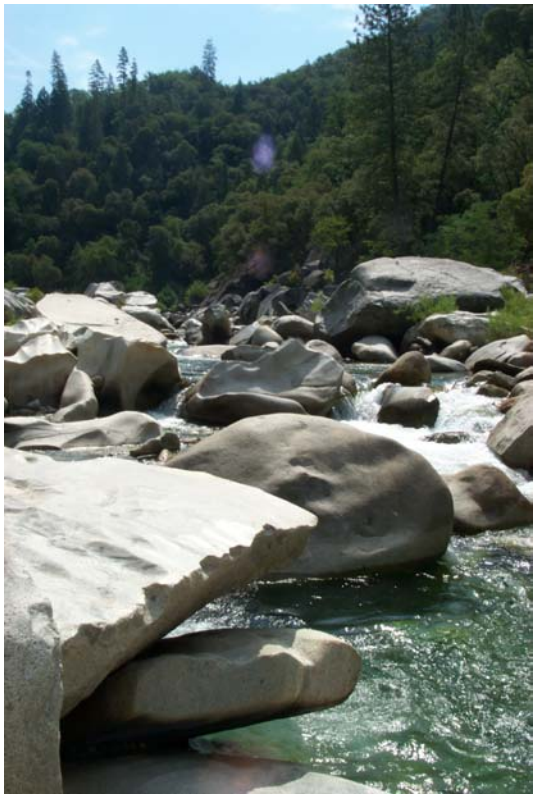
DRAFT REPORT
Fish Health Monitoring of Fall Chinook and Steelhead in the Yuba and Feather Rivers (2002-2003)



Kimberly True
United States Fish and Wildlife Service
California-Nevada Fish Health Center

Oroville Facilities Relicensing Environmental Work Group
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CONSERVING AMERICA'S Fisheries

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ABSTRACT

The California- Nevada Fish Health Center, with the support of the California Department of Fish & Game (CDF&G) and the California Department of Water Resources (DWR), conducted fish health surveys in the Yuba and Feather Rivers to determine what major fish pathogens are present in the watershed and how they are distributed geographically and among different fish species.

Of particular interest is the presence or absence of the fish virus, Infectious Hematopoietic Necrosis Virus (IHNV), which is endemic to the Sacramento basin and a significant fish pathogen of cultured and wild chinook salmon (*Oncorhynchus tshawytscha*). Unique strains of IHNV, FR2 and FR3, have been identified in the Feather River State Fish Hatchery (FRSFH) production program in recent years (Pers.comm Dr. Bill Cox, CDFG 2003). These unique viral strains caused higher rates of mortality in hatchery-reared Steelhead (*Oncorhynchus mykiss*) and there is concern that virulent strains of IHNV could impact natural populations of steelhead and chinook in the Feather and Yuba Rivers.

Infectious Hematopoietic Necrosis virus was not detected in over 1,500 juvenile fish tested in the Feather and Yuba Rivers. Virology was performed primarily on fall chinook juveniles, a limited number of steelhead, and common non-salmonid fish species. Other fish pathogens including *Renibacterium salmoninarum, Yersinia ruckeri, Aeromonas hydrophila, Pseudomonad spp* and *Ichthyopthirius multifilis, and Lernaea* were detected in juvenile fish. *Pseudomonad* infections in hardhead (*Mylopharodon conocephalus*) in the Yuba River in 2003 and *Ichthyopthirius multifilis* detected in juvenile fall chinook in the Feather River in 2003 occurred at significant numbers of organisms, or high prevalence levels in fish populations, to pose a health risk for these species.

IHN viral testing was conducted on returning fall chinook adults sampled during carcass surveys on Feather River, Yuba River and Clear Creek from Oct-Nov 2003. IHNV was detected in fall chinook adults from the Feather River at an incidence of 45.6%, from the Yuba River at 27.8%; and in Clear Creek at 45.6%. Historical data for Coleman NFH (Battle Creek) is also reported as a mean incidence of IHNV of 55.5% from the sampling period of 1993-2003.

Dr. Ron Hedrick of the University of California, Davis (UCD) is in the process of strain typing the viral isolates obtained from adults tested in this study using polyclonal antibodies developed against the FR2 and FR3 IHNV strains. This work will determine if the endemic strain of IHNV or more virulent strains (FR2 and FR3) are present in the adult chinook populations. Antibody studies will be followed by sequence analysis of the G gene in a subset of the viral isolates.

STATUS OF FISH POPULATIONS

The Yuba and Feather Rivers are major tributaries to the Sacramento River and are essential watersheds in California for natural production of Chinook salmon and steelhead. CALFED, along with numerous stakeholders are in the process of evaluating the feasibility of removing dams that block adult salmon migration. Removal of existing dams could open up large amounts of natural habitat, thereby increasing natural production by chinook salmon and steelhead, and potentially aiding in the rate of recovery for these important species.

Declining chinook populations in the Central Valley have prompted intense restoration efforts in the Yuba River as Chinook salmon are a valuable resource and key element of the State's aquatic biodiversity. The lower Yuba River supports fall, late-fall, and spring-run (state and federally listed threatened) Chinook salmon and steelhead trout (state and federally listed threatened).

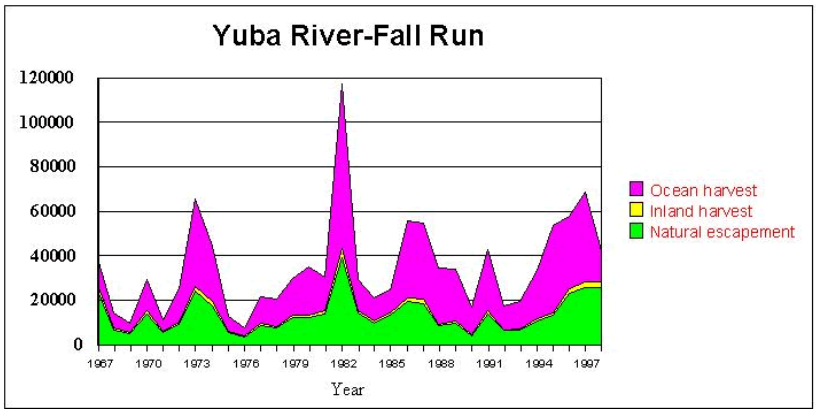
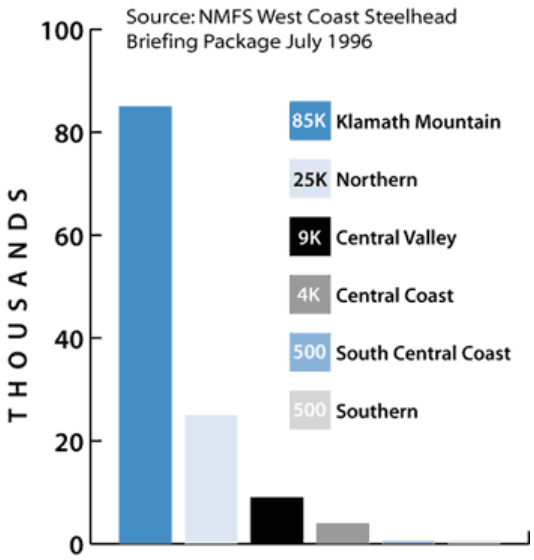


Figure 1. Fall Chinook natural production and escapement.
Source: Anadromous Fish Restoration Program (AFRP) website.

The Yuba River supports a self-sustaining population of steelhead and is essentially the only wild steelhead fishery remaining in the Central Valley (AFRP 2004). Statewide, numbers of steelhead have fallen to less than 50% of their populations of 30 years ago. Sacramento and San Joaquin River system populations are significantly reduced to fractions of their 1960's levels with dams blocking 90% of their spawning habitat. A 1996 briefing by National Marine Fisheries Service (NMFS), a division of the National Oceanic and Atmospheric Administration (NOAA), estimated the total run size of steelhead for the Central Valley Evolutionary Significant Unit (ESU) to be approximately 9000 (CalTrout 2004).



Wild stocks are mostly confined to upper Sacramento River tributaries such as Deer, Mill and Antelope Creeks and the Yuba River. The National Marine Fisheries Service has listed steelhead as Threatened or Endangered in nearly every river they inhabit within California. Steelhead are listed as Threatened in the Yuba River (ESU12 – Central Valley).

Figure 2. Estimated California Steelhead Populations by ESU
Source: Caltrout website.

Multi-year, comprehensive fish health assessments are needed in the Yuba and Feather Rivers to ascertain the distribution and effect of fish disease on anadromous and resident fish populations in these important watersheds. Health and fitness of juvenile salmon out-migrants are major determinates of their performance and ability to survive early ocean rearing. Infectious disease during the critical out-migrant period can exhaust energy reserves and impair immune function. Disease can influence survival directly (mortality) and indirectly by affecting fitness during early ocean rearing (predator avoidance, saltwater adaptation, etc.). It is also important to understand the health status of resident populations in the Yuba and Feather River. Resident trout and non-salmonid species can act as disease carriers or reservoirs of infection than can affect juvenile salmonids, as well as returning adults.

The information obtained from this study provides baseline data for fish health management by providing a better understanding of pathogen prevalence and physiological condition of Chinook and steelhead juveniles and adults in the Yuba and Feather River. This information can assist the CALFED Bay Delta program and stakeholders in determining the current risks associated with fish disease. The potential impacts to the health of anadromous fish populations under current conditions will provide a baseline for any future watershed restoration activities.

In addition to supporting CALFED's feasibility studies under the Upper Yuba River Science Program, this study builds on data collected from natural fish populations under the USFWS-National Wild Fish Health Survey (NWFHS). The NWFHS is a national fish health initiative to determine the prevalence and distribution of major fish pathogens in wild fish populations throughout the United States. Fish health data is maintained in a national database established in 1998 and maintained by Montana State University Environmental Statistics Group. Fish health data is accessible, via the internet, to researchers, resource managers, stakeholders, and the public to allow queries of fish health findings by geographical area (Hydrological Unit Code or HUC), species, or positive pathogen findings. The NWFHS website can be accessed at http://wildfishsurvey.fws.gov/

Study Objectives

The objectives of this study are:

1. Determine the presence and distribution of major fish pathogens in the Yuba and Father River in natural Fall Chinook, steelhead and non-salmonid fish populations (Table 1). Pathogens of interest include the major diseases of salmonid fish species and pathogens of regional interest (Table 2).

2. Compare pathogen distribution in natural fish populations in the Yuba and Feather River and assess risks associated with known pathogen epizootics at Feather River SFH.

3. Determine if a unique strain of IHNV (FR –Type 2) detected at Feather River State Fish Hatchery (FRSFH) in 1999 and 2000 is present in natural chinook and steelhead juvenile, and adult populations.

4. Determine the prevalence of IHNV in returning fall chinook adults in the Feather and Yuba Rivers.

5. Determine the prevalence of IHNV in fall chinook adults returning to Clear Creek and Battle Creek (from hatchery monitoring records) to compare IHNV prevalence in the upper Sacramento basin to the Yuba and Feather River watershed.

6. Assist with strain-typing analysis of all IHN viral isolates collected during the study. Submit viral isolates to Dr. Ron Hedrick of UCD to perform serological strain typing of viruses to determine distribution and movement of viral serotypes within the basin.

7. Include pathogen survey data from the Yuba and Feather River in the National Wild Fish Health Survey database to provide baseline fish health information about these important watersheds.

Table 1. Non-salmonid fish species examined in the Feather and Yuba River.

Fish Species	**Common Name**
Mylopharodon conocephalus	Hardhead
Micropterus dolomieu	Smallmouth bass
Lepomis macrochirus	Blue gill
Catostomus occidentalis	Sacramento sucker
Notemigonus crysoleucas	Golden shiner

Table 2. Major fish pathogens and diseases tested for under the National Wild Fish Health Survey (NWFHS).

Fish Pathogens	**Fish Disease Common Name or Abbreviation**
Infectious Hematopoietic Necrosis virus	IHN
Infectious Pancreatic Necrosis virus	IPN
Viral Hemorrhagic Septicemia virus	VHS
Oncorhynchus Masou virus[1]	OMV
Largemouth Bass virus[2]	LMBV
Renibacterium salmoninarum	Bacterial Kidney Disease (BKD)
Yersinia ruckeri	Enteric Red Mouth (ERM)
Aeromonas salmonicida	Furunculosis
Flavobacterium columnare[3]	Columnaris
Edwardsiella ictaluri[2]	Enteric Septicemia
Ceratomyxa shasta[3]	Salmonid Ceratomyxosis
Myxobolus cerebralis	Whirling Disease
Bothriocephalus acheilognathi	Asian Tapeworm
Other Parasites[4]	Parasitic infections
Numerous ectoparasites and internal parasites and associated diseases[4]: *Ambiphyra, Epistylis, Chilodonella, Ichthyobodo necatix (Costia) , Ichthyopthirius multifilis(ICH), Gyrodactylus, Lernaea (anchor worm), Nanophyetus (salmon poisoning fluke).*	

Note: **1. Not found in North America to date.**
2. Family or species-specific pathogen tested for if species are examined (Largemouth bass and catfish were not collected in this study).
3. Pathogen of Regional Interest (PRI) under the NWFHS.
4. External and internal parasites detected by microscopic examination and/or histology.

