Whirling Disease Management Plan for the Upper Feather River Basin

A Cooperative Project:
Sierra Institute for Community and Environment
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Executive Summary

The Upper Feather River Basin Fisheries Assessment and Restoration Strategy (Rogers et al., 2018) identified *M. cerebralis* management as a priority concern. This Management Plan, hereafter referred to as "The Plan", summarizes the research findings and recommendations of a Technical Advisory Group convened to address the pathogen in the Upper Feather River Basin (the Basin). The Plan has two primary elements, a summary of Whirling Disease ecology relevant to the Basin; and a summary of management approaches considered and recommended by the Whirling Disease Task Group (WDTG), a collaborative group of resource managers representing a variety of agencies, organizations, and institutions.

Whirling Disease Ecology

The Plan summarizes Whirling Disease information in the context of conditions in the Upper Feather River Basin focused on these topics:

- Susceptibility of *M. cerebralis* hosts
- Locations of *M. cerebralis* within the watershed
- Identification of *M. cerebralis* vectors
- Fish health practices at federal, state and privately-owned hatcheries
- Environmental characteristics conducive to *M. cerebralis* establishment
- Lessons learned from other locations about management for *M. cerebralis*.

Management Elements

The Plan includes six primary approaches to managing for Whirling Disease in the watershed. Those elements and related key actions are summarized below.

Basin-wide risk Assessment Model: A model was developed to predict the likelihood of *M. cerebralis* presence. Overall rating of risk was derived by combining assessments of pathogen introduction and pathogen establishment. Ratings informed development of other Management Plan elements.

Public awareness: The lack of public awareness about the presence of *M. cerebralis* in the Feather River and about measures necessary to reduce its spread led the WDTG to recommend preparation of a Public Outreach and Communication Plan, which is a component of the Management Plan.

Equipment Decontamination Protocols: Uncertainty about the effectiveness of equipment decontamination protocols used by agencies and organizations doing aquatic work in the watershed to prevent the spread of *M. cerebralis* led to a review of existing protocols, recommended protocols, and recommended practices for planning and conducting aquatic surveys.

Whirling Disease Resistant Fish: Trout strains with demonstrated resistance to Whirling Disease have been planted with success in other areas. A preliminary approach to identifying suitable strains and planting locations was developed.

Monitoring: Knowledge of the extent of *M. cerebralis* presence throughout the watershed is incomplete as a result of limited surveys. A plan for *M. cerebralis* monitoring in the watershed is presented.

Meadow Restoration Project Risk Assessment: The potential contribution of meadow restoration projects in providing suitable habitat for *M. cerebralis* is a concern. A tool was developed to evaluate the risk of meadow improvement projects in contributing to Whirling Disease.

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Cover Photograph

Goodrich Creek, just upstream of Highway 32 Crossing. By Michael Kossow.

Whirling Disease Management Plan for the Upper Feather River Basin

The Upper Feather River Basin Fisheries Assessment and Restoration Strategy identified *M. cerebralis* management and control as a restoration priority (Rogers et al., 2018). Implementation of the Strategy prompted formation of a public-private Whirling Disease Task Group (WDTG). The group's purpose was to identify needs and the priority management options, relative to Whirling Disease for resource managers in the watershed. The Plan relies on research and management techniques applied elsewhere. Results of that work are the basis of this Plan.

1.0 Background

The Upper Feather River Basin (Figure 1) lies upstream of Lake Oroville, a reservoir operated as part of the California State Water Project. The 3,200 km^2 watershed extends east to the Sierra Nevada Crest and the Diamond Mountains and northeast into the Cascade Range. Mount Lassen is the highest point in the drainage. The Basin includes several branches: North Fork, East Branch of the North Fork, Middle Fork, South Fork, and the West Branch. Anthropogenic activities in the Upper Feather River include timber harvesting, mining, agriculture, hydroelectric dams and reservoirs, and recreational uses such as boating and angling. The majority of these activities continue to influence current watershed conditions.

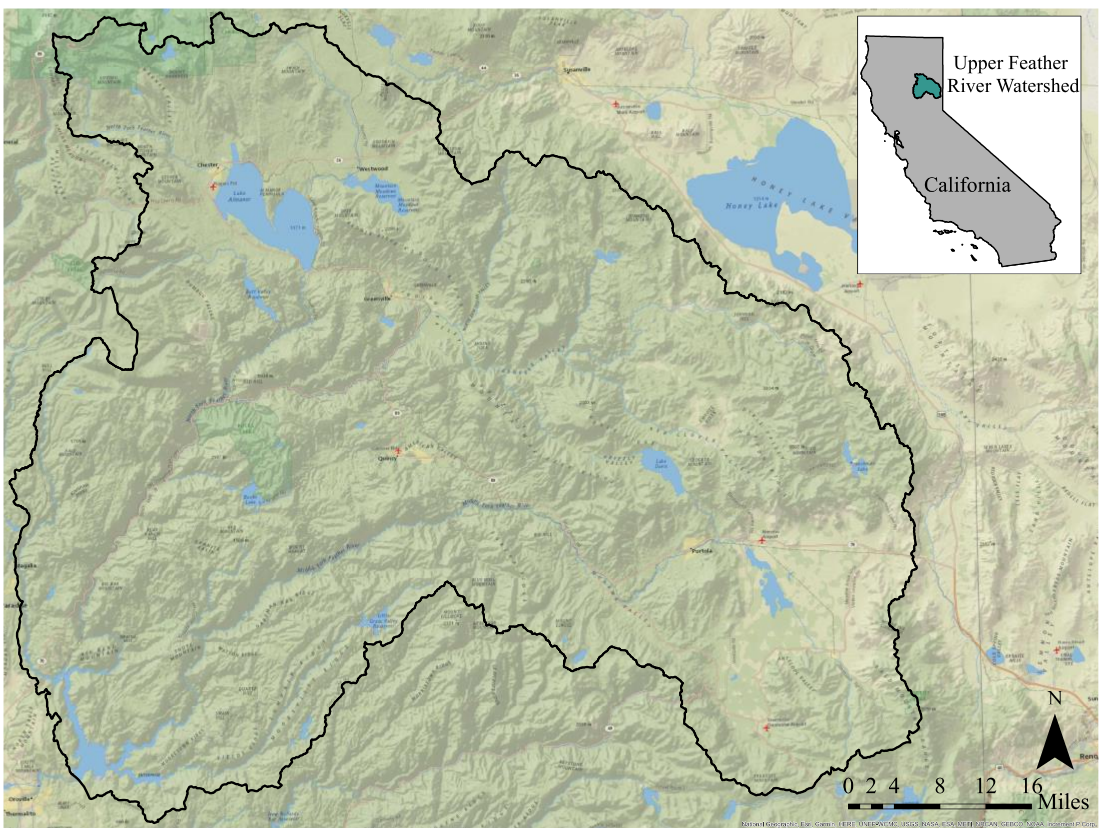

Figure 1. Upper Feather River Basin in Northern California

The Upper Feather River Basin is a priority for restoration and protection due to its importance as a major source of water in California. A long history of anthropogenic impacts in the watershed has resulted in degraded watershed conditions in some areas which, among other concerns, have significantly impacted fisheries. Examples of factors influencing basin condition and the health of its fisheries include hydroelectric developments, the fragmentation of aquatic habitats, pollution and sedimentation resulting from roading and management activities, water diversions for agricultural use, and the introduction of aquatic invasive species (AIS) including the etiological agent of Whirling Disease, *M. cerebralis*.

Myxobolus cerebralis was first detected in the Basin in 1984 and has contributed to rainbow trout (*Oncorhynchus mykiss*) declines in some areas, though the impact of *M. cerebralis* in the watershed is not well understood. *M. cerebralis* was first detected in California in 1965 at a private hatchery in Monterey County and eventually spread throughout the state (Modin, 1998). It became well-established in the Eastern Sierra from the Lahontan Basin to the Owens Valley Basin. Despite its rapid progression across the state, California did not experience the widespread devastation of wild trout populations observed in other Western states. Recent surveys show that *M. cerebralis* continues to persist in the Basin at several locations.

2.0 Development of the Whirling Disease Management Plan

2.1 Whirling Disease Task Group

The WDTG comprises resource managers and researchers representing several agencies and nonprofit organizations with interest or management responsibilities in the Upper Feather River Basin. Specifically, the group includes representatives from the California Department of Fish and Wildlife (CDFW), California Department of Water Resources (DWR), Plumas County Fish & Game Commission, Maidu Summit Consortium (MSC), Feather River Chapter, Trout Unlimited (FRTU), University of California, Davis (UC-Davis), Washington State University (WSU), Feather River Land Trust (FRLT), California Trout (CalTrout), and the Plumas (PNF), Lassen (LNF), and Tahoe National Forests (TNF). WDTG members were identified based on their interest and/or expertise in Feather River fisheries or Whirling Disease management and control efforts. The WDTG met on four occasions throughout the development of the Plan to identify, explore, and recommend Whirling Disease information needs and priority actions.

The WDTG was presented with Whirling Disease information and actions employed elsewhere to manage for Whirling Disease. Next, additional information needs and potential management approaches were identified by the group. All information gaps were considered a priority for inclusion in the Plan. Priority management actions were selected for further study to determine their applicability to Whirling Disease concerns in the Upper Feather River Basin. Following further investigation and discussion, priority management actions for implementation in the watershed were selected.

2.2 Potential Management Approaches

Numerous approaches to managing for Whirling Disease were investigated and discussed by the WDTG. Ultimately, six were recommended for inclusion in the Plan. These are listed below. (discussion of is provided in Section 5):

- development of a Basin-wide risk assessment model
- outreach and education
- assessment of equipment decontamination protocols
- resistant salmonid strains
- monitoring
- project-level risk assessment for meadow restoration projects

Additional management actions that were identified and evaluated are reservoir release flow manipulation, increasing public involvement in *M. cerebralis* monitoring, the formation of a rapid response team for fish diseases, and planting *T. tubifex* strains resistant to *M. cerebralis*. These topics were not recommended as management approaches, for the reasons discussed below.

2.2.1 Pulse Flows

Flow manipulation is a technique currently being used on the Klamath River to control outbreaks of another salmonid pathogen, *Ceratonova shasta*. *C. shasta* is a myxozoan parasite which, like Whirling Disease, requires two hosts: a salmonid and a polychaetae worm. High flows remove the fine sediment habitat of worm hosts as well as the worms themselves. The increase in flow also dilutes the concentration of the infective life stages of the pathogen, decreasing the likelihood that they will come into contact with salmonid hosts (True et al., 2015).

Although there are distinct similarities between the *C. shasta* and *M. cerebralis* life cycles that may make flow management applicable to *M. cerebralis*, it was not recommended as a management tool for *M. cerebralis* in the watershed. Potential flow manipulation is restricted to stream reaches below dams. Only one of these, Indian Creek below Antelope Lake, is known to be infected by *M. cerebralis*. Given the extent of interagency coordination necessary and the impact that high flows could have on other fish and aquatic organisms, this management approach was not considered high priority by the WDTG.

2.2.2 Public Involvement in *M. cerebralis* Monitoring

Citizen science programs for invasive species and fish population monitoring are widespread and have been successful tools for increasing the monitoring capacity of agencies. However, previous citizen science programs have not been directed towards the monitoring of pathogens because the methodologies do not lend themselves to simple field procedures.

As an endoparasite, *M. cerebralis* presents specific challenges for monitoring. Identification of the pathogen often requires the euthanasia of fish, and usually laboratory analysis of *M. cerebralis* from euthanized hosts or free *M. cerebralis* life stages. However, it is important to note that new advances in collection methodologies such as environmental DNA (eDNA) might provide an avenue for future citizens science programs. Although a citizen science program is considered to be beyond the scope of the current Management Plan, it could be considered for the more general monitoring of AIS or other pathogens in the Upper Feather River Basin in future efforts. Agencies currently encourage public involvement in reporting fish and wildlife concerns on official webpages.

2.2.3 Rapid Response Teams

Rapid response teams have been used to address AIS concerns as well as outbreaks of aquatic diseases. They have been developed across the country for early detection and response to control AIS threats (Kolby et al., 2015). Teams have comprised fish and wildlife professionals with the training and expertise to confirm identification of AIS or diseases rapidly (i.e., early detection). This allows for the initiation of actions to contain or eradicate the threat (i.e., early response).

A rapid response plan for AIS has been developed in California (California Department of Fish and Game, 2008). The Fish Health Laboratory (CDFW) serves as a rapid response team for fish health concerns in wild populations. Due to this existing rapid response structure, it was determined that no further development of rapid response was necessary.

2.2.4 Introduction of Resistant *T. tubifex* Lineages

Resistant *T. tubifex* strains have been used in management and control efforts for Whirling Disease (Nehring et al., 2018). Most lineages of *T. tubifex* are not susceptible to the pathogen and are thought to act as biofilters for myxospores, i.e., they remove myxospores from the environment through consumption, but do not produce triactinomyxons (TAMs). Research has shown that resistant *T. tubifex* may out-compete infected susceptible worms and produce a shift in host community composition after introduction that reduces TAM production. Along with other management actions, including salmonid removal for at least 3 years and filling waterbodies known to produce high numbers of TAMs, this action has been effective in removing the pathogen (Nehring et al., 2018).

The WDTG did not consider the introduction of resistant *T. tubifex* a priority in the Basin. This recommendation was based on variable success of introductions elsewhere. Results indicated that over time, streams planted with resistant *T. tubifex* eventually shifted from planted resistant lineages back to susceptible lineages. Uninfected susceptible *T. tubifex* outcompete resistant lineages (D. Winkelman, Unit Leader, Colorado Cooperative Fish and Wildlife Research Unit, personal communication). Another concern for this management tool is that planting lineages of *T. tubifex* resistant to *M. cerebralis* could result in introduction and/or disease outbreaks of other pathogens. An additional challenge with this approach is fish removal. Removal of this host is key to eradication of *M. cerebralis,* but fish removal is impractical in most locations in the watershed.

2.2.5 Other Aquatic Pathogens

Other aquatic pathogens were not the focus of this Management Plan, but increased knowledge of the distribution of other aquatic pathogens in the watershed is a need. Recent surveys have identified locations of additional pathogens in the Basin (Rogers et al., 2018; E. Soto, 2019) and will serve as a foundation to improve understanding of AIS distribution. The WDTG recommended an increased effort to investigate the distribution of other pathogens.

3.0 Whirling Disease Ecology

One of the first tasks of the WDTG was to identify information needs. *M. cerebralis* research relevant to those questions is summarized in the sections that follow. To provide context, topics are addressed in relation to the Upper Feather River Basin. The WDTG identified six primary information gaps:

- Susceptibility of *M. cerebralis* hosts
- Locations of *M. cerebralis* within the watershed
- Identification of *M. cerebralis* vectors
- Fish health practices of federal, state, and privately-owned hatcheries
- Environmental characteristics conducive to *M. cerebralis* establishment
- Lessons learned about management for *M. cerebralis* from other areas

3.1 *M. cerebralis* Life Cycle

M. cerebralis has a multiple-host life cycle with two obligate hosts: the oligochaete *Tubifex tubifex* and a representative of the salmonid family (Beauchamp et al., 2002; 2006) (Figure 2). The pathogen has two life stages, the myxospore that develops and multiples primarily within the cartilage of hosts, and the TAM that develops and multiplies in the epithelial lining of the digestive tract of *T. tubifex*. The myxospore settles to the stream bottom and infects *T. tubifex* when consumed. TAMs are buoyant and float in the water column until they locate a salmonid host. Upon contact the TAM injects an infective sporoplasm into the epithelial layers of the fish. The sporoplasm eventually migrates to the cartilage. Myxospores are primarily released into the environment following fish mortality.

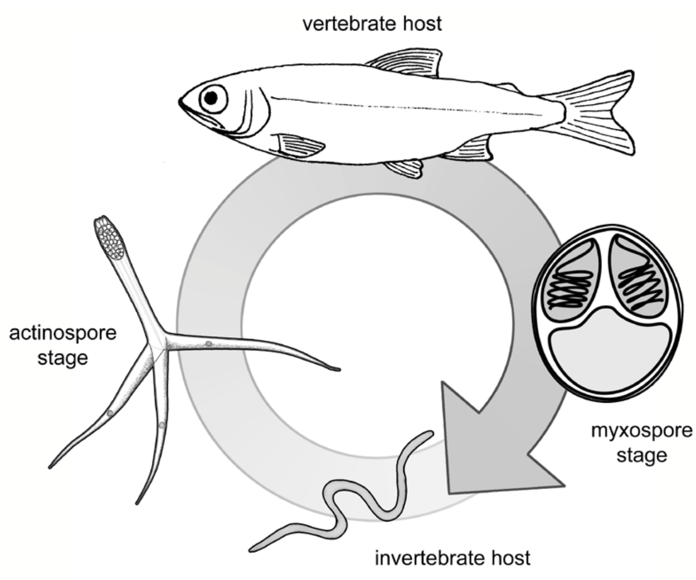

Figure 2. The life cycle of the pathogen *M. cerebralis* (Credit: Stephen Atkinson)

3.2 Salmonid Hosts

3.2.1 Host Symptoms

The symptoms experienced by salmonid hosts include black-tail, cranial and spinal deformities, rapid circular swimming behavior (i.e., "whirling"), and mortality. Symptoms result from histological damage caused by the pathogen's digestion of cartilage tissue and compression of spinal tissue from inflammation due to host's immune response (MacConnell & Vincent, 2002). Multiple factors determine the severity of symptoms. These include salmonid species and life stage and TAM exposure dose (Hedrick et al., 2003).

3.2.2 Host Susceptibility

Susceptibility to *M. cerebralis* is measured by evaluations of histological damage, presence or severity of clinical Whirling Disease symptoms, and the amount of myxospores produced in hosts following similar TAM exposure doses. Although many salmonids serve as hosts, some are significantly more vulnerable to infection than others.

Of the species present in the Upper Feather River Basin, rainbow trout, Eastern brook trout, *Salvelinus fontinalis*, and kokanee salmon, *Oncorhynchus nerka*, are more susceptible to the pathogen (Table 1). Wild rainbow trout strains in particular appear to exhibit the worst pathology and produce the highest number of myxospores (Sarker et al., 2015). In comparison, German brown trout, *Salmo trutta*, exhibit the clinical signs of Whirling Disease more rarely and produce far fewer spores (Hedrick et al., 1999). Susceptibility ratings (Table 1) were originally developed and presented by MacConnell & Vincent (2002) and found in Sarker et al. (2015). Both sources provide comprehensive summaries of salmonid susceptibility to *M. cerebralis*.

Younger trout are more susceptible to the pathogen than adult fish, and the susceptibility window is species-dependent. For example, Chinook salmon, *Oncorhynchus tshawytscha*, have a susceptibility window of up to 3 weeks (Sollid et al., 2003) while rainbow trout susceptibility spans a 9-week period (Ryce et al., 2004). Younger age classes are more susceptible. They experience greater histological damage, are more likely to exhibit the Whirling Disease symptoms, and produce more myxospores (Sarker et al., 2015)

Table 1. Susceptibility of salmonids to Whirling Disease (*present in the Upper Feather River Basin). From Sarker, et al (2015), MacConnell and Vincent (2002).

Genus	Species	Common Name	Susceptibility Rating
Oncorhynchus	*mykiss**	rainbow trout	3
	aguabonita	golden trout	2-3
	clarki	cutthroat trout	2-3
	*nerka**	kokanee salmon	3
Salvelinus	*fontinalis**	brook trout	2
	namaycush	lake trout	0
Salmo	*trutta**	brown trout	1

Susceptibility Rating	Description
0 = resistant	No spores develop
1 = partial resistance	Clinical symptoms are very rare, only develop after exposure to very high parasite doses
2 = susceptible	Clinical disease is common at high parasite doses or when very young, but demonstrate greater resistance at small parasite exposure doses
3 = highly susceptible	Clinical disease common

3.3 *Tubifex tubifex* Hosts

3.3.1 Host Symptoms

In contrast to symptoms observed in salmonid hosts, *T. tubifex* infected with *M. cerebralis* apparently experience mild effects and low mortality. However, infected individuals demonstrate lower weight and abundance (Stevens et al., 2006) and appear be at a competitive disadvantage with uninfected *T. tubifex* (D. Winkelman).

