted from implementation of the BiOp. Therefore, any proposed management guidance should be in addition to that on the premise that we don't want to create new problems as we solve others. Text remains unaltered.
Klamath Water Users Association	Page 4	Second full paragraph:		We recommend the document identify anticipated sources of funding including whether there is any expectation it would be reimbursable by Project Contractors?	This is beyond the scope of this document.
Klamath Water Users Association		Control Measures Eliminated - #1		The dewatering alternative is dismissed summarily, and it appears that reasons 1 and 2 for dismissal may be the same. The alternative that was considered is not thoroughly described, but the potential benefits of any alternative should be fully analyzed. Natural variability of the river system involved very low flows. Variations of the alternative could also be considered in combination with other actions.	Our technical information, as discussed in the Guidance Document, indicated that dewatering would be ineffective and entail high risk to existing ecosystem function. In particular (as noted in the Guidance Document in the dewatering discussion) newer work has shown that infected polychaetes reside in deeper pools due to the settling rate of the spores. See polychaete memo Figure 4 Page 8).

DTAT Guidance Document Comments and Responses					
Submitting Party	**Comment Location**			**Comment**	**Disposition**
	Page #	**Paragraph**	**Sentence**		
Klamath Water Users Association	Page 5	Control Measures Eliminated - #3		This measure is too readily dismissed. The prospect of dam removal may or may not be "imminent.	If it becomes apparent that dam removal will either 1) not happen, or 2) be delayed for a significant period of time; then yes, we agree this option should be re-examined. Footnote added.
Klamath Water Users Association	Page 6	First full paragraph		KWUA appreciates the acknowledgement of variable hydrologic conditions here. We think that acknowledgement is lost in other areas of the document.	The Guidance document makes many references to this, in particular in discussion around implementation of Management Guidance #2 and 3 which involve releases of high flows from Iron Gate Dam.
Klamath Water Users Association	Page 7	Management Guidance 1 - "Description"		Acknowledgement of hydrologic conditions should be made here. The unprecedented consecutive years of drought could be a factor in the "recent scarcity of these types of flows". Also, flow conditions in 2016, and their current relevance, has not been considered here. It is also difficult to understand how the logic supporting that this action should be implemented every single winter, and more so because there is apparently uncertainty about effectiveness.	The Guidance Document did not attempt to attribute low flows to any single cause, but rather to link the lack of high flows with high numbers of polychaetes and then high mortalities of C. shasta. As discussed in the document, we believe there is a solid link between flow, polychaetes, C shasta spore density and finally fish mortality.
Klamath Water Users Association	Page 7	Management Guidance 1 - Last full paragraph		Given the Draft Guidance's frequent reference to the Klamath Project, we believe it is important here to recognize that the period since 2000 has seen curtailments and constriction on irrigation diversion that were not characteristic of the 35 years prior to 2000.	This is beyond the scope of the document and inconsistent with the fact that we are not targeting any specific source or group for water needed downstream.
Klamath Water Users Association	Page 8	Figure 3		The Draft Guidance appears to support that pre-2001 river management was beneficial to the system. This should be discussed and evaluated in the document.	Specifically, the Guidance Document notes the higher frequency of high flows in that time period and combines that knowledge with 1) the high densities of polychaete worms in the upper Klamath River below Iron Gate Dam, and 2) recent polychaete habitat modeling that clearly links hydraulic conditions during periods of high flow to subsequent polychaete distribution. (see Polychaete Memo).
Klamath Water Users Association	Page 9	Management Guidance 2		KWUA appreciates the acknowledgement here of how hydrology could be relevant to this recommendation. Potential implications for other stakeholders in the system of this and any other recommendation should be described. KWUA also notes that the occurrence of flows described as deep flushing flows is strongly related to natural conditions? Biological Opinions have dictated micromanagement of the system (and provided less water for Project operations) over the last 16 years.	Potential implications for other stakeholders in the system cannot be described, because it is not known how (or if) the Bureau of Reclamation will implement this recommendation.
Klamath Water Users Association	Page 10	Management Guidance 3		In the "Support" discussion, the Draft Guidance alludes to decreased flows in recent history without mention to natural hydrologic conditions (i.e. drought). The document implies that these decreased flows are the result of management decisions rather than natural hydrologic conditions. Figure 3 helps illustrate that drought plays a major role.	We believe that both changed management and drought conditions are responsible for lower winter flows. For example, the flood control curve at Upper Klamath Lake has changed significantly in recent years. Text remains unaltered.
Klamath Water Users Association	Page 11	Sixth full paragraph		Klamath Project contractors should be part of FASTA and should be consulted.	This is a topic to take up with the Bureau of Reclamation who runs the FASTA process
Klamath Water Users Association	Page 11	Seventh full paragraph		This action is not legal, feasible, or prudent in most or all years. Also, as noted elsewhere, effects on other interests and resources has not been considered.	The difficulties of implementation are clearly acknowledged in the Guidance Document which only calls for the Bureau of Reclamation and PacifiCorp to "look for opportunities" to implement high flows.
Klamath Water Users Association	Page 11	Eighth full paragraph, first sentence "support"		There is a lack of documentation that this is fact. What role has natural hydrology played in the reproductive success of *C. shasta* ?	The 2013 BiOp has a detailed section that discusses how fish disease is affected by flows and how flows are affected by the operation of the Klamath Project. Clearly the Guidance Document does not lay blame for the disease situation at the feet of any user group, but does identify low flow conditions caused in part by drought conditions that have lead to high infection rates of sampled fish. Text remains unaltered.
