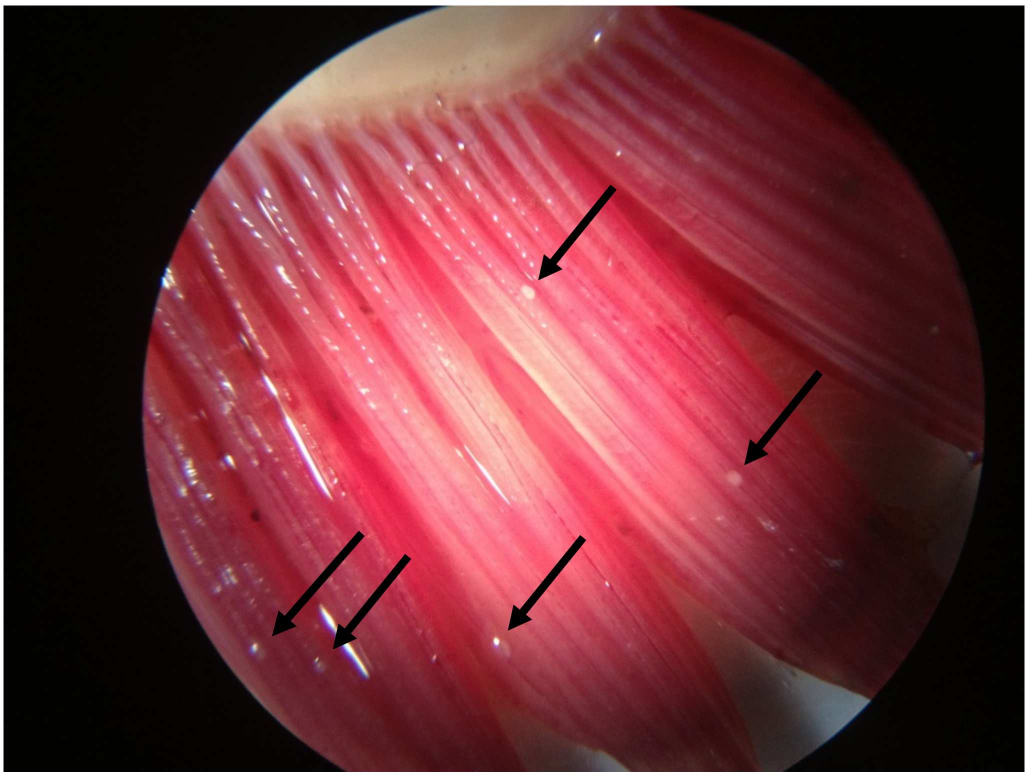
An Outbreak of *Ichthyophthirius multifiliis* in the Klamath and Trinity Rivers in 2014

Final Report

Michael Belchik[1]
Yurok Tribal Fisheries Program



August 2015

[1] 23001 Highway 96 Weitchpec Route. Hoopa, CA 95546 mbelchik@yuroktribe.nsn.us

Contents

List of Figures

List of Tables

Acknowledgements

I would like to acknowledge the support of the Yurok Tribe, the Tribal Council and the staff who helped me prepare this report. In particular, I acknowledge the hard work and dedication of my staff who collected much of the field data. I would also like to thank the Hoopa Valley Tribe for collecting and sharing crucial ich data from the lower Trinity River. I am indebted to the staff of US Fish and Wildlife Service who made many helpful editing suggestions and critiques. I would also like to thank Dr. Joshua Strange of Stillwater Sciences for review of early drafts and kind encouragement, and Dr. Peter Baker also of Stillwater Sciences for his assistance on statistical analysis. Finally, I would like to thank the Bureau of Reclamation for funding this effort. Any conclusions reached in this report are the author's, and do not necessarily reflect the views or conclusions of any person or entity who helped review early versions of this report.

Citation

The proper citation for this report is:

Belchik, M.R. 2015. An Outbreak of *Ichthyophthirius multifiliis* in the Klamath and Trinity Rivers in 2014. Yurok Tribal Fisheries Program Data Series Report. 56pp.