3.3.2 Host Susceptibility

Susceptibility in *T. tubifex* hosts is primarily measured through TAM production. Of the five lineages of *T. tubifex* in North America, two are susceptible to the pathogen: lineage I and III (see Table 2). Lineage III worms are particularly susceptible to *M. cerebralis* with high TAM production observed in infected individuals (Zielinski et al., 2011). Lineage I worms have a mixture of susceptible and resistant individuals with mostly resistant phenotypes in which no

TAMs develop (Beauchamp et al., 2005; Beauchamp et al., 2006). The remaining North American *T. tubifex* lineages are not known to develop TAMs (Beauchamp et al., 2005; Beauchamp et al., 2006).

In the Upper Feather River Basin, *M. cerebralis* and *T. tubifex* surveys have shown that susceptible lineages are present. Surveys have demonstrated that lineages I and III are both found in *M. cerebralis*-positive streams (Weber et al., 2012; Richey et al., 2018).

Table 2. Susceptibility of North American *T. tubifex* lineages

North American Lineage	Susceptibility
I	1
III	2
IV	0
V	0
VI	0

Susceptibility Rating	Description
0	TAMs do not develop
1	Lineage is comprised of susceptible and resistant individuals with mostly resistant phenotypes
2	Lineage is comprised of mostly susceptible individuals and produce high numbers of TAMs

3.4 *M. cerebralis* Detections in the Upper Feather River Basin

M. cerebralis was first detected in the Basin in 1984 in an unknown location in Rock Creek (the location was not properly documented). There are also records of *M. cerebralis* detection in Last Chance Creek, though this location was also not properly documented. Subsequently the pathogen was detected in Yellow Creek, and the North Fork of the Feather River (near Milk Ranch Creek) during surveys conducted between 1984 and 2000. Since 2000, surveys have detected *M. cerebralis* in Goodrich Creek, Indian Creek, Lights Creek, and Hungry Creek. eDNA surveys by UC Davis in 2019 detected the pathogen for the first time in Round Valley Reservoir, and at several locations along the North Fork of the Feather River (E. Soto, 2019).

M. cerebralis presence in Yellow Creek has received considerable attention because Yellow Creek is designated as a Wild Trout steam by CDFW. Rainbow trout numbers had declined significantly by 2000, generally corresponding to *M. cerebralis* detection in 1997. All resident trout species (brown, rainbow, and brook trout) tested positive for the pathogen and showed signs of Whirling Disease during surveys between 1997 and 2000. Surveys conducted in 2011 and 2016 found the pathogen persisting in Yellow Creek (Mehalick & Weaver, 2011; Rogers et al., 2018) and that the fishery has become brown trout dominated (Mehalick & Weaver, 2011).

These detections of *M. cerebralis* are based on limited survey information. The need for more information on *M. cerebralis* presence in the Basin is addressed in the monitoring plan.

3.5 *M. cerebralis* Vectors

Both human-assisted and natural dispersal vectors for *M. cerebralis* are relevant in the Upper Feather River Basin. In the U.S., human-assisted dispersal, via fish planting, has been linked to *M. cerebralis* introductions (Modin, 1998). Natural dispersal also plays an important role, based on anecdotal evidence used by resource managers to explain patterns of pathogen introductions they observed (T. Horton, Region 3 Fisheries Manager, Montana Fish, Wildlife, & Parks, personal communication).

Five factors are most likely to contribute to *M. cerebralis* dispersal in the Basin. Human-assisted dispersal is suspected of occurring through fish transfers and aquatic recreation (angling and boating). Natural dispersal vectors of concern include birds, mammals, and fish. Each of these factors is discussed below.

The myxospore life stage is the main focus of the discussions most resilient to environmental change and is most likely to persist and disperse. Myxospores are capable of surviving outside of hosts for several months. The TAM life stage is more fragile and survives for a much shorter time frame: approximately 6-15 days at 7-15ºC (El-Matbouli et al., 1999).

3.5.1 Human Assisted Dispersal

3.5.1.1 Fish Planting

Human movement of infected hatchery-reared salmonids, primarily trout, is the only *M. cerebralis* vector demonstrated in North America. The prevalence of infection among wild salmonid populations and myxospore concentrations in aquatic systems has been shown to correspond to the locations of fish planting (Thompson & Nehring, 2000). Asymptomatic hatchery-reared salmonids infected with *M. cerebralis* contributed significantly to pathogen dispersal.

In California, fish stocking, particularly the planting of infected trout, has resulted in *M. cerebralis* introductions. This discovery, and the spread of other pathogens, resulted in changes to state hatchery operations. These included updated facilities to remove environments conducive to *M. cerebralis* and more stringent fish health practices in state-operated hatcheries. There is some evidence that privately-owned trout hatcheries have implemented changes. However, it is not necessary for these facilities to meet the same requirements as state-operated hatcheries.

3.5.1.2 State Operated Hatcheries

In the Upper Feather River Basin, CDFW plants trout in multiple areas with high recreational use. These locations include Antelope Lake, Bucks Lake, Lake Almanor, Frenchman Lake, and the NF Feather River. Trout are planted at a catchable size to support recreational fishing. State-operated Darrah Springs and Crystal Lake Hatcheries are the suppliers for CDFW trout planting in the Basin (J. Rowan, State Hatchery Manager, CDFW, personal communication).

CDFW has implemented a number of precautionary measures to prevent the spread of *M. cerebralis* and other pathogens. Annual certifications of fish health are required for state-operated hatcheries. In order to recertify, salmonid populations for each hatchery are tested for *M. cerebralis* and other pathogens of concern (M. Adkison, Statewide Fish Health Coordinator, CDFW, personal communication). During a facility inspection, a subsample from each lot of the most susceptible species in each water source is inspected. Sample size varies to achieve a 95% confidence in detection, given a pathogen prevalence level of infection ≥ 5%. During spawning, broodstock are tested for viruses and bacteria. Throughout the growing cycle (approximately 6-14 months), fish undergo routine inspections by hatchery staff and fish pathologists.

The detection of *M. cerebralis* in CDFW hatcheries in recent years highlights the importance of pathogen testing in state-operated hatcheries. In 2015, trout from the Darrah Springs Hatchery were discovered to be infected with *M. cerebralis* when they were transferred to the Mt. Shasta Hatchery. Trout from all facilities were quarantined and *M. cerebralis* testing was conducted. The infected trout could not be stocked in California waters and so were repurposed as a food supply. Stocking of *M. cerebralis*-positive trout in California is only permitted in designated *M. cerebralis*-infected waters such as those of the Eastern Sierra. A few weeks after the discovery of *M. cerebralis* in trout from the Darrah Springs Hatchery population, fish from Hot Creek Hatchery in the Eastern Sierra also tested positive. The hatchery continued normal operations during this infection as it was in a *M. cerebralis*-positive region.

Additional mitigation efforts included a progression away from the use of earthen-bottomed rearing ponds that provide a suitable environment for the pathogen (Allen & Bergersen, 2002) and use of resistant strains. CDFW has recently developed a broodstock of a *M. cerebralis*-resistant rainbow trout strain (Hofer rainbow) for planting in *M. cerebralis*-positive areas. This strain is currently grown at two CDFW hatcheries: Hot Creek Hatchery and Mt. Shasta Hatchery (J. Rowan).

3.5.1.3 Hatcheries Outside California

CDFW requires that all salmonid life stages imported from outside the state are certified disease-free. The diagnostic procedures for pathogen detection for out-of-state hatcheries follow the same American Fisheries Society professional standards ("Bluebook: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens") as employed for CDFW testing.

These efforts allow the agency to monitor infections of hatchery-reared salmonids from out-of-state and state-operated facilitates and increase the likelihood of detecting the pathogen in asymptomatic infected hatchery populations.

3.5.1.4 Private Hatchery Operations

Privately-owned hatcheries that contribute to trout planting in the watershed include the Chester High School (CHS) Hatchery in Chester, the Feather River College (FRC) hatchery in Quincy, and Mount Lassen Trout Farm that primarily operates at Paynes Creek. Mount Lassen Trout Farm is the main source of private hatchery fish in the Basin, followed by the FRC hatchery (A.

Mouser, Environmental Scientist for Plumas/Sierra County Fisheries, CDFW, personal communication). The CHS hatchery program is a much smaller operation. Trout from this facility are planted locally in Lake Almanor (D. Tognotti, Aquaculture Teacher and Fish Hatchery Director, CHS, personal communication). Until 2010, the American Trout and Salmon Company in Susanville also planted trout in a few locations in the Basin, including Goodrich Creek and the Chester area.

Privately-owned hatcheries are not required to attain CDFW annual certifications. Development of effective disease protocols and testing for pathogens is at the discretion of the individual hatchery. Privately-owned hatcheries may voluntarily participate in CDFW fish health certifications. If *M. cerebralis* is detected, the hatchery must undergo a 90-day quarantine period. Further, hatcheries with infected fish are not permitted to plant those fish in California except in infected streams in the Eastern Sierra. Economic risks posed by participation in the CDFW fish health certification discourage private hatchery engagement (D. McFarland, Owner and Fish Hatchery Manger, American Trout and Salmon Company, personal communication).

Regular testing for *M. cerebralis* and other pathogens does not occur in the local privately-owned hatchery operations. The CHS Hatchery does not conduct regular pathogen testing, but the facility undergoes annual inspections by a CDFW representative (D. Tognotti). The FRC hatchery does not conduct testing but does alert CDFW when there are obvious signs of infection in brood stocks (A. Mouser). The American Trout and Salmon Company ceased fish planting in 2010 but had voluntary participated in CDFW fish health certifications (D. McFarland).

The lack of pathogen testing at privately-owned facilities represents a significant concern for *M. cerebralis* spread, particularly for asymptomatic infected hatchery populations. Review of limited information on pathogen testing revealed positive *M. cerebralis* detections have occurred at two privately-operated hatcheries. A Mount Lassen Trout Farm facility tested positive from 1985-1987. The American Trout and Salmon Company facility tested positive in 2010 during the CDFW fish health certification process (D. McFarland). In response to *M. cerebralis* presence, the American Trout and Salmon Company installed concrete raceways to reduce future risk of *M. cerebralis* establishment.

Continued positive detections in state-operated hatcheries suggests that salmonids in private operations may also be exposed to *M. cerebralis*. Asymptomatic fish can be unknowingly planted if regular pathogen testing does not occur at the hatchery. *M. cerebralis*-exposed fish from privately-owned hatcheries could be a significant source of myxospore dispersal in the Basin.

3.5.2 Recreation

Recreational use also is thought to have played a role in pathogen dispersal. Any recreational activity with the potential to move water or sediment could contribute to pathogen transport and pathogen spread, if receiving waters provide environmental conditions that maintain myxospore or TAM viability.

The Basin supports a variety of aquatic recreation activities that are popular locally and also attract visitors from outside the region. Of these, angling and boating pose the greatest risk of *M. cerebralis* dispersal.

3.5.2.1 Angling

Myxospore transport via activity and equipment has been the focus of concern related to recreational fishing. Surveys in Montana found widespread movement of *M. cerebralis* by anglers across aquatic systems over short periods of time. Anecdotes from resource managers in California (M. Adkison, Statewide Fish Health Coordinator, CDFW, personal communication) and other western states (T. Horton) describe patterns of pathogen detections consistent with *M. cerebralis* movement by anglers.

Anglers can transport myxospores by moving infected fish or fish parts. Angler gear has been linked to myxospore transport. Felt-soled wading boots retain viable myxospores longer than other commonly used sole materials (e.g., nylon, neoprene, rubber) (Gates et al., 2008). Myxospores are not removed from waders that are rinsed with water pressure equal to a residential garden hose.

Research on gear cleaning practices of anglers has raised concerns. For example, surveys in Montana found anglers transport an average of 22g of sediment on boots and waders. Forty percent of anglers reported that they occasionally, rarely, or never clean equipment between uses (Gates, 2007). Transport of sediment on gear can move myxospores and may also facilitate pathogen introductions by carrying infected *T. tubifex*.

The popularity of recreational fishing in the Upper Feather River Basin suggests this vector might play a large role in *M. cerebralis* spread. Further, the public is largely unaware of AIS threats in the Basin. This increases the likelihood that anglers will not take appropriate precautions to avoid *M. cerebralis* spread.

3.5.2.2 Boating

Boating also may play a role in *M. cerebralis* transport, though contribution to pathogen spread is more uncertain than the risk from angling. Boats may serve as *M. cerebralis* vectors via retention of standing water, such as engine cooling water, which can maintain relatively constant temperatures (Arsan & Bartholomew, 2008; Zielinski & Bartholomew, 2009). Engine cooling water may maintain myxospore or TAM viability for a longer period of time compared to bilge or ballast water (Johnson et al., 2001). However, we found no direct evaluation of *M. cerebralis* spore survival in conditions similar to engine cooling water. This is a possible vector for *M. cerebralis* introductions in all reservoirs except Round Valley Reservoir, where motors are not permitted.

3.5.3 Natural Dispersal

Natural dispersal of *M. cerebralis* may occur when myxospores are transported by predators of infected fish (Arsan & Bartholomew, 2009) or when infected fish swim within or among aquatic systems. Whirling Disease is likely to make infected salmonids more susceptible to predation, and dispersal of myxospores through predation may be a significant *M. cerebralis* vector. Avian and mammalian predators can move myxospores between systems that are not hydrologically connected, releasing viable myxospores into uninfected waters with suitable *T. tubifex* habitat. The following discussion focuses on myxospore survival after salmonid host predation as myxospore survival on fur and feathers is unknown.

3.5.3.1 Birds

Research regarding the contributions of wildlife to *M. cerebralis* spread focused on the transport of myxospores via predation by piscivorous birds. Movement of myxospores by birds has been implicated as a vector where *M. cerebralis*-positive water bodies were not known to receive hatchery reared fish, are not commonly frequented by or easily accessible to anglers, and/or are not hydrologically connected to wild salmonid populations. These sites include high mountain lakes in Colorado (D. Winkelman) and streams in Utah above fish barriers (W. Cavender, Director of the Fisheries Experiment Station, Utah Division of Wildlife Resources, personal communication).

Anecdotal evidence of avian contribution to *M. cerebralis* spread has prompted multiple investigations into myxospore viability following passage through the digestive tracts of various bird species. These studies have shown that myxospores are viable following consumption by three species: great blue herons, *Ardea herodias,* (Koel et al., 2010), black-crowned night herons, *Nycticorax nycticorax*, (Taylor & Lott, 1978), and mallards, *Anas platyrhynchos*, (El-Matbouli & Hoffmann, 1991). Myxospores are not viable following host consumption by American white pelicans, *Pelecanus erythrorhynchos*, or double-crested cormorants, *Phalacrocorax auratus* (Koel et al., 2010)

All three bird species known to pass viable myxospores in feces occur in the Basin, where they may contribute to *M. cerebralis* dispersal. Studies of food retention time (Stone et al., 1978) and flight speeds (Alerstam, 2003; Kerlinger, 2008) of piscivorous birds suggest that avian vectors could rapidly transport *M. cerebralis* to hydrologically unconnected subwatersheds. Bird dispersal of myxospores was thought to be the cause of *M. cerebralis* introductions at one local hatchery (D. McFarland).

3.5.3.2 Mammals

Evaluating the contribution of mammals to *M. cerebralis* introductions is difficult. To our knowledge, only one study has investigated myxospore viability following passage through mammals’ digestive tracts. In that study, myxospores were not viable following consumption by mice (*Musculus musculus*) (El-Matbouli et al., 2005). There is no additional information regarding the ability of piscivorous mammals to act as vectors.

If myxospores remain infective to *T. tubifex* hosts following ingestion by mammals, they would likely transport spores over shorter distances than birds. Studies of food retention time (Szymeczko & Skrede, 1990) and movement of piscivorous mammals (Reid et al., 1994; Harrington et al., 2014) present in the Basin suggest that myxospore transport distances would be relatively short, e.g., otter, *Lontra canadensis,* and mink, *Neovison vison*. American black bears, *Ursus americanus,* could move over greater distances in the watershed (Stratman et al., 2000) but are less likely to deposit myxospores in aquatic systems.

3.5.3.3 Fish

Salmonids in the Basin demonstrate some susceptibility to the pathogen and are likely contributors to myxospore movement. The movement of resident fish is restricted by anthropogenic activities that have fragmented aquatic habitats, including an extensive road network and several hydroelectric and recreational dams. Oroville Dam prevents anadromous fish from reaching the Basin. Infected fish may disperse myxospores over considerable distances in connected aquatic habitats inside the Basin. Increases in habitat connectivity (i.e., fish passage improvements) could increase risk of pathogen dispersal through fish movement.

Movement of myxospores also may occur through the predation of infected salmonids by other fish. One study has shown that myxospores are able to survive passage through the digestive system of Northern pike, *Esox lucius* (El-Matbouli & Hoffmann, 1991). Although this species is not native to California and does not currently occur in the Basin, it suggests that myxospores can survive fish predation. Predation of infected salmonids could increase the rate at which myxospores are returned to a system. Consumption by other fish is likely to result in a direct transfer of myxospores to the water.

3.6 Environmental Characteristics which Support *M. cerebralis* Establishment

Stream flow, substrate type (Krueger et al., 2006), and stream temperature influence pathogen establishment. A "permissive environment" has attributes that support *T. tubifex* communities and provide an opportunity for *M. cerebralis* life stages to locate and complete development in both hosts. Low stream velocity, silt and clay substrates, and stream temperatures within the optimal spore development range are important factors in *M. cerebralis* establishment. Organic enrichment also may contribute to elevated populations of *T. tubifex* and increase the risk of pathogen establishment.

3.6.1 Permissive Environment Characteristics

3.6.1.1 Velocity

Flow conditions influence *T. tubifex* habitat and survival and salmonid infection. Low velocities (Hallett & Bartholomew, 2008), low flow volume (Neudecker et al., 2012) and the absence of flushing flows (Bartholomew et al., 2007) have been associated with increased salmonid infection. Hallett and Bartholomew (2008) reported salmonid infection prevalence is two times greater during lower flow velocities (similar to lake or stagnant pool environments) compared to higher velocities (similar to summer flow in backwater pools and shallow stream margins) under laboratory conditions. TAM production did not differ between the two flow regimes, suggesting infection rates were related to the ability of infective stages to reach their hosts. The authors suggested that myxospores were able to settle in the sediment more quickly where they were consumed by *T. tubifex*. TAMs also may have been better able to attach to salmonids in the slow flow regime: more TAMs without their sporoplasms were found in the slow flow. Additionally, *T. tubifex* survival was four times greater in the slow flow regime. The slow velocity allowed for the accumulation of fine particulate materials and increased water temperatures associated with optimal *T. tubifex* habitat. This suggests more *T. tubifex* were available for the myxospore life stage when velocity was low.

3.6.1.2 Substrate

Substrate is important for pathogen establishment due to its role in supporting *T. tubifex*. Fine sediment (silt or clay) is the preferred habitat of *T. tubifex* (Sauter & Güde, 1996) which likely explains why more infected worms are found in silt and clay compared to other substrates (Krueger et al., 2006). Susceptible lineage III worms prefer finer particulates compared to resistant lineages (Winkelman et al., 2005). Further, fine sediment conditions may increase pathogen proliferation, as *T. tubifex* maintained in silt and mud release more TAMs than those housed in sand (Arndt et al., 2002; Blazer et al., 2003) or leaf litter (Blazer et al., 2003).

Increased sediment delivery to streams can result in increased fine material in channel substrates. Human activities that increase sediment delivery and enhance habitat for *M. cerebralis* include road systems and grazing. Road-related sediment delivery has been associated with increased TAM production (Mcginnis & Kerans, 2013). Grazing channels and riparian areas can increase sediment delivery to streams already characterized as permissive environments (i.e., meadows).

3.6.1.3 Water Temperature

Development of *M. cerebralis* life stages is temperature-dependent. The optimal temperature range for TAM development in *T. tubifex* is 10-15ºC (El-Matbouli et al., 1999) while 12-17ºC is optimal for myxospore development in salmonids (Halliday, 1976). Spore development within these optimal temperatures ranges translates to 52-121 days at 10-15ºC for myxospore development (Hedrick & El-Matbouli, 2002) and 88 days at 15ºC for TAM maturation and release from *T. tubifex* (Kerans et al., 2005).

Optimal stream temperatures for the *M. cerebralis* life cycle often result in cyclical shedding of spores when stream temperature increases during spring and summer (Thompson et al., 1999). Release of TAMs into warming waters during July and August is correlated with the seasonal period when susceptible salmonid life stages are readily available (Nehring et al., 2003). Optimal temperature for *T. tubifex* (10-15ºC) is also associated with the highest level of TAM production (Blazer et al., 2003; El-Matbouli et al., 1999). Given the influence of temperature on the *M. cerebralis* life cycle, it is not surprising that the most severe cases of Whirling Disease in rainbow trout have been observed when stream temperatures are between 10-15°C (Gilbert & Granath, 2003).