Klamath Water Users Association	Page 12	"Uncertainties"		The expressed uncertainty is significant. The potential cost to Upper Klamath Lake, the Klamath Project, Upper Basin irrigators, and the refuge is not uncertain.	Noted
Klamath Water Users Association	Page 16	Conclusion		The Conclusion, particularly the last two paragraphs, is obviously direct advocacy, and does not square with characterizations of the process as only technical or informational in nature.	The penultimate paragraph discusses Tribal trust, and is simply a statement of fact; the Federal Agencies to have a responsibility in that regard. The last paragraph simply speaks to the necessity of acting quickly due to ongoing high mortality rates. Both of these are well supported, and the authors of the Guidance Document do not agree that re-stating well supported factual information is advocacy.
Klamath Water Users Association		General Comment		There appears to not have been an opportunity for the Klamath Tribes to engage or have a seat at the table in developing this document. What is the role or position of the Klamath Tribes regarding this Guidance Document?	The Klamath Tribes did provide comments on this document.
Klamath Water Users Association		General Comment		There are considerable reference to altered flow regimes, including over the last 16 years. Klamath Project contractors have had decreased certainty and generally less water over that same period of time than they have had historically.	Noted

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
Klamath Water Users Association		General Comment		The Draft Guidance does not explain the process for creation of the DTAT or any subgroup that prepared the Draft Guidance Document. We believe this should be provided. There is reference on the first page to potential "[Others]" as authors. This apparent "sign on" process does not seem consistent with the concept of this paper as the product of a strictly technical analysis. Nor do several statements throughout the document.	noted
Klamath Water Users Association		General Comment		Overall we find the document incomplete. We understand the document specifically and purposefully did not discuss other socio-economic factors, affects on other stakeholders or species. Still, to conclude that some of these flow regimes are "physically possible and achievable" is not correct.	We disagree, and believe we completely addressed the purpose of the document, which was to identify Management Guidance to address severe disease conditions below Iron Gate Dam.
Klamath Water Users Association		General Comment		Any meaningful Guidance document must be based on a feasibility evaluation with a cost benefit analysis. This document does not include that information and therefore should be retitled to something like "Disease Management Concepts Assessment."	The term "Guidance" was a result of discussions with Federal Fish Management Agencies.
NMFS	1	2		Replace: "......served as a surrogate to determine rate of Coho infection." WITH "....served as the best surrogate available at the time for determining the estimated degree of Coho infection."	It seems to be the best surrogate currently as well. Text remains unaltered.
NMFS	1	2	1	Add "May through July" before 2014	Agree
NMFS	2	1	1 "Research described......."	add "adult salmonid" in front of carcass densities	Agree
NMFS	2	1	last of paragraph	add "salmon" after "(SONCC)"	Agree
NMFS	4	2	"Members of the DTAT....."	add "with" in front of Scott Foote	Agree
NMFS	5	1st under "Control Measures......" heading	1. Dewatering......	put "are" before killed	Agree
NMFS		2nd under "Control Measures......" heading	2. Manual carcass remvoval......... - 2nd to last word of paragraph	"recommended below" instead of "recommend below"	Do not agree.
NMFS	6	1	General comment	1. Seems there may be a reliance on faster in-season turn-around time on POI and spore monitoring, and this may not be possible to mobilize soon. Also could be issues with 'preliminary data' being used for 'if/then' water management decisions?	Our discussions with OSU researcher indicates that timing would be consistent with that outlined in our document.
NMFS	6	1	General comment	2. Might want to review polychaete habitat discussions and check for consistency with the associated Tech. Memo.?	Text remains; we believe it is supported by Tech Memos and BiOp.
NMFS	6		5.........	typo - change Dilution to dilution	agree
NMFS	6	The data summarized......	1st sentence	Change M. speciose to M. speciosa	
NMFS	6	The first three.......	last sentence of paragraph	Change "....guidance actions significantly...." to "guidance actions may significantly....."	ok
NMFS	7	Description:......	last sentence	recommend considering downramping rates on a case by case basis	Clarifying language added
NMFS	7	Support:.....	2nd to last sentence	replace "recent years" with 2014 and 2015	ok
NMFS				replace "The Polychaete Memo describes how populations of polychaetes can be kept in check by mobilizing sediments, and the Geomorphic Memo describes what flows are needed to accomplish various geomorphic objectives." WITH "The Polychaete Memo describes how polychaetes distribution is related to substrate and hydraulics, and the Geomorphic Memo describes what flows are needed to accomplish various geomorphic objectives"	Text changed and clarified.
NMFS	9	under Management Guidance 2:	NOTE: If the deep.....	This is a bit confusing and could use clarificaiton	Text changed and clarified.
NMFS	9			There are three minor typos in the rest of recommendation	Text changed and clarified.
NMFS	10	Management Guidance 3		Comments include: "Uncertain if flows of this magnitude can be engineered due to infrastructure and safety constraints." AND "Unclear to me how #3 is different than #2" AND "Agree that it may be dependent on accretions as the hydraulic capacity at Link dam may be ~ 10 K cfs. Also, this may be duplicative with Management Guidance 2. It is probably realistic to consider when drought conditions may preclude implementation of both 2 and 3."	A sentence can be added at end of Description: "Furthermore, such an event may need to occur during times of high accretion between Link River Dam and Iron Gate Dam, due to outflow limitations at Link River Dam".
NMFS		Management Guidance 3	Support: 2nd and 3rd sentences	Change: "The Polychaete Memo describes how these stable flow conditions have contributed to a proliferation of M. speciosa. Provision of these flows will reduce......" TO "The Polychaete Memo describes how these stable flow conditions leads to increased polychaete habitat Provision of these flows will likely reduce...."	Text changed and clarified.
NMFS	10	Management Guidance 3	Adaptive management section:	Recommend deleting first part of first sentence: "Ideally, it would be desirable to monitor"	Text changed and clarified.
NMFS	10	Management Guidance 4	Objective: 1st sentence	Should change "....when 80% of the juvenile outmigration is ...." TO "...when 80% of the juvenile Chinook outmigration is ...."	Text changed and clarified.