Abstract

In September 2014, an outbreak of I*chthyophthirius multifiliis* (ich) occurred in the Klamath and Trinity Rivers in migrating adult salmonids. The Yurok Tribal Fisheries Program monitored the progress of this parasitic disease organism throughout the time and space of the fall migration period in the lower 44 miles of Klamath River and at Iron Gate and Trinity River Hatcheries. Ich levels climbed until they reached high levels, however no mortality event was observed in the Klamath or Trinity Rivers, nor was a diseased state observed in these fish. Salmon observed at hatcheries at the upper end of the migration range within the Klamath and Trinity Rivers had almost no ich on them. The evidence collected in 2014, as well as the known biology of ich and the migrating adult salmonids, indicate a strong likelihood that increased flows reduced the severity of this outbreak.

Purpose of Report

The purpose of this report is to document an outbreak of *Ichthyophthirius multifiliis* (ich) that occurred in the lower Klamath River in the fall of 2014 along with associated environmental conditions including flow, water temperature and information about the timing, magnitude, and pre-spawn mortality of the fall-run Chinook and coho salmon runs. Ich (pronounced "ick") was the primary pathogen responsible for the fish kill event of 2002 in the lower Klamath River (USFWS 2003) which resulted in the death of more than 34,000 migrating adult salmonids. The majority of those deaths occurred within the boundaries of the Yurok Reservation. Preventing another fish kill is a primary management objective of federal, state, and tribal fisheries managers and co-managers on the Klamath River. This report summarizes fish disease monitoring data of ich in the Klamath River from 2014, associated environmental conditions, as well as information about the fish run. This report also summarizes "lessons learned" based on the scientific data collected in 2014, and provides information about flow and flow management in the Klamath and Trinity Rivers with regard to ich. This report does not make specific flow management recommendations for the future.

Introduction

Following the lower Klamath River fish kill event of 2002, the Yurok Tribal Fisheries Program (YTFP) began monitoring in September 2003 for the prevalence of *Ichthyophthirius multifiliis* (Fouquet 1876) in fall-run Chinook salmon in the lower Klamath River as well as an associated organism *Flavobacterium columnare* that causes the disease columnaris.

Ich is a fresh-water ciliated protozoan parasite native to Eurasia, but now found throughout the world. It is believed to be ubiquitous in the Klamath River, however in most sampling years it has been at levels below detection threshold using the techniques described below. Ich infections cause damage to the skin and gills of numerous fish species, including salmonids. Large-scale outbreaks occur in rivers and lakes when there is a combination of suitable environmental conditions and susceptible fish. Suitable river conditions for an ich outbreak are low flows and low turnover rates, congregations of susceptible fish, and presence of the disease organism; ich outbreaks may worsen if water temperatures are elevated. Fish become especially susceptible when they are stressed and in high densities (Matthews 2005, Dickerson and Dawe, 1995; Bodensteiner, 2000).

Elevated water temperatures are not necessary for an ich outbreak. For example, significant ich mortality occurred in British Columbia in low flow spawning channels at 13 to 15°C (Traxler et al., 1998), which is lower than water temperatures experienced by migrating salmon and steelhead in the Klamath and Trinity Rivers. High water temperatures can favor ich outbreaks by; 1) increasing the speed at which ich reproduces, 2) decreasing immune function and increasing stress levels of susceptible fish, and 3) possibly increasing the number of theronts each ich tomont can produce (Forwood et al 2015, Ewing 1986).

In addition to the fish kill in the Klamath River in 2002, fish kill events attributed to ich have happened in Butte Creek, California in 2003 and 2004 (Ward 2004), the Babine River and tributaries in British Columbia, Canada in 1994 and 1995 (Traxler 1998), the Nanaimo River in British Columbia (Hop Wo 2003), in Vallvidrera Creek in Spain (Maceda-Veiga et al, 2009), Lake Titicaca in Nicaragua (Wurtsbaugh and Tapia 1988), and in multiple locations in Great Britain (Hewlett et al 2009). Overall however, the occurrence of fish kills in natural systems involving salmonids from ich world-wide appears to be infrequent, although it causes heavy losses every year in aquaculture systems and in the aquarium trade (Matthews 2005).