3.6.1.4 Organic Enrichment

Organic enrichment is not a requirement for pathogen establishment but eutrophication supports high densities of *T. tubifex* (Blazer et al., 2003) and high TAM production (Allen & Bergersen, 2002; Thompson & Nehring, 2000). Grazing can be a significant source of organic enrichment

(Eby et al., 2015). The potential for organic enrichment related to cattle activity in the Basin is high due to private rangeland and active US Forest Service (USFS) allotments in the Basin's meadows and valleys.

3.6.1.5 Examples of Permissive Environment

Low gradient channels characterized by low stream velocities and substrate dominated by fine particulates are strongly associated with *M. cerebralis* presence (Eby et al., 2015). Lakes, reservoirs, and ponds also have been identified as permissive environments for pathogen establishment. Beaver ponds have a particularly strong relationship with *M. cerebralis* establishment and proliferation due to the combination of low velocities, fine sediment deposition and organic enrichment (Schisler & Bergersen, 2002)

4.0 *M. cerebralis* Risk Assessment

Overview

The persistence of *M. cerebralis* in the Upper Feather River raises concerns about its distribution and effect on salmonid populations. A risk assessment was developed to evaluate these concerns. Models have been used successfully to evaluate the probability of *M. cerebralis* occurrences and Whirling Disease outbreaks in other aquatic systems. Our assessment combines the potential for *M. cerebralis* introduction and establishment to derive relative ratings for *M. cerebralis* presence. A diagram of the process is shown in Figure 3.

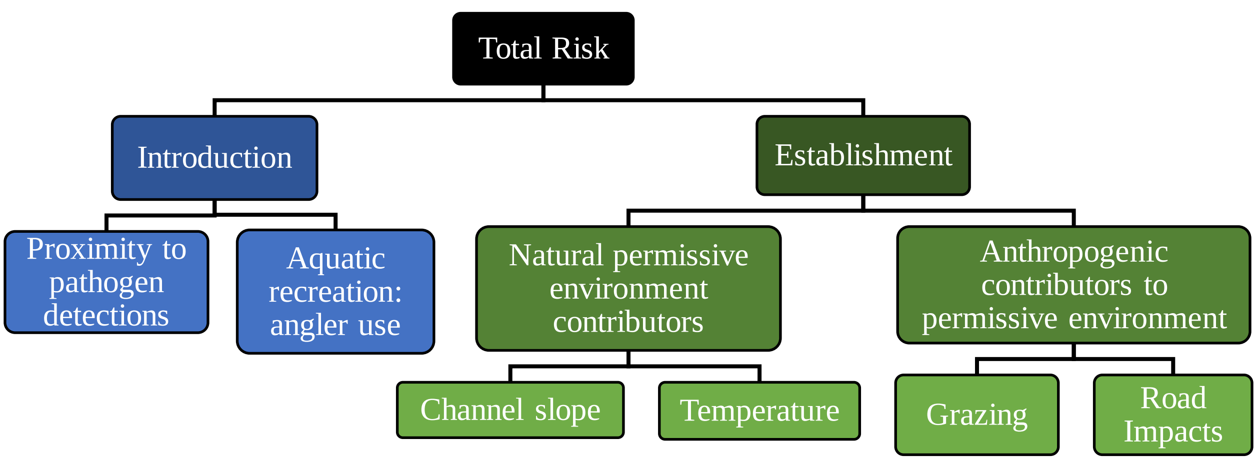

Figure 3. Diagram of the risk assessment including *M. cerebralis* introduction and establishment factors

The assessment was applied to each HUC 12 subwatershed in the Basin (Figure 4). HUC 12s are typically 10,000-20,000 acres and generally encompass the area contributing to third order streams. This scale is widely used in watershed assessments and analyses of watershed condition. All analyses were carried out in Microsoft Excel version 16.16.20 and ArcGIS version 10.1.

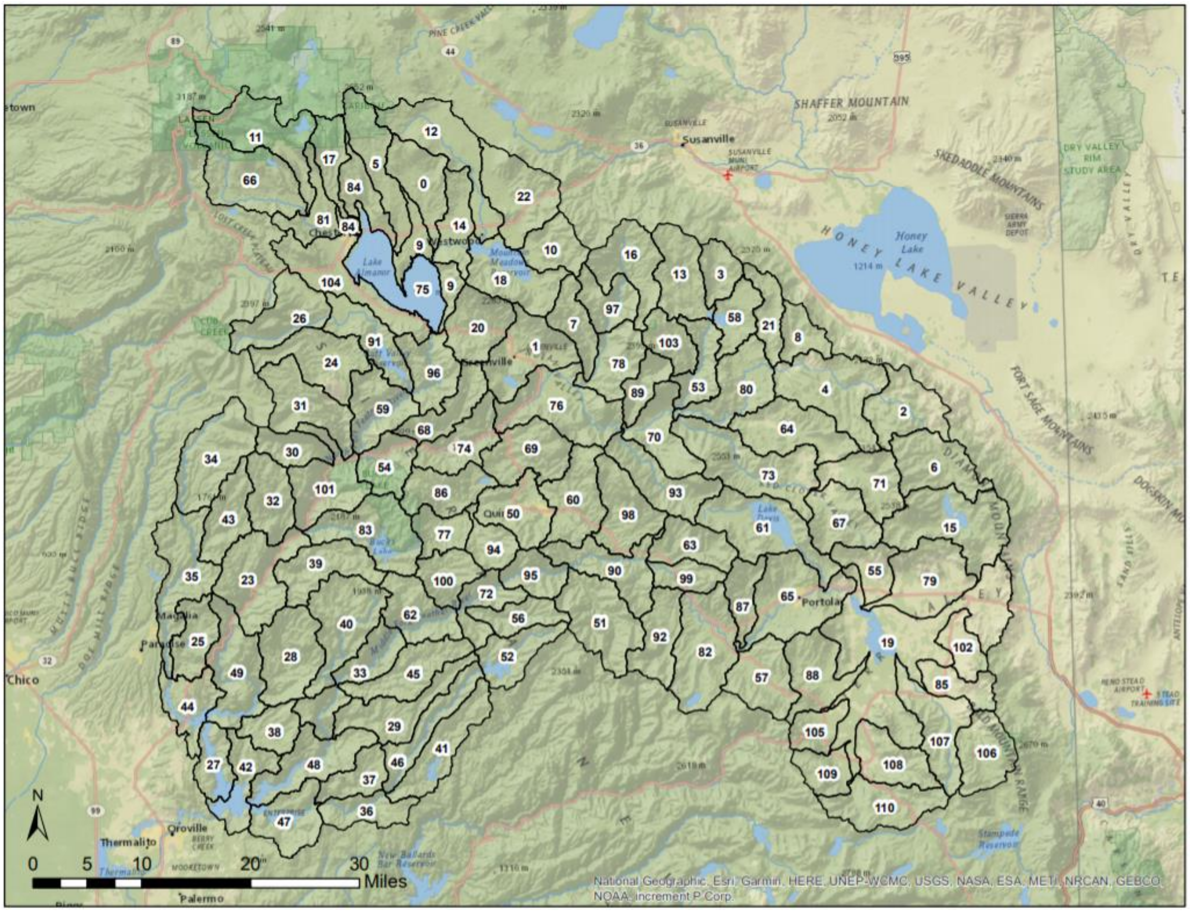

Figure 4. Upper Feather River subwatersheds (HUC 12). Key to subwatersheds is provided in Appendix A.

4.1 *M. cerebralis* Introduction

The risk of *M. cerebralis* introduction was based on two contributors: proximity to known *M. cerebralis* detections and aquatic recreational use.

4.1.1 Proximity

The rating for proximity to subwatersheds with positive *M. cerebralis* detections was based on *M. cerebralis* detections. Subwatersheds with positive *M. cerebralis* detections from at least two years were assigned the highest risk score (3). Multiple positive detections indicate areas that support the pathogen overtime, so these subwatersheds are rated as high risk for both introduction and overall risk, exclusive of the other model elements. Subwatersheds with detections in only 2019 were rated as moderate risk due to temporal variability.

Table 3. Factors and scoring for introduction and establishment

Introduction Parameter	Attribute	Metric	Score
Proximity to positive detections	Location of detections	Detection in subwatershed	Classed as high risk
		Neighboring (or positive sample in 2019)	2
		> 1 subwatershed removed	1
Aquatic recreation	Angler use	High use	3
		Moderate use	2
		Low use	1
Establishment Parameter	**Attributes**	**Sub-watershed Metric(s)**	**Score**
Natural permissive environment characteristics	Length (miles) of streams with slope ≤ 2% and August stream temperature 12-15ºC	> 6	10
		2 < 6	8
		1 < 2	6
		> 0 < 1	4
		0	2
Anthropogenic contributors to permissive environment	Grazing on low gradient channels (miles)	> 10	5
		> 3-10	4
		> 0-3	3
		0	2
		No grazing in subwatershed	1
	Road impact	> 0.94	5
		0.28 > score < 0.94	4
		-0.20 > score < 0.28	3
		-0.65 > score < -0.20	2
		score > - 0.65	1

Subwatersheds with pathogen presence also present a risk of transferring *M. cerebralis* to other areas. Subwatersheds adjacent to those with positive *M. cerebralis* detections were considered to be at moderate risk of introduction (from the existing adjacent detection) and were assigned a risk score of 2. The evaluation assumes there is fish habitat connectivity between watersheds, though it is likely some may be blocked by road crossings or other barriers. Proximity also accounts for potential transport by birds or mammals. Subwatersheds not containing *M. cerebralis* or adjacent to *M. cerebralis* detections were assigned a score of 1 (Table 3). Data are provided in Appendix B and results shown in Figure 5.

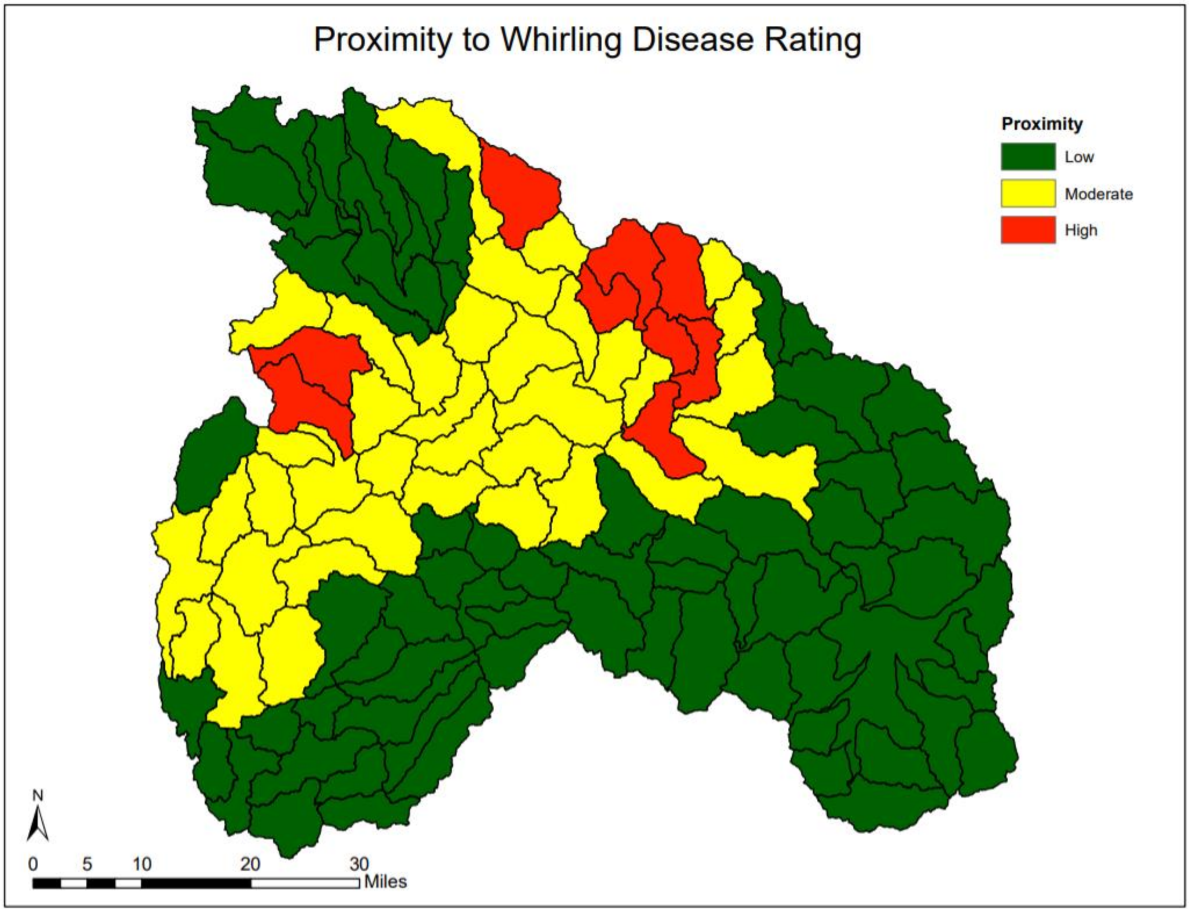

Figure 5. Subwatershed Whirling Disease proximity ratings

4.1.2 Angler Use

To quantify the risk of *M. cerebralis* introduction associated with aquatic recreation, the level of angling use in each subwatershed was rated as high (3), moderate (2) or low (1) (Table 3). These ratings were based on proximity of developed recreation sites to lakes, streams, and rivers and estimates of angling use based on expert knowledge provided by members of the WDTG (Michael Kossow and Ken Roby, FRTU). Although the Upper Feather River Basin hosts a variety of aquatic recreational opportunities, angler use was chosen due to its previous association with *M. cerebralis* introductions. Proximity to developed recreation sites also correlates strongly with boating, the other primary aquatic recreational activity of concern. This approach was taken because data on angler movements and angler use was available for only a few subwatersheds. Data are provided in Appendix B and results shown in Figure 6. Results reflect the highest use in and around the Basin's reservoirs and along the portion of the NF Feather with highway access. Areas easily accessible to highway traffic also result in high use ratings along portions of the Middle Fork.

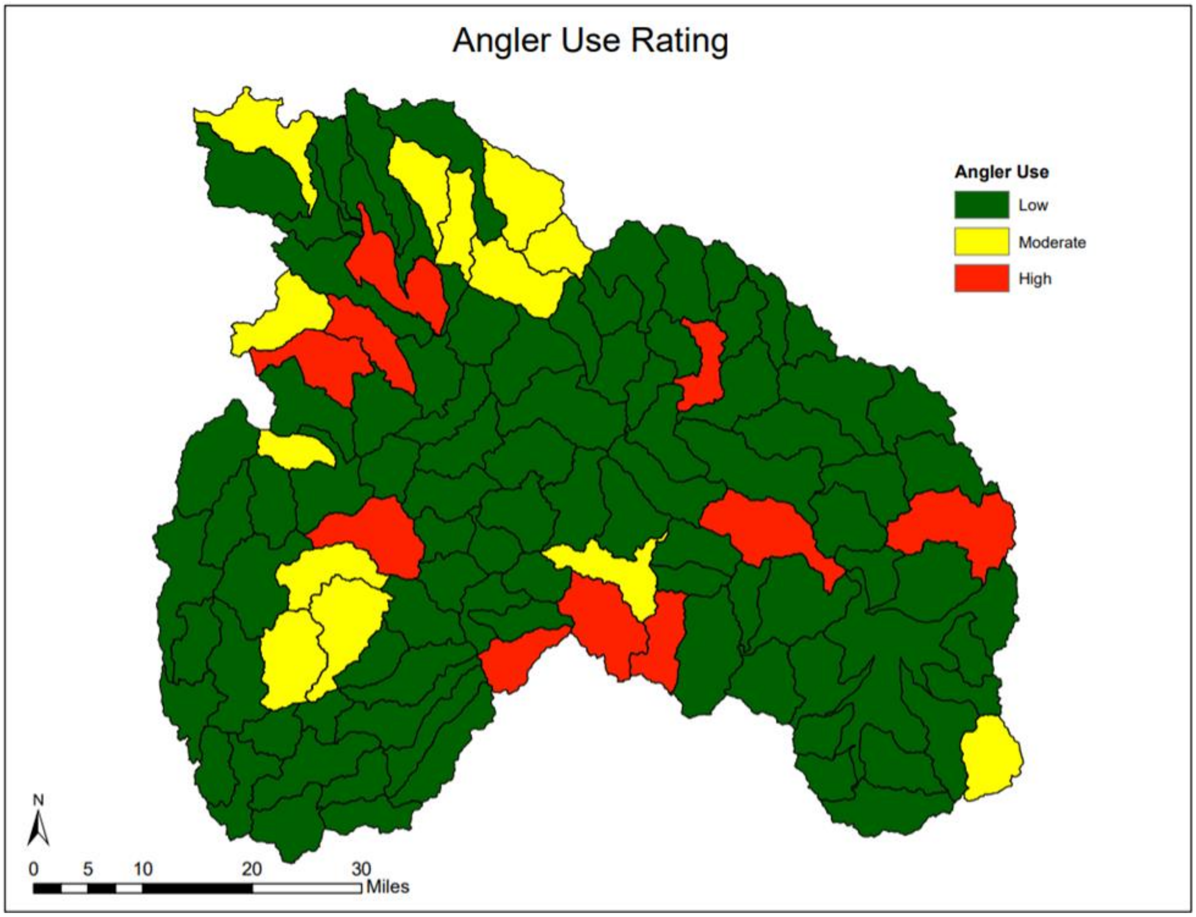

Figure 6. Angler use ratings

4.2 *M. cerebralis* Establishment

Habitats with low velocities, permissive temperatures, and channel substrate with high levels of fine sediment are key attributes of *M. cerebralis* establishment potential. Two characteristics were selected to represent these factors: stream slope and stream temperature regime. Indicators of road impacts and a measure of potential grazing impacts were also included in the model, due to linkages with accelerated sediment delivery and organic enrichment. All four factors have previously been shown to exhibit influence on *M. cerebralis* establishment capabilities.

An additional influence on *M. cerebralis* habitat is wildfire, due to increased sediment delivery following some high-intensity fires. Recovery from wildfire in terms of sediment production is variable, and we found it difficult to account for this in the model and did not include it as an element. However, managers should appreciate the risk of sediment delivery to potential *M. cerebralis* habitat and consider this risk in managing following wildfires.

4.2.1 Natural Contributors to Permissive Environment

Ratings of natural permissive environment components (i.e., temperature and channel slope), were calculated using the NorWeST Stream Temperature Regional Database and Modeling

Procedure (Issak et al. 2017; http://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html). Originally developed to assess the suitability of Bull Trout habitat, the NorWeST modeling system estimates stream temperatures during the month of August. August represents a critical time period for Bull Trout. The model uses ten predictor variables known to significantly influence average August stream temperatures, including elevation, canopy cover, drainage area, and stream slope. The stream temperature data that inform this model came from thousands of stream sites across the Western United States, supplied by multiple partners, providing a robust dataset. It is important to note that anthropogenic influences of stream temperature such as logging and grazing are not included as predictors in the NorWeST model.

The average August stream temperatures metric from the NorWeST Model was used as a proxy for the temperature regime that supports the completion of the *M. cerebralis* life cycle. As discussed earlier (Permissive Environment Characteristics, section 3.6.1), use of this metric allowed identification of streams with both appropriate average temperature and channel slope (≤ 2%) in the NorWeST database. Further, the month of August represents a period of high salmonid infection risk due to TAM release from *T. tubifex*.

Combining temperature and slope provided a rough screen for where *M. cerebralis* permissive environments might exist. Only streams with both projected average August stream temperature between 12 and 15ºC and a slope of ≤ 2% were used in the establishment risk rating. This temperature range was chosen because it represents the overlap between the optimal stream temperatures required for myxospore and TAM development during the spring and summer months. A ≤ 2% channel slope was used as an indicator for streams that might provide suitable habitat for *M. cerebralis*, namely those with channel substrates with high percentages of fine particles.

The length of stream segments containing both the stream temperature and slope conditions was calculated for each subwatershed. Subwatersheds with any streams with the appropriate temperature and slope characteristics were considered to have increased risk. Subwatersheds with no stream segments with target temperature and slope conditions were scored as 1 on a 5-point scale (Table 3). The remaining four categories were assigned by first dividing the distribution of values into quartiles and then rounding these breaks to whole miles (Table 3). Data are provided in Appendix B and results shown in Figure 7. The influence of stream temperature is the most obvious result. Higher elevation subwatersheds with high ratings will maintain water temperatures suitable to both rainbow trout and *M. cerebralis*. These subwatersheds nearly form a rim around the crest of the Basin.