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
NMFS	11	Description: 1……	1…..	Not sure what the basis is for the 5 spores/l threshold. Seems that in the past this threshold has been related to coho salmon. ALSO The influence of IGD releases often diminishes further downstream.	The following language was added to document:"For emergency flow releases to be effective, dilution flows need to occur as disease is worsening but before excessive levels of lethal doses are occurring in juvenile fish. Hallet (2012) found the lethality threshold of 40% was reached at 10 spores per liter for Chinook but only 5 spores per liter for coho salmon. This level of mortality is extremely high with small changes in mortality rates translating to a large difference in overall survival rates (Fujiwara 2014). Actinospores are generally released when temperatures are above 10°C, and remain viable (able to infect salmon) at temperatures ranging from 11 to 18ºC (Foott et al. 2006). A lethal combination of spores and temperatures is occurring at the trigger threshold of 5 spores per liter and 13 degrees C and given lag times of sampling of a week and likely trajectories given an examination of past trends, this was thought to be a prudent precautionary approach to avoid excessive mortality rates. Reclamation in the 2013 BiOp proposed flow increases for the Klamath River downstream of IGD to dilute actinospore concentrations within 24 hours of receiving information that disease thresholds have been met and used 5 spores per liter of Type II as a threshold (2013 BiOp page 41) so while this was only implemented once and with meager flows, this is a previously contemplated action. The unspecified genotyping referenced in the memo is in part to allow for much more rapid turnaround time for results (reduction of 2-4 days of process time), which would allow for more effective implementation for fish and water conservation. It is not disputed that there is sufficient information to assess C. Shasta through genotyping and that data would still be collected. Chinook salmon are the disease surrogate for coho in the ITS and incredibly important to tribal fisheries so should be protected during these actions along with ESA listed coho salmon.
NMFS	11	Description 2….. (in regard to "….exceeds 20% in aggregate for the preceding week at the Kinsman Rotary Screw Trap."		Not sure that POI estimates can be provided in real time.	Per discussions with Kim True, USFWS CA-NV Fish Health Center, a week is tight, but possible, especially, if a process is implemented that gets the samples to the fish health lab sooner.
NMFS	11	3. (in regard to 80% of the expected wild run)	The juvenile catch at the Kinsman rotary screw trap is estimated to have not exceeded 80% of the expected wild run	Not sure that this can be currently estimated in real time.	USFWS Biometrician thinks this can be estimated, without relying upon real-time downstream migrant trap data.
NMFS	11	criteria #4	"The daily average water temperature has reached 13C at Seiad Valley".	Recommend deleting criteria #4	done
NMFS	11	When disease……		replace "….readiness prepare" with "…..readiness to prepare"	done
NMFS	11	"Maintain flows….."	Maintain flows at 3,000 cfs until spore or POI at Kinsman Trap sampling indicates a decrease in disease levels .	Given sample turn-around time, this would always be at least a week, perhaps more.	TRUE
NMFS	11	"Maintain flows….." (last sentence)	"After initiating this action, evaluate the efficacy of specific flow targets and adjust….."	May want to consider duration also.	text added
NMFS	11	"Support:….. "	2nd sentence	Replace: "to rearing and migrating juvenile fish, causing significant population" WITH "to rearing and migrating juvenile fish, contributing to significant population"	change made
NMFS	11	"Support:….. "	last sentence of first paragraph "Spring flows similar to average natural flow conditions are required to make meaningful reductions in C. shasta transmission during critical outmigration periods."	This statement could use a support citation?	sentence deleted
NMFS	12	"The concentration of….."	2nd sentence of paragraph	"….(modified with velocity)". REPLACE with "….(modified with volume and velocity)".	text changed
NMFS	12	"The concentration of….."	This is consistent with the observation of reduced disease infection rates and mortality estimates in wetter years with higher flows as opposed to drier years and lower flows	Add to this sentence: "…., although water temperature plays a role also".	done
NMFS	12	"The concentration of….."	Increased flows also increase water vilocity and have been shown to reduce transmission rates.	This statement could use a supporting citation. Is this based on lab studies, or river studies?	Citation added
NMFS	12	"Despite limited……"	Spore concentrations of 5 spores per liter (Hallett et al., 2012) and prevalence of infection of 20% (True et al., 2016) are strong indicators of impending and realized high disease rates.	Is this a quote from the previous citations?	Citations are provided
NMFS	12	Both outmigration…..	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.	Is this being proposed for in-season managemetn considerations?	Yes, prior to 80% of the estimated natural run having migrated from the Upper Basin and 20% of prevalence of infection of captured fish exceeding 20% in aggregate for the preceeding wad at the Kinsman trap.
NMFS	12	"Uncertainties:…."	…..low termperatures often coincide with higher flows……	Not necessarily true, not certain but not aware of any correlation between low temps and higher flows AND Is this generally true for releases above turbine capacity?	Text remains; modified by the word "often", which is true in rainy, wet spring periods, or times of abundant snowmelt runoff.
NMFS	13	Management Guidance 5:	General	It may make sense to combine Management Guidance 1 and 5	This should be conidered by disease experts, such as the Disease Management Recommendations Team, things to consider include: 1) potential scour of redds, 2) time of the year that the polychaetes are most vulnerable, 3) time of year that would minimize recolonozation by polychaetes prior to spring
NMFS	13	Support:…..	last of 1st paragraph	delete "entrain" and replace with "strand"	text changed
NMFS	13	Uncertainties	2nd sentence	replace decipher with discriminate	text changed to "distinguish"
NMFS	13	Adaptive Management……. 3rd paragrpah	1st sentence	replace "identified" with "identifiable"	done
NMFS	13	Adaptive Management……. 4th paragrpah	2nd sentence	estimate "quantify" with "estimate the"	done
NMFS	14	Managemetn Guidance 6	Description: #3 "Consider reduction……"	Manage this season?	Need to consider in collaboration with Co-managers, however it is worth noting that the release in 2017 is likely to be < 2,000,00 due to low egg take of brood 2016.