After the fish kill event in in the lower Klamath River in 2002, fisheries experts and managers from federal, state and tribal entities discussed possible management actions, including increased flows, that could be implemented to lessen or eliminate the chances of another fish kill. The Bureau of Reclamation provided additional flows from Trinity Reservoir in 2003, 2004, 2008, 2009, 2012, 2013 and 2014 to decrease the likelihood of another fish kill event. Because of the high levels of mortality experienced in 2002, the Yurok Tribal Fisheries Program (YTFP) initiated a monitoring effort in the lower Klamath River to generally monitor the health of migrating adult salmonids and to detect any ich outbreak before it reached lethal levels. In 2014, this monitoring program detected ich in migrating fish. Those results are presented in this report. The year 2014 was the first year since 2002 that ich was observed in significant amounts on fish in the Klamath River[2].

Ichthyophthirius multifiliis (ich)

[NOTE: much of the background information in this section is from Matthews 2005, and Dickerson and Dawe 1995].

Ichthyophthirius multifiliis (ich) is a single celled protozoan ectoparasite[3] that infects teleost fishes throughout the tropical and temperate regions of the world and north to the Arctic Circle. Ich has caused significant losses in natural systems as well as aquaculture settings and in the aquarium trade; therefore it has been studied extensively.

Life Cycle of Ich

Ich has a direct lifecycle that does not involve a secondary host (Figure 1). The lifestage that feeds on a fish and causes a disease state is called a trophont. Trophonts feed on fish tissue and grow to up to 750 microns (μ), approximately the size of a grain of salt. Trophonts embed themselves in the epithelium of

[2] Ich was observed in 2003, but at very low levels in adult salmon.

[3] An ectoparasite is one that lives on the external surface of its host organism.

the skin or gill tissue as they reach maturity, and when the trophont has completed its growth cycle, it detaches and becomes a tomont.

After drifting free for a short period of time (less than 24 hours), tomonts attach to substrate and encyst. Tomonts have a preference for settling on light colored substrate (Matthews 2005). Tomonts undergo internal cell fission and produce 250-1000 tomites each, depending on environmental variables and the length of time the trophont was able to stay attached to the fish. Ewing (1986) showed that tomite production doubled when temperatures increased to 24°C from 21°C, and Forwood et al (2015) found a modest increase in theront production between 21° and 24°C. Another study, however, found a slight decrease in tomite production at 24°C compared to 21°C (Aihua and Buchmann, 2001).

Tomites break free from the cyst and become free-swimming theronts that then actively seek a fish to attach to. The free-swimming theront is short lived, and must immediately find a fish host. Theront viability decreases significantly after 12 hours at 20°C, and decreases to near zero after 22.5 hours (Matthews 2005). Thus, infection of new fish must take place relatively near where tomonts have encysted on the bottom of the river or other substrate and have released their theronts. Theronts are small and not visible to the naked eye (approximately 25-60 µ), covered with cilia and can swim very rapidly. Theronts are positively attracted to light at the surface of the water, and when a theront attaches to a fish (generally on the dorsal exterior surface, or the gill), it immediately burrows into the epithelium and begins its transition to the trophont life stage, thus completing the life cycle of ich. Up to 50% of theronts perish within 10 minutes of attempting to burrow into the epithelium of a potential host fish (Ewing 1986).