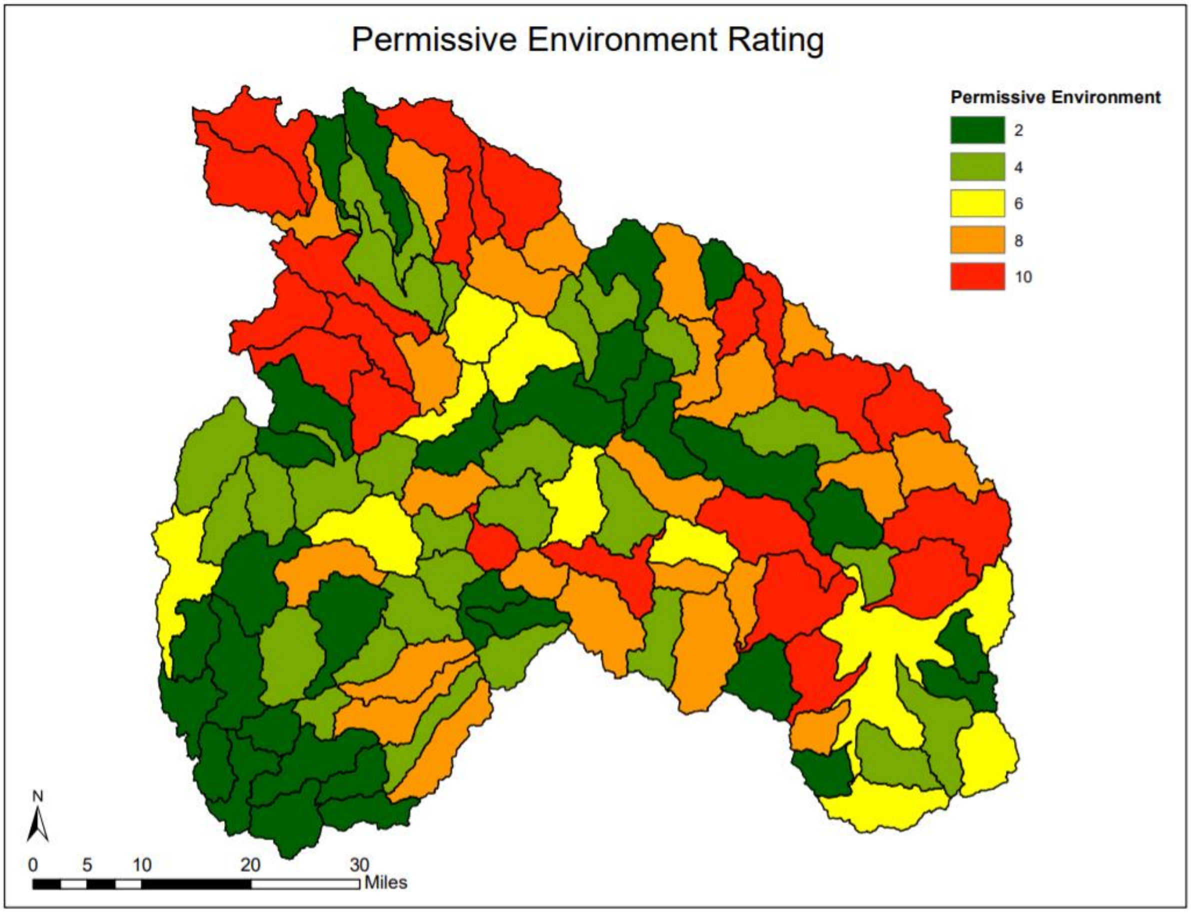

Figure 7. Permissive environment rating (based on suitable stream temperature and channel slope)

M. cerebralis detections in streams with target temperature and channel slope conditions suggest the metric is a reasonable predictor of *M. cerebralis* establishment. It is important to note that *M. cerebralis* is known to persist outside of the target temperature and slope conditions, though this is uncommon. Further, our approach gives no or low weight to microhabitats (short stream segments and low gradient habitat in steeper streams). Therefore, we assume the combined stream temperature and slope metric to be an underestimation of available *M. cerebralis* habitat in each subwatershed. However, the metric does contribute to providing a relative rating of establishment potential between subwatersheds.

4.2.2 Anthropogenic Facilitators of Permissive Environment

4.2.2.1 Roads

The road risk rating was derived from a road impact metric developed during an assessment of watershed condition in the Basin (Rogers et al., 2018). The road impact metric combines two road characteristics for each subwatershed. The first was the number of road crossings. The second was "near stream road density," calculated as the percentage of roaded acres within the area derived by calculating 30m corridors on each side of all stream channels in the subwatershed. The two metrics were combined using the Environmental Evaluation Modeling

System (EEMS). EEMS is a tree-based fuzzy-logic model. Fuzzy-logic models are mathematical means of representing vagueness and impreciseness in data. The EEMS, specifically, is designed to allow for the combination of data from different sources and different domains (Conservation Biology Institute, 2013). Within EEMS, stream-road crossings and near-stream road density data were converted into a combined value system in which subwatershed road impacts were delineated on a numeric scale of -1 to +1, with -1 indicating high road impact and +1 indicating low road impact. For ease of understanding, these values were multiplied by -1 so that high values represent high impact. A 5-point rating scale was applied according to natural breaks in the distribution of the road impact EEMS ratings across subwatersheds, using the natural break function in ArcGIS (Table 3). Data are provided in Appendix B and results shown in Figure 8. Results display that the highest road impacts generally following the mid-elevation, coniferous forest-dominated areas of the Basin, reflecting the historic roading of these areas to provide access for timber harvest.

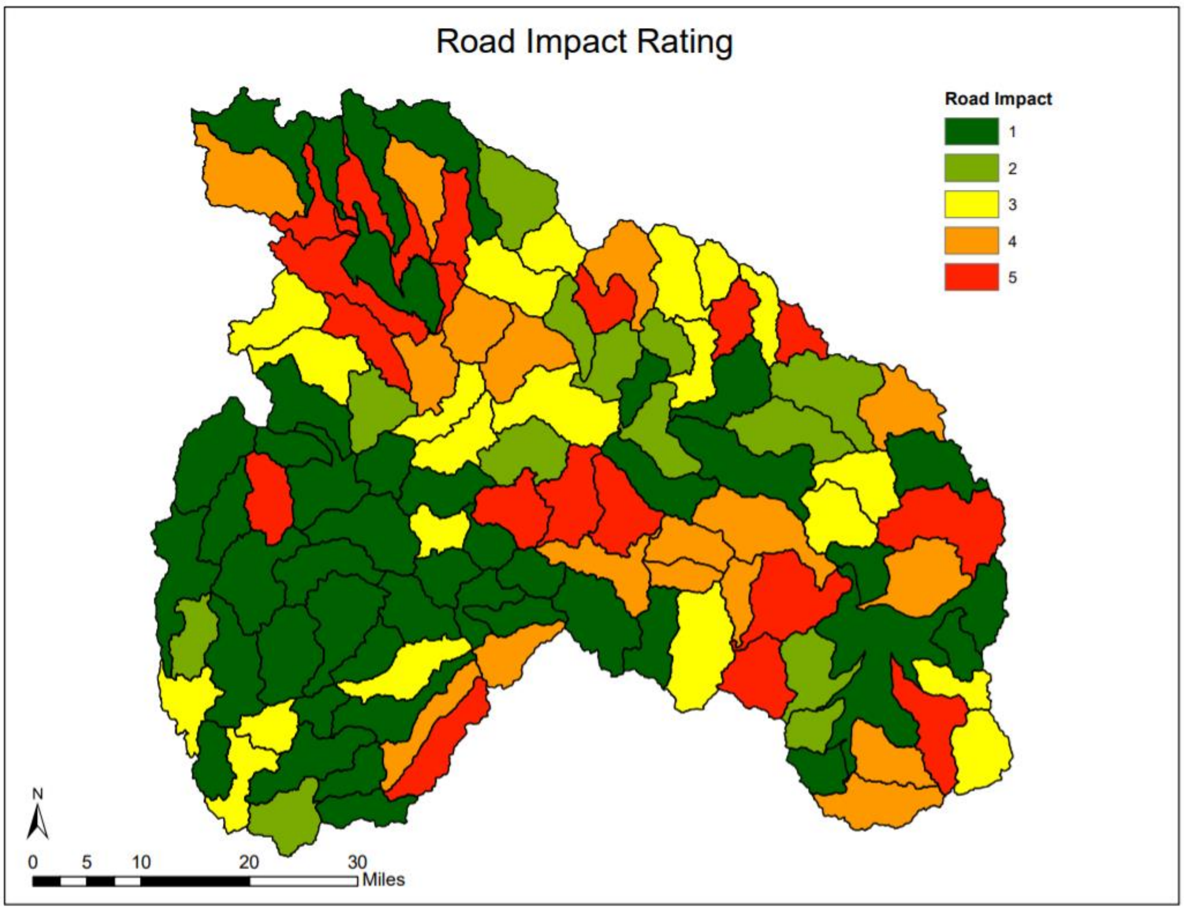

Figure 8. Road impact rating

4.2.2.2 Grazing

The assumption of increased risk from grazing is due to the strong association between the sediment delivery and organic enrichment related to livestock activity and high densities of infected *T. tubifex*. The contribution of rangeland to *M. cerebralis* establishment potential was rated based on the presence, or absence, of grazing within a subwatershed and the amount of low-gradient stream channels within those subwatersheds where grazing was evident. The USFS grazing allotment spatial database was used to identify subwatersheds on public lands that were grazed. Only allotments identified as active were considered to be grazed. Google Earth was used to determine if private lands were grazed, based on characteristics such as fencing and trailing. Personal knowledge of the area relative to grazing was also employed. Scoring for this element (Table 3) ranged from 1 (no grazing) to 5 (grazing with more than 8 miles of low gradient channel present). A score of 2 was assigned to subwatersheds where active allotments were present but no low gradient channels were identified. Estimates of the amount of low gradient channel came from the NorWeST database. Note these were low-gradient channel lengths, not the combined channel length-stream temperature value described earlier. Low-gradient lengths were adjusted in subwatersheds with low-gradient river reaches (e.g. NF Feather River). Rocky channel banks in these reaches are not sensitive to grazing impacts and were not counted. Data are provided in Appendix B and results shown in Figure 9. Results reflect current and historic grazing use of the east side of the Basin where large meadow systems dominate.

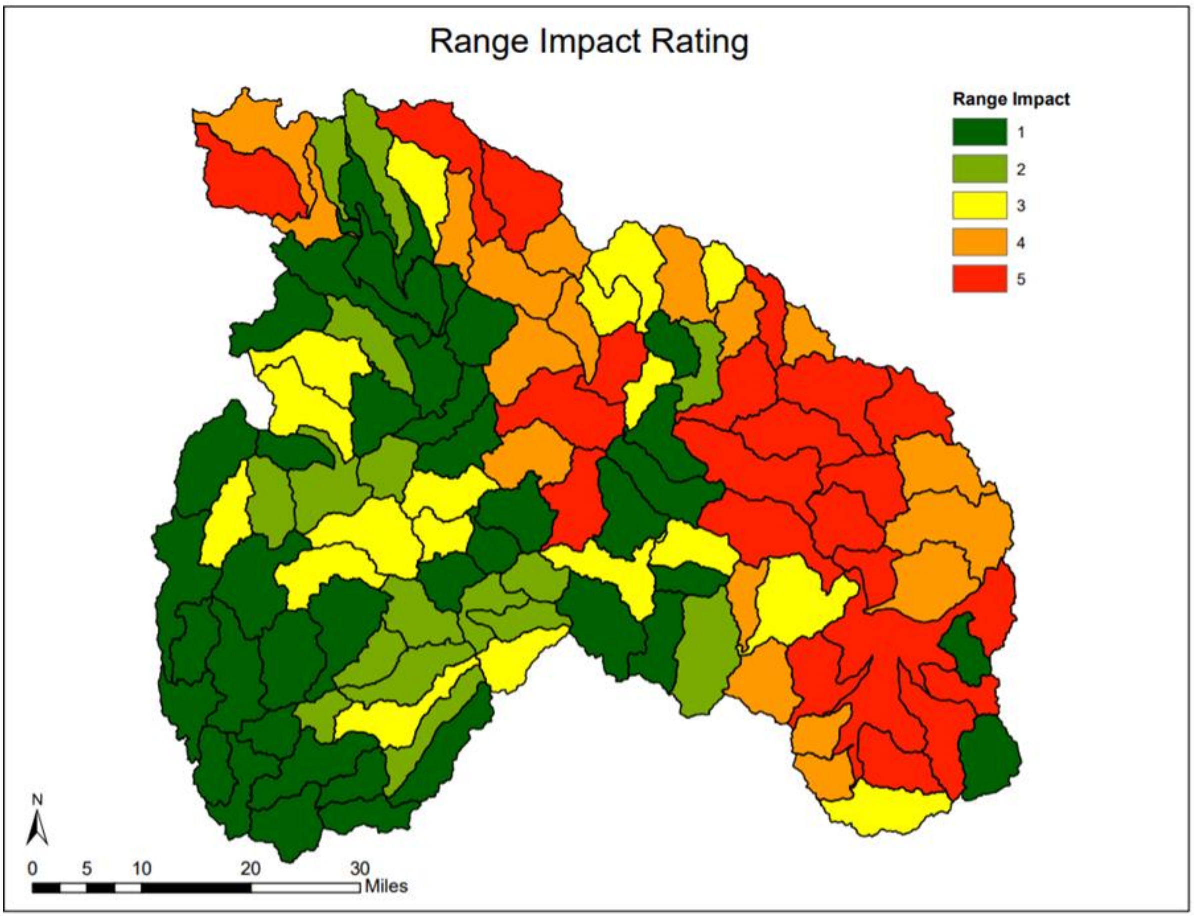

Figure 9. Range impact rating

4.3 Rating Scheme

4.3.1 Introduction

Subwatersheds with established *M. cerebralis* presence (n=12) were rated high for both introduction and overall risk. Remaining subwatersheds were placed in low, moderate or high risk categories. Subwatersheds with scores of 1 for both angling and proximity (total score of 2) were rated as low risk. Subwatersheds with cumulative scores of either 3 or 4 were rated as moderate risk. Watersheds with cumulative scores of 5 were classed as high risk. This was the result in subwatersheds with high angler use adjacent to waters with known detections. Introduction data are provided in Appendix B and results are shown in Figure 10.

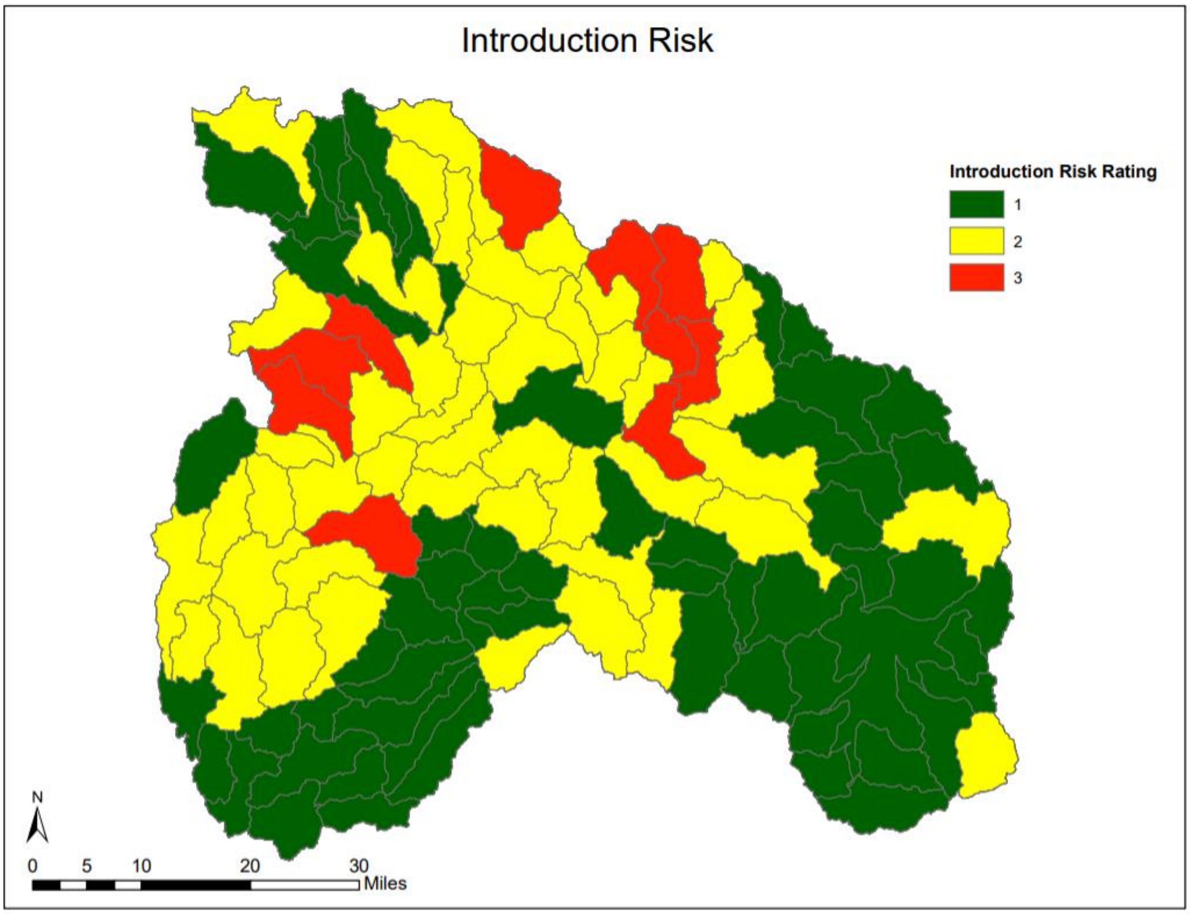

Figure 10. Introduction ratings

4.3.2 Establishment

The contribution of the temperature and slope metric was weighted by a factor of two (resulting in scores of 2, 4, 6, 8, or 10, Table 3) in calculating the establishment rating. This was done because a) both factors were considered among the strongest predictors of *M. cerebralis* permissive environment, and b) subwatersheds with high slope and temperature scores correlated well with the location of *M. cerebralis* detections. Scores for the three elements were added to produce cumulative scores ranging from 4 to 20. Subwatersheds were classified into risk categories based on the following rule set. Subwatersheds with scores of 4-8 are classified as low risk, those with scores of 9-13 as moderate risk, and those with scores of 14-20 as high risk. Establishment date are provided in Appendix B and results are shown in Figure 11.