NMFS	15	Support section. Paragraph 5	3rd	replace: "…myxospores that likley infect polychaetes" with "….myxpspores that can can infect polychaets"	done

DTAT Guidance Document Comments and Responses

Submitting Party	Comment Location			Comment	Disposition
	Page #	Paragraph	Sentence		
NMFS	15	Support section. Paragraph 5	2	Replace ".....myxospores from juveniles...." with ".......myxospores in juveniles....."	done
NMFS	15	Support section. Paragraph 6	last sentence	"Benson (2014) also suggests the potential for hatchery Chinook Salmon to contribute" Insert "juvenile" in front of Chinook Salmon	done
NMFS	16	Conclusion	first paragraph	insert "incidental" in between "NMFS take statement"	done
NMFS	16	Conclusion - first paragraph	first paragraph	Replace "These high rates of infection play a major....." WITH "These high rates of infection likely play a major....."	don't agree; supported by citation (Fujiwara et al.) as noted in introduction.
Klamath Tribes	General			cooler water temperatures, higher late spring and summer flows, and a more typical substrate distribution prior to dam construction helped keep C. shasta at bay historically	Agreed; see "long term measures" section regarding dam removal on page 5.
Klamath Tribes	General			the recommended high flows are much higher than they would have been historically (pre-dam)	Do not agree. Guidance measures 1-3 call for flows consistent with high flow years in the past (see Geomorphic Memo for full analysis). Dilution flows (Guidance measure 4) calls for a reinitiation of high snowmelt flows which may or may not be higher than natural flows in the past. This Guidance Measure is an emergency action to alleviate acute disease conditions in real time as they are occurring.
Klamath Tribes	General			Some may argue that dams allow for higher flows for diseased salmon and are therefore essential, we do not agree with this assessment and this should be explained in the document.	We agree; the Secretarial Determination reports show unequivocally that the dams have only limited ability to change flows. See dam removal section on Page 5 of the Guidance Document.
Klamath Tribes	General	guidance measures 1-5		are these presented in order of priority?	The Guidance Measures are not presented in order of priority, although it should be noted that measures 1-3 and 5 are preventative measures intended to prevent disease conditions to become acute in the first place.
Klamath Tribes	General	Guidance 1		How was a 72 hour flow duration arrived at? The geomorphic tech memo? Figure 3 indicates that past years saw several days of sediment mobilizing flows, so is 72 hours sufficient?	The management guidance is to provide for surface flushing. It is assumed that longer periods of time will provide more complete movement and sorting of sediment. 72 hours was selected as a reasonable amount of time in which these processes are given time to occur.
Klamath Tribes	General			calling for a single 72 hour surface flushing flow of ~6,000 cfs despite "strong evidence to suggest that frequent surface flushing flows minimize available habitat for polychaetes" (emphasis added). Based on this statement, shouldn't this guidance be recommending more than a single surface flushing flow? If I am misunderstanding the recommendation (i.e., the guidance is recommending several surface flushing flows), perhaps clarifying this point in the document would be helpful.	Management Guidance 1 is called for at least once per year. Will add language clarifying that it is acceptible to have more than one event per year. It is assumed that, in wetter years, flows greater than 6,030 will occur more frequently than once per year.

	Disease Management Planning Effort - Guidance Document Comments USFWS Comments				*AFWO Review of our initial response to comments. Some responses by the DTAT changes subsequently.*	
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USFWS Klamath Falls	7	2	Specifically, provide a flow of at least 6,030 cfs from Iron Gate Dam for a 72 hour period	How was this time period for elevated flows determined? Please reference the technical memos or otherwise give justification for this time period.	Please see Uncertainties in this section: "In general, a longer duration event will accomplish more of the objective than a shorter duration, because more of the suspended sediments flush out of the river system, rather than being re-deposited further downstream."	We further suggest the authors cite page 5 of the Spore Technical Memo as it relates to a justification for events of longer duration... "*One hypothesis for the unique spatial pattern of spore concentrations observed in 2016 relates to the 11,200 cfs Iron Gate Dam discharge event occurring March 2016. This event could have dislodged and moved high numbers of polychaete worms downstream in the drift and these redistributed worms, if infected, may have contributed to the relatively high spore concentrations observed in the lower river (J. Alexander, pers. comm).*"
USFWS Klamath Falls	8	1	The estimated two year recurrence interval (Geomorphic Memo page 6) of 6,030 cfs was selected	Please justify making a flow with a 50/50 chance of occurring in any given year into an annual flow. This does not seem to be supported by the technical memos, nor is it within the nature of a two year recurrence interval flow	Supporting language added to support subsection of this proposed action. "We have recommended this flow to occur every year until the end of the BiOp or dam removal occurs for two reasons: 1)The reduction in surface flushing flows experienced since the year 2000, relative to the 35 years prior, has resulted in fine sediment and suspended sediments accumulating on the bed of the river and 2) the polychaete memo explains how higher flows and increased substrate instability (such as that caused by higher flows) dislodges polychaete worms and decreases polychaete Weighted Usable Area. The reduction in high flow occurrence and subsequent accumulation of fine sediments is a concern because high densities of M. speciosa have been observed in such deposits (Polychaete Memorandum page 3). These sediments may provide prime feeding grounds for M. speciosa, given that in addition to being sessile suspension feeders they likely also have the flexibility to feed on organic matter in deposited sediments (Polycheate Memo)."	See AFWO Comments on Synthesis Report
USFWS Klamath Falls	8	1	However, there is strong evidence to suggest that frequent surface flushing flows minimizes available habitat for polychaetes	Please cite either technical memos or other relevant documentation to support this statement.	Citation added (Polychaete tech memo pages 4 and 5 support this statement)	Also see AFWO Comments on Synthesis Report