FISH PATHOGENS AND DISEASE TRANSMISSION

Disease is the culmination of various defects, abnormalities, deficiencies and injuries as they occur at the cellular and tissue level resulting in clinically apparent dysfunction. Cellular injury leads to changes in structure and function of tissues and organs. The changes in function are recognized as symptoms or clinical signs. The more subtle changes in structure at the cellular and tissue level are recognized as morphological lesions and histological changes. Disease can result from infectious agents, nutritional deficiencies, toxicants, environmental factors, or genetics (Plumb 1994).

Infection versus Disease

Infectious disease involves the reproduction and transmission of a causative organism (virus, bacteria, parasite, and fungi) from one host to another, resulting in an abnormal number of animals becoming infected. When effects and/or numbers of the organism result in impaired physiological function or performance, the infection has progressed to an epizootic disease. Endemism is the continued presence or persistence of a pathogen in a population or geographical area and may, or may not, be accompanied by clinical signs of disease. Infectious organisms are of two basic types: obligate and facultative. Obligate pathogens require a fish host to reproduce and survive for any length of time. Facultative organisms occur in the environment where they can survive and reproduce freely.

It is important to differentiate between infection – the presence of an infectious organism, and the conditions that manifest as disease. Fish may be infected, and carriers of an organism without being diseased. Many organisms are normally present in the aquatic environment, but do not cause disease. Under certain conditions, when an environmentally induced host-pathogen imbalance occurs, facultative organisms infect a normally resistant host and cause adverse pathology (Plumb 1994). Because disease is the interruption or dysfunction of normal physiological processes that are necessary for growth and survival, it generally involves major organs or primary physiological function (i.e. normal respiration by the gills, ion exchange in the kidney, etc).

Hatchery and Wild Fish Interactions

Hatchery and wild fish interactions can be controversial topics in fish health and natural resource management. While disease in the hatchery setting is often initiated by transmission of pathogens from wild fish (Oliver 2000,Wedemeyer 1996), hatchery dynamics can amplify disease and may contribute to increased numbers of pathogens in the natural environment. The specific mechanisms related to disease transmission between hatchery and wild fish are poorly understood.
It is important to study disease interactions between hatchery and wild fish in both controlled laboratory studies and in the natural environment in order to define the risks associated with hatchery diseases to natural fish populations.

Disease susceptibility to infectious organisms differs for wild fish in a natural environment compared to the artificial conditions that exist in a hatchery setting.
Stress is the most significant factor in the hatchery setting, and plays a major role in susceptibility to fish pathogens. Fish reared in hatchery settings are exposed to adverse environmental conditions including elevated temperatures, poor water quality, high density and frequent handling. These factors taken individually, and especially cumulatively, can cause significant strain on the defense mechanisms of the immune system. Many diseases of hatchery fish are associated with, or enhanced by, the stress associated with hatchery environment.

Wild fish are typically in equilibrium with endemic pathogens when environmental conditions are relatively normal, and natural outbreaks are rarely observed (Wedemeyer 2001). However,

environmental degradation and elevated water temperatures in natural settings pose many of the same stressors experienced by hatchery fish. It is equally important to our understanding of fish disease interactions to profile the health status of natural chinook and steelhead in order to understand what factors contribute to infection and disease progression. By understanding fish health in both hatchery and natural populations, we can determine what, if any, impacts hatchery fish may have on natural populations.

IHNV Transmission Studies

The U.S. Fish and Wildlife Service (USFWS) through the California-Nevada Fish Health Center (Ca-Nv FHC) has studied IHNV transmission at Coleman National Fish Hatchery (CNFH) for several years to determine the risks to natural fish populations below the hatchery in Battle Creek and in the Sacramento River. While experiments conducted on clinically infected Chinook indicated significant numbers of viral particles are shed from infected fish, disease transmission to healthy fish has been difficult to demonstrate (unpublished data, Foott and True 1996).

Surveys of 377 natural fall chinook fry in the upper Sacramento River in 1996 (Foott 1996) and 203 fall chinook sampled from the Red Bluff Diversion Dam RST in 1997 (True, unpublished data) did not detect IHNV, indicated this virus is not common in juvenile chinook.

Further studies to determine the minimum age that fish become infected with IHNV indicate that Chinook salmon become susceptible to infection by virus as yolk-fry. While young fish may be infected with low levels of viral agents, fry did not develop signs of clinical disease, nor progress in their infection levels over an 8 week period. This study also demonstrated that groups of fish subjected to stress mimicking hatchery handling did have increased titers of virus by approximately 10-fold compared to non-stressed groups. Even with higher viral titers, stressed fish failed to develop disease in two studies repeated over a 16 week period (unpublished data, True 1999).

More recent studies (Foott 2000) designed to mimic hatchery and wild fish interactions employed natural fish that were placed in co-habitation with fish artificially-infected with IHNV. Natural fish did not become infected or diseased despite a study design that included manipulations with the density of fish in individual units and the ratios of infected fish to natural fish per tank.

In summary, the Ca-Nv FHC has conducted several studies to determine the transmissibility of IHNV to natural chinook. Fall chinook have not demonstrated a high degree of susceptibility to infection with IHNV at the viral doses that would be expected in a river environment. Abstracts of these studies can be found in Appendix C, or on the Ca-Nv FHC website (http://www.r1.fws.gov/canvfhc/nwfhsman.htm).

Viral Strains and Genetic Analysis of Viral Traffic

Infections with IHNV have resulted in significant mortality at Feather River State Fish Hatchery (FRSFH) during early operation of the facility and up until the late 1970's (Pers. comm. Tresa Veck, CDFG 2004). IHNV epizootics have occurred more recently in 1998, 2000, 2001, causing significant losses of chinook salmon (DWR 2001). In 2000, a unique strain of IHNV, FR Type 2, was isolated from wild chinook exhibiting clinical signs below the FRSFH in snorkel surveys conducted by DWR. In 2002, FR Type 2 IHNV caused significant mortality in steelhead at FRSFH (Pers. comm. Dr.Bill Cox, CDFG 2002).

When unique or virulent strains of virus occur, genetic techniques tell much about the relatedness of a viral isolate to its predecessors in a watershed or larger geographical region. The literature contains several examples where genetic analysis is utilized to demonstrate the establishment of new viral strains or movement of existing strains in previously non infected chinook populations (Anderson 2000, Kurath 2003). Researchers at USGS-Western Fisheries Research Center in Seattle, Washington have also been successful in developing genetic techniques to analyze the phylogenetic relationship of viral isolates in the Columbia River basin (Kurath 1993). Previous work done by Dr. Kurath has shown dramatically different patterns of virus evolution under varying environmental conditions (Kurath 1999). This information can be analyzed from an epidemiological perspective to help identify viral sources following disease outbreaks, and demonstrate geographical movement of IHNV within a basin.

The close proximity of the Feather River to the Yuba watershed adds to the level of concern for potential impacts from IHNV, or other hatchery related diseases, to natural fish populations in this adjoining watershed. It is logical to hypothesize that IHNV can move both upstream and downstream in a watershed with migrating infected fish (Busch 1983, Groberg 1983). The primary concern is that this apparently virulent isolate could spread to other hatchery facilities and/or infect naturally produced steelhead in the Feather and Yuba Rivers. Therefore, for this study, it is important to fully characterize all IHNV isolates that are recovered from anadromous fish populations.

Dr. Ron Hedrick, University of California Davis (UCD) has been studying the FR Type 1 and 2 strains of IHNV to gain a better understanding of virulence factors and susceptibility of different salmonid hosts. Viral isolates obtained in this study will be submitted to UCD for strain typing analysis. Dr. Hedrick's laboratory will utilize polyclonal antibodies in serum neutralization assays, and has developed 3 antibodies against the IHNV type strain for the Sacramento River and the two unique FR strains. Sequence analysis of the mid G gene of the virus will also be preformed on a subset of the viral isolates. This strain typing work is an important step to understanding the prevalence, distribution and potential movements of IHNV within the basin.

Importance of Fish Health Monitoring

It is important to establish a baseline of the prevalence and distribution of IHNV and other significant fish pathogens in a watershed. Baseline information and continued monitoring is necessary to determine the changes that occur in prevalence and distribution of pathogens both spatially (geographical distribution and movement) and temporally (seasonality). Identification of strain types of IHNV by molecular methods can provide information about changes in the viral genome and virulence (LaPatra 1993a) . Molecular tools have been an extremely useful management tool in the Columbia River and similar work should be performed in the Sacramento basin.

For endemic pathogens such as *Infectious Hematopoietic Necrosis virus*, *Renibacterium salmoninarum* and *Ceratomyxa shasta*, the likelihood that these pathogens will persist is high now that they are established in the region. The severity of infection and distribution in the Yuba River is not well known (Pers.comm. Dr. Bill Cox, CDFG 2002). For facultative, or opportunistic organisms, environmental conditions can play a much larger role in the spatial and temporal changes that are observed. Comprehensive fish health monitoring can provide current data on fish diseases and help to elucidate the environmental conditions that lead to impaired fish health and disease susceptibility.

MAJOR FISH PATHOGENS

Specific pathogens that are vertically transmitted, difficult to control, or that become endemic in a fish population or watershed are of major concern. Significant diseases for salmonids include: Infectious Hematopoietic Necrosis (IHN) virus, *Renibacterium salmoninarum* (Bacterial Kidney Disease) and *Ceratomyxa shasta*. These pathogens are endemic to the Sacramento basin and have been routinely isolated from juvenile and adult chinook salmon at federal and state fish hatcheries including Coleman National Fish Hatchery (CNFH) on Battle Creek and the Feather River State Fish Hatchery (FRSFH) in Oroville.

Infectious Hematopoietic Necrosis Virus (IHNV)

Infectious hematopoietic necrosis virus (IHNV) is a significant viral pathogen of both cultured and wild salmon and trout throughout western North America (Williams and Amend, 1976; Groberg and Fryer, 1983; Meyers et al., 1998). IHNV is the type species of the newly recognized aquatic rhabdovirus genus *Novirhabdovirus* (Walker 2000) and causes acute disease in cultured and wild fish salmonids in both freshwater (Groberg and Fryer 1983, Williams and Amend 1976) and saltwater (Kent et al, 1988). IHNV is virtually endemic to all watersheds (Wolf 1988) in North America that support salmonid populations, and is endemic to chinook populations in several major rivers in Northern California (Sacramento, San Joaquin, and Feather River). IHNV is routinely isolated from adult stocks returning to state and federal hatcheries of the Sacramento basin and is routinely recovered from wild spawning salmonids with no clinical signs of IHNV(Mulcahy et al 1983, 1987. LaPatra et al. 1991a; Meyers1998). The virus is transmitted horizontally through the water column and vertically via egg surfaces. Epizootics in hatchery populations of Fall and Late-fall chinook cause significant losses. Steelhead juveniles are generally considered non-susceptible to the specific strain of IHNV (type IV) found in California, as compared to strains found elsewhere in the Pacific Northwest, with the exceptions noted for the FR-2 strain.

***Renibacterium salmoninarum* - Bacterial Kidney Disease (BKD)**

Another significant fish disease, termed Bacterial Kidney Disease (BKD), is caused by the gram-positive bacteria *Renibacterium salmoninarum* and is vertically transmitted, within the ova, from the female adult to progeny. *Renibacterium salmoninarum* causes Bacterial Kidney Disease (BKD) in both hatchery and natural salmonid stocks (Fryer, 1981 and Bullock, 1988) and is a chronic infection which can be systemic but primarily impairs normal kidney function. The most significant impairment caused by this bacterium is the inability of infected smolts to osmoregulate properly as they transition to salt water, resulting in smolt mortality and/or poor early ocean survival.

Ceratomyxa shasta - Ceratomyxosis

The parasite *Ceratomyxa shasta* is included in the taxonomic class *Myxosporea* (Hoffman 1999) and causes the disease referred to as Ceratomyxosis in salmonids. The parasite causes acute inflammation of the intestine and visceral organs leading to death (Bartholomew 1989). *C.shasta* has a complicated life cycle with two life stages, which includes development of an actinosporean in the aquatic worm *Manayunkia speciosa* and a myxosporean stage that develops in salmonids. The distribution of the polycheate likely defines the geographical distribution of this pathogen (Bartholomew 2001).

OTHER PATHOGENS

A parasite of concern is another myxosporidean *Myxobolus cerebralis* which causes Whirling Disease (Markiw 1992b). This parasite has two life stages involving a polycheate worm (Tubifex tubifex) and salmonids. The parasite infects the epithelium of young fish, and then migrates

through neural tissue and the spinal column where it infects soft cartilage in the brain prior to bone ossification. The parasite undergoes asexual mitosis in the cartilage and forms myxosporean spores (Moeller 2001). While Whirling Disease has garnered much concern and study in the Rocky Mountain states, the effects of the disease have been less severe in California salmonid populations (Modin 1998).

YUBA RIVER

The Yuba River watershed is a major tributary to the Sacramento River system and essential water resource in Northern California. From it's headwaters in the Sierra Nevada, the river is comprised of three forks; the North, the Middle, and the South.