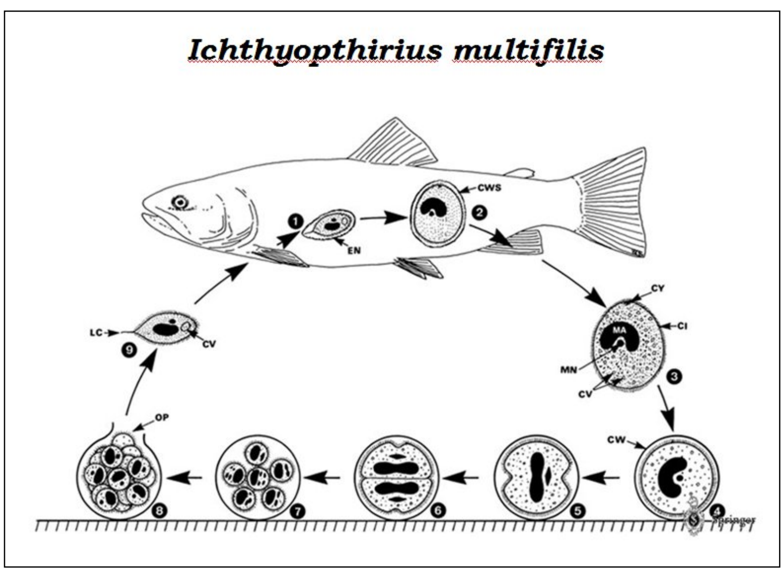


Figure 1: Life cycle of Ich showing the parasitic trophonts stages (#1 and 2), the mature ciliated trophont stage (#3) attaches to benthic substrate before dividing into tomites (#6-8), which are then released as the ciliated theront stage (#9) that must actively swim and find a suitable host within approximately 24 to 72 hours. (Figure and caption from Strange 2010b).

Timeline of Events in 2014

- July 17, 2014: YTFP begins adult fish sampling in the lower Klamath River, primarily at Blue Creek refugia. High water temperatures preclude adult fish presence at other locations in the Klamath River mainstem away from thermal refuge areas.
- July 24, 2014: Yurok Tribe captures a pink salmon at the Pecwan Creek thermal refugia area with suspicious spots on gills. Pictures and a gill imprint were taken. Pictures showed possible ich, but gill imprints were negative.
- July 26, 2014: YTFP begins its adult salmonid monitoring project in the lower Klamath, below the Trinity River confluence, to look for ich.
- August 21-September 10, 2014: Several more fish with suspicious spots on gills are collected by YTFP personnel. Gill imprints[4] are taken.
- August 23, 2014: Start of Lewiston Dam pulse flow release on the Trinity River.
- September 12, 2014: Laboratory examination by California-Nevada Fish Health Center (CNFHC) of adult salmon captured by YTFP confirms presence of ich on fish gills from August 21 and 27, and September 10, 2014.
- September 12, 2014: Increased flows at Lewiston Dam are concluded.
- September 13, 2014: YTFP captures 9 fish near Tectah Creek of which 7 have ich, one at a "severe" level (greater than 30/gill arch). CNFHC is contacted and agrees to come to the lower Klamath on Monday Sept 15 for confirmation sampling.
- September 14, 2014: Increased flows from Iron Gate Dam arrive at Weitchpec for Yurok Boat Dance ceremony. The flows begin to subside that evening.
- September 15, 2014: YTFP captures 26 adult Chinook salmon for examination by CNFHC. Ich levels exceed emergency flow criteria (Appendix 2), and the Bureau of Reclamation implements emergency flows from Trinity Reservoir the following day.
- September 17-Oct 8, 2014: 137 more adult Chinook salmon are sampled at various locations on the Yurok Reservation showing a significant increase of ich organisms over time.
- September 18, 2014: Ich noted on Trinity River Hatchery spring Chinook salmon at Lewiston (Kwak 2014a).
- October 4, 2014: Iron Gate Dam flows increased from 1000 cfs to 1700 cfs in response to continued high levels of ich infection observed in migrating adult Klamath River Chinook salmon.
- October 14-November 13, 2014: An additional 90 gill samples from adult Chinook salmon are examined at Iron Gate Hatchery, Shasta Racks, and Trinity Hatchery. These fish show light levels of ich infection.
- October 15, 2014: Iron Gate Dam flows returned to 1000 cfs.
- Mid-October to mid-January: collection of pre-spawn mortality data from spawning surveys for Chinook and coho salmon.

Goals and Objectives of Ich Sampling

The goal of the sampling program for ich in 2014 was to determine when and where ich increased to levels detectable using sampling techniques described below. Once ich was detected, objectives

---

[4] A gill imprint is made by blotting a wet fresh fish gill onto a glass slide and fixing it with a preservative so it can be examined under a microscope at a later time. Ich organisms on the gill stick to the glass of the slide where they can be observed later.

included: 1) determine how ich infection intensity changed over time at several specific locations, and 2) determine the geographic extent of the ich infection of adult fall Chinook in the lower portions of the Klamath and Trinity Rivers.