USFWS Klamath Falls	9	3	Description of guidance (2)	What other potential ecosystem level effects may happen with this type of regime? Please address, with appropriate citation, the science supporting a net benefit from these flows to the overall ecosystem, as an imbalanced ecosystem could create unforeseen difficulties for the very species which we are trying to protect	See discussion on page 2 of Geomorphic Memo for thorough discussion on this topic complete with references. This information, combined with the analysis proving that the past 15 years has lacked these high flows supports the premise that flows of this magnitude (i.e. 11,250 cfs), and provided at least every other year for the duration of the BiOp or until dam removal will place the system <u>in balance</u>, rather than in a state of imbalance as asserted in the comment.	The reviewer infers that the current highly-altered and often over-allocated water system in the upper basin is in balance and raises concern that deviation from current water management practices could result in an "imbalanced ecosystem". We suggest referencing page 3 of the Fish Technical Memo in your response, which states that "*When environmental conditions are significantly altered... the change will most often favor the parasite...compared to the host (Webster et al. 2007)*" and that "*the parasite adapts more quickly to environmental change than the host, causing the parasite-host equilibrium to shift out of balance (Thompson 1994).*" The Fish Technical Memo also states that "*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*",
USFWS Klamath Falls	9	4	NOTE: if the deep flushing event as described in Management Guidance 1 does not result in flows above 6,030 cfs for at least 72 hours, it will not fulfill the fine sediment management guidance action (#1)	This note has been the source of consternation in discussions with other reviewers. I read this as saying is that we can kill two birds with one stone (i.e. guidance 1 and 2), but if the ramping rates for guidance 2 preclude a flow exceeding 6030 cfs for 72 hours, the peak flow of guidance 2 will not fulfill guidance 1. Suggest clarification of this note and the purpose for its insertion here	Commentor was correct in assertion that the meaning of the note was to clarify the "kill two birds with one stone" but an unfortunate typographical error has contributed to confusion. The wording has been changed to clarify using a positive assertion. "NOTE: if the deep flushing event as described in Management Guidance 2 does result in flows over 6,030 cfs for at least 72 hours, it will also fultill the surface flushing as described in Management Guidance 1.	Important comment and clarification.

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USFWS Klamath Falls	10	4	Management Guidance 3 (general comment)	Who will determine when these flows are available and the timing in which they should occur? Suggest clarifying within the body of this guidance as to potential decision-making process and if further hydrological modeling will be needed to define this decision-making process. Further, suggest addressing "inherent unpredictability and public safety factors" further. Are these flows intended to manifest as flood releases in wet springs, a la 2016? If so, this should be stated. Otherwise, this guidance is very similar to #3.	The Guidance 3 description states that it is the Bureau of Reclamation and PacifiCorp that would "look for opportunities" to implement this action. Have added "and other fisheries co-managers" after PacifiCorp to clarify that they should reach out to a broader technical audience. Specifying the exact circumstances for each case where this might be implemented is (as noted in the Guidance Document) complex and should be handled on a case-by-case basis.	Our understanding is that the draft "Guidance Document" was prepared by the Tribes to provide science-based actions for consideration by managers that would lower the incidence of C. shasta in juvenile Chinook and Coho salmon in the Klamath River, independent of consideration of other demands on the Basin's limited water resources and/or agency obligations. Consistent with our understanding, we suggest the Tribes refer to the document titled Final_Revised Disease Technical Advisory Team Scoping Document_092916.pdf prepared by BOR, the Klamath Falls Fish and Wildlife Office, and NMFS which states " *Once the Guidance Document is completed, the Bureau of Reclamation will coordinate with the National Marine Fisheries Service and the U.S. Fish and Wildlife Service to determine how to best apply the information presented in the Guidance Document to Klamath Project operations...*".
USFWS Klamath Falls	11	1	Hold in reserve 50 TAF…	It is highly unlikely that 50 TAF can be "held in reserve" at any time in the year, much less the period annually in which water is at its most over-allocated in this system. This effectively means that 50 TAF can be requested and must be made available if the below criteria are evident, regardless of other obligations or needs. This would coopt all inflows to the lake at a critical time. Suggest a reduction in the amount requested for "reserve," which would also require adjustment of flow schedule below	Added statement in the Introduction section explaining that the purpose of this management guidance was to identify conditions in the mainstem river that would alleviate the high mortalities experienced due to disease in juvenile salmonids. The question of "how" to make these things happen is beyond the perview and scope of the document. We acknowledge that the system is complex, and variables interact with each other in unpredictable ways. none the less, we believe it is physically possible to deliver this quantity of water in the specified time period.	We concur with the authors' response and suggest they add the reference Final_Revised Disease Technical Advisory Team Scoping Document_092916.pdf prepared by BOR, the Klamath Falls Fish and Wildlife Office, and NMFS which states " *Once the Guidance Document is completed, the Bureau of Reclamation will coordinate with the National Marine Fisheries Service and the U.S. Fish and Wildlife Service to determine how to best apply the information presented in the Guidance Document to Klamath Project operations...*".
USFWS Klamath Falls	11	2	Spore concentrations exceed 5 spores (non?specified genotype) per liter for the preceding sample based on qPCR from water filtration samples at any sampling station;	Non-specified genotype is worrisome, given Sascha Hallett's work on genotypic specificity of C. shasta infection in a given species of salmon. There is obvious and universal interest in protecting the tribal trust species (i.e. Chinook), but the primary focus currently is on the listed Coho. Dr. Hallett's work should not be disregarded in this document. Suggest allowing specification of genotype so that species-specific threat may be more effectively evaluated, given other factors at play in the river.	We are recommending flows when the spore count reaches 5/L of any type, on the premise that 1) 5/L is indicative of a rising trend, 2) distinguishing DNA type takes precious extra days, and (?) 3) high concentrations of type 1 can mask detection of type 2.	There is no potential for TI masking TII at the concentration levels being discussed here. Dr. Hallett's work is extensively utilized in this document.