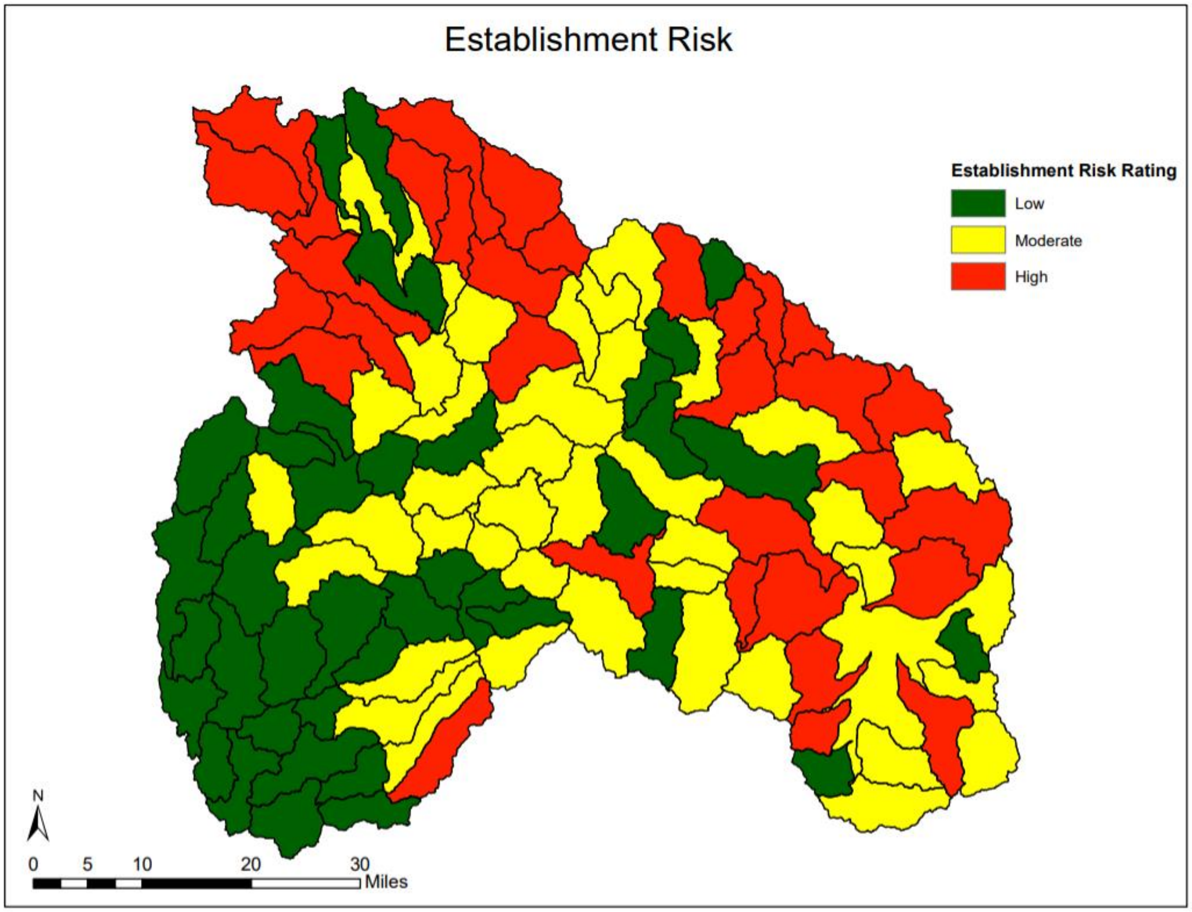

Figure 11. Establishment ratings

4.3.3 Whirling Disease Risk

Results for introduction and establishment were combined to derive an overall rating of Whirling Disease risk for each subwatershed. The simple rule set established to classify subwatersheds as low, moderate, or high risk is shown in Table 4. Establishment scores influenced the overall ratings more than the introduction scores. The reasoning was that *M. cerebralis* would be unable to complete its life cycle (and cause disease) without appropriate habitat. Subwatersheds with Whirling Disease persistence received ratings of high for both introduction and overall watershed risk.

Table 4. Whirling Disease risk rating scheme

Establishment Rating	Introduction Rating	Watershed Risk Rating
High	High	High
High	Moderate	High
High	Low	Moderate
Moderate	High	High
Moderate	Moderate	Moderate
Moderate	Low	Moderate
Low	High	Moderate
Low	Moderate	Low
Low	Low	Low

4.4 Results

4.4.1 Introduction

Subwatersheds with high ratings are clustered around sites with documented infections. Two subwatersheds with high ratings (Upper Yellow Creek and Indian Creek) are sites of both infection and high angling use. It is noteworthy that no subwatersheds in the West Branch, Middle Fork or South Fork are rated as high (Figure 10). This is due to *M. cerebralis* surveys being limited to the North Fork.

4.4.2 Establishment

The majority of subwatersheds contain some habitat that can support the *M. cerebralis* life cycle. Most subwatersheds are in the moderately low or moderate risk classes (Figure 11). All major drainages within the watershed contain subwatersheds at high risk for *M. cerebralis* establishment. Few subwatersheds are in the low risk category. This suggests *M. cerebralis* has widespread establishment potential and may be present in areas that have not been surveyed.

Permissive temperature and slope parameters were good predictors of *M. cerebralis* establishment. Several subwatersheds where previous surveys detected the pathogen, such as Cold Stream-Indian Creek, Lone Rock Creek-Indian Creek, Upper Yellow Creek, and Goodrich Creek-Frontal Mountain Meadows Reservoir have correspondingly high establishment potential according to this metric (Figure 7). This indicates that *M. cerebralis* may have the potential to establish at other locations in these subwatersheds. In contrast, the results indicate subwatersheds along the lower North Fork of the Feather River have little habitat with temperature and slope conditions to support *M. cerebralis*. Some sites in this area tested positive in the third year of a multi-year survey. The lack of preferred habitat for *M. cerebralis* in these subwatersheds may provide an explanation for the variability in *M. cerebralis* detections between survey years.

The road and grazing components show high risk across the Basin. Very few areas are rated low in both these categories. Large sections of subwatersheds in the eastern portions of the Basin include active grazing allotments. This suggests that many areas could be experiencing localized sediment delivery and organic enrichment facilitating *T. tubifex* habitat. All subwatersheds with known positive *M. cerebralis* detections experience some degree of effect associated with either active grazing allotments or the road system.

4.4.3 Whirling Disease Risk

Subwatersheds at high risk for *M. cerebralis* are located in both the North and Middle Forks of the Feather River (Figure 12). Clusters of subwatersheds with high risk ratings, but without *M. cerebralis* detections surround subwatersheds with positive detections, high angler traffic, and high establishment potential. Establishment potential in *M. cerebralis* positive subwatersheds is a risk to the spread of *M. cerebralis* in that system. Goodrich Creek-Frontal Mountain Meadows Reservoir, Upper Yellow Creek, Cold Stream-Indian Creek, and Lone Rock Creek-Indian Creek all neighbor multiple high risk subwatersheds where *M. cerebralis* has not yet been detected. Very few subwatersheds are considered low risk. Clusters of low risk subwatersheds are located in the South Fork, Middle Fork and around Oroville Reservoir.

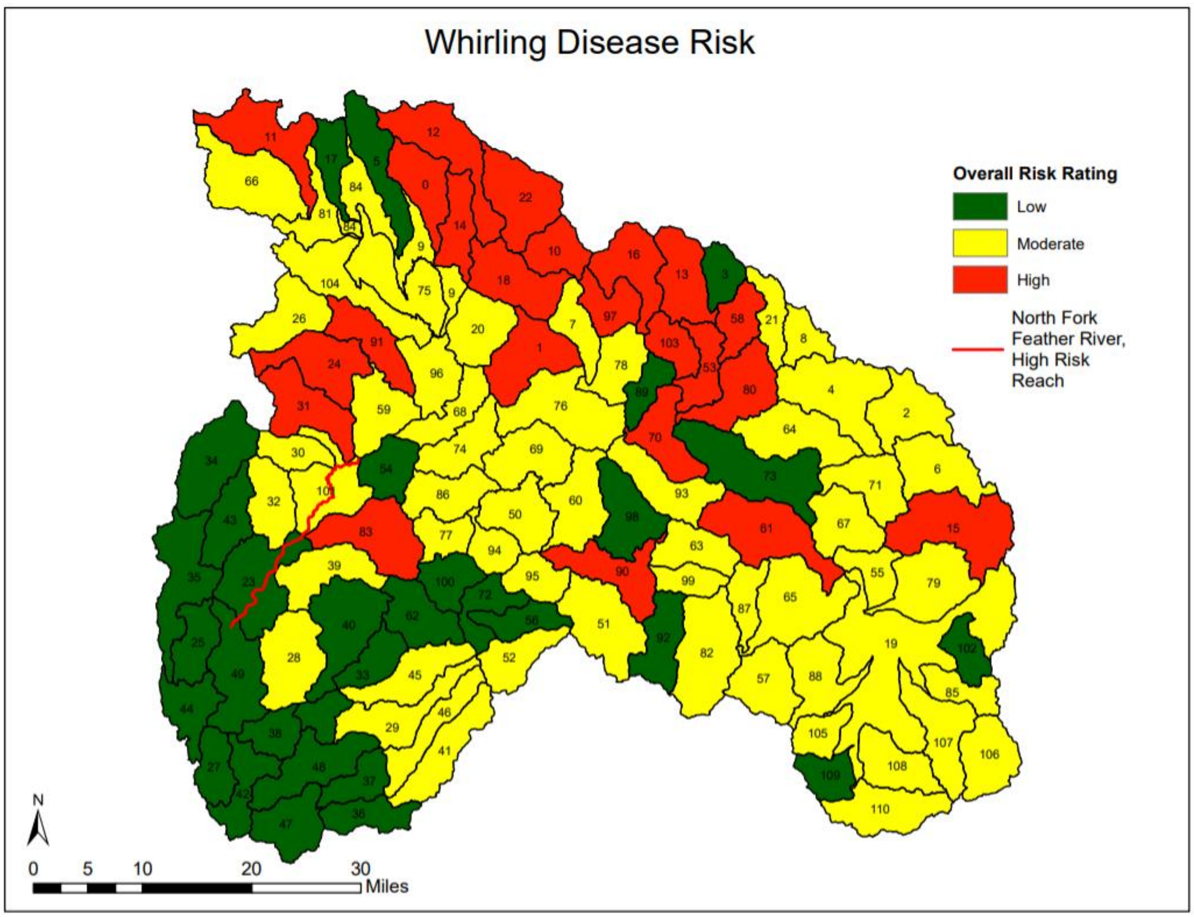

Figure 12. Whirling Disease risk

Subwatersheds without *M. cerebralis* detections with high establishment potential represent sources of *M. cerebralis* that can be transported to hydrologically connected and adjacent unconnected subwatersheds. *M. cerebralis* is most likely to be present in subwatersheds in close proximity to subwatersheds with both positive detections and high establishment potential. This is reflected in high risk ratings for subwatershed clusters in the headwaters of the North Fork Feather River (Hamilton Branch, Warner Creek) and in multiple Middle Fork subwatersheds (Table 5).

Table 5. Subwatersheds without *M. cerebralis* detections with high Whirling Disease risk

Drainage	Subwatersheds at High Risk
North Fork Feather River	Antelope Creek
North Fork Feather River	Dry Creek-Hamilton Branch
North Fork Feather River	Robbers Creek
North Fork Feather River	Rock Creek-Hamilton Branch
North Fork Feather River	Mtn. Meadows Creek-Frontal Mtn. Meadows Reservoir
North Fork Feather River	Mountain Meadows Reservoir
North Fork Feather River	Warner Creek
North Fork Feather River	Butt Valley Reservoir-Butt Creek
North Fork Feather River	Lower Wolf Creek
North Fork Feather River	Soldier Creek-Butt Creek
North Fork Feather River	Poison-Last Chance Creek
North Fork Feather River	Bucks Creek
Middle Fork Feather River	Poplar Creek
Middle Fork Feather River	Big Grizzly Creek
Middle Fork Feather River	Frenchman Lake-Little Last Chance Creek

Wildfire was not included in the assessment model. It is worth noting that most (33) of the subwatersheds rated as having moderate risk ratings would be elevated to high risk if the permissive environment rating was increased from moderate to high. It's likely that a high-intensity wildfire that burned large portions of a subwatershed would result in an increase in sediment delivery sufficient to create a more attractive environment for *T. tubifex*. Managers should consider such risks in post fire management in subwatersheds rated as moderate.

The use of subwatersheds as the scale of analysis to evaluate the risk of Whirling Disease in the Basin proved to be efficient and effective, with one exception. In most cases, the attributes used to describe the risk of introduction and establishment at the subwatershed scale are linked, geographically and hydrologically, with stream habitat within the subwatershed. This is not the case with the main stem of the NF Feather River where several detections of *M. cerebralis* are located. This section of the river lies downstream of several subwatersheds (e.g., Rush Creek, Mill Creek, Chipps Creek, Milk Ranch Creek) but does not lie within them. In fact, the upstream area contributing flow and otherwise influencing this stretch of the North Fork is huge, on the order of hundreds of thousands of acres. We have reconciled this problem by classing the subject reach in the North Fork as high risk (Figure 12). This recognizes the presence of Whirling Disease in the reach but does not attribute its presence to any subwatersheds.

5.0 Priority Management Actions

Introduction

The WDTG identified six management elements to respond to presence of *M. cerebralis* in the Upper Feather River Basin. These are:

1. Basin -level risk assessment
2. Whirling Disease outreach and education
3. Best practices for aquatic surveys and equipment decontamination protocols
4. Monitoring, with an emphasis on defining distribution of *M. cerebralis*
5. Resistant salmonid strains
6. Project-scale risk assessment for restoration projects in meadows with streams.

Each management issue is discussed below, except the project-scale assessment, which is presented as a stand-alone document in Appendix C.

5.1 Whirling Disease Outreach and Education

The WDTG felt strongly that improved education of anglers and the general public was needed to increase support for management actions and better implement practices aimed at reducing the spread of the pathogen by anglers and boaters. The Outreach and Education plan developed for the Upper Feather River Basin is based on lessons learned from those addressing Whirling Disease in other areas, and from outreach and education work on other AIS. Key components of this work include promotion of Whirling Disease literacy and awareness, and the encouragement of adherence to stewardship guidelines to prevent *M. cerebralis* spread.

5.1.1 Whirling Disease Communication Plan

Whirling Disease outreach and education in the Upper Feather River Basin combines the recommended outreach and awareness tools and literacy approaches of the WDTG. The specific components of the communication plan are described below with the identification of key facilitators who could improve delivery of the components.

Locations recommended for various outreach materials were based on the Risk Assessment. The logic is that information is most needed at locations with the highest potential to spread the pathogen. These are subwatersheds where *M. cerebralis* is present and those with high risk for introductions and establishment. This risk is in part due to the presence of developed recreation facilities that attract anglers. Whirling Disease posters, signage and other outreach tools can be used to supplement each other. For instance, posters require preexisting platforms to be displayed. USFS campgrounds have kiosks to meet this need. New permanent signage outside developed sites will require coordination for placement, construction and long-term maintenance.

5.1.1.1 Plan Elements

1. Whirling Disease signage for placement at key locations such as boat ramps and campgrounds and planning long-term maintenance of signage. Coordination with the USFS, CDFW, DWR, and other agencies and organizations is needed for sign placement.
   a. Whirling Disease and general AIS signage are proposed at locations where angling and boating, specifically motorboats, are common. These are the recreation areas around Antelope Lake, Lake Almanor, Butt Lake, Bucks Lake, Lake Davis, Frenchman Lake, and Round Valley Reservoir; as well as rest areas on the NF Feather River (Gansner Bar, North Fork, Queen Lily, Shady Rest) and campgrounds in Tásmam Kojóm (Humbug Valley).

2. Coordinate with CDFW to include Whirling Disease information on preexisting AIS posters for placement at key locations. Key locations are those outlined for signage as well as additional developed campgrounds, according to the subwatershed risk assessment ratings. Coordination with the USFS for placement of posters in informational kiosks at campgrounds is needed.
   a. Priority recreation areas for posting beyond those recommended for permanent signage are the Greenville, Soldier Meadows, and Warner Creek campgrounds.
   b. Additional coordination with PNF and LNF to provide posters at PNF Headquarters and Ranger District offices.

3. Develop Whirling Disease brochures and informational rack cards to provide at bait shops, angling retailers, and agency personnel for direct distribution to recreational water users. Coordination with the USFS and tourism centers for placement of outreach materials is needed.
   a. The distribution of brochures and rack cards should occur in the locations identified for permanent signage as well as tourism information centers such as the Indian Valley Chamber of Commerce, Eastern Plumas Chamber of Commerce, and Portola Visitors Center & Williams House Museum.
   b. Coordination with PNF and LNF to provide brochures and rack cards at PNF Headquarters and ranger district offices would also facilitate the distribution of Whirling Disease information.

4. *M. cerebrali*s detection map to increase awareness among targeted outreach groups and agency personnel.
   a. Currently coordinating with CDFW to include this information on the fishing regulations map or a Whirling Disease informational page to target angler audiences and to provide a link to the fishing regulations map during sport fishing license purchase.

5. Coordinate with WDTG members to include Whirling Disease information on agency/organization websites and develop and utilize other media to support the on-the-ground outreach tools.
   a. Coordinate with CDFW for the inclusion of Whirling Disease information on the existing AIS webpage.

b. Coordinate with WDTG agencies and organizations to include informational webpages or links to preexisting information on the CDFW AIS informational website.
c. Coordinate with local radio stations, podcasts, and newspapers to highlight Whirling Disease information during key time periods such as the beginning of freshwater sport fishing season. Radio and newspaper announcements regarding Whirling Disease would be effective if conducted in population centers near high-risk zones in the watershed. This includes Chester, Portola, and Greenville. Expanding the scope of participation in radio announcements and podcasts to local programs in larger population centers outside of the watershed is recommended to increase awareness for target tourist audiences. The Barbless Fly-fishing Podcast based in Chico is an example.

6. Partner with existing aquatic educational classroom programs to develop Whirling Disease programs/activities to target audience groups including FRTU's Trout in the Classroom Program and the aquaculture program at CHS.
   a. Classroom education programs are recommended throughout the watershed. Chester, Westwood, and Greenville are all located in and around clusters of high risk subwatersheds in the North Fork of the Feather River. In the Middle Fork, Portola is also located within and nearby the high risk subwatersheds in that drainage.
   b. Whirling Disease information could be included in Plumas Unified School District's (PUSD) "Year of the Fish" (4^{th} grade) curriculum.
   c. Quincy and Loyalton are both within lower risk subwatershed clusters, however, education at all school centers would better serve the wider purpose of Whirling Disease Literacy and compliance with stewardship guidelines.

7. Inventory additional existing AIS outreach efforts for future planning and partnership to facilitate the development of a more comprehensive outreach campaign for the watershed.
   a. Identify other existing education and outreach programs to address gaps and overlaps and determine new program needs.

5.1.2 Basis of the Approach

Priority outreach tools and considerations for Whirling Disease identified by the WDTG have been used in successful outreach campaigns. Specifically, these efforts found simple messaging, identifying and targeting key audiences, and developing an effective media approach as key contributors to a successful outreach plan. These and other key lessons learned, which informed the Feather River Plan, are summarized briefly below.

5.1.2.1 Whirling Disease Literacy Information

Previous outreach campaigns have identified five main factors as integral components to increase literacy (Alberta Environment and Parks, 2018):

1. The history of Whirling Disease in North America and its effect on salmonid populations
2. The *M. cerebralis* life cycle
3. The symptoms of the disease in wild salmonids
4. The locations of pathogen detections

5. The locations of areas vulnerable to *M. cerebralis* establishment

5.1.2.2 Public Stewardship Guidelines

Public stewardship guidelines provide the public with the tools to reduce their own contribution to the spread of *M. cerebralis* (as well as other AIS).

Primary stewardship guidelines are:

1. Equipment
    a. Minimizing contact of personal items with water, including vehicles, boots, etc.
    b. Following the rules of "clean, drain, dry":
        i. Cleaning equipment that has contacted water
        ii. Draining any water collected in equipment from the recreational waterbody
        iii. Drying equipment completely (preferably in sunlight) prior to use in a new waterbody
    c. Avoiding the use of personal equipment that is difficult to clean appropriately according to the principles of clean, drain, dry (i.e., felt-soled waders)
2. Movement of infected hosts
    a. Avoiding the movement of fish or fish parts between bodies of water
    b. Properly disposing of fish parts in waste receptacles to avoid myxospore introductions through plumbing systems
    c. Avoiding the use of *T. tubifex* as bait

5.1.2.3 Simple Messaging

Simple messaging provides easy-to-remember headlines that promote the prevention of AIS spread. Examples relevant to the development of Whirling Disease outreach materials in the Upper Feather River Basin are "Clean, Drain, Dry" for equipment decontamination and "Keep Wheels Out of Water." Additionally, promoting public engagement through emphasis of ownership of a shared resource like the phrase "Protect Our Waters," can be particularly effective (K. Staigmiller, Fish Health Coordinator, Montana Fish, Parks, and Wildlife, personal communication). CDFW's AIS program utilizes simple messaging tools in its current AIS program, providing a framework for Whirling Disease outreach materials.

5.1.2.4 Targeting Key Audiences

Targeting information to specific audiences can engage key groups in the prevention of AIS spread. Particular considerations in choosing target audiences are their ability to contribute to introductions, as well as their ability to promote your message. Based on these considerations, anglers, recreational boaters, and young audiences (K-12) were identified as key audiences for Whirling Disease outreach and education in the Upper Feather River Basin.

Angler movement has been associated with *M. cerebralis* introductions. This group, along with recreational boaters, represent key audiences to encourage compliance with stewardship guidelines due to their potential to contribute to AIS movement. Their engagement also facilitates the spread of Whirling Disease information across the angling and boating communities through word of mouth.

Engaging young audiences not only encourages their personal compliance in AIS stewardship guidelines, it can impact the stewardship behavior of adult family members and friends. As the future stewards of natural resources, this group has a long-term influence on and interest in the protection of water resources from AIS.