Materials and Methods

Study Sites

Adult fall-run Chinook salmon were collected from several locations in the lower Klamath River and the Trinity River just above the confluence with the Klamath River on the Yurok Reservation (Figure 2). Additional fish were collected from Iron Gate Hatchery (river mile 190) on the Klamath River, and from Trinity River Hatchery at Lewiston Dam (river mile 110). Samples were collected by the Karuk Tribe at Ishi Pishi Falls, and by the Hoopa Valley Tribe on the Hoopa Valley Indian Reservation. The results from the Karuk Tribe's sampling are not presented in this report.

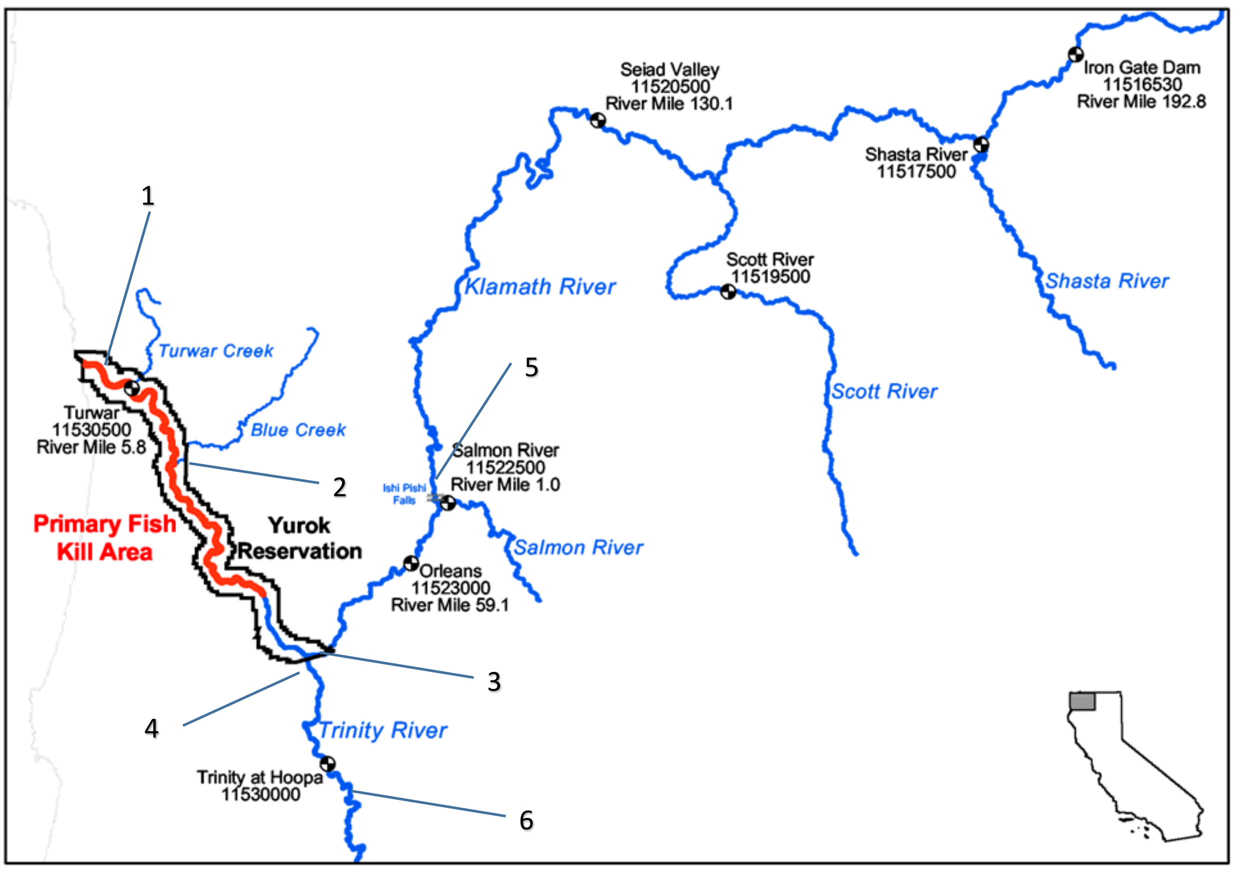


Figure 2: Study area for ich sampling in 2014. River gages are shown as well as sampling locations. Site 1: Old 101 bridge in Klamath estuary. Site 2: Blue and Tectah Creeks (sites are about 5km apart and were treated as a single site for analysis). Site 3: Klamath River just above Trinity River confluence. Site 4: Trinity River just above confluence with the Klamath. Site 5: Ishi Pishi Falls on the Klamath River. Site 6: Hoopa Valley Indian Reservation on the Trinity River.

Fish Collection

For fish collected on the Yurok Reservation, adult salmon were captured with set or drifted monofilament gillnets 30ft to 100ft long, 12 ft deep, and mesh size 7 ¼in. Some adult salmonids in July and early August were captured with hook and line, spears, or dip nets. Drift gillnet sets were conducted by setting a net perpendicular to the main current of the river, and allowing the net to float downstream with the current for approximately 100-150m. Stationary gill net sets were typically deployed at the upstream end of an eddy feature. The float line of the net was secured to the bank and the net was stretched at an angle to the flow of the river. Stationary sets were fished for two to seven hours per day, and field crews tended the net for the entire duration of the set, removing and processing salmon as they became entangled. Most sampling took place between late afternoon and midnight, but as the ich outbreak progressed, fishing took place at all times of the day.

For fish captured on the Yurok Reservation, the fish were immediately gutted and put on ice after the gills were removed for examination for ich. All fish were distributed to Yurok Tribal elders. All fish were captured from the mainstem Klamath and Trinity Rivers; no fish were captured from any other tributaries. Sampling efforts on the Yurok Reservation stopped as the run began to wane on the reservation, as the majority of fish had moved upriver.