USFWS Klamath Falls	11	2	Spore concentrations exceed 5 spores (non?specified genotype) per liter for the preceding sample based on qPCR from water filtration samples at any sampling station;	These criteria will be met in 9/10 years, effectively creating a new minimum flow for the river for much of the spring/summer. This appears to set the bar quite low for justification of these flows. Suggest reevaluating the criteria upon which these flows are based.	These criteria are based on the conclusion in the BiOp that 5 spores/L of type 2 and 10 spores/L of type 1 cause an estimated 40% mortality in Coho and Chinook juvenile salmon respectively (BiOp at 341-342). While it is acknowledged that this is a significant amount of water, it is also apparent that mortalities from this disease are a "key factor limiting salmon recivery in the Klamath River" (BiOp at 341). Furthermore, given the restrictions placed on implementation of this measure (water must be >13C, less than 80% of the outmigration complete), it is not at all clear that 9/10 years would require additional flows.	Has the guidance document and/or logic progression been clarified? NOTE: Yes
USFWS Klamath Falls	12	1	Increased flows also increase water velocity and have been shown to reduce transmission rates.	This also seems to be a tenuous connection, given the current science outlined in the tech memo. Please cite with the appropriate support or remove.	Citation to be added.	Ray, R.A., and J. L. Bartholomew. 2013. Estimation of transmission dynamics of the Ceratomyxa shasta actinospore to the salmonid host. Journal of Parasitology 140:907–916. doi: 10.1017/S0031182013000127.

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USFWS Klamath Falls	12	5	low temperatures often coincide with higher flows	This is anecdotal. I've not seen hydrologic research to support this, though I'd imagine there is likely some correlation. Perhaps a study should be commissioned to demonstrate the veracity of this statement in the Klamath River?		As reported by Dettinger et al. (2015) "Lower flows can concentrate pollutants, increase stream temperatures, and reduce dissolved oxygen" which they attribute to, in part, increased air temperatures that result in increased ET, decreased soil moisture, increase demand, a lower thermal capacity (mass) and therefore increased diurnal temperature fluctuations, etc. Van Vliet at al. 2012 reported a high sensitivity of water temperature to discharge of large rivers during warm, dry conditions. The authors reported that decreases in discharge of 25% and 50% resulted in significantly lower maximum water temperatures in the winter and significantly higher maximum water temperatures during the summer, which they attribute to decreased thermal capacity and increased sensitivity to atmospheric warming and cooling.
USFWS Klamath Falls	14	2	negative interactions between hatchery/natural fish in the Klamath River, while meeting the two objectives listed above	What are these negative interactions? Are they disease related? Is that disease something other than C. shasta?		My opinion is that the negative interactions of hatchery/wild populations is common knowledge and does not require citation.
USFWS Klamath Falls	16	3	Adaptive Management and Monitoring Considerations for (MG6)	Perhaps language could be added here to the effect of, "The disease management team recognizes that these measure are outside of the purview of BoR and the Services. However, given the need for implementation of these measures, we strongly suggest a concerted effort between CDFW and the Yurok, Karuk, and Hoopa Tribes; with input from Reclamation, the Services, and the Klamath Tribes; to see these measures implemented at the IGD hatchery	The guidance document identifies management actions that may alleviate C. shasta conditions on the Klamath without regard to implementation. This Guidance Document does not demand that Bureau of Reclamation implement any of these actions. Text will remain unaltered.	We agree with the authors' response and further suggest they cite the Final Disease Technical Advisory Team Scoping Document prepared by BOR, the Klamath Falls Fish and Wildlife Office, and NMFS which states " Once the Guidance Document is completed, the Bureau of Reclamation will coordinate with the National Marine Fisheries Service and the U.S. Fish and Wildlife Service to determine how to best apply the information presented in the Guidance Document to Klamath Project operations...".
Bureau of Reclamation	General			Additional detail needs to be provided for the scientific bases of timing, duration, and frequency intervals of events for *all* management options. For example, if a management action is recommended every year, please more closely tie the recommended timing, duration, and frequency back to the technical memoranda.	The technical memos did not, nore were they intended to, contain specific details regarding recommendations to address juvenile disease issues in the Klamath. The memos were to provide the scientific basis of current knowledge regarding *C. shasta*, from which guidance recommendations could be developed. If addtional details are needed to further refine management measures to minimize C. shasta, then that should be considered by a Disease Managemetn Recommendations Team comprised of Co-Managers.	The response does not appear to address the review comment. For example, in our comments on the report we suggest the authors provide a more through explanation as to why a 2-yr return period flow would be recommended every year, and suggest a justification.
Bureau of Reclamation	General			The four USFWS C. shasta technical memos provide a logical basis to conclude that regulation of the Klamath River, particularly storing water during winter and spring, may exacerbate C. shasta infection rates for outmigrating salmon downstream of Iron Gate Dam (IGD) during some years. These impacts would be most pronounced in the first 15-20 river miles downstream of IGD where flow conditions are principally driven by IGD water releases. However, a functional relationship between IGD discharge and disease conditions (i.e. fish infection or mortality rates) in the Lower Klamath River, which would be necessary to quantify the impact of KP operations on disease-induced fish mortality, has not been developed. Therefore, the outcome of the flow management measures proposed in the Guidance Document is highly uncertain, and these measures should be regarded as investigational.	It is likely that disease conditions in the Upper Klamath River affect disease conditions in the Lower Klamath River, whether it be from dislodged/infected polychated worms, free floating actinospores, or fish that become infected in the Upper Klamath and die to release myxospores downstream. We recognize that there is still much to learn regarding the dynamics of C. shasta in the Klamath River, which is why adaptive management is highlighted as being so important in the Synthesis Memo	We suggest the authors add that the prevalence of infection has been most pronounced during drier water years such as 2005 (prior to the May spill event), 2009, 2014, and 2015. During these dry water years, the discharge of the Klamath River below Iron Gate accounts for a greater proportion of the mainstem Klamath River discharge and therefore, has an increased influence on the total discharge of the mainstem Klamath River at locations located downriver as compared to wetter water years. Also important to note that contributions of Iron Gate Releases to of the overall Q of the Klamath River located at sites downriver varies considerable with date. As an example, see attached figure (10) from Beeman et al. 2008.