5.1.2.5 Utilizing Multiple Media Platforms

As a general rule, having a multi-media approach expands the breadth of audience groups likely to hear the message (Alberta Environment and Parks, 2018). Evaluating how to most effectively reach targeted audiences with the desired information is an essential step in establishing effective outreach campaigns. Media platforms identified as priorities by the WDTG are Whirling Disease signage indicating pathogen presence in the watershed, pamphlets and posters provided to angling retailers and tourism centers, radio announcements, an *M. cerebralis* detection map for the watershed, educational webpages on agency websites, and educational events and programs.

5.2 *M. cerebralis* Equipment Decontamination Protocols

Adoption and implementation of effective *M. cerebralis* aquatic survey guidelines and equipment decontamination protocols for those conducting work in the Upper Feather River Basin will reduce the risk of spreading the pathogen to uninfected waters.

5.2.1 Aquatic Field Work Best Management Practices

Best Management Practices (BMPs) for planning, timing, and conducting aquatic field work most applicable to the prevention of *M. cerebralis* spread were derived from those developed by CDFW, USFS (Region 4), and Alberta Parks and Environment. The CDFW and USFS protocols are intended to address risks with all AIS. The Alberta protocols were developed specifically for Whirling Disease.

1. Plan ahead and use available information to determine where AIS species are and if aquatic work will require entry or equipment contact into waters with positive AIS detections.

2. Whenever possible, avoid aquatic work in multiple waterbodies in the same field day. If movement between multiple waterbodies is necessary, clean and disinfect all equipment (waders, nets, etc.) prior to each use in different waterbodies.

3. Whenever practical, sampling and other aquatic work should be accomplished upstream to downstream to reduce the risk of transporting AIS to uninfected upstream areas.

4. Avoid transferring fish, fish parts, sediment, and water between drainages or between unconnected waters within the same drainage.

5. If equipment decontamination cannot be done on site, contaminated equipment should be transported in sealed plastic bags or otherwise isolated to keep separate from clean gear.

6. Avoid the use of equipment that cannot be appropriately decontaminated (i.e., felt-soled waders) if suitable alternatives are available. For a full list of materials evaluated in terms of their suitability for equipment decontamination techniques, see the Alberta Government's Equipment List for Decontamination Purposes.

7. Whenever possible, dedicate watercraft, vehicles, or sensitive equipment that cannot be sterilized according to the *M. cerebralis* equipment decontamination protocols by drainage OR designate these equipment types according to drainages with and without AIS detections.

8. Avoid off-highway and motor vehicle entry into waterbodies or wetlands when possible. If unavoidable, clean mud and organics according to the AIS equipment decontamination protocol.

5.2.2 *M. cerebralis* Equipment Decontamination

An *M. cerebralis* decontamination protocol has been developed by the USFS Intermountain Region (Region 4): "Guide to Preventing Aquatic Invasive Species Transported by Wildland Fire Operations" (see the USFS Region 4 Aquatics Invasive Species website). The CDFW has also developed AIS decontamination protocols (which can be found on the AIS page of the CDFW website).

AIS protocols for CDFW and the USFS include the following steps:

1. Clean all mud and debris from equipment and watercraft:
    a. For equipment: use a stiff scrubbing brush to effectively remove debris. Ensure that small crevices in boots and other equipment are appropriately cleaned.
    b. For watercraft, remove all mud, plants, and other debris prior to leaving the boat launch.

2. Drain any standing water from watercraft including the motor, motor cooling system, live wells, and bilges.

The next step is to disinfect equipment. QUAT is one option, mixed according to label directions. Additional methods are a 5% bleach or 1% sodium hypochlorite solution. The latter solutions appear to be more than effective for *M. cerebralis*. CDFW calls for drying equipment for at least 48 hours, which is effective for both *M. cerebralis* life stages. In acknowledgement of concerns regarding the current protocols' effectiveness for the AIS present in California, CDFW is currently updating their AIS decontamination protocols, and USFS representatives have also expressed interest in updating current protocols if needed.

Other state agencies have adopted the CDFW decontamination protocols for fieldwork in aquatic systems, and CDFW requires adherence to their AIS protocols for recipients of aquatic scientific collection permits. These agencies (or their contractors) do most of the aquatic field work in the

Basin. Therefore, once completed, the updated CDFW AIS protocol will play an important role in preventing *M. cerebralis* spread.

Effective decontamination procedures have been compiled including the required QUAT concentrations, freezing time, and hot water temperatures (Table 6). To prevent the spread of *M. cerebralis*, all equipment, including wading equipment, dive equipment, sampling equipment, and watercraft, must be decontaminated using at least one of the protocols listed.

Table 6. Decontamination protocols

Method	Conditions and Exposure Time*
Hot water wash (high pressure washing unit or submersion)	90ºC (195ºF) for 10 minutes (Myxospores) 75°C (167°F) for 5 minutes (TAMs)
Drying	24-hour exposure, preferably in sunlight
Bleach, 6% sodium hypochlorite (NaCIO)	Exposure to 1% bleach solution (5000 ppm of available chlorine) for 10-15 minutes Mixing instruction: • 1.1 oz bleach per 1-gallon water • 2.2 Tablespoons bleach per gallon water • 0.9-gallon bleach per 100 gallons water *Contact time* = 15 minutes
Quaternary ammonium compounds	Exposure to 1500 ppm Quat compounds 1. 4.6% Sanicare Quat128® solution Mixing instructions: • 6.4 oz per 1-gallon water • 5 gallons per 100 gallons water *Contact time* = 10 minutes. OR 2. 3.1% Sparquat256® solutions Mixing instructions: • 4.3 oz per 1-gallon water • 3.4 gallons per 100 gallons water *Contact time* = 10 minutes OR 1.8% Green Solutions High Dilution 256® solution Mixing instructions: • 2.5 oz per 1-gallon water • 1.9 gallons per 100 gallons water *Contact time* = 10 minutes
Freezing**	Exposure to -20ºC for 1 week

Source (Hedrick et al., 2008)

*Time Periods represent minimum exposure time frames

**Conflicting reports on freezing with myxospores potentially remaining viable beyond the 7-day time period (El-Matbouli et al., 2005)

5.3 Resistant Salmonid Strains

Planting resistant strains of susceptible trout in the Upper Feather River may be an important tool where *M. cerebralis*-related population declines have occurred. The pathogen contributes to habitat fragmentation by preventing susceptible species from utilizing streams, and it gives non-native salmonids, such as brown trout, a competitive advantage.

Impacts of Whirling Disease on trout populations in the western United States are significant but, in most cases, not all individuals are infected. In time, many populations recover to some degree. This response raises the promise of finding native fish with resistance to the pathogen for purposes of accelerating recovery of infected populations.

In addition to finding resistant fish in locally infected streams, salmonids resistant to *M. cerebralis* have been raised in hatcheries and then released in infected waters to supplement populations reduced by the disease. Resistant fish also reduce the risk of salmonid infection because they carry lower densities of myxospores than infected fish. Reduced myxospore production acts to disrupt pathogen proliferation and decrease salmonid TAM exposure dose (D. Winkelman).

The WDTG identified three options for the use of resistant salmonid strains in the Upper Feather River Basin. They are (a) use of non-local resistant wild rainbow trout strains, (b) use of a hatchery-reared resistant Hofer strain, (c) study of resistant strains from local infected waters.

5.3.1 Non-local Strains

Resistant rainbow trout strains discovered since the introduction of *M. cerebralis* in North America are the Harrison Lake rainbow trout from Montana (Hedrick et al. 1998; Hedrick et al., 2003) and the Gunnison River rainbow trout from Colorado (Fetherman et al., 2018). In general, these wild resistant strains seem to demonstrate less resistance to the pathogen than hatchery-raised strains. The WDTG's primary concern with non-local strains are potential impacts posed by introducing fish with genetics evolved in other bioregions. For this reason, use of these strains is not recommended in the Feather River Basin.

5.3.2 Hofer Rainbow Trout

Hofer rainbow trout are highly resistant to *M. cerebralis*. Also referred to as German rainbow trout, due to its history in European hatcheries where *M. cerebralis* is endemic, Hofer rainbows developed a resistance to the pathogen during a period of over 100 years following their initial introduction in the late 1800s. Its long history as a hatchery fish has domesticated the strain. It is fast-growing and survives well in the hatchery environment (Fetherman et al. 2018).

In California, the Hofer rainbow strain has been produced at Hot Creek Hatchery for several years to support recreational fishing opportunities in the Eastern Sierra. The first year of Hofer production at the Mt. Shasta hatchery is this year: 2020. The current availability of the resistant Hofer strain in CDFW hatcheries and its resistance makes this strain an option for stocking in the Upper Feather River Basin.

5.3.3 Local Resistant Strains

Use of resistant strains from local streams is attractive because it reduces concerns for introducing fish whose genetic make-up evolved elsewhere. Fish with locally evolved genetics might provide more lasting and successful population recovery. To pursue this option, investigations of the resistance of the resident susceptible salmonid species from infected streams would be conducted to determine if they possess natural resistance. This would involve testing fish for exposure to TAMs in a laboratory. Evaluation of resistance would be gained by comparing myxospore production, histological damage, and Whirling Disease symptoms in the wild fish with resistant and susceptible strains.

If a resistant wild strain were identified, broodstock and rearing of fish for planting would have to be developed at CDFW or local (Chester HS, Feather River College) hatcheries.

5.3.4 Current Planting of Hatchery Fish in the Watershed

Several locations on the 2020 CDFW trout planting schedule are streams with positive *M. cerebralis* detections (Table 7). The Basin has portions of two CDFW regions, the North Central and Northern (Lassen County) regions. To plant resistant rainbows in priority locations (areas with *M. cerebralis* detections), the CDFW Fisheries Branch for each region must be consulted and provided with a justification for planting resistant rainbows.

Table 7. CDFW 2020 scheduled plants in subwatershed with *M. cerebralis* detections

Region	Planting Location	Subwatersheds (HUC 12)
1	Lower Goodrich Creek	Goodrich Creek-Frontal Mountain Meadows Reservoir
2	North Fork, Belden	Mosquito Creek-North Fork Feather River
2	Antelope Lake	Antelope Creek and Lone Rock Creek-Indian Creek
2	Round Valley Reservoir	Lower Wolf Creek

Four factors impact availability of resistant fish plants from CDFW hatcheries: new allotments, production need, coordination with CDFW and timing. New allotments would require additional funding. The 2020 planting schedule indicates a relatively modest initial production need for Hofer rainbows. Unless current Hofer allotments are redirected from other regions, it is assumed that increased production (and funding) would be needed to meet additional requests. If the quantity needed is beyond current capacity, broodstock may need to be increased. It is likely that it would be 4-6 years before additional catchable fish would be ready (J. Rowan).

Priority areas in the Upper Feather River Basin for CDFW planting of resistant rainbows to support recreational fishing opportunities are those with confirmed detections of *M. cerebralis*. Current understanding of *M. cerebralis* impacts on susceptible salmonid populations in these areas is limited. A necessary first step would be to survey salmonid populations to assess intensity of infections among the resident salmonid population, the age structure of the population, and the spatial distribution of susceptible species. Locations with detections and high establishment potential (indicating possible high TAM exposure) include Upper Yellow Creek, Goodrich Creek, and Indian Creek.

6.0 Monitoring

Introduction

This monitoring plan was developed to address two primary needs. The first is a means to check on and document the degree to which recommended management actions are implemented. The other need, to better understand both the distribution and persistence of Whirling Disease in the Basin, is addressed in the *M. cerebralis* monitoring section. It is presumed that the WDTG will continue to meet and will be responsible for implementation of this Plan.

6.1 Implementation Monitoring

Monitoring questions are framed around the six management elements included in this plan.

6.1.1 Basin Level Risk Assessment

The assessment has been completed. The WDTG will meet annually, or more often, to assess whether management agencies are employing the Plan. At this time, needs to update the Plan will be discussed.

6.1.2 Whirling Disease Outreach and Education

The WDTG will meet annually, or more often, to assess whether the following elements of the Outreach and Education Plan have been implemented. Results will be summarized by the WDTG and shared with WDTG members.

a. Whirling Disease signage has been produced.
b. Whirling Disease signage has been placed at boat ramps and campgrounds identified in the WD Communication Plan.
c. AIS signage that includes Whirling Disease has been produced.
d. AIS signage has been posted at the locations identified in the plan.
e. Whirling Disease information signs have been produced.
f. Information signs have been installed at Yellow Creek campground and Lone Rock Campground.
g. Whirling Disease brochures have been produced.
h. Whirling Disease brochures have been distributed to bait shops and angling retailers as identified in the plan.
i. Whirling Disease brochures have been distributed to PNF Headquarters and PNF and LNF Ranger District offices in the Basin.
j. Whirling Disease informational rack cards have been produced.
k. Whirling Disease informational rack cards have been distributed to bait shops and angling retailers as identified in the plan.

l. Whirling Disease informational rack cards have been distributed to PNF and LNF Headquarters and ranger district offices in the Basin.
m. A *M. cerebrali*s detection map has been produced.
n. The *M. cerebrali*s detection map is used by CDFW online through fishing regulations or Whirling Disease informational page.
o. WDTG has shared Whirling Disease information on local radio stations or via podcasts.
p. FRTU will broaden the Trout in the Classroom Program to include discussion of Whirling Disease.
q. FRTU has worked with CHS to include discussion of Whirling Disease in CHS' aquaculture program.
r. FRTU has coordinated with PUSD to include Whirling Disease in the school's "Year of the Fish" curriculum.

6.1.3 Best Practices for Aquatic Surveys and Organization Equipment Decontamination

The WDTG will meet annually, or more often, to assess whether management agencies are employing the survey BMPs and decontamination protocols. At this time, needs to update the BMPs and protocols will be discussed

6.1.4 Resistant Salmonid Strains

The WDTG will annually, or more often, report to its membership on any plans to plant resistant trout in Basin waters. If planting is anticipated, the WDTG will develop appropriate monitoring questions to evaluate implementation effectiveness.

6.1.5 Project-scale Risk Assessment for Restoration Projects in Meadow Streams.

The WDTG will meet annually, or more often, to assess meadow restoration projects in the Basin implemented in the previous year. The WDTG will review those projects to determine (a) if the project scale risk assessment was used during project planning, (b) if the assessment was properly applied; and (c), if the assessment's determination informed project design.

6.2 Monitoring for *M. cerebralis*

M. cerebralis has been present in the Basin for at least 35 years. Though not documented, it is assumed that initial testing for the pathogen followed observations of trout displaying Whirling Disease symptoms. Subsequently, use of eDNA as a tool to test for the pathogen was developed. eDNA provides a cost-effective method to survey for *M. cerebralis* and has been employed (along with other methods) in recent years in surveys for the pathogen at several locations in the Basin. Recognizing the connection between increased sediment delivery and suitable habitat for Whirling Disease, the PNF used wildfire settlement funding to survey stream substrates for *M. cerebralis* in areas impacted by large wildfires in 2015-16 and 2018-19. This provided information about pathogen's distribution in some tributaries (including Yellow Creek) to the North Fork Feather River (Chips Fire) as well as tributaries to the East Branch, North Fork

Feather River (Moonlight Fire). The FRTU also conducted a Basin-wide survey for the pathogen in stream water during 2016 and sampled for eDNA at 83 locations.

The result of prior eDNA sampling is an incomplete picture of Whirling Disease presence in the Basin. Information on Whirling Disease ecology in this report provided the WDTG a clearer picture of suitable habitat for Whirling Disease in the Basin, and the risk assessment has provided risk ratings of subwatersheds' potential to support the pathogen. The WDTG's priorities for monitoring focused on three issues: 1) better understanding of Whirling Disease distribution, 2) a better understanding of pathogen persistence where it has been detected, and 3) the relative effectiveness of sampling techniques. More specifically, past sampling has employed both sediment and water column collections for eDNA analysis, but the relative effectiveness of the two techniques remains unclear.

The monitoring task group crafted an approach to address three monitoring questions:

1. Is Whirling Disease occurrence greater in subwatersheds rated as high in the risk assessment?

2. Does *M. cerebralis* persist in infected waters?

3. Which is the more cost-effective eDNA technique for detecting *M. cerebralis*: stream substrate sediment or water column collections?

6.2.1 Timing

We propose a 9-year monitoring plan beginning in 2021 or 2022.

Environmental DNA, fish collection, and sentinel fish sampling will be conducted in August and September to increase the likelihood of detections.

6.2.2 Sample Methods

6.2.2.1 Environmental DNA

6.2.2.1.a Water Column

At each sample site, two 1-L stream water samples, treated as replicates, followed by one 1-L negative control composed of distilled water, will be collected and filtered. Water will be filtered through a sterile, disposable Nalgene Analytical Test Filter Funnel. At the time of this writing, a 47mm diameter, 0.45 µm pore size cellulose nitrate filter membrane is required. This standard will be evaluated prior to sampling, if a larger pore size is used, sample volume may increase. The filter funnel will be connected to a vacuum pump. Water samples will be collected with a gloved hand using whirl packs, and the gloves will subsequently be disposed of as waste. Collected water will then be poured into the filter funnel. The pump will be engaged until 1-L of stream water has passed through the filter membrane. If filters become clogged with algae or sediment, multiple filters will be used. The amount of sample run through each filter will be noted. After filtering, the filter will be allowed to dry momentarily, removed from the disposable funnel with sterile forceps, and, placed into a labeled coin envelope. The envelope will subsequently be stored in a sealed Ziploc bag along with approximately 2.5 oz. of indicating silica gel desiccant beads and stored out in a cooler.

Water samples will be collected at arm's length from the stream bank to reduce the potential contamination resulting from entering the water at each site. If it is necessary to enter the water to reach a given designated sample site, care will be taken not to enter the water at any point upstream of the actual sample collection site. All wetted field gear will be decontaminated. The precise location of actual sample collection will be recorded using a handheld GPS unit. All equipment retained for reuse (vacuum pumps, flasks, tubing) will be decontaminated.

6.2.2.1.b Sediment

Sediment samples will be collected manually by digging into substrate directly with a sterile, 50-mL conical tube. Samples will be frozen, then stored at -80°C until processing. Further details on the procedure are provided in Richey, et al (2018).

6.2.2.2 Population Surveys

Sample protocol summarized here is taken from Thurow (1994), where more detailed information is available. Where feasible, surveyors should move upstream. Snorkelers will enter the water downstream from the reach unit to be surveyed and proceed upstream slowly while avoiding sudden movements. Fish will be counted as the snorkeler passes them so duplicate counts are avoided. Fish will be counted by species and size class. Sizes can be estimated by approaching fish, aligning their snout and tail with adjacent objects, and measuring that distance with a rule or marked glove.

Depending on stream size, one or two snorkelers will conduct the survey. One snorkeler will be used if a single observer is able to see from bank to bank. The observer will count all fish in the entire sampling unit. Depending on the characteristics of the unit, the snorkeler can proceed up the center of the unit and count fish by zigzagging outward to both banks. Care should be taken to search for fish throughout the unit, including the margins, and to inspect all cover components (such as undercut banks, substrate, and organic debris). If the water is too deep or turbulent to zigzag and visibility is adequate, the observer will move up one bank of the unit and count all fish to the other bank. In water too deep to count upstream, the observer will float down the center of the unit and count all fish from bank to bank, remaining as motionless as possible.

To avoid recounting fish, observers should stay adjacent to each other, move at the same speed, and only count fish that pass them. If two snorkelers are used, the unit is divided, and snorkelers will use one of three techniques. First, where feasible, the unit will be divided in half. Snorkelers will begin in the center of the unit, move upstream shoulder to shoulder, and count all fish between themselves and the bank. Second, if the unit is too deep or turbulent to allow that approach, snorkelers can use natural breaks and features such as boulders to divide the unit. Snorkelers will then count all fish in their portion of the unit. Third, in water too deep to move upstream, two snorkelers will lock hands and float down the center of the unit, counting all fish from their shoulders to the bank.

With either one or two observers, fish will be counted by species and size class. Counts will be recorded on a PVC cuff or slate and later transferred to a data sheet. After completing counts, observers or other crew members will estimate the surface area of the snorkeled unit, and then record the total length of the unit and measure the width at three or more equally spaced intervals. The surface area can be estimated either by multiplying the length times a mean width or by calculating the area of individual segments and pooling them for a total area estimate. The density of fish is expressed as the number of fish per 100 m^2.

Observations will also be made regarding the number of fish demonstrating Whirling Disease symptoms: fish with black-tail, skeletal deformities, and exhibiting whirling motions. Population characteristics to evaluate infection impact include fish density, fish age class distribution, and species presence. Specific to age class, studies in *M. cerebralis* positive areas show young-of-the-year not surviving to older stages due to the timing of TAMs finding susceptible juvenile salmonids (Alberta Environment and Parks, 2018).