The forks flow west through rugged canyons toward their confluence in California's northern Central Valley. The North Fork flows first into New Bullards Bar Reservoir, then is joined by the Middle Fork 5 miles below the New Bullards Bar Dam. The South Fork is born from run off out of Lake Angela at Donner Pass in the Sierra Nevada, and runs 64 miles before joining the other two forks at Englebright Reservoir. In total, the watershed drains over 1,300 square miles before flowing into the Feather River at Marysville, CA. The Feather River then joins the Sacramento River as it heads toward the San Francisco Bay and the Pacific Ocean.

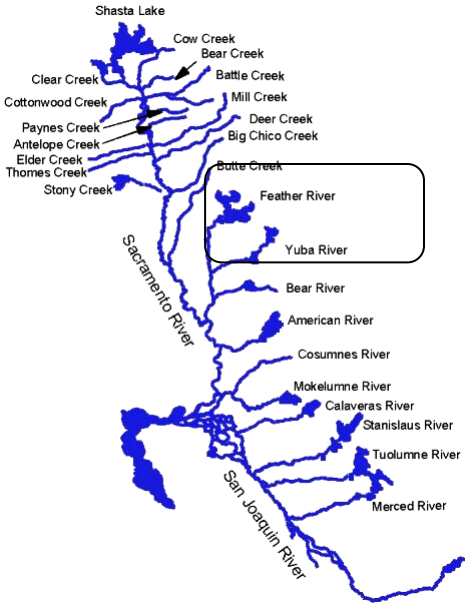


Figure 3. Map of Feather and Yuba Rivers in Sacramento River basin.

The Yuba watershed is a vital resource for wild chinook and steelhead fisheries, drinking and irrigation water for many municipalities, hydroelectric power, and recreational use in a 39-mile stretch of the South Yuba designated as Wild and Scenic in 1999.

Ecological Impacts to the Yuba Watershed

Hydraulic Mining

Historically, the Yuba was the most heavily mined basin in the Sierra Nevada. Mining was halted, due to severe environmental degradation, in the mid 1900's. The changes to normal hydrological function that occurred in the Yuba River as a result of the late 1800s hydraulic mining was one of the most dramatic environmental events in California history. Thousands of acre-feet of sediment entered the river channel, causing the Yuba River channel near Yuba City to rise 90 feet, which led to repeated flooding for decades (Mount 1954).

Effects of mining are still seen today in perturbed hydrology, heavy sedimentation and the accumulation of arsenic and mercury contaminants in certain tailings in the Yuba. Several of the dams on the Yuba were built to contain sediment or control flooding that resulted from hydraulic mining operations.

Dams

The Yuba River system has become one of the most dammed and diverted water systems in the Sierra Nevada. Twenty significant dams have been built throughout the watershed. The largest are the New Bullards Bar Dam, and Englebright Dam which forms the Englebright Reservoir.

The North Yuba begins below Yuba Pass near State Highway 49 at an elevation of 6701 feet. It runs near the state highway as far down the valley as Downieville, where it heads westward to New Bullards Bar Reservoir.

The Middle Yuba originates from snow runoff gathered at Jackson Meadows Reservoir in Sierra County. It flows through steep, narrow canyons to the 75 ft. Our House Dam, just south of Camptonville. There it is diverted into a 3.8 mile-long tunnel that conveys the water to Oregon Creek, as described in two agreements with the Federal Regulatory Commission (FERC) and California Department of Fish and Game(CDFG). Middle Yuba and Oregon Creek water is diverted into a second 1.2 mile-long tunnel that flows into New Bullards Bar reservoir.

Bullards Bar Reservoir is the 11th largest reservoir in California, holding nearly 1 million acre-feet of water from Upper Yuba, Middle Yuba and Oregon Creek. In the 16 mile-long reservoir, water from the North Yuba and Middle Yuba are combined and utilized for hydropower generation at New Colgate Powerhouse at Bullards Bar Dam, operated by the Yuba County Water Agency (YCWA). Bullard Bar Dam was built in 1969 primarily for flood control. Major flooding occurred in the valley near Marysville and Yuba City in 1950, 1955, 1964, 1986, and 1997.

South Yuba flows through Placer and Nevada counties as it is joined by numerous small and large creeks on its way to Bridgeport. There it reaches it's confluence with the Middle Yuba, and is joined by the North Yuba (out of New Bullards Bar Reservoir). The Yuba flows into Englebright Reservoir, which was created by the 180-foot Englebright Dam built by the U.S. Army Corps of Engineers (USACE) in 1941. The original purpose of the Englebright dam was to trap gold mining debris to keep them out of the lower river. However, it also completely blocks 39 miles of suitable fish habitat on the South Yuba and 16 miles on the North and Middle Yuba Rivers. Englebright Dam was retrofitted after its construction for hydropower generation. Water is moved through two tunnels through turbines at the Narrows 1 Powerhouse owned and operated by PG&E, and Narrows 2 Powerhouse which is owned and operated by Yuba County Water Agency.

The three rivers are combined as they flow through the Yuba Goldfields and reach the Daguerra Point Dam (built by USACE in 1906) 12.3 miles below Englebright Dam. Neither Englebright nor Daguerra Dam provide flood control: both dams were built as debris dams, however Daguerra Dam now serves as a diversion site for irrigation canals.

Non-Native Fish Species

Biologically, the Yuba River system, including the associated reservoirs, holds a number of cold and warm water fish species, many of which have been introduced and are popular for recreational fishing. Hydraulic mining in the Yuba watershed has greatly impacted the diversity and number of fish species present. Habitat was transformed from shady, pool and riffle streams into long, exposed runs. The South Yuba presently contains only remnant populations of pikeminnow (*Ptychocheilus grandis*), hardhead (*Mylopharodon conocephalus*) and suckers (*Catostomus sp.*) (Moyle 2002). Other native species are missing altogether (Gard 1994).
Englebright Lake and the upper river support brown trout (*Salvelinus fontinalis*), resident rainbow trout (*Oncorhynchus mykiss*), largemouth (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) , channel catfish (*Ictaluris punctatus*) and pikeminnow.

Water quality

Water quality in the Yuba River watershed has improved since the heavy sedimentation and contamination that occurred during the mining era. Efforts are under way to protect aquatic species

and humans from exposure to contaminants including actions such as listing by the Environmental Protection Agency (EPA) of the Lava Cap Mine Site as a Superfund National Priority Site.

In 2001, elevated levels of fecal coliform (*Enterococcus* bacteria) were identified in the South Fork of the Yuba, at Edward's Crossing. Public access and swimming were closed for a period of time, and the cause of the coliform contamination remains unknown (Pers. Comm. Janet Cohen, South Yuba River Citizens League-SYRCL 2002).

Other concerns about water quality in the Yuba system include fluctuating water levels and temperatures as related to migratory salmonids. To encourage the populations of these native species, certain minimum flow and maximum temperature requirements are needed to keep their spawning and rearing grounds available and adequate.

Fish Health in Natural Populations

The major concerns for this watershed involve hatchery and wild fish interactions in terms of disease transmission. These issues are being addressed by this study and in the current FERC re-licensing studies.

Despite the above listed issues of contamination, habitat degradation, and water quality, fish health in the Yuba river system appears to be relatively good. Major disease outbreaks in natural adult or juvenile fish populations have not been reported. It should be noted, however, that natural disease outbreaks are seldom observed and comprehensive fish health monitoring will be needed to ascertain the health status of fish populations in the Yuba River.

Adult pre spawn mortality of fall Chinook in the Yuba river and Feather River needs further study as well. In recent years, up to 30% mortality has occurred in returning adults when water quality (primarily defined by temperature and dissolved oxygen) was relatively good compared to previous years (Pers. comm. Stephanie Theis, Jones and Stokes 2004). Fish health assessments of returning adults can determine if pre spawn mortality is related to infectious processes or contaminants as determined by histological exams.

FEATHER RIVER

The lower Feather River is located within the Central Valley of California and drains the western slope of the Sierra Nevada. The reach of river below Oroville Dam to the confluence with the Sacramento River is low gradient and consists of several structures. These include the Thermalito Forebay, Afterbay, and the Fish Barrier Dam (rm 67) at the Feather River State Fish Hatchery (FRSFH). The state hatchery in Oroville which was constructed by Department of Water Resources to mitigate for habitat loss in the upper Feather River.
Lake Oroville was created with the completion of the Oroville Dam in 1967, and has a capacity of approximately 3.5 million acre feet. The reservoir is multi-use providing flood control, municipal water supply, hydropower, and recreation.

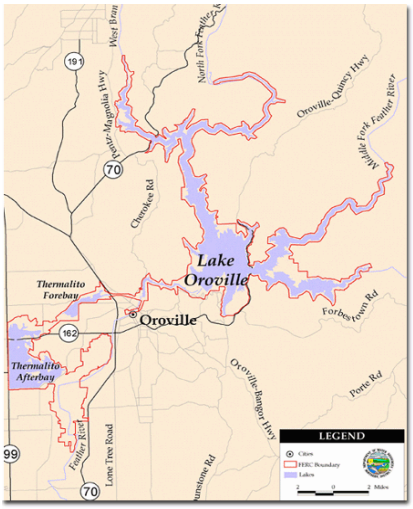


Figure 4. Upper Feather River drainage (Source: DWR–Oroville Facilities re-licensing website.

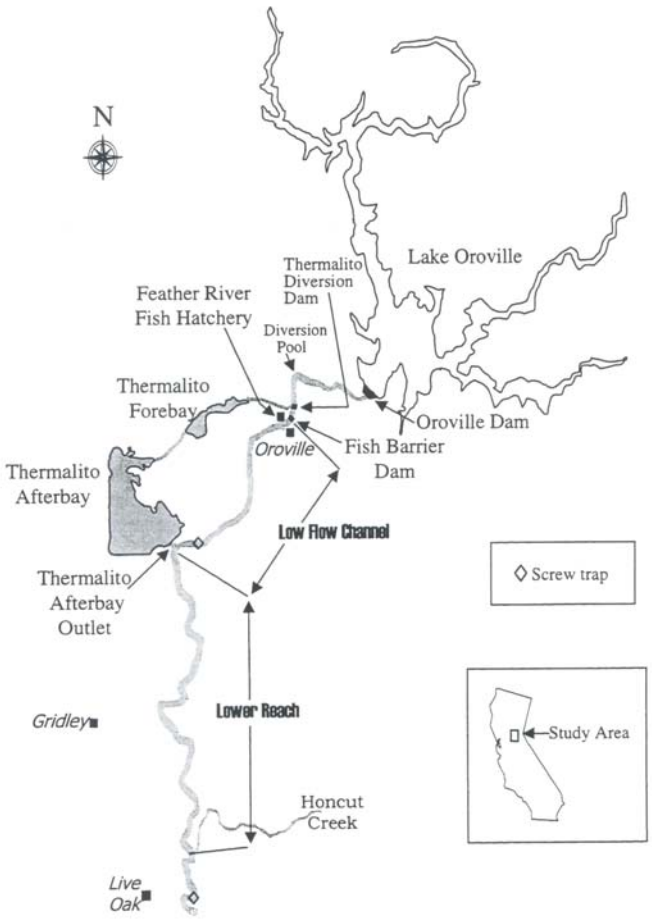


Figure 5. Feather River study site.
Source: DWR –Feather River Study 1997-1998.

The majority of water released from Lake Oroville is diverted at Thermalito Diversion Dam into the Power Canal and Thermalito Forebay.

Hydropower is produced as water flows from the Forebay to the Afterbay and then is returned to the Feather River via the Thermalito Afterbay Outlet. The remainder of flow, approximately 500 cubic feet per second (cfs) is released into the historic river channel termed the Low Flow Channel where natural production of chinook salmon occurs. The Fish Barrier Dam is the upper limit for upstream migrating fish.

Rotary screw traps are operated by DWR just above the Thermalito Afterbay outlet (Thermalito RST- approximately rm60) and just upstream of the Gridley boat launch (Gridley RST – approximately rm 50). The majority of juvenile salmon collected by these traps are parr, and averaged from 27-115mm, indicating that most of the fall chinook emigrate below the low flow channel well before smoltification.

The California-Nevada Fish Health Center (CaNv FHC) assessed fish health and tested for major fish pathogens in natural juvenile fall chinook (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) in the Yuba River. Fish were collected by Rotary Screw Trap (RSTR), operated by CDFG, from February through July in 2002.

Non-salmonids, limited to Hardhead (*Mylopharodon conocephalus*), were collected from the SF Yuba near Purdon Crossing in July 2002.

Fish health assessments continued in the Yuba River in 2003. Fall Chinook, steelhead and non-salmonids were collected by beach seine and electro-fishing on the main stem (Hwy 20 bridge), the SF (Hwy49 bridge) and the MF Yuba (Hwy 49 bridge) from April through July.

Adult fall Chinook were collected in cooperation with carcass surveys conducted on the Yuba by Jones & Stokes from October through November in 2003. Thirty kidneys were collected from carcasses in three reaches:Parks Bar, Rose Bar and Daguerra Dam. A total of ninety fish were sampled to assess the prevalence and distribution of IHNV in returning adults.

Juvenile fall chinook were collected by rotary screw trap in the Feather River, operated by DWR, from February through March in 2002.