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Bureau of Reclamation	General			The relationship between coho salmon smolt survival and flow has been quantified (Beeman et al. 2012), but this relationship includes all sources of fish mortality, not just disease. Beeman et al. (2012) did not quantify latent effects of disease that may occur after fish reach the ocean, but water temperature is the primary determinant of disease-induced mortality, and ocean temperatures are much cooler, suggesting that survival rates of infected fish would likely increase upon ocean arrival. Though latent effects of disease infection are a concern, it was generally believed that in-river survival conditions between IGD and the ocean were adequately described by the Beeman et al. (2012) study. One interpretation of the study findings is that small operational changes should not be expected to significantly reduce juvenile coho mortality. Instead, large flow events, such as those associated with large storms, were the most influential throughout most of the Lower Klamath River. The information described in the four USFWS disease technical memos also supports the idea that large flow events are needed to meaningfully alter disease conditions and subsequent fish mortality.	Of the flow related control measures identified in the Synthesis Memo to minimize *C. shasta* levels in the Klamath River, four of five measures are focused on interrupting the life cycle of C. shasta, primarily by disrupting polychaete worms (an obligate part of C. shasta life cycle), polychaete habitat, or infection of polycaetes by myxospores released from rotting carcassess. The effectiveness of these control measures to impact polychaetes was not been assessed by the Beeman study. The control measure that consist of the provision of emergency spring dilution flows if certain criteria are met, could somewhat be related to the findings of the Beeman study. Whether increasing flows to 3,000 cfs (and then going to 4,000 cfs if necessary) is considered a "small operational change" or "large flow event" is up to interpretation, however the amount of water required to meet these levels during the April/May time period can vary substantially, depending upon hydrologic conditions. During some wetter water years, these flow levels would be met without any flow augmentation.	We suggest the authors also mention the Coho Salmon survival study the Service initiated in 2005 and summarized in the Beeman et al. 2012 multi-year report. This study addressed survival for outmigrating natural and hatchery-produced Coho Salmon smolts and not for Coho Salmon fry. These two life history stages of development are likely to have a much different migration pattern and therefore, exposure history and parasite dose in the mainstem Klamath River.
Bureau of Reclamation	General			Please consider assessing the potential role of increasing flows from key tributaries of the Klamath River below IGD in reducing C. shasta POI. Although outside of Reclamation's jurisdiction, we feel that increasing cool water flows from key tributaries could reduce spore production and improve upstream passage into the tributaries. Concentrating efforts on increasing flows from IGD ignores the potential role tributaries could play at reducing actinospore concentrations in the mainstem Klamath River and increasing usage of those habitats by juvenile coho salmon.	It is not clear what time of year is being recommending for increasing flows in tributaries, given the reference to fish passage, however we agree that cool water accretions from Klamath River tributaries are critical for the health of the Klamath River ecosystem and could help to ameliorate disease conditions in the Klamath River to some extent. However, the magnitude of water diverted within tributaries downstream of Iron Gate Dam is relatlively small compared to IGD discharge - while the impact of these diversions is extreme within the tributaries, the impact to the mainstem Klamath flow is much less (but still important).	Note that while generally true, not all tributaries provide "cool water flows" at all times of the season. For example, the Shasta River near its confluence can, at times, be warmer than the mainstem Klamath River just upstream of its confluence with the Shasta River (see graph from 2016).
Bureau of Reclamation	4	5		"Spore dilution" is mentioned as an objective of one of the DTAT's flow management recommendations. However, spore dilution via flow augmentation has not been shown to appreciably decrease infection rates in Klamath River salmon (2014, tech memo). Infection rates are most strongly correlated with water temperature, especially in cases where spore concentrations are high. Lab studies have also shown that temperature is the key factor for predicting mortality of infected fish, which means releasing more warm water above the tubine capacity at IGD may not appreciably (if at all), affect mortality rates.	In the spring time, is there an inverse relationship between water flow from IGD and water temperature? Data is not as plentiful to assess the relationship between IGD Q and disease levels as we would like, however the spore technical memo did show a substantial decline in prevalence of infection from C. shasta from a substantial flow event in 2005 (Figure 4), and there was a moderate decline in C. shasta spore levels from an increase in Q to 1,900 cfs in 2014 (Figure 6).	In addition to the spore data the authors refer to in their response, we suggest the Tribes discuss the abrupt increase in disease-induced mortality of young-of-year Chinook Salmon that was observed in juvenile outmigrant trap catches at the Bogus, I-5, and Kinsman fish trap sites below IGD beginning on April 29, 2004 (Chamberlain and Williamson 2006) as reported by Hetrick et al. (2009). By early May 2004, mortality approached 50% for unclipped young-of-year Chinook salmon captured at the Kinsman, Happy Camp, and Persido Bar trap sites located further downstream. From June 2 to June 18, mortality observed in daily catches of Chinook Salmon at the Kinsman site ranged between 51% and 88%. During this period, flows below IGD averaged 1,810 cfs for April, 1,290 cfs for May and dropped to 942 cfs for June, with daily mean values ranging from 2,060 - 802 cfs.