Similar techniques were used on the Hoopa Valley Reservation, and some fresh-caught fish gillnetted or otherwise captured by tribal members were also examined.

Examination for Ich

Field Counts Using Dissecting Microscope

Upon capture, adult salmonids (mostly fall-run Chinook salmon and some steelhead) were examined externally with the unaided eye or a hand lens for evidence of ich or a columnaris infection, general body condition, and adipose fin clips (ad-clips), indicating hatchery origin. Fork lengths were measured, and if the fish was ad-clipped, the head was collected and frozen for later coded-wire tag extraction. Samplers then removed the outside (first) gill arch from the left and right sides of individual fish and placed them in clear plastic bags for examination. Individual ich organisms on the gill arches were counted as soon as possible, typically within an hour of removal, but sometimes as much as two or three hours later. If immediate examination was not possible, the samples were placed on ice in a plastic bag for later processing at the laboratory. Counts obtained from gill samples left on ice for several hours were very similar to those from fresh gills. Ich counts from both left and right gill arches were reported. The fish was then immediately placed on ice for later distribution to Yurok Tribal elders for consumption. Data collected included:

1. Date of capture
2. A unique serial number for each fish (Sample #)
3. Location
4. Species
5. Fork length (cm)
6. Sex
7. Any tags or marks (adipose fin clip, maxillary clip, etc)
8. Left and right side columnaris severity (0-4)

9. Counts of ich organisms on gills (left and right gill arches)

Gill arches (Figure 3) were examined under a dissecting scope at 10X power using an oblique light source such as a bright LED flashlight or natural ambient light. Only the external surfaces of the filaments were examined for ich, and the gill filaments were not pulled apart or otherwise dissected. Trophonts on the surface of the gill or embedded but visible were counted (Figure 4). The initial objective was to ascertain whether or not 30 ich/gill arch was or was not exceeded[5], however as the numbers of ich organisms per gill climbed throughout the sampling period, the counting procedure was altered to include a complete surface count.

Prior to September 13 when ich counts were low, all organisms were counted. On September 13, the count was