Adult fall Chinook were collected in cooperation with carcass surveys conducted on the Feather River by DWR on October 27, 2003. Eighty-seven kidney tissues were collected from carcasses located below Feather River SFH

Additional adult fall Chinook were collected from Clear Creek, near Redding on October 29, 2003. 46 kidneys were collected from chinook carcasses and tested to assess the prevalence and distribution of IHNV in returning adults.

The methods used to collect, process, and test fish tissues are standardized throughout the country for the National Wild Fish Health Survey (NWFHS). The detailed procedures and laboratory protocols can be found in The National Wild Fish Health Survey Procedures Manual (True 2000) at the following websites:

NWFHS	http://fisheries.fws.gov/FHC/FHCNational.htm.
CaNv Fish Health Center	http://www.r1.fws.gov/canvfhc/nwfhsman.htm

Organosomatic Indices

A subset of twenty individual fish were weighed (0.1 g) and measured (total length, mm) to determine condition factor (KTL = W/L^3x10,000).

Parasitology

Fish were then examined externally and internally for clinical signs of disease and any organ abnormalities. Mucus samples (skin scrape) and gill tissues were examined for parasites and general morphology with light microscopy at 40-450x magnification.

Bacteriology

A sample of kidney tissue from fish of appropriate size was streaked onto 100 mm petri plates, or 20 x 125 mm test tube slants, of Brain Heart Infusion Agar (BHIA) and incubated at room temperature for 72 hours. If growth appeared on the BHIA media, isolated colonies were subcultured onto new BHIA plates to supply bacteria for phenotypic characterization and presumptive identification. Subcultured isolates were screened for bacterial fish pathogens by standard microscopy (Gram characteristics, morphology and motility) and appropriate biochemical tests. Bacterial isolates that were ubiquitous in freshwater and without associated clinical signs were identified to a general group, while those that were potential fish pathogens such as *Aeromonas salmonicida*, or *Yersinia ruckeri* were examined to a presumptive identity. Corroborative testing of positive bacterial isolates was done by Fluorescent Antibody Testing (FAT).

Renibacterium salmoninarum by ELISA

Kidney tissue from fish of appropriate size was removed and diluted 1:8 with Phosphate Buffer Saline (PBS) with Tween 20, homogenized, and separated by centrifugation. The samples were then loaded onto 96-well plates assayed by Enzyme Linked Immunosorbent Assay (ELISA) for the presence of *Renibacterium salmoninarum* antigen (Pascho 1987) . The ELISA was run in replicate when the quantity of kidney tissue from individual fish was sufficient. The absorbency values, measured as optical density(OD), were averaged for replicate wells. Individual fish with ELISA OD values greater than 2 standard deviations above the negative reference control OD, and up to 0.200, were defined as low level infections, 0.201-.999 moderate level, and values of 1.00 or higher were considered high infection levels. Corroborative testing of positive ELISA samples was done by Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction (confirmation testing for *R.salmoninarum*)

Kidney samples are tested using Quantitative PCR, which detects and quantifies the specific P57 DNA sequences from *Renibacterium salmoninarum* (Chase 1998, Maniatis 1982). When Rs DNA is present, the quantity of DNA is increased with each amplification cycle of the assay and exceeds

normal background levels of fluorescence. The cycle number when this occurs provides relative quantification of DNA present in the original kidney tissue.

Positive Controls used in the assay are cultured *R.salmonarum* cells (ATCC 33206) diluted 1:5 in PBS and extracted in the same manner as kidney sample sets.

Virology

Samples of kidney and spleen, or visceral tissue in the case of smaller fish, were removed from each fish to assay for the prevalence of Infectious Hematopoietic Necrosis Virus (IHNV), Viral Hemorrhagic Septicemia Virus (VHSV), and Infectious Pancreatic Necrosis Virus (IPNV) using accepted cell culture techniques (True 2000, AFS Blue Book 2003). Kidney and spleen tissues from 3 fish were pooled into one sample, but occasionally 4-5 fish were pooled when the total number of fish was not a multiple of three. For cell culture assay, tissue samples were weighed and diluted to 1:10 in Hank's Balanced Salt Solution (HBSS) and homogenized with a Stomacher 80 Lab Blender®. Samples were centrifuged at 5000 x g for 15 m and then 1.0 mL of the supernatant was combined with 1mL of HBSS supplemented with antibiotics and antimycotic (200 IU mL-1 penicillin G, 200 IU mL-1 streptomycin, 0.5 µg mL-1 amphotericin B and 40 µg mL-1 gentamycin). Final sample dilutions of 1:20 and 1:100 were inoculated onto confluent Chinook Salmon Embryo 214 (CHSE-214), Epithelioma Papillosum Cyprinid (EPC), and Fat Head Minnow (FHM) cell lines in replicate wells of 48-well plates. Samples were incubated on a platform rocker for 30-60 m at 15°C and then 0.5 mL Minimum Essential Media (MEM) with 5% Fetal Bovine Serum (FBS) was added to each well. Plates were incubated at 15°C for 21 d and were examined bi-weekly for evidence of cytopathic effects (CPE). Corroborative testing of positive viral results utilized Immunohistochemistry techniques (Drolet 1993) using a Diagxotics (Wilton, CT) universal antibody (14D) against IHNV and a Vectastain Laboratories® (Burlingame, CA) Horseradish Peroxidase Kit.

Myxobolus cerebralis (Whirling Disease)

Screening for *Myxobolus cerebralis*, the causative agent of Whirling Disease, was done by Pepsin-Trypsin Digest (PTD) of cranial elements consisting of bone and cartilage. Sampled salmonids were decapitated and the heads grouped into pools of 5 fish, then frozen until laboratory analysis could be performed. The heads were heated in a 60°C water bath for 60 m, so that the cranial elements could be removed from the soft flesh. The cranial elements were then ground in a blender and placed in a pepsin solution of 20 mL g-1 of tissue, and incubated at 37°C for 40 m. The samples were centrifuged, supernatant removed, and digested in a solution of trypsin at 20 mL g-1 of tissue. Samples were incubated at room temperature on a rocker plate for 30 m. The larger remaining particles were filtered through cheesecloth and the samples were centrifuged a final time, prior to discarding the supernatant. A small amount of water was added to the pelleted preparation to provide adequate solution volume in which samples could be examined by phase contrast microscopy at 200-400x. If tissues were positive for myxosporean parasites, corroborative testing was done by PCR.

Histology

During field dissection, target organs were rapidly removed from the fish, or whole fish were fixed in Davidson's Fixative or Prefer Fixative (Anatech, Battle Creek, MI) for 24-48 hours. Tissues were processed for 5 m paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X) for internal and intracellular

parasites, and tissue changes associated with disease. Presence or absence of the metacercarial stage of *Nanophyetus salmonicola* (presumptive) in the posterior kidney was noted. If typical *C. shasta* trophozoites were observed in the intestine the infections were rated as light or heavy based on the number of parasites observed (>10 parasites in section = heavy).

2002 Yuba River – Juvenile Monitoring on the Main stem (RST) and SF Yuba at Purdon Crossing

Fall Chinook and steelhead were sampled at the main stem Yuba RST, RM 6 near Marysville (N39.10.55.92; W121.30.23.22) on Feb 12, Mar 21, and May 14. During the March period, the RST was capturing approximately 500-1000 fall Chinook per day, and 100-200 fish/day on May 14.
Few Steelhead were collected by RST on Mar 21 (n=3) and May 14 (n=6). Two Bluegill were collected by RST on Mar21 and examined for viruses and bacteria and found negative. Hardhead were the only species of fish collected by beach seine and electrofishing at Purdon Crossing on the south fork the Yuba on July 11.

Figure 6. Rotary screw trap on Yuba River, near Marysville. Photo: K.True (USFWS)

Fall Chinook

Juveniles collected at the Marysville RST appeared normal and growing well between February and May 2002. With the exception of some skin abrasions and scale loss, fish were in good conditions and very few abnormalities were noted (see organosomatic datasheet – Appendix A).

Fish had normal fat scores indicative of natural fish, and grew an average of 13mm in the period from Feb 12 to Mar 21. Condition factor (TL-KTL) also increased from 0.559 to .636 during this period, indicating that fish were gaining weight as well as length.

Only 1 fish was observed with anemia on Mar 21. Anemia is a common clinical sign of viral infection, however virus was not detected in the kidney and spleen tissues.

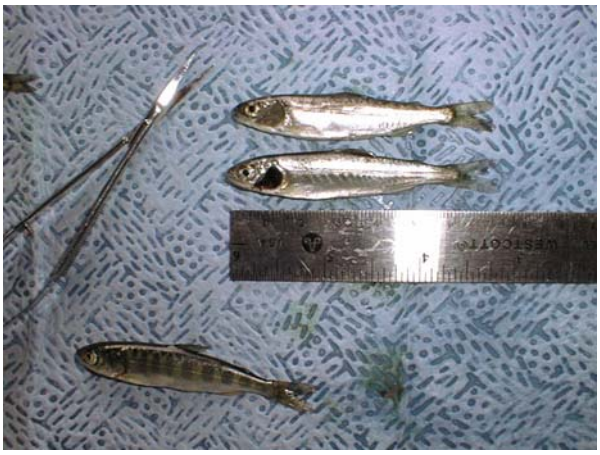


Figure 7. Fall Chinook with anemic gills (top) and normal gills (bottom). Photo: K.True (USFWS)

Other abnormalities included coagulated yolk syndrome in a few fish sampled Feb 12. Coagulated yolk is generally caused by trauma to alevins during a sensitive period prior to complete absorption of the yolk sac. High flows that occurred in the Yuba the week prior, may have mechanically traumatized fish within redds, or displaced sac-fry into the river earlier than they would have normally emerged.

Parasitology exams were conducted on the larger fish collected Mar 21 by standard skin and gill scrapes and microscopic examination at 200-600x using phase contrast. Internal organs and the intestinal tracts were also examined for helminths and spores of *Ceratomyxa shasta*. No parasites were observed on the skin, gill, abdominal cavity or in the intestine.

Histology was performed on 4 fish and no inflammatory response or lesions associated with the coagulation yolk were noted. No internal parasites were observed histologically (see pathology reports, Appendix B).

Figure 8. Fall chinook smolts from Yuba RST (May 14 2002) Photo: K.True (USFWS)

Fish collected on May 14, 2003 were well developed smolts and appeared in excellent condition. Forklengths ranged from 61-89mm, with an average length of 70.8mm. Twenty fish were examined by organosomatic index and no abnormalities were noted.
Laboratory testing was negative for all major fish pathogens (Table 3)

Table 3. Summary of Sample Location and Assays Performed for fall chinook in the Yuba River in 2002

Sample Date	Location	No. Fish Examined	Assays Performed	Test Results	Remarks
Feb 12	Main stem RST (Marysville)	20	Organosomatic Index	Normal	Coag yolk observed 2/20 Darkened peduncle 1/20 Some scale loss/fin erosion 2/20 Fat scores normal (natural fish)
		415	Virology	Negative	
		4	Histology	Normal	No internal parasites
		30	Pepsin-Trypsin Digest (Whirling disease)	Negative	
Mar 21	Main stem RST (Marysville)	30	Organosomatic Index	Normal	Severe anemia 1/30
		245	Virology	Negative	
		10	Parasitology	Negative	No externals or *C.shasta*
May 14	Main stem RST (Marysville)	20	Organosomatic Index	Normal	
		40	Virology	Negative	
		30	Bacteriology	Negative	
		30	Pepsin-Trypsin Digest (Whirling disease)	Negative	
Total No. Fish Examined per Assay:					
			Organosomatic Index	**70**	
			Virology	**700**	
			Histology	**4**	
			Bacteriology	**30**	
			Digest – Whirling Disease	**60**	

Steelhead

Sample numbers of steelhead were very low due to inefficiency of RSTs to capture this species. A total of 3 fish were examined on Mar 21 ranging in size from 75-99mm. Six fish were examined on May 14, with size ranges of 81-122mm. Fish appeared normal on both collection dates with the exception of some pectoral fin erosion in one fish. Fish were large enough to sample blood for hematocrits (percent of whole blood that is comprised of red blood cells). Values were within normal ranges of 35-45% packed red blood cells. The mean hematocrit value increased from 36% on Mar 21 to 44% on May 14, however with the small sample sizes of fish collected, it cannot be determined if this increase was a significant change in percentage of red blood cells.

Figure 9. Steelhead collected from Yuba River RST Mar 2002 Photo: K.True (USFWS)

ELISA testing showed elevated OD values indicative of the presence of soluble P57 protein of *Renibacterium salmoninarum* in the 9 fish sampled. Polymerase Chain Reaction using primers specific to *R.salmoninarum* confirmed the presence of DNA in the steelhead. Soluble protein alone can indicate prior exposure, but not necessarily active infection with *R.salmoninarum*. However, the confirmation of bacterial DNA of *R.salmoninarum* indicates that fish are actively infected with this bacteria. However, the infection level was very low and does not pose a significant health risk.