Bureau of Reclamation	8	3		While there is considerable discussion to support the 6,030 cfs flow, the true target is the reduction in the host population where the scientific basis included in the Guidance Document is that there is "strong evidence to suggest". This does not provide the "predicted outcome" assumed in the adaptive management consideration section on page 4. Clear definition is needed. Please include.	Not clear what this comment is asking or recommending. The predicted outcome of the surface flushing flows is the mobilization of surface sediments, reduction in available polychaete habitat, and resultant reduction in polychate densities. (Why didn't we include infected polychaetes?)	A strict focus on infected polychaetes is probably not required as justification for the flow recommendation.
Bureau of Reclamation	11	1	Mangement Guidance 4: Emergency Provision Flow to dilute spores	In general, Reclamation believes the thresholds listed for detemining whether to implement a diluting flow are difficult to quantify, especially given the dynamic nature of natural systems. This has the potential to make implementation of a diluting flow incredibly difficult. For example what tool or approch is available and accepted as a means to estimate whether 80% of the expected wild run has passed the Kinsman, Scott and Shasta? It doesn't seem like this threshold is easily quantifiable, especially in real time (i.e., how do we know what 80% is until we get to the end?). Looking back at past data, water temperature and prevalance of infection are also highly dynamic, leading to the possibility of an on-again/off-again management action.	USFWS staff (operators of the Kinsman trap) have indicated that they can develop a method for estimating when 80% of the run has passed the trap site - this would be independent of real-time trapping information. Dynamic flows in the spring time would not necessarily be a bad thing.	USFWS staff have indicated that they will investigate the development of a method for estimating when 80% of the run has passed the trap site, and have suggested that the results of this investigation are likely to be successful.
Bureau of Reclamation	11		Mangement Guidance 4: Emergency Provision Flow to dilute spores	The thresold criteria for Kinsman rotary screw trap is specific to wild fish (to not exceed 80%), but the criteria number 2 regarding overall POI does not specify whether the POI is all fish captured, or just wild? Please clarify.	The 80% is specific to wild fish emigration, the POI level pertains to all fish sampled prior to when 80% of the wild run is estimated to have passed the Kinsman trap site.	The authors response is correct.

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Bureau of Reclamation	11	1		Smolt trapping data at Bogus Creek (USFWS 2002-2005) showed that coho salmon smolts migrated through the most upstream reach of the Lower Klamath River between March 1 and May 1, nearly a month prior to the increase in C. shasta spore concentrations recorded in 2005 (Spore Memo, Figure 4). If coho emigration regularly precedes spore concentration increases, dilution strategies would not be effective.	In addition to some coho smolts being in the Upper Klamath River during May, there are also young of year coho in the Upper Klamath at this time; rearing and/or re-distributing from one area to another. For example see USFWS report titled *Arcata Fisheries Data Series Report DS 2016-47 Summary of Abundance and Biological Data Collected During Juvenile Salmonid Monitoring on the Mainstem Klamath River Below Iron Gate Dam, California, 2014* (David et al, 2016) - available on USFWS Arcata web site.	Observed timing at the most upstream portion of the river does not estimate mainstem rearing or migration timing.
Klamath Water Users Association	General			The Draft Guidance describes the 2014 and 2015 situation as critical, and refers to an immediate dire need. We believe parties would benefit from a more complete understanding of the consequences of C. shasta for overall populations of coho or other species through time and over the range of conditions that exist in the river, and the relative role of disease and other factors affecting populations, including at the present time.	Please see Fujiwara et al. 2011, True et al. 2013 as cited in the Fish Infection Memo (attached as appendix to the Guidance Document) for a peer-reviewed fully developed analysis of population effects. Although this does not include the most recent data, it discusses population level effects to chinook and coho salmon.	We suggest the authors also refer to Tables 1 and 2 in the Fish Technical Memo.
Klamath Water Users Association	Page 2	First full paragraph:		The focus here falls to the operation of the Klamath Project; KWUA encourages a broader perspective. The flows affected by Klamath Project operations also supplement natural flow conditions in certain times of year. Additionally, the last sentence in the paragraph makes an assumption regarding infection rates in coho salmon. We are not aware of data on this issue and ask that it be provided if it exists and is available.	The language and conclusions in this paragraph are largely drawn from the 2013 BiOp. (NOTE: should we include language addressing this issue?	Exact quotes might not be necessary, but instead a more directed citation (page, section, paragraph numbers, etc.).
NMFS				replace "The Polychaete Memo describes how populations of polychaetes can be kept in check by mobilizing sediments, and the Geomorphic Memo describes what flows are needed to accomplish various geomorphic objectives." WITH "The Polychaete Memo describes how polychaetes distribution is related to substrate and hydraulics, and the Geomorphic Memo describes what flows are needed to accomplish various geomorphic objectives"	see comments above	USFWS suggested a similar change.
NMFS	11	Description: 1……	1…..	Not sure what the basis is for the 5 spores/l threshold. Seems that in the past this threshold has been related to coho salmon. ALSO The influence of IGD releases often diminishes further downstream.	see comments above	The authors could copy/paste responses provided above to other reviewers here. USFWS has also noted concerns about threshold levels.
NMFS	11	3. (in regard to 80% of the expected wild run)	The juvenile catch at the Kinsman rotary screw trap is estimated to have not exceeded 80% of the expected wild run	Not sure that this can be currently estimated in real time.	USFWS Biometrician thinks this can be estimated, without relying upon real-time downstream migrant trap data.	USFWS statistician has agreed to investigate a method, but the investigation could include using real-time downstream migrant trap data (but cannot rely upon detection probability reliant population estimates).
NMFS	12	"The concentration of….."	Increased flows also increase water vilocity and have been shown to reduce transmission rates.	This statement could use a supporting citation. Is this based on lab studies, or river studies?	we should cite the study that found this or delete	Here is the citation for velocity vs transmission: Ray, R.A., and J. L. Bartholomew. 2013. Estimation of transmission dynamics of the Ceratomyxa shasta actinospore to the salmonid host. Journal of Parasitology 140:907–916. doi: 10.1017/S0031182013000127.