6.2.2.3 Sentinel Fish Surveys

This technique uses hatchery-reared salmonids (susceptible species and ages) held in cages in the stream reach for a set time period (e.g. two weeks) (Eby et al., 2015; Richey et al., 2018). The cages hold the fish in place and expose them to TAMs. Following exposure, the fish are collected, euthanized, and analyzed for myxospores, to determine both pathogen presence and the degree of TAM exposure.

Sentinel fish surveys provide information on the spatial distribution of *M. cerebralis* establishment in the surveyed area and can direct resource managers to point sources of TAM production. This is done through the association between a) confirmed observations of the *M. cerebralis* myxospore life stage in sentinel fish hosts (i.e., *M. cerebralis* presence), b) measures of myxospore burdens (i.e. high myxospore loads = high TAM exposures) at the chosen survey locations, and c) visual inspections of sentinel fish for Whirling Disease symptoms upon collection.

6.2.3 *M. cerebralis* Distribution

Step 1- In years 1, 3, 5, and 7, samples will be collected (water column eDNA) in eight randomly selected subwatersheds that were rated as high risk in the risk assessment (of 23 subwatersheds rated as high), 14 subwatersheds rated as moderate (of 56 subwatersheds rated as moderate) and eight rated as low (of 32 subwatersheds rated as low). Because they contain no low gradient channel reaches (NorWeST model), The Little North Fork Feather River, Onion Valley, and Sucker Run subwatersheds will be excluded from sampling (all are rated as low risk).

Sampling will be conducted at three low-gradient ($\leq$ 2%) stream reaches in each selected subwatershed. Selected stream reaches will be at least 100m in length. The low-gradient reach located furthest downstream in the subwatershed will be sampled. The second site will be randomly selected from a population of remaining reaches that meet the 100m length and ≤ 2% gradient criteria. If 100m reaches are not available, 50m reaches will be used. The third site will be randomly selected from reaches that are not proximate to the previously selected sites (if any such reaches remain in the sample pool). Replicate samples will be collected at the downstream end of the low gradient reach. A control will be collected at each site. At river sites, samples will be taken from the first three flatwater habitat units upstream of the subwatershed border.

Step 2- In years 2, 4, 6, and 8, in watersheds with positive results in year 2, samples will be collected from each low gradient reach at least 100m in length, at the downstream extent of the habitat. Two samples will be collected at each site. If three 100m reaches do not exist, a 50m reach criterion will be applied.

6.2.4 *M. cerebralis* Persistence

Step 1- In years 1, 4, and 7, in each watershed rated as infected with *M. cerebralis* (Table 8), Step 2 for *M. cerebralis* Distribution will be applied, and the samples will then be replicated. Selection of these subwatersheds was based on locations where Whirling Disease was detected by at least two survey efforts. Subwatersheds where infections are shown to persist based on sampling described in 6.2.2 will be added in years 4 and 7, as appropriate.

Step 2- In years 2, 5, and 8, for reaches with positive readings, fish population surveys (electrofishing or snorkeling) will be conducted. Reporting will include density, age class distribution, and WD symptoms for each salmonid species. Presence of other fish species will be noted.

Step 3- In years 3, 6, and 9, for reaches with poor density and size class distribution, or absence of rainbow trout, sentinel fish sampling will be conducted. If selected reaches are less than 100m, the entire reach will be surveyed. It the reach is greater than 100m, a 100m section will be randomly selected for the survey. Existing fish survey information from LNF and PNF will be used to define the expected condition on which to define "poor" density and size class distribution.

Table 8. *M. cerebralis* persistence monitoring stations

Subwatershed Map ID	Water Body (subwatershed)
53	Indian Creek (Cold Stream)
24	Upper Yellow Creek
16	Upper Lights Creek

6.2.5 eDNA Sediment and Water Column Collections

In years 1 and 3, in sites in high risk subwatersheds, sediment samples for *M. cerebralis* eDNA analysis will be collected. Results from co-located water column and sediment sample sites will be evaluated at the end of year 3. If there is a clear indication that sediment sampling is the more effective technique, sampling in remaining years will be shifted to sediment.

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Appendix A - Subwatershed (HUC 12) Listing

Map ID	HUC12	NAME
0	180201210205	Rock Creek- Hamilton Branch
1	180201220502	Lower Wolf Creek
2	180201220201	Ferris Creek-Last Chance Creek
3	180201220301	Boulder Creek
4	180201220204	Willow Creek-Last Chance Creek
5	180201210402	Bailey Creek
6	180201230101	Lookout Creek-Little Last Chance Creek
7	180201220403	Cooks Creek
8	180201220202	Cottonwood Creek
9	180201210405	Almanor Peninsula-Frontal Lake Almanor
10	180201210201	Mountain Meadows Creek-Frontal Mountain Meadows Reservoir
11	180201210101	Warner Creek
12	180201210203	Robbers Creek
13	180201220303	Lone Rock Creek-Indian Creek
14	180201210206	Dry Creek-Hamilton Branch
15	180201230102	Frenchman Lake-Little Last Chance Creek
16	180201220401	Upper Lights Creek
17	180201210401	Benner Creek
18	180201210204	Mountain Meadows Reservoir
19	180201230310	Sierra Valley Channels
20	180201220501	Upper Wolf Creek
21	180201220203	Clarks Creek
22	180201210202	Goodrich Creek-Frontal Mountain Meadows Reservoir
23	180201210606	Camp Creek-North Fork Feather River
24	180201210501	Upper Yellow Creek
25	180201210703	Concow Creek
26	180201210301	Soldier Creek-Butt Creek
27	180201210804	Potter Ravine-North Fork Feather River
28	180201210801	French Creek
29	180201230704	Fall River
30	180201210601	Chips Creek
31	180201210502	Lower Yellow Creek
32	180201210604	Rock Creek- North Fork Feather River
33	180201230705	Brush Creek-Middle Fork Feather River
34	180201210701	Last Chance Creek-West Branch Feather River
35	180201210704	Little West Fork West Branch Feather River-West Branch Feather River
36	180201230604	Oroleve Creek-South Fork Feather River
37	180201230605	Sucker Run
38	180201210802	Berry Creek
39	180201210605	Grizzly Creek

40	180201230702	Little North Fork of Middle Fork Feather River
41	180201230602	Lost Creek
42	180201230707	East Fork Canyon Creek-Feather River
43	180201210702	Big Kimshew Creek
44	180201210705	Dark Canyon-West Branch Feather River
45	180201230703	South Branch Middle Fork Feather River
46	180201230603	Rock Creek-South Fork Feather River
47	180201230606	Oregon Gulch-South Fork Feather River
48	180201230706	Frey Creek-Middle Fork Feather River
49	180201210803	Chino Creek-North Fork Feather River
50	180201220804	Mill Creek-Spanish Creek
51	180201230503	Nelson Creek
52	180201230601	Little Grass Valley Reservoir-South Fork Feather River
53	180201220305	Cold Stream-Indian Creek
54	180201220903	Mill Creek-East Branch North Fork Feather River
55	180201230308	Mapes Canyon
56	180201230506	Onion Valley Creek
57	180201230403	Sulphur Creek
58	180201220302	Antelope Creek
59	180201210408	Mosquito Creek-North Fork Feather River
60	180201220702	Taylor Creek-Greenhorn Creek
61	180201230401	Big Grizzly Creek
62	180201230701	Willow Creek-Middle Fork Feather River
63	180201230501	Long Valley Creek
64	180201220205	Squaw Queen Creek
65	180201230404	Humbug Creek-Middle Fork Feather River
66	180201210102	Willow Creek-North Fork Feather River
67	180201220102	Upper Red Clover Creek
68	180201220901	Rush Creek
69	180201220805	Tollgate Creek-Spanish Creek
70	180201220603	Ward Creek-Indian Creek
71	180201220101	Dixie Creek
72	180201230508	Dogwood Creek-Middle Fork Feather River
73	180201220103	Lower Red Clover Creek
74	180201220902	Soda Creek-East Branch North Fork Feather River
75	180201210406	Lake Almanor
76	180201220604	Hough Creek-Indian Creek
77	180201220801	Meadow Valley Creek
78	180201220404	Lower Lights Creek
79	180201230309	North Channel Little Last Chance Creek
80	180201220206	Poison Creek-Last Chance Creek
81	180201210103	Louse Creek-North Fork Feather River
82	180201230406	Frazier Creek-Middle Fork Feather River

83	180201210602	Bucks Creek
84	180201210403	Mud Creek-Frontal Lake Almanor
85	180201230306	Town of Loyalton
86	180201220803	Silver Creek-Spanish Creek
87	180201230402	Willow Creek
88	180201230307	Carman Creek
89	180201220601	Hosselkus Creek
90	180201230504	Poplar Creek-Middle Fork Feather River
91	180201210302	Butt Valley Reservoir-Butt Creek
92	180201230405	Jamison Creek
93	180201220602	Little Grizzly Creek
94	180201220802	Rock Creek- Spanish Creek
95	180201230505	Washington Creek-Middle Fork Feather River
96	180201210407	Clear Creek-North Fork Feather River
97	180201220402	Middle Lights Creek
98	180201220701	Estray Creek-Greenhorn Creek
99	180201230502	Jackson Creek-Middle Fork Feather River
100	180201230507	Bear Creek
101	180201210603	Milk Ranch Creek-North Fork Feather River
102	180201230305	Correco Canyon
103	180201220304	Hungry Creek
104	180201210404	Marian Creek-Frontal Lake Almanor
105	180201230304	Turner Creek
106	180201230201	Badenaugh Canyon-Smithneck Creek
107	180201230202	Bear Valley Creek-Smithneck Creek
108	180201230303	Lemon Canyon-Perry Creek
109	180201230302	Hamlin Creek
110	180201230301	Bonta Creek-Cold Stream

APPENDIX B - Feather River Basin Whirling Disease Risk Assessment Data

Wshd #	Subwatershed	Establishment							Introduction			Subwatershed Rating
		Range Miles	Range Rating	T/S Miles	T/S Rating	Rds Score	Rds Rating	Rating	Angling	Proximity	Rating	
0	Rock Creek- Hamilton Branch	5.0	3	5.0	8	0.68	4	High	2	1	Mod	High
1	Lower Wolf Creek	15.2	4	1.1	6	0.52	4	High	1	3	Mod	High
2	Ferris Creek-Last Chance Creek	10.1	5	9.3	10	0.65	4	High	1	1	Low	Moderate
3	Boulder Creek	1.3	3	0.0	2	0.24	3	Low	1	2	Mod	Low
4	Willow Creek-Last Chance Creek	23.4	5	6.4	10	-0.09	2	High	1	1	Low	Moderate
5	Bailey Creek	2.7	2	0.0	2	-0.41	1	Low	1	1	Low	Low
6	Lookout Creek-Little Last Chance Creek	5.9	4	4.2	8	-0.43	1	Moderate	1	1	Low	Moderate
7	Cooks Creek	5.5	4	0.7	4	-0.19	2	Moderate	1	2	Mod	Moderate
8	Cottonwood Creek	5.2	4	3.3	8	1.00	5	High	1	1	Low	Moderate
9	Almanor Peninsula-Frontal Lake Almanor	0.1	1	0.1	4	1.00	5	Moderate	1	1	Low	Moderate
10	Mountain Meadows Creek-Frontal Mountain Meadows Reservoir	5.4	4	4.2	8	0.20	3	High	2	2	High	High
11	Warner Creek	9.1	4	8.6	10	-0.98	1	High	2	1	Mod	High
12	Robbers Creek	22.5	5	18.8	10	-0.47	1	High	1	2	Mod	High
13	Lone Rock Creek-Indian Creek	7.6	4	5.6	8	0.15	3	High	1	3	High	High
14	Dry Creek-Hamilton Branch	7.5	4	7.5	10	1.00	5	High	2	1	Mod	High
15	Frenchman Lake-Little Last Chance Creek	9.6	4	9.5	10	0.67	5	High	3	1	High	High
16	Upper Lights Creek	0.3	3	0.0	2	0.39	4	Moderate	1	3	High	High
17	Benner Creek	0.1	2	0.0	2	-0.46	1	Low	1	1	Low	Low
18	Mountain Meadows Reservoir	4.7	4	4.4	8	0.22	3	High	2	2	High	High
19	Sierra Valley Channels	139.2	5	0.9	6	-0.48	1	Moderate	1	1	Low	Moderate
20	Upper Wolf Creek	1.8	1	1.4	6	0.65	4	Moderate	1	2	Mod	Moderate
21	Clarks Creek	14.1	5	11.2	10	0.16	3	High	1	1	Low	Moderate
22	Goodrich Creek-Frontal Mountain Meadows Reservoir	24.7	5	22.1	10	-0.05	2	High	2	3	High	High
23	Camp Creek-North Fork Feather River	10.4	1	0.0	2	-0.45	1	Low	1	3	High	Low
24	Upper Yellow Creek	9.7	3	9.7	10	0.59	3	High	3	3	High	High
25	Concow Creek	6.2	1	0.0	2	-0.07	2	Low	1	2	Mod	Low

Wshd #	Subwatershed	Establishment							Introduction			Subwatershed Rating
		Range Miles	Range Rating	T/S Miles	T/S Rating	Rds Score	Rds Rating	Rating	Angling	Proximity	Rating	
26	Soldier Creek-Butt Creek	9.7	1	9.7	10	0.29	3	High	2	2	High	Moderate
27	Potter Ravine-North Fork Feather River	0.2	1	0.0	2	-0.85	1	Low	1	1	Low	Low
28	French Creek	6.0	1	0.8	4	-0.57	1	Low	2	2	High	Moderate
29	Fall River	3.2	3	3.2	8	-0.19	1	Moderate	1	1	Low	Moderate
30	Chips Creek	0.0	1	0.0	2	-1.00	1	Low	2	2	High	Moderate
31	Lower Yellow Creek	0.6	3	0.0	2	-0.62	1	Low	1	3	High	High
32	Rock Creek- North Fork Feather River	1.2	2	0.7	4	0.68	5	Moderate	1	2	Mod	Moderate
33	Brush Creek-Middle Fork Feather River	11.7	2	0.1	4	-0.86	1	Low	1	1	Low	Low
34	Last Chance Creek-West Branch Feather River	0.5	1	0.5	4	-0.76	1	Low	1	1	Low	Low
35	Little West Fork West Branch Feather River-West Branch Feather River	8.2	1	1.9	6	-0.47	1	Low	1	2	Mod	Low
36	Oroleve Creek-South Fork Feather River	1.6	1	0.0	2	-0.62	1	Low	1	1	Low	Low
37	Sucker Run	0.0	1	0.0	2	-0.72	1	Low	1	1	Low	Low
38	Berry Creek	2.2	1	0.0	2	0.29	3	Low	1	1	Low	Low
39	Grizzly Creek	2.8	3	2.6	8	-0.75	1	Moderate	2	2	High	Moderate
40	Little North Fork of Middle Fork Feather River	0.0	1	0.0	2	-0.44	1	Low	2	1	Mod	Low
41	Lost Creek	2.5	1	2.1	8	1.00	5	High	1	1	Low	Moderate
42	East Fork Canyon Creek-Feather River	0.5	1	0.0	2	0.05	3	Low	1	1	Low	Low
43	Big Kimshew Creek	1.5	3	0.8	4	-0.85	1	Low	1	2	Mod	Low
44	Dark Canyon-West Branch Feather River	0.4	1	0.0	2	0.16	3	Low	1	1	Low	Low
45	South Branch Middle Fork Feather River	2.6	2	2.6	8	0.10	3	Moderate	1	1	Low	Moderate
46	Rock Creek-South Fork Feather River	1.6	2	0.6	4	-0.63	4	Moderate	1	1	Low	Moderate
47	Oregon Gulch-South Fork Feather River	0.6	1	0.0	2	-0.30	2	Low	1	1	Low	Low
48	Frey Creek-Middle Fork Feather River	0.1	1	0.0	2	-0.88	1	Low	1	1	Low	Low
49	Chino Creek-North Fork Feather River	5.6	1	0.0	2	-0.50	1	Low	1	2	Mod	Low
50	Mill Creek-Spanish Creek	11.1	1	0.6	4	0.94	5	Moderate	1	2	Mod	Moderate
51	Nelson Creek	5.2	1	4.6	8	-0.79	1	Moderate	3	1	High	Moderate
52	Little Grass Valley Reservoir-South Fork Feather River	0.6	3	0.6	4	0.42	4	Moderate	3	1	High	Moderate
53	Cold Stream-Indian Creek	5.1	2	5.0	8	0.23	3	Moderate	3	3	High	High

Wshd #	Subwatershed	Establishment							Introduction			Subwatershed Rating
		Range Miles	Range Rating	T/S Miles	T/S Rating	Rds Score	Rds Rating	Rating	Angling	Proximity	Rating	
54	Mill Creek-East Branch North Fork Feather River	7.7	2	0.1	4	-0.90	1	Low	1	3	Mod	Low
55	Mapes Canyon	12.4	5	0.3	4	-0.41	1	Moderate	1	1	Low	Moderate
56	Onion Valley Creek	0.0	2	0.0	2	-1.00	1	Low	1	1	Low	Low
57	Sulphur Creek	4.8	4	0.0	2	1.00	5	Moderate	1	1	Low	Moderate
58	Antelope Creek	7.3	4	6.0	10	1.00	5	High	1	2	Mod	High
59	Mosquito Creek-North Fork Feather River	8.3	1	8.3	10	-0.12	2	Moderate	1	3	Mod	Moderate
60	Taylor Creek-Greenhorn Creek	9.7	5	1.2	6	1.00	5	Moderate	1	2	Mod	Moderate
61	Big Grizzly Creek	12.1	5	11.8	10	0.39	4	High	3	1	High	High
62	Willow Creek-Middle Fork Feather River	5.6	2	0.3	4	-0.63	1	Low	1	1	Low	Low
63	Long Valley Creek	2.4	3	1.3	6	0.41	4	Moderate	1	1	Low	Moderate
64	Squaw Queen Creek	19.0	5	0.3	4	-0.20	2	Moderate	1	1	Low	Moderate
65	Humbug Creek-Middle Fork Feather River	27.5	3	16.6	10	1.00	5	High	1	1	Low	Moderate
66	Willow Creek-North Fork Feather River	21.2	5	20.1	10	0.47	4	High	1	1	Low	Moderate
67	Upper Red Clover Creek	11.8	5	0.0	2	0.03	3	Moderate	1	1	Low	Moderate
68	Rush Creek	1.6	1	1.4	6	0.25	3	Moderate	1	2	Mod	Moderate
69	Tollgate Creek-Spanish Creek	12.0	4	0.1	4	-0.28	2	Moderate	1	3	High	Moderate
70	Ward Creek-Indian Creek	6.2	1	0.0	2	-0.18	2	Low	1	3	High	High
71	Dixie Creek	11.0	5	2.4	8	0.22	3	High	1	1	Low	Moderate
72	Dogwood Creek-Middle Fork Feather River	6.3	2	0.0	2	-0.82	1	Low	1	1	Low	Low
73	Lower Red Clover Creek	24.4	5	0.0	2	-0.79	1	Low	1	2	Mod	Low
74	Soda Creek-East Branch North Fork Feather River	10.3	1	0.0	2	0.18	3	Low	1	3	High	Moderate
75	Lake Almanor	0.5	1	0.0	4	-0.83	1	Low	3	1	High	Moderate
76	Hough Creek-Indian Creek	35.1	5	0.0	2	0.28	3	Moderate	1	3	Mod	Moderate
77	Meadow Valley Creek	2.3	3	0.3	4	0.09	3	Moderate	1	1	Low	Moderate
78	Lower Lights Creek	11.0	5	0.0	2	-0.30	2	Moderate	1	2	Mod	Moderate
79	North Channel Little Last Chance Creek	34.4	4	10.2	10	0.44	4	High	1	1	Low	Moderate
80	Poison Creek-Last Chance Creek	23.0	5	2.0	8	-0.52	1	High	1	2	Mod	High
81	Louse Creek-North Fork Feather River	4.1	4	4.0	8	1.00	5	High	1	1	Low	Moderate