Other bacteria, *Aeromonas hydrophila* (1/6 fish) and *Micrococcus spp*. (1/6 fish) were cultured from steelhead sampled on May 14. *Aeromonas hydrophila* is generally considered an opportunistic bacteria that infects fish when they are handled and stressed, such as in a hatchery setting, or when environmental conditions are degraded. Steelhead had been held in the trap for an extended period of time (over 24 hours) to provide samples for the fish health assessments. Holding these steelhead for this period may have compromised the immune system sufficiently to permit opportunistic bacterial infections, or the fish may have harbored these bacteria prior to capture. No clinical signs of bacteremia (swollen kidney or internal hemorrhaging) were observed in the fish on field exam, and despite isolation of bacteria in 15% of the fish tested, these fish did not appear clinically diseased.

Noteworthy for the May 14 collection of steelhead is the observation that all fish examined, size range of 81-122 TLNG(mm) were females with clearly discernable ova. This indicates the initiation of the reproductive cycle for these presumed steelhead yearlings collected in the main stem Yuba.

Table 4. Summary of Sample Location and Assays Performed for steelhead in the Yuba River in 2002

Sample Date	Location	No. Fish Examined	Assays Performed	Test Results	Remarks
Mar 21	Main stem RST (Marysville)	3	Organosomatic Index	Normal	Hct range: 32-42 (mean=36)
		3	Virology	Negative	
		3	Bacteriology (culturable)	Negative	
		3	Bacteriology/ELISA (Bacterial Kidney Disease)	Negative	
		3	Parasitology	Negative	No externals or *C.shasta*
		3	Pepsin-Trypsin Digest (Whirling disease)	Negative	
May 14	Main stem RST (Marysville)	6	Organosomatic Index	Normal	Hct range: 32-55 (mean=44) Moderate gill hyperplasia 1/3 All females with developing gonads
		6	Virology	Negative	
		6	Bacteriology (culturable)	+1/6 +1/6	*Aeromonas hydrophila* *Micrococcus spp.*
		6	Bacteriology/ELISA (Bacterial Kidney Disease)	+1/6	*Renibacterium salmoninarum* (Confirmed by PCR)
		6	Parasitology	Negative	
		6	Pepsin-Trypsin Digest (Whirling disease)	Negative	
Total No. Fish Examined per Assay:					
			Organosomatic Index	**9**	
			Virology	**9**	
			Bacteriology (cultured)	**9**	
			Bacteriology/ELISA	**9**	
			Parasitology	**9**	
			Digest – Whirling Disease	**9**	

Hardhead – South Fork Yuba at Purdon Crossing

Thirty-five Hardheads (*Mylopharodon conocephalus*) were collected at Purdon Crossing in July 2002 by beach seine, cast net, and electrofishing. The site had very little cover in the month of July, some deep pools but primarily slow, shallow reaches where water temperatures reached 75.8F (ambient air temperatures were >100F). Hardhead were the only species observed with the exception of a few unidentified larval fish. This site was selected due to the presence of fecal coliforms in 2001 (*Enterococcus spp*) which closed the area to public use. While we would not normally expect to isolate coliforms (enteric bacteria of warm blooded mammals) from poikilothermic fish, the elevated summer water temperatures could possibly support these bacteria in the water column for limited periods of time.

Figure 10. Purdon Crossing – South Fork Yuba
Photo: K.True (USFWS)

Virology was negative for the 35 fish tested. *Pseudomonad spp*. bacteria were cultured from 10/35 (29%) indicating systemic infection by this opportunistic bacterium which is most likely due to the environmental stress associated with the elevated water temperature.

Yuba 2003 – Juvenile Monitoring on the Main stem and Upper Tributaries

Main stem Yuba

Fall Chinook were sampling on the main stem Yuba at the highway 20 bridge on Apr 1 and June 11, 2003. Non-salmonids were sampled from the upper tributaries on Jul 23 at the highway 49 bridge on the SF and on the MF of the Yuba river.

Fall Chinook Juveniles

Juveniles were collected by beach seine at the Hwy 20 bridge on the main stem on Apr 1 (N39.13.205' W121.19.949'). Large numbers of juveniles were holding in back eddy areas within remnant side channels which provided decreased flows and some woody debris cover.

Over 290 fish were sampled for virus and 60 fish were tested for bacteria. Twenty fish were examined by organosomatic index. Fish were healthy and averaged 45.5mm in fork length. Fat scores were low, but within expected range for natural fish. Histology was performed on 10 fish and no significant health problems or parasite infections were detected, including *C.shasta* (organosomatic index and pathology report - Appendix A and B).

Figure 11. Beach seining main stem Yuba near Hwy 20 bridge Photo:K.True (USFWS)

Fish were sampled again on Jun 11 at this site, and 205 fish were tested for virus, 30 for bacteria and a 20 fish for organosomatic index. Virology was negative, however the bacterium *Yersinia ruckeri* was isolated from 1/30 fish tested.

Steelhead Juveniles

Sixty steelhead juveniles were collected by beach seine at the Hwy 20 bridge on the main stem on Jun 11. Twenty fish were examined for organosomatic index, and 60 fish for virology. Fish were too small (mean forklength 44.5mm) to perform bacteriology, ELISA, or test for *Myxobolus cerebralis* (Whirling Disease).

Non-salmonids

Sacramento sucker (*Catostomus occidentitalis*) were also collected by beach seine on Jun 11, 2003. Twelve fish were tested and found negative for viral and bacterial pathogens.

Figure 12. Field exams of rainbow trout collected from the Yuba RST
Photo: K.True (USFWS)

Table 5. Summary of Sample Location and Assays Performed for all fish species in the main stem Yuba River in 2003

Sample Date	Location	Spp and No. Fish	Assays Performed	Test Results	Remarks
Apr 1	Main stem (Hwy 20 bridge)	**Chinook** 20	Organosomatic Index	Normal	
		291	Virology	Negative	
		30	Bacteriology	Negative	
		10	Histology	Normal	No internal parasites
Jun 11	Main stem (Hwy 20 bridge)	**Chinook** 20	Organosomatic Index	Normal	
		205	Virology	Negative	
		30	Bacteriology	+1/30	*Yersinia ruckeri*
Total No. Fall Chinook Examined per Assay:					
			Organosomatic Index	**40**	
			Virology	**496**	
			Histology	**10**	
			Bacteriology	**60**	
Jun 11	Main stem (Hwy 20 bridge)	**Steelhead** 20	Organosomatic Index	Normal	
		60	Virology	Negative	
Jun 11	Main stem (Hwy 20 bridge)	**Sucker** 12	Virology	Negative	
		12	Bacteriology	Negative	

Upper Tributaries – SF and MF Yuba River

Rainbow trout and Non-salmonids

Fish were sampled by electrofishing on the South Fork of the Yuba near Hwy 49 bridge on Jul 23. Limited numbers of rainbow trout (*Oncorhynchus mykiss*), small mouth bass (*Micropterus dolomieui*) and hardhead were collected at two sites approximately ½ mile and 1 mile upstream of the Hwy 49 bridge. No clinical signs of disease were present in any of the fish sampled, and all laboratory tests were negative (Table 6).

Non-salmonid fish species were also collected by electrofishing on the Middle Fork of the Yuba at the intersection of Hwy 49, near the town of North San Juan. Sacramento sucker, small mouth bass and hardheads were sampled and found negative for fish viruses. The Sacramento suckers had *Aeromonas hydrophila* and *Pseudomonad spp.* bacteria cultured from kidney tissue from individual fish. With the small sample size per fish species and lack of clinical signs of disease upon field examination, the finding of these common bacteria is probably not significant to the health status of the non-salmonid fish populations in the upper tributaries.

Figure 13. SF Yuba near Hwy 49.

Table 6. Summary of Sample Location and Assays Performed for all fish species in the upper Yuba tributaries in 2003

Sample Date	Location	Spp and No. Fish Examined	Assays Performed	Test Results	Remarks
Jul 23	South Fork (Hwy 49 bridge)	**Rainbow** 1	Virology	Negative	
		1	Bacteriology	Negative	
		1	Pepsin-Trypsin Digest (Whirling Disease)	Negative	
		Hardhead 21	Virology	Negative	
		21	Bacteriology	Negative	
Jun 23	Middle Fork (Hwy 20 bridge)	**Sucker** 12	Virology	Negative	
		12	Bacteriology	+1/12 +1/12	*Aeromonas hydrophila* *Pseudomonas spp.*
		Hardhead 16	Virology	Negative	
		16	Bacteriology	Negative	
		Small mouth bass 5	Virology	Negative	
		5	Bacteriology	Negative	
Total No. Fish Sampled by Species:			**Rainbow trout**	1	
			Hardhead	**37**	
			Sacramento Sucker	**12**	
			Small mouth bass	**5**	

Feather River 2003 – Juvenile Monitoring (RST) of Fall Chinook salmon in the Low Flow Channel (LFC) above Thermalito Afterbay

Juvenile fall Chinook salmon were collected at the RST, operated by Department of Water Resources (DWR) in the low flow channel just upstream of the Thermalito Afterbay (TAB) on Feb 19, Mar 11 and Mar 27 and from the Gridley Boat Launch (GBL) on Mar 27.

Fish were just buttoning-up (sac fry) on Feb 19 when the trap was averaging 5000 fish per day. Juveniles appeared normal and growing well between February and late March 2003 with an average forklength of 37.8 and 47.9mm, and condition factor of 0.82 and 0.83, respectively.

By Mar 27, only 8 fall Chinook were captured in the RST and 2/8 of these fish had clinical signs of white spot disease caused by the ciliate *Ichthyopthirius multifilis,* commonly referred to as Ich. Parasite loads of this ciliate were moderate to heavy on the two fish infected. Because of the low sample numbers at the TAB RST, additional fish were collected from the next site downstream, the Gridley Boat Launch (rm 42), where traps were still collected ~200/day. The sample size needed to detect fish pathogens at a 5% prevalence is 60 fish (Ossiander 1973).

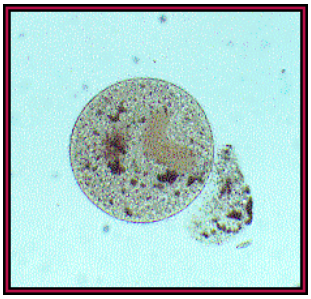


Figure 14. ***Ichthyopthirius multifilus*** **protozoan parasite of the skin and gills.**

Fish at the Gridley site appeared normal, and were comparable in size and condition factor to fish sampled at the low flow channel above TAB.
Ich was not observed on fish from this site.
Viral samples were collected from 153 fish and 30 fish were tested for culturable bacteria. Fish were too small to test for *Renibacterium salmoninarum* by ELISA (Bacterial Kidney Disease).
See summary of test results - Table 7.

Histology was performed on 10 fish from the TAB RST on Feb19 and ten fish from the GBL RST on Mar 27. Ich was also seen in histological sections of fish collected Mar 27 . No other significant abnormalities or lesions were observed in the fish sampled. *Ceratomyxa shasta* was not observed in either sample set (Appendix B – Pathology Reports).

Table 7. Summary of Sample Location and Assays Performed for fall chinook in the Feather River in 2003

Sample Date	Location	No. Fish Examined	Assays Performed	Test Results	Remarks
Feb 19	Thermalito RST (above TAB)	20	Organosomatic Index	Normal	
		153	Virology	Negative	
		10	Histology	Normal	No internal abnormalities or parasites
Mar 11	Thermalito RST	150	Virology only	Negative	Samples were collected and shipped by DWR RST crew
Mar 27	Thermalito RST	8	Virology	Normal	
		8	Parasitology exam	+2/8	*Ichthyopthirius multifilus*
Mar 27	Gridley Boat Launch RST	20	Organosomatic Index	Normal (as noted)	1 fish - hemorrhaged mandible 1 fish with anemia)
		60	Virology	Negative	
		30	Bacteriology	Negative	
Total No. Fish Examined per Assay:					
			Organosomatic Index	**40**	
			Virology	**371**	
			Histology	**10**	
			Bacteriology	**30**	

IHNV Surveys of Returning Adult Chinook salmon in the Yuba, Feather and Clear Creek

To ascertain the incidence of IHNV in natural fall Chinook adults, kidney samples were collected from carcasses during the fall of 2003 and assayed for this virus from the Yuba River, Feather River and from Clear Creek, near Redding. Tissues were tested for all common fish viral pathogens.

Returning fall Chinook adults were sampled during carcass surveys on the main stem Yuba River conducted by Jones and Stokes on Oct 28, Nov 5 and Nov 6. Fish were collected from the upper reach, middle and lower reach from the Narrows near Englebright Dam to the confluence with the Feather River.

Adult chinook were collected from Clear Creek by Ca-Nv FHC staff. Clear Creek data, as well as historical data from Feather River SFH and Coleman NFH is included in this report to provide a comparison of IHNV incidence in the major tributaries of the upper Sacramento basin.

Yuba River

Fish were collected on the Yuba River in the three reaches (Rose Bar, Parks Bar, and Daguerra Point Dam) that comprise the main stem below Englebright Dam. Thirty fish were collected from each reach from Oct 27 through Nov 6. Kidney tissue was placed in an antibiotic solution to eliminate bacterial and fungal organisms and then assayed on cell culture for typical cytopathic effects (CPE) of fish viruses. Samples that were positive for CPE were confirmed with immunohistochemistry to identify IHNV using a universal antibody against all strains of IHNV.

Viral isolates were amplified by passage on EPC cell lines and stored at -70C. A sub-set of viral isolates was submitted to Dr. R. Hedrick at University of California, Davis (UCD) for strain typing analysis.

Adult escapement data for 2003 was provided by Stephanie Theis of Jones and Stokes. See Appendix D for spawning escapement and CWT recoveries for the Yuba River in 2002.

Table 8. Fall Chinook Adult Escapement by Reach – Yuba River 2003

Reach Description	Total Adult Escapement	No. Adult: No. Grilse
Rose Bar: Narrows to Hwy 20 bridge	9,193	8811/ 382
Parks Bar: Hwy 20 bridge to Daguerra Point Dam	11,731	11,072/ 659
Daguerra Point Dam: DPD to Simpson Lane	7,973	7,735/ 238
Totals:	**28,897**	**27,618/ 1279 (4.4%)**

Table 9. Summary of IHNV isolated from Yuba River adult Fall Chinook.

Collection Date (Lab Case No.)	Site Description (Lat/Long - DMS)	No Positive (Tissue Culture)/ No. Sampled	Percent Positive for viral CPE	No. Confirmed with IHC (% Positive by IHC)
10/28/2003 03-152	Rose Bar (upper reach) 391309N; 1211755W	7/30	23.3	7/10 (70)
11/05/2003 03-155	Parks Bar (middle reach) 391316N; 1211949W	6/30	20	6/6 (100)
11/06/2003 03-156	Daguerra Pt Dam (lower reach) 391051N;1213044W	12/30	40.0	12/13 (92)
	Totals:	**25/90**	**27.8**	**25/29 (86.2)**

Feather River

Adult Chinook were sampled from the Feather River in the low flow channel just below Feather River SFH on Oct 27. Eighty-seven fish were sampled in the same manner as described above and viral isolates were submitted to UCD for strain serotyping.

Table 10. Summary of IHNV isolated from Feather River adult Fall Chinook.

Collection Date (Lab Case No.)	Site Description (Lat/Long - DMS)	No Positive (Tissue Culture)/ No. Sampled	Percent Positive for viral CPE	No. Confirmed with IHC (% Positive by IHC)
10/27/2003 03-151	Feather River (below FRSFH) 393059N;1213313W	15/83	18.1	15/16 (93)
	Totals:	**15/83**	**18.1**	**15/16 (93.8)**

Clear Creek

Adult Chinook were sampled from Clear Creek in reach 5 and 6 on Oct 29, 2003. Forty-six fish were sampled in the same manner as previously described and viral isolates were submitted to UCD for strain serotyping.

Table 11. Summary of IHNV isolated from Clear Creek adult Fall Chinook.

Collection Date (Lab Case No.)	Site Description (Lat/Long - DMS)	No Positive (Tissue Culture)/ No. Sampled	Percent Positive for viral CPE	No. Confirmed with IHC (% Positive by IHC)
10/29/2003 03-150	Clear Creek (reach 5 and 6) 402952N;1222928W	21/46	45.6	21/21 (100)
	Totals:	**21/46**	**45.6**	**21/21 (100)**

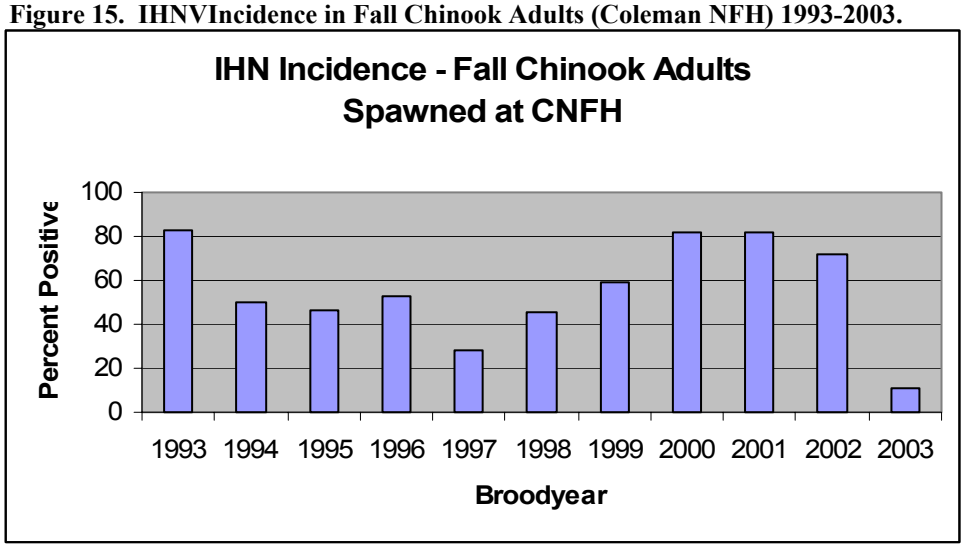
A total of 65 IHN viral isolates were submitted to Ron Hedrick at UCD (Yuba River 29, Feather River 15, and Clear Creek 21). Dr. Hedrick is using antibodies against the mid-glycoprotein (G) gene of IHNV to determine the relatedness of viral isolates and to determine if the IHN viral strains isolated from chinook adults in this study are similar to the FR-2 strain which has caused mortality in steelhead reared at FRSFH. Results of the Dr. Hedrick's strain evaluation work are pending at the time of this report.

Historical data from CNFH spawning operations indicates that the incidence of IHNV in fall chinook adults returning to the hatchery averaged 55 % over the past 11 years. The incidence has ranged from 11.4% in October-November 2003 to 82.6% in 1993.

Table 12. IHNV Incidence in Fall Chinook Adults Spawned at Coleman NFH

Brood Year	% Pos Males	% Pos Females	Total No. Fish Sampled (Pool size)	IHNV Incidence
2003	3	33	114 (3-pool)	11
2002	70	73	180 (3-pool)	72
2001	70	93	180 (3-pool)	82
2000	80	83	180 (3-pool)	82
1999	50	67	180 (3-pool)	59
1998	40	50	90 (3-pool)	45
1997	25	32	120 (1-pool)	28
1996	53	53	60 (1-pool)	53
1995	48	44	78 (1-pool)	46
1994	33	59	125 (1-pool)	50
1993	92	80	208 (1-pool)	83

Figure 15. IHNVIncidence in Fall Chinook Adults (Coleman NFH) 1993-2003.



Historical data from FRNFH spawning operations indicates that the incidence of pre-spawn mortality in fall chinook adults received at the hatchery averaged 17.7% in the period of 1993-2003. Pre-spawn mortality ranged from 3 to 36% in male fish and 4 to 38% in females.

Table 13. Pre-spawn Mortality (PSM) in Fall Chinook Adults returned to FRSFH, 1993-2003.

Brood Year	% PSM Males	% PMS Females	Total PSM
2003	11.8	17.6	14.6
2002	20.8	24.9	22.6
2001	19.7	26.0	22.4
2000	22.3	22.6	22.4
1999	14.9	13.3	14.1
1998	35.6	38.0	36.7
1997	10.8	15.5	12.9
1996	18.4	14.3	16.2
1995	14.6	27.4	21.4
1994	2.7	4.1	3.3
1993	9.6	8.4	8.9

DISCUSSION

VIRUSES

Major fish viruses, including IHNV, IPNV, VHSV, and OMV were not detected in juvenile fall chinook, steelhead or non-salmonid fish species during a two-year fish health monitoring study (2002-2003) of the Yuba and Feather rivers.

Yuba River - Juvenile Fall Chinook, Steelhead and Non-salmonids

In the Yuba River, IHNV was not detected in over 700 fall chinook juvenile salmon tested in 2002, and 490 fish tested in 2003 for a total of 1196 fish sampled. Virus was not detected in 69 steelhead and 47 non-salmonids fish species (hardhead, small mouth bass, Sacramento sucker) tested during the study. The sample sets for virology included large numbers of fall chinook, often over 200 fish per sample date. At this level of testing, there is a 95% confidence interval that IHNVwould be detected if it occurred at a prevalence of infection (POI) level of 2% in this population. Testing at a POI of 2% will generally detect IHNV in carrier fish; animals without clinical signs of disease. The large sample sets and repeated sample dates from Feb-May 2002 and Apr-Jun 2003 provide a high level of confidence that IHNV is not present in natural fall chinook juveniles in the Yuba River. Sample sets of steelhead and non-salmonids were small compared to the chinook sample sets and therefore cannot provide the same level of testing sensitivity.

Feather River - Juvenile Fall Chinook

In the Feather River, fall chinook were sampled from Feb 19-Mar 27, in 2003. Over 370 fish were tested for IHNV. Only two chinook throughout the study period exhibited general clinical signs (anemia and hemorrhaging) that may have been indicative of viral infection, however these fish tested negative for viral infection. These clinical signs are general and can be attributable to other pathological or physiological conditions. All virology testing, with the exception of Mar 27, consisted of sample sets large enough to provide detection of virus if it were present in the population at 2% POI.

BACTERIA

Yuba River - Juvenile Fall Chinook, Steelhead and Non-salmonids

Other bacterial fish pathogens were detected in the Yuba River. In 2002, 10 of 35 hardheads collected from Purdon Crossing on the SF of the Yuba tested positive for *Pseudomonad spp.* infections. While the fish lacked clinical signs of bacteremia and appeared normal on field exam, 29% of the fish had culturable bacteria in the kidney, a fairly high prevalence of infection. This level of *Pseudomonad* infection in the Hardhead population would most likely lead to disease as water temperatures (75.8F in July 2002) continued to increase, throughout the mid and late summer period.

Enterococcus bacteria, or other fecal coliforms, were not detected in SF Yuba in Hardhead. Bacterial fecal coliforms (fecal bacteria from warm blooded mammals) would not normally be expected to infect warm or cool water fish species, however previous detection of *Enterococcus* in water samples were detected in 2002 and these findings generated public health concerns. Ruling out this bacteria in resident fish populations is important in light of the highly elevated water temperatures and the recreational use of this area in the SF of the Yuba River by the public.

In 2003, 1 of 6 steelhead tested positive for *Renibacterium salmoninarum*, the bacterium responsible for Bacterial Kidney Disease. The OD value by ELISA indicated a low level of *R. salmoninarum* antigen present in the kidney tissue and further testing by Polymerase Chain Reaction confirmed the presence of specific *R.salmoninarum* DNA. Other bacteria isolated from steelhead include *Micrococcus spp* and *Aeromonas hydrophila*. None of the fish examined exhibited clinical signs of disease and these bacterial organisms can be opportunistic fish pathogens.
One fall chinook salmon collected in May from the main stem site near Hwy 20 tested positive for *Yersinia ruckeri,* the bacteria responsible for Enteric Redmouth disease in cultured fish.

No fish pathogens were detected in the other non-salmonid species tested during this study, including hardhead (n=16) and Sacramento sucker (n=12) collected in the main stem Yuba in 2003, and small mouth bass (n=5) collected from the middle fork in 2003.

Feather River - Juvenile Fall Chinook
No bacterial pathogens were detected in the Feather River in juvenile chinook sampled in 2002.

PARASITES
Yuba River - Juvenile Fall Chinook, and Steelhead
Significant parasites, including *Ceratamyxa shasta* and *Myxobolus cerebralis* were not detected in 84 chinook juveniles tested in 2002 and 2003. Sample size for microscopic or histological examination was relatively small for chinook (n=24) and steelhead (n=9), however no internal parasites or abnormalities were observed. Whirling Disease spores (*Myxobolus cerebralis*) were not observed in 60 fall chinook and 9 steelhead heads processed by Pepsin-Trypsin Digest and examined microscopically. While the sample size for steelhead was small, past surveys conducted in Battle Creek using the Pepsin-Trypsin Digest method detected *M.cerebralis* spores in small sample sets when the parasite was present in low to moderate numbers (True 1999).

Feather River - Juvenile Fall Chinook
Fall chinook collected from the TAB-RST on Mar 27 2003 had significant numbers of the parasite *Ichthyopthirius multifilis* (Ich) observed microscopically on the skin and gill of 2/8 fish examined. Histological examination also detected trophozoites, presumed also to be *Ichthyopthirius multifilis,*on the skin and gill. Water temperature was relatively low (57F/13C) at this sampling site in March. Ich is a temperature dependant pathogen, with an optimum temperature range for the infective stage of approximately 13-24C (Lom 1992). This parasite could be debilitating on juvenile chinook in the Feather River if the levels observed on gill tissue continued to rise with increasing water temperatures throughout late spring and summer.

ADULT CARCASS SURVEYS – IHNV

Returning adult chinook salmon were tested from the Yuba River, Feather River, and Clear Creek in the Fall of 2003. Historical data from Coleman National Fish Hatchery has been included to provide information, and a relative reference of IHNV incidence in a hatchery populations compared to the natural populations in the Yuba River and Clear Creek.

Yuba River – Adult Chinook

The incidence of IHNV detected in returning adult chinook to the Yuba River averaged 27.8% in individual fish collected in carcass surveys conducted between Oct 28-Nov 6, 2003. Fish were sampled from 3 reaches of the main stem below Englebright Dam. Viral prevalence was similar in the 30 fish collected from Rose Bar at 23% compared to 30 fish collected from the middle reach at Parks Bar at 20%. Viral incidence was highest in the lowest reach, Daguerra Point Dam, at 40%.

Viral incidence generally increases with density of adult fish, migration distance in large river systems, and temporal distribution related to spawn timing (higher incidence in the later period of the run). While fish densities were not significantly different for the 3 reaches tested, IHNV prevalence was higher in the lowest reach at 40%, despite the lower density of adults observed in this reach of the river. Rose Bar (upper reach) was sampled October 28, and Parks Bar (middle reach) and Daguerra Point Dam (lower reach) were sampled approximately one week later (Nov 5 and 6), so temporal differences in viral incidence would not be expected to be significant. Migration distance would not be expected to be a significant factor in viral incidence in the Yuba as the distance from the lower reach to the upper reach is relatively small and less than 20 river miles. Total escapement was 9,193 for Rose Bar, 11,731 for Parks Bar, and 7,973 for the lowest reach from Daguerra Point Dam to Simpson Lane (Table 14).

Table 14. Viral Incidence and Total Escapement of Fall Chinook in the Yuba River 2003

Reach Description	Total Adult Escapement	Percent Pos IHNV
Rose Bar: Narrows to Hwy 20 bridge	9,193	23
Parks Bar: Hwy 20 bridge to Daguerra Point Dam	11,731	20
Daguerra Point Dam: DPD to Simpson Lane	7,973	40
	Total Adults: 28,897	**Mean Percent Positive: 27.8%**

Feather River – Adult Chinook

Incidence of IHNV in Feather River adult chinook was 18.1% in 83 individual fish collected on Oct 27 from just below the hatchery ladder near FRSFH.

Clear Creek – Adult Chinook

The incidence of IHNV in adult chinook returning to Clear Creek was 45.6% in 46 individual collected Oct 29 from reach 5 and 6.

Coleman National Fish Hatchery – Historical Data for Adult Chinook

Routine testing of adults returning to CNFH on Battle Creek over the entire run period detected IHNV in 11% of the 114 fish tested in 3-fish sample pools. Viral incidence at CNFH has ranged from the this low incidence in 2003, to as high as 83% of returning adults. Historically, CNFH IHNV incidence averaged 55.5% in the period from 1993-2003.

This data from carcass surveys conducted in 2003 suggests that incidence of IHNV is actually higher in the upper Sacramento tributaries in both hatchery and natural populations (CNFH and Clear Creek) compared to the Yuba and Feather rivers. Data from Yuba River and Clear Creek

comprises a single year of adult testing and more monitoring would be needed to determine if this observation is representative of the overall geographical distribution of IHNV in the Sacramento basin tributaries. It should also be noted that routine testing at CNFH consists of viral testing of 3-fish pools after 1997 (see Table 12). Pooled samples can skew the percent positive towards a higher incidence for this population when compared to single sample testing.

It is interesting to note that data collected in carcass surveys for 2003, indicate that both the hatchery stock at CNFH and the presumed "hatchery origin" stock in the Feather River have lower viral incidences at 11% and 18% than the natural adults returning to the Yuba at 28% and Clear Creek at 46%. Again, in light of historical IHNV incidence at CNFH which averages 56%, further monitoring would be needed to determine if this trend holds over time.

RISK OF DISEASE TRANSMISSION TO NATURAL POPULATIONS

In terms of IHNV transmission to natural fish from hatchery fish straying into the Yuba River, the escapement data and viral incidence in returning adults does not suggest a significant risk to natural populations. FRSFH fall chinook have been identified by coded wire tag (CWT) recovery in carcass surveys on the Yuba river, as well as fish from Mokulumne, Merced and Nimbus SFH. In 2002, 56 CWT were recovered and 25 were determined to be of FRSFH origin (Pers. comm. Stephanie Theis, Jones and Stokes 2003). In 2003, a total of 60 CWT fish were recovered from a total escapement estimate of over 28,000. If viral incidence in straying FRSFH adult Chinook is similar to the incidence of adults returning to the FRSFH hatchery at 18%, then the number of infected adults in the Yuba would comprise a very small proportion of the spawning population.

IHN VIRAL STRAIN TYPING

Viral strain typing work will be completed by the Dr. Ron Hedrick of UCD. Using antibody serotyping and genetic sequencing, all viral isolates detected in this study will be tested to determine what strain types of IHNV exist in natural and hatchery populations in the Sacramento River tributaries. The majority of viral isolates detected in this study were confirmed as IHNV by the California-Nevada Fish Health Center, using immunohistochemistry techniques and a universal antibody (14D) from DiagXotics Laboratories. One isolate from the Feather River and 4 isolates from the Yuba River did not confirm using this universal antibody, which may indicate that these isolates have slightly different or altered epitopes than the wild type strain. Similar results have been observed in past testing of IHNV isolates from CNFH fall chinook adults when the 14D antibody is used in the immunoblot technique, a similar immunological confirmation method. In those studies, approximately 30% of CNFH IHNV isolates were not recognized by the Diagxotics 14D universal antibody (unpublished data, True 1994).

The California strain of IHNV is less virulent than geographic serotypes found in the Columbia River, Alaska, or the Hagerman Valley in Idaho. It has not been determined whether unconfirmed IHNV isolates represent unique strains of IHNV, or whether the universal antibody simply does not recognize all variants of the IHNV found in California. Dr. Hedrick's work will help determine whether these "unusual isolates" are significant in terms of the virulence factors and geographic distribution.

PRE-SPAWN MORTALITY

Pre-spawn mortality was estimated to be less than 1% in Yuba River adults, in 2003. However, in the past 2 of 8 years, pre-spawn mortality has reached 30% of returning adults. During these periods, fish appeared healthy and water quality conditions were relatively normal for the Yuba River system (Pers. comm. Stephanie Theis, Jones and Stokes 2004). Adult mortality prior to

spawning has not been investigated from a fish health perspective and further study is needed to determine if the cause is infectious or environmental in nature.
The Feather River SFH has also experienced significant pre-spawn mortality in the low flow channel and FRSFH holding units. Many of these mortality events can be attributed to increased densities and elevated water temperatures in specific years (Pers. comm. Tresa Veck, CDFG 2004).

SUMMARY

The risk of IHNV transmission to natural fish populations in the Yuba and Feather River from operation of the Feather River State Fish Hatchery appears to be low.

IHN virus was not been detected in juvenile fish populations in the Yuba and Feather Rivers in this two-year study. A total of 1567 Chinook, steelhead or non-salmonid species have been tested. IHNV was not present in large sample sets of fall Chinook in the Yuba River: 700 tested in 2002 and 496 in 2003. Similar findings were observed in the Feather River, where testing at a prevalence of infection level of 2% did not detect IHNV. At this level of testing, it is reasonable to conclude that juvenile chinook are not infected, nor carriers, of IHNV in the Yuba and Feather Rivers. If the virus is present, it is at extremely low prevalence levels and does not pose a significant risk to natural juvenile chinook populations.

Viral incidence in natural adult populations is not significantly different from natural populations in the upper Sacramento tributaries, such as Clear Creek. Viral incidence in Yuba River natural adults is actually higher than adult Chinook returning to CNFH and FRSFH based on the data obtained in the 2003 carcass survey. Relatively few FRSFH chinook CWT adults are recovered in the Yuba carcass surveys, indicating the likelihood of viral transmission between these stocks, or to subsequent progeny of natural spawners, is quite low.

Parasitic infections with *Ichthyopthirius multifilis* and *Lernaea* pose some risk to Feather River fall Chinook juveniles as water temperatures increase throughout summer. Other bacteria, including *Yersinia ruckeri*, and opportunistic *Pseudomonad* infections could also pose risks of epizootics under unfavorable environmental conditions. *Renibacterium salmoninarum* was detected in one steelhead, but at very low levels, and does not pose a significant health risk.

No other significant fish pathogens were present in juvenile or adult Chinook, steelhead, or non-salmonid fish species.

FURTHER STUDIES

1. Fish health assessments of resident fish populations in Englebright and New Bullards Bar Reservoirs are needed to determine if significant fish pathogens occur in these bodies of water and pose fish health risks to Chinook populations in the lower Yuba River.

2. Determine cause of significant pre-spawn mortality events in the Yuba river and determine if pre-spawn mortality is associated with individual stocks in each tributary (Yuba and Feather Rivers) or attributable to basin wide conditions for returning adult Chinook salmon.

3. Continue monitoring chinook and steelhead under the National Wild Fish Health Survey to build upon the baseline data provided in this study and expand our knowledge of changes in the fish health status of important natural fish populations in the Sacramento basin. Understanding the relationship between disease pathogens and wild fish populations provides a biological basis for management decisions regarding restoration efforts, fish passage above barriers, stocking programs of native and non-native fish species, and other fisheries activities in these two watersheds.

4. Continue to monitor incidence of IHNV in natural adult Chinook populations in the upper Sacramento River basin through carcass surveys. Extend surveys sites for adult chinook and continue strain typing studies to determine the prevalence and movement of this pathogen within the Sacramento basin

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I would also like to thank the staff of the California-Nevada Fish Health Center for assistance with field collections and laboratory testing: Joshua Rietsma, Lyn Rosten, Ron Stone, and Rick Harmon.

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PATHOLOGY REPORT

US Fish & Wildlife Service phone 530-365-4271

CA-NV Fish Health Center fax 530-365-7150
24411 Coleman Hatchery Rd
Anderson, CA 96007

FHC Case No.**:** **2002-014 Feb12 2002** Submittal date**:**
Sample Collector**:** Sample Site(s): **Yuba River RST (Mryvl)**
Histological specimen examiner**: J. Scott Foott**
Species: **Chinook** Age: **fry**
Tissues: **sagittal sections of 7 fry**

Fixative: Davidson (X) , PREFER-ETOH ( ), 10%BF ( ), ZFIX ( ), Bouins ( )
Stains: Hematoxylin & eosin (X), PAS ( ), Iron ( )
Block No. 4275 -4279 Block / slide deposition: FHC

Blood Smear (Number): ND Bloodsmear Stain: Lieshman-Giemsa ( ), DiffQuick( )
Clinical chemistry: ND

Summary

Liver, gill, intestine, and kidney are normal in all 7 fish. No parasites were seen. While yolk was observed in the peritoneal cavity, no inflammation response or yolk composition difference suggestive of coagulated yolk was observed in 4 fish.

PATHOLOGY REPORT

US Fish & Wildlife Service phone 530-365-4271

CA-NV Fish Health Center fax 530-365-7150
24411 Coleman Hatchery Rd
Anderson, CA 96007

FHC Case No.**: 2003-33 Feb 19 2003** Submittal date**:**
Sample Collector**:** Sample Site(s): **Feather River RST (TAB)**
Histological specimen examiner**: J. Scott Foott**
Species: **Chinook** Age: **fry**
Tissues: **sagittal sections with multiple tissues, not all organs found in every section**

Fixative: Davidson (X) , PREFER-ETOH ( ), 10%BF ( ), ZFIX ( ), Bouins ( )
Stains: Hematoxylin & eosin (X), PAS ( ), Iron ( )
Block No. 4275 -4279 Block / slide deposition: FHC

Blood Smear (Number): ND Bloodsmear Stain: Lieshman-Giemsa ( ), DiffQuick( )
Clinical chemistry: ND

Summary

Sagittal sections of 10 fish examined (2/block) revealed no significant lesions or abnormalities. A large ciliate parasite (Ich?) with macronucleus was observed on the gill of 1 salmon but was not associated with gill damage. Another fish had mild inflammation of the visceral adipose tissue. No *C. shasta* was observed in any intestinal section (0 of 9).

PATHOLOGY REPORT

US Fish & Wildlife Service phone 530-365-4271

CA-NV Fish Health Center fax 530-365-7150
24411 Coleman Hatchery Rd
Anderson, CA 96007

FHC Case No.**: 2003-44 April 01 2003** Submittal date**:**
Sample Collector**:** Sample Site(s): **Yuba River**
Histological specimen examiner**: J. Scott Foott**
Species: **Chinook** Age: **fry**
Tissues: **sagittal sections with multiple tissues, not all organs found in every sec tion**

Fixative: Davidson (X) , PREFER-ETOH ( ), 10%BF ( ), ZFIX ( ), Bouins ( )
Stains: Hematoxylin & eosin (X), PAS ( ), Iron ( )
Block No. 4280 -4284 Block / slide deposition: FHC

Blood Smear (Number): ND Bloodsmear Stain: Lieshman-Giemsa ( ), DiffQuick( )
Clinical chemistry: ND

Summary

Sagittal sections of 10 fish examined (2/block) revealed no significant lesions, parasitic infection or abnormalities. Two fish had mild inflammation of the visceral adipose tissue with 1 animal showing lipofuscin pigment associated with the inflammation. This clinical picture is reminiscent of higher water temperature smolt migration in the Delta and Klamath R. No *C. shasta* was observed in any intestinal section (0 of 10).

APPENDIX C – Abstracts of Transmission Studies conducted at Ca-Nv FHC

1996 Investigation Report (Foott)
Survey of natural fall-run chinook alevin and swim-up fry from Battle creek and the Upper Sacramento River for Infectious Hematopoietic Necrosis Virus (IHNV). December 1995-January 1996. U.S. Fish and Wildlife Service, Anderson, CA.

Abstract - Over 377 Fall-run Chinook (FCS) alevins or 30-35mm fork length "swim-up" fry were collected by beach seine from 6 sites over a 28 day period (07DEC95-05JAN96). Virological assays of 2-5 fish pooled tissue samples from these fish did not demonstrate replicating virus (including IHNV) during 14-18 day incubation periods on epithelioma papulosium cyprinid cell cultures (EPC) held at 15C. FCS adults returning to Coleman NFH in 1995 had a 46% incidence of IHNV infection and the redd area surveyed in Battle creek had numerous FCS adult carcasses. This data suggests that IHNV infection was quite rare (if present) among this age group of natural Fall-run chinook in spite of the probability of horizontal transmission from the carcasses of IHNV+ parents.

Note: Under the National Wild Fish Health Survey, an additional 203 fall chinook were sampled from Mar-Apr in 1997 in the upper Sacramento River from rotary screw traps operated by Red Bluff Fish and Wildlife Office. IHNV was not detected in this sample of natural out migrating fall chinook juveniles.

1996 unpublished data (Foott and True)
Shedding Study of *Infectious Hematopoietic Necrosis Virus* (IHNV) from clinical moribund Fall Chinook, during an epizootic at Coleman NFH.

Moribund fall chinook were collected from Coleman NFH during an IHNV epizootic. Fish with clinical signs of IHNV (showing exophthalmia, darkened, riding high in water column), were placed in individual 100 mL beakers of sterile water. At 1, 10, and 30 minutes, a 10 ml water sample was taken and tested by tissue culture for number of plaque forming units (PFU). After 30 minutes, a mucus scraping and kidney sample were collected from each fish and tittered for IHNV. Virus was shed rapidly, within 1 minute, into the water and increased in quantity (1000-2000 PFU / mL) over the 30 minute period. Mucous sampled at 30 minutes contained high concentrations of virus at (10^4 - 10^5 PFU /ml), which is approximately 100 times higher than the quantity of virus shed directly into the water. Viral titers of the kidney were higher than mucous levels (10^6 - 10^7 PFU /ml).
It is unknown if short contact between fish infected with this level of virus with non-infected fish is sufficient to transmit virus and produce an infection. The high virus titer of the mucus could be a significant "inoculum" if fish make direct contact (nip or exhibit piscivory) with moribund fish.

1999 unpublished data (True)
Minimum dose of *Infectious Hematopoietic Necrosis Virus* (IHNV), and minimum age of Fall Chinook salmon to induce clinical signs of viral infection.

Summary - Transmission studies were conducted from January through April 1999 to determine the viral dose and incubation period required for fall Chinook fry to become infected with IHNV. The study challenged young fall Chinook (1200 and 600 fish/pound) with viral doses on 10^1-10^4 plaque-forming units per milliliter (PFU/ml) by 1-minute bath immersion. Following challenge, fish were subjected to stress 3 times per week by de-watering rearing units for 30 seconds. Prevalence of IHNV was monitored in all subsequent mortality and in sub-sets of each group (subclinical fish) at 3 and 7 weeks post challenge. The study demonstrated that IHNV caused low-level mortality (.02-.07%) within 2 weeks at higher doses (10^3 and 10^4 pfu/ml) in both stressed and non-stressed groups. However, fish did not develop clinical signs of disease despite high levels of infection in the subsampling testing conducted at weeks 3 and 7. Prevalence of IHNV in the 10^3 and 10^4 groups (both stressed and non-stressed) ranged from 28-71% at week 3, and 13-67% at week 7. Overall, the cumulative percent mortality during the study due to IHNV, was higher at 16.7% in the 10^4 stressed group when compared to 12.5% in the non-stressed group. Stress fish also had higher titers (an increase of 1 log or 10x) of virus in the kidney tissue compared to non-stressed fish at the same challenge dose.

2000 Investigational Report (Foott, Nichols and Harmon)
Lack of experimental evidence for IHNV transmission from infected hatchery salmon to natural Chinook salmon in the Sacramento River. U.S. Fish and Wildlife Service, Anderson, CA.

Abstract - Coleman National Fish Hatchery (CNFH) has a long history of infectious hematopoietic necrosis (IHN) disease in its juvenile chinook salmon (Oncorhynchus tshawytscha) that can result in high fish mortality and the subsequent release of large numbers of IHNV exposed juveniles. The transmission of IHNV to wild or natural chinook populations in the Sacramento River system from infected hatchery fish is a concern for resource managers. In this study, natural chinook juveniles were cohabitated with experimentally IHNV-infected hatchery chinook at ratios of 1:1, 1:10, and 1:20 for either 5 minutes or 24 hours. Additional natural chinook salmon were held in cages within the exposure tanks. During the 7 d post-exposure rearing period, a portion of each natural group was stressed daily. These exposures were designed to simulate brief and "worst case" natural fish contacts with a massive hatchery release of infected fish. Virus was not detected by tissue culture assays from any natural chinook in the 3 experiments. The inability to detect virus in the tissues of exposed natural fish, regardless of their duration of exposure, ability to directly interact with infected fish, or post-exposure stress indicates a low ecological risk to natural populations if infected hatchery fish are released into the Sacramento River. Unique characteristics of the host – pathogen relationship should be evaluated for each situation when developing risk assessments.

APPENDIX D – Yuba River 2002 Spawning Escapement Survey CWT Recoveries

Head Tag	Location	Sex	Recovery Date	CWT Code	Run	Brood Year	Hatchery	Release Site	Stock Name	Date Released	Tagged	Untagged	Agency
36122	Rose Bar	F	10/1/02	0501020713	Fall	1998	Feather R Hatchery	Georgianna Slough	Feather River	3/30/99	26248	0	CDWR
36123	Rose Bar	F	10/1/02	0601060902	Spring	1998	Feather R Hatchery	Crockett	Feather River	5/24/99	50473	0	CDWR
36124	Rose Bar	F	10/1/02	0601060904	Spring	1998	Feather R Hatchery	Crockett	Feather River	6/4/99	50713	0	CDWR
36137	Rose Bar	M	10/1/02	062631	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50877	0	CDWR
36138	Rose Bar	F	10/1/02	0601060904	Spring	1998	Feather R Hatchery	Crockett	Feather River	6/4/99	50713	0	CDWR
36139	Rose Bar	F	10/1/02	0601060902	Spring	1998	Feather R Hatchery	Crockett	Feather River	5/24/99	50473	0	CDWR
36140	Rose Bar		10/1/02	062682	Spring	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/26/01	47742	2832	CDWR
36115	Parks Bar	F	10/2/02	062670	Fall	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	5/22/01	31384	2146	CDWR
36125	Parks Bar	M	10/2/02	062631	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50877	0	CDWR
36130	Parks Bar	M	10/2/02	0601060906	Spring	1998	Feather R Hatchery	Crockett	Feather River	6/4/99	53958	0	CDWR
36136	Parks Bar	M	10/2/02	062664	Fall	2000	Feather R Hatchery	Wickland Oil Net Pen	Feather River	4/30/01	202096	718675	CDFG
36131	Rose Bar		10/3/02	062664	Fall	2000	Feather R Hatchery	Wickland Oil Net Pen	Feather River	4/30/01	202096	718675	CDFG
36132	Rose Bar	M	10/8/02	060215	Fall	1998	Mokelumne R Fish Ins	Crockett	Mokelumne River	7/2/99	95203	775696	EBMD
36141	Rose Bar	F	10/8/02	062655	Fall	1999	Feather R Hatchery	West Sacramento	Feather River	4/10/00	25005	0	FWS
36162	Rose Bar	F	10/8/02	100000		2000					0	0	
36163	Rose Bar	F	10/8/02	062653	Fall	1999	Feather R Hatchery	West Sacramento	Feather River	5/1/00	20926	0	FWS
36121	Rose Bar	F	10/9/02	062681	Spring	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/23/01	47742	2832	CDWR
36142	Parks Bar		10/9/02	062673	Fall	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/23/01	46642	3189	CDWR

Head Tag	Location	Sex	Recovery Date	CWT Code	Run	Brood Year	Hatchery	Release Site	Stock Name	Date Released	Tagged	Untagged	Agency
36159	Parks Bar		10/9/02	062680	Spring	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/26/01	47742	2832	CDWR
36126	Daguerre	F	10/10/02	0601060905	Spring	1998	Feather R Hatchery	Crockett	Feather River	6/4/99	51333	0	CDWR
36135	Rose Bar	F	10/15/02	062663	Fall	1999	Mokelumne R Fish Ins	Mokelumne R, Mouth	Mokelumne River	4/21/00	24250	0	SJRG
36168	Rose Bar	F	10/15/02	062631	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50877	0	CDWR
36134	Rose Bar	F	10/16/02	062631	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50877	0	CDWR
36171	Parks Bar	M	10/16/02	062633	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	51964	0	CDWR
36172	Parks Bar	F	10/16/02	062634	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50928	0	CDWR
36127	Daguerre	F	10/17/02	0601060906	Spring	1998	Feather R Hatchery	Crockett	Feather River	6/4/99	53958	0	CDWR
36170	Rose Bar	F	10/17/02	100000		2000					0	0	
36173	Parks Bar	F	10/17/02	062632	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50893	0	CDWR
36167	Daguerre	F	10/21/02	062665	Fall	2000	Feather R Hatchery	Wickland Oil Net Pen	Feather River	5/31/01	142204	718675	CDFG
36116	Rose Bar	F	10/22/02	062638	Fall	1998	Feather R Hatchery	Crockett	Feather River	6/11/99	50827	0	CDWR
36120	Rose Bar	F	10/22/02	100000		2000					0	0	
36128	Rose Bar	F	10/22/02	100000		2000					0	0	
36129	Rose Bar	F	10/22/02	060215	Fall	1998	Mokelumne R Fish Ins	Crockett	Mokelumne River	7/2/99	95203	775696	EBMD
36169	Rose Bar	F	10/22/02	062940	Fall	1999	Tiburon Net Pens	Tiburon Net Pens	Feather River	8/26/00	28888	0	TYEE
36174	Rose Bar		10/22/02	062671	Fall	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	5/22/01	31575	2159	CDWR
36117	Parks Bar	M	10/23/02	064404	Fall	1999	Merced R Fish Facil.	Jersey Pt, San Joaquin R	Merced River	4/20/00	25824	0	CDFG
36118	Parks Bar	M	10/23/02	062673	Fall	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/23/01	46642	3189	CDWR
34342	Parks Bar	F	10/30/02	064916	Fall	1998	Mokelumne R Fish Ins	New Hope Landing	Mokelumne River	5/28/99	51042	1208854	EBMD
36199	Daguerre	M	11/1/02	100000		2000					0	0	

Head Tag	Location	Sex	Recovery Date	CWT Code	Run	Brood Year	Hatchery	Release Site	Stock Name	Date Released	Tagged	Untagged	Agency
36198	Rose Bar	M	11/5/02	062675	Fall	2000	Feather R Hatchery	Rodeo Minor Port	Feather River	4/26/01	42704	2920	CDWR
36200	Rose Bar		11/5/02	062941	Fall	2000	Tiburon Net Pens	Tiburon Net Pens	Feather River	8/25/01	41819	12	TYEE
36119	Daguerre	M	11/7/02	060247	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	51366	0	EBMD
36143	Rose Bar	F	11/12/02	100000		2000					0	0	
36177	Rose Bar		11/12/02	100000		2000					0	0	
36176	Parks Bar	F	11/13/02	064921	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	25200	0	EBMD
36165	Rose Bar	M	11/19/02	0601061002	Fall	1999	Merced R Fish Facil.	Jersey Pt, San Joaquin R	Merced River	5/1/00	24661	0	CDFG
36166	Rose Bar	F	11/19/02	064920	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	25162	0	EBMD
36175	Rose Bar	M	11/19/02	064921	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	25200	0	EBMD
36194	Rose Bar		11/19/02	062716	Fall	2000	Mokelumne R Fish Ins	West Sacramento	Mokelumne River	4/26/01	25384	128	CDFG
43201	Daguerre	M	11/21/02	062663	Fall	1999	Mokelumne R Fish Ins	Mokelumne R, Mouth	Mokelumne River	4/21/00	24250	0	SJRG
36164	Daguerre	M	11/24/02	064920	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	25162	0	EBMD
43203	Rose Bar	F	11/25/02	064918	Fall	1998	Mokelumne R Fish Ins	New Hope Landing	Mokelumne River	5/13/99	49804	0	EBMD
43204	Parks Bar	F	11/26/02	060257	Fall	1999	Mokelumne R Fish Ins	New Hope Landing	Mokelumne River	9/26/00	51076	185329	EBMD
43202	Parks Bar	M	11/29/02	064921	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	25200	0	EBMD
43205	Rose Bar	M	12/3/02	060248	Fall	1998	Mokelumne R Fish Ins	Sherman Isl Op Jersy	Mokelumne River	5/21/99	49740	0	EBMD
36144	Parks Bar	F	12/4/02	065457	Fall	2000	Nimbus Fish Hatchery	Wickland Oil Net Pen	American River	6/15/01	99102	285185	CDFG