Wshd #	Subwatershed	Establishment							Introduction			Subwatershed Rating
		Range Miles	Range Rating	T/S Miles	T/S Rating	Rds Score	Rds Rating	Rating	Angling	Proximity	Rating	
82	Frazier Creek-Middle Fork Feather River	15.3	2	5.7	8	0.06	3	Moderate	1	1	Low	Moderate
83	Bucks Creek	2.1	3	1.1	6	-0.59	1	Moderate	3	2	High	High
84	Mud Creek-Frontal Lake Almanor	0.4	1	0.3	4	1.00	5	Moderate	1	1	Low	Moderate
85	Town of Loyalton	10.8	5	0.0	2	0.03	3	Moderate	1	1	Low	Moderate
86	Silver Creek-Spanish Creek	4.9	3	2.8	8	-0.55	1	Moderate	1	2	Mod	Moderate
87	Willow Creek	4.5	4	3.4	8	0.52	4	High	1	1	Low	Moderate
88	Carman Creek	27.1	5	8.6	10	-0.23	2	High	1	1	Low	Moderate
89	Hosselkus Creek	0.9	3	0.0	2	-0.74	1	Low	1	2	Mod	Low
90	Poplar Creek-Middle Fork Feather River	13.6	3	13.2	10	0.64	4	High	2	1	Mod	High
91	Butt Valley Reservoir-Butt Creek	7.5	2	7.5	10	0.97	5	High	3	2	High	High
92	Jamison Creek	1.4	1	0.2	4	-0.35	1	Low	3	1	High	Low
93	Little Grizzly Creek	4.4	1	4.1	8	-0.60	1	Moderate	1	2	Mod	Moderate
94	Rock Creek- Spanish Creek	6.4	1	6.4	10	0.68	1	Moderate	1	1	Low	Moderate
95	Washington Creek-Middle Fork Feather River	7.0	2	3.9	8	-1.00	1	Moderate	1	1	Low	Moderate
96	Clear Creek-North Fork Feather River	4.1	1	4.1	8	0.61	4	Moderate	1	2	Mod	Moderate
97	Middle Lights Creek	2.5	3	0.4	4	0.98	5	Moderate	1	3	Mod	High
98	Estray Creek-Greenhorn Creek	1.3	1	0.1	4	1.00	5	Low	1	1	Low	Low
99	Jackson Creek-Middle Fork Feather River	5.3	1	5.3	8	0.58	4	Moderate	1	1	Low	Moderate
100	Bear Creek	0.1	1	0.1	4	-0.48	1	Low	1	1	Low	Low
101	Milk Ranch Creek-North Fork Feather River	10.9	2	0.0	4	-0.84	1	Low	1	3	High	Low
102	Correco Canyon	6.8	1	0.0	2	-0.66	1	Low	1	1	Low	Low
103	Hungry Creek	0.7	1	0.6	4	-0.13	2	Low	1	3	High	High
104	Marian Creek-Frontal Lake Almanor	9.2	1	7.0	10	1.00	5	High	1	1	Low	Moderate
105	Turner Creek	7.6	4	4.3	8	-0.07	2	High	1	1	Low	Moderate
106	Badenaugh Canyon-Smithneck Creek	1.1	1	1.1	6	0.08	3	Moderate	2	1	Mod	Moderate
107	Bear Valley Creek-Smithneck Creek	15.4	5	0.1	4	1.00	5	High	1	1	Low	Moderate
108	Lemon Canyon-Perry Creek	18.3	5	0.9	4	0.56	4	Moderate	1	1	Low	Moderate
109	Hamlin Creek	3.2	4	0.0	2	-0.37	1	Low	1	1	Low	Low

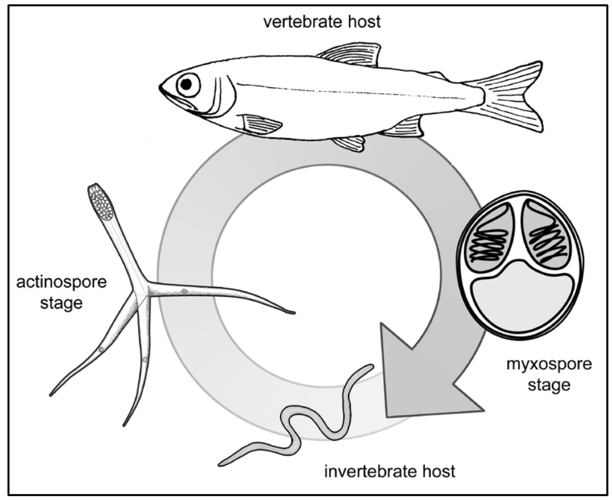
Wshd #	Subwatershed	Establishment								Introduction				Subwatershed Rating
		Range Miles	Range Rating	T/S Miles	T/S Rating	Rds Score	Rds Rating	Rating		Angling	Proximity	Rating		
110	Bonta Creek-Cold Stream	1.7	3	1.7	6	0.49	4	Moderate		1	1	Low		Moderate

APPENDIX C - Whirling Disease Risk Assessment: Meadow Restoration Projects

Sierra Institute for Community and Environment and Feather River Chapter, Trout Unlimited (August 2020)

Whirling Disease: the *Myxobolus cerebralis* Life Cycle

Whirling Disease causes black-tail, cranial and spinal deformities, rapid circular swimming behavior (i.e. “whirling”), and mortality in susceptible salmonids. The etiological (disease-causing) agent of Whirling Disease is the myxozoan parasite *M. cerebralis*. *M. cerebralis* has a multiple host life cycle with two obligate hosts: the oligochaete *Tubifex tubifex* and most salmonid species (Whipps et al., 2004). The life stages of the pathogen are 1) the myxospore which develops and multiplies primarily within the cartilage of salmonid hosts and 2) the triactinomyxon (TAM) which develops and multiplies within the epithelial lining of the digestive tract of *T. tubifex* hosts.



Overview

M. cerebralis was first detected in the Upper Feather River Basin in 1984 and surveys have detected *M. cerebralis* in the Basin since. Likely areas within the Basin for *M. cerebralis* presence include low gradient meadows. These areas support the environmental characteristics that allow *M. cerebralis* to complete its life cycle and become established in a system. Previously, risk assessment models have been used to evaluate the probability of *M. cerebralis* occurrences in aquatic systems using known contributors to *M. cerebralis* introduction (vectors) and the ability of the pathogen to establish (suitable environmental conditions). This assessment uses known contributors of *M. cerebralis* introduction and establishment to evaluate the relative risk of a potential project. In turn, the relative risk is used to guide the design of meadow restoration projects. This risk rating includes uncertainty associated with *M. cerebralis* presence by considering pathogen presence (or absence) at the project site within the project subwatershed and adjacent subwatersheds. Since low gradient streams provide suitable habitat for both *M. cerebralis* and its hosts, the best way of demonstrating low project risk is to demonstrate that the pathogen is not present at the project site or in nearby waters. Contributors to *M. cerebralis* introduction and establishment risk are discussed below, followed by a diagram of the assessment. Use the risk rating to inform meadow restoration project design.

Risk Contributors

M. cerebralis Vectors

Recreation Traffic

M. cerebralis life stages can be transported on equipment or within water or sediment collected in or on recreational equipment (waders, watercraft, etc.), as well as through movement of infected fish and fish parts.

Salmonid Planting
The movement of hatchery-reared fish has played a large role in the introductions of *M. cerebralis* throughout North America, and is the only vector established to have a causal link with *M. cerebralis* introductions.

Fish Movement
Infected fish can move myxospores. Increased fish movement may be associated with improved habitat connectivity, which is, in turn, associated with increased risk. Therefore, complete barriers to fish movement (no upstream fish movement), such as dams and many culverts, can prevent the spread of the pathogen from downstream sources. Only complete barriers should be considered in this assessment since infected fish movement and introductions can occur with partial barriers. For any fish passage component of a project, *M. cerebralis* surveys to address pathogen presence and the extent of establishment above and below the barrier should be considered (Zielinski & Bartholomew, 2009).

M. cerebralis Presence and Proximity to Positive Detections
Waters with *M. cerebralis* detections present the highest risk for salmonid population impacts. Waters near *M. cerebralis* detections present lesser risk. The project area can be exposed to *M. cerebralis* infections in nearby systems through downstream TAM movement and upstream or downstream fish movement. Birds, mammals and anglers can transport the pathogen between unconnected systems. The assessment assumes this risk is higher when infections are present in waters close to the project area.

***M. cerebralis* Establishment**

Host Presence
The assessment assumes susceptible salmonid species are present in low gradient streams associated with meadows. This is based on widespread presence of multiple trout species throughout the Feather River Basin. *T. tubifex* is also be assumed to be widespread, and meadow environments are known to facilitate desirable habitat conditions for the worm. Lentic habitats provide optimal *T. tubifex* habitat.

Permissive Environment
The environment most conducive to *M. cerebralis* establishment is characterized by a combination of low velocity flow (stagnant or still water), fine sediment substrate (silt/clay), and temperatures within the optimal ranges for TAM and myxospore development. Slow velocity flow and fine sediment substrate are preferred habitat of *T. tubifex* and facilitate *M. cerebralis* and host overlap. Meadow streams provide suitable substrate and flow characteristics, and they are considered high risk if permissive temperature conditions also exist. Organic enrichment from livestock activity facilitates high densities of *T. tubifex* hosts and has been previously associated with *M. cerebralis* presence.

Results from the NorWeST Stream Temperature Regional Database and Modeling Procedure (Isaak et al., 2016) were used to determine risk associated with stream temperature. Stream segments with projected average August stream temperatures between 12 and 15ºC are considered high risk. Segments with this condition are highlighted in red on the map on page 5. This temperature range was chosen because it represents the overlap between the optimal stream temperatures required for myxospore and TAM development. The average August stream temperatures metric from the NorWeST Model is considered an approximate measure of the permissive temperature regime as specific numbers of degree days are needed for pathogen development. August often represents a period of high salmonid infection risk due to TAM release from *T. tubifex* hosts being triggered by warmer water temperatures.

Instructions

1. Follow the Meadow Restoration Project Risk Categorization Diagram to assess risk via pathogen presence and permissive environment, while addressing uncertainty.

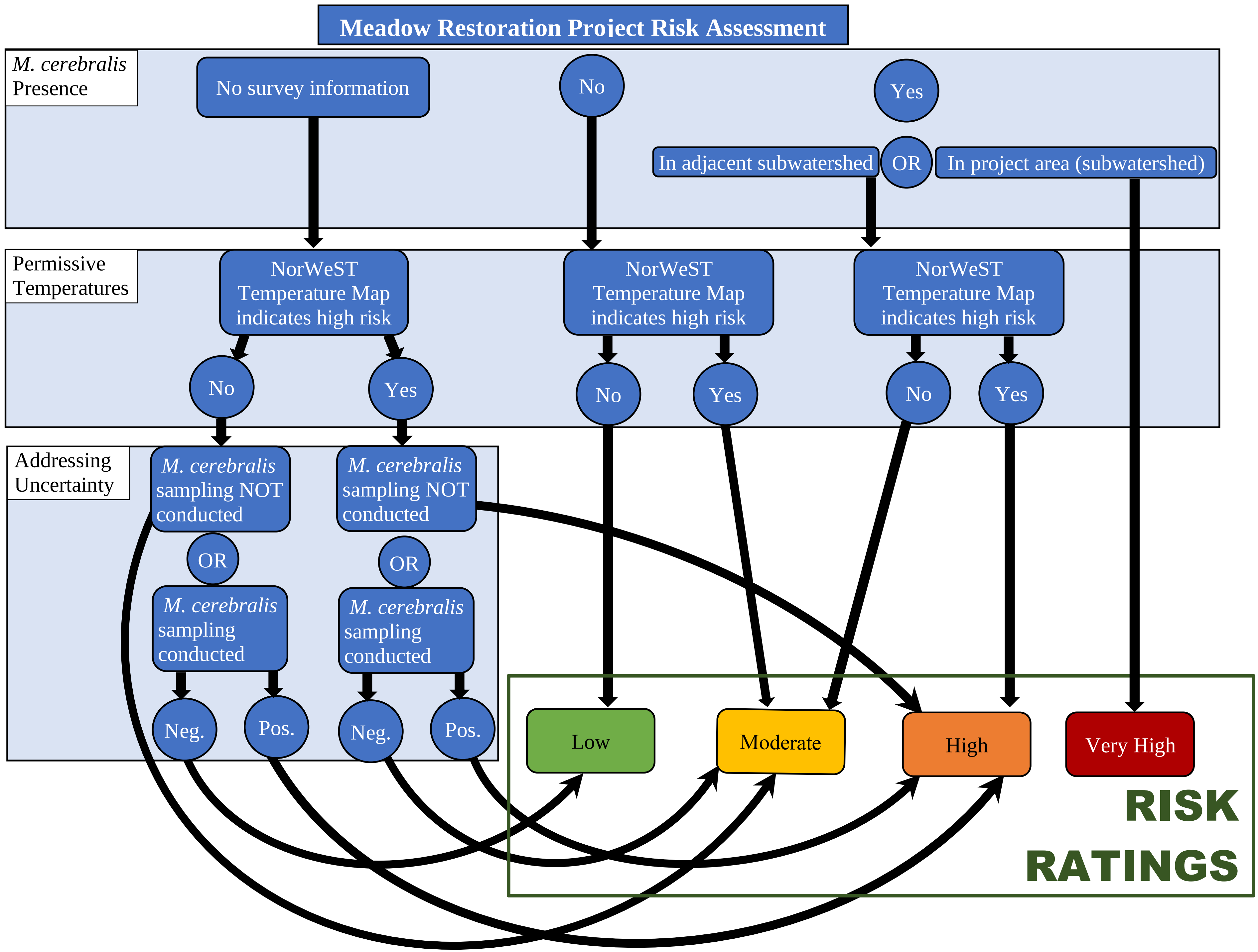
    a. *M. cerebralis* detections: use previous survey information to determine if the pathogen has been detected in the project area. A "no" result is based on negative tests conducted within the past two years.

    b. Permissive temperatures: use the Upper Feather River Basin NorWeST Stream Temperature Model on page 5.

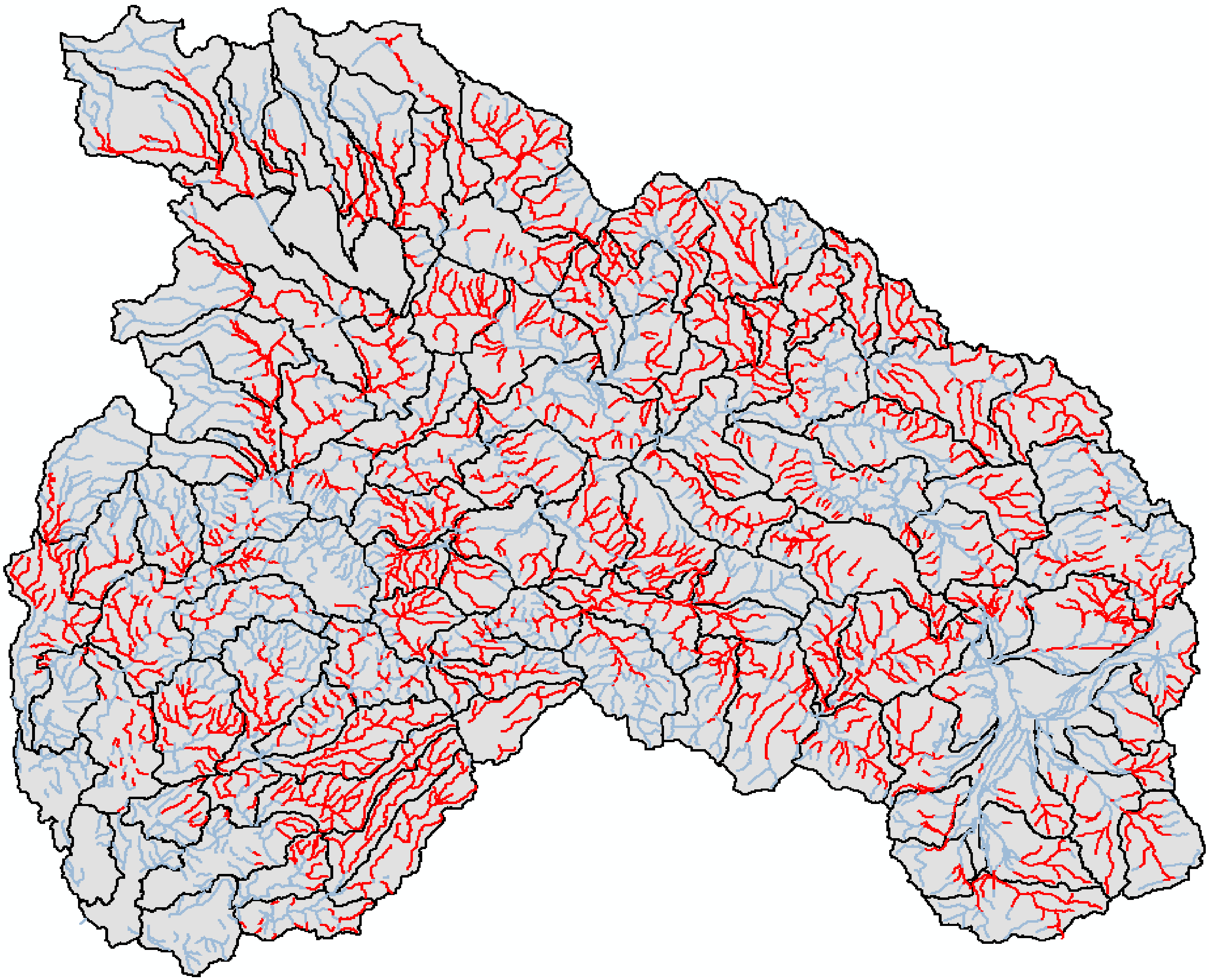
    c. Uncertainty: uncertainty associated with *M. cerebralis* presence can be addressed by conducting surveys for the pathogen.

2. Review the General Risk Modifiers ***and*** Fish Passage Risk Modifiers to determine additional contributors on project risk.

3. Based on risk level (low, moderate, high, very high), consider Meadow Restoration Design Considerations to avoid or reduce risk. Recommendations were based on the influence of flow velocity on substrate and the *M. cerebralis* life cycle (Hallett & Bartholomew, 2008).



Meadow Restoration Project Risk Assessment
M. cerebralis Presence
No survey information
No
Yes
In adjacent subwatershed
OR
In project area (subwatershed)
Permissive Temperatures
NorWeST Temperature Map indicates high risk
No
Yes
NorWeST Temperature Map indicates high risk
No
Yes
NorWeST Temperature Map indicates high risk
No
Yes
Addressing Uncertainty
M. cerebralis sampling NOT conducted
OR
M. cerebralis sampling conducted
Neg.
Pos.
M. cerebralis sampling NOT conducted
OR
M. cerebralis sampling conducted
Neg.
Pos.
Low
Moderate
High
Very High
RISK RATINGS



NorWeST Stream Temperature Model Risk Map for the Upper Feather River Watershed: the stream segments with temperatures between 12 and 15ºC are highlighted in red to indicate high risk. If the project area is within the red segments, it is high risk for this factor. Reservoirs are not included in the map.

Risk Modifiers

<u>General Risk Modifiers</u>

If one or more of the following are applicable to the project area, increase risk rating category by one (e.g., Meadow Restoration Project Risk Assessment value of "Moderate" within a range allotment would be categorized as "High").

There will be livestock in stream channels or riparian areas post implementation.

Planted salmonids can access the project area (i.e. there are no complete fish barriers between a downstream stocking location and the project area or fish are stocked upstream of the project area).

The project plan is designed to increase recreational use. Specifically, the project plan will improve or add campgrounds, boating launches, and/or other features which encourage recreational use.

M. cerebralis decontamination protocols will not be utilized during implementation.

<u>Fish Passage Risk Modifier (for complete barriers only)</u>

If fish passage improvements are planned for the project area – increase risk category by one if:

M. cerebralis surveys have not been conducted in the project area.

M. cerebralis surveys are conducted in the project area and:

1. *M. cerebralis* is detected below, but not above, a fish barrier.
   OR
2. *M. cerebralis* is detected above and below a barrier, but the extent of *M. cerebralis* establishment and/or infection risk above a barrier is very low compared to below the barrier.

Meadow Restoration Design Considerations by Risk Categories:

Very High:
The project planners should consider other project locations.
The project design should not result in an increase in ponds or other lentic habitat.

The project design should reduce low velocity habitat and sediment delivery by at least

High:
The project design should result in a reduction of low velocity habitat and sediment delivery.

Moderate:
The project design should not increase the amount of low velocity habitat or sediment delivery.

Low:
No Whirling Disease design considerations are necessary.

Acknowledgements

Michael Kossow of Trout Unlimited and Kyle Rodgers of Sierra Institute for Community and Environment provided essential guidance, support, and assistance in the formulation and finalization of this Meadow Restoration Project Risk Assessment Guide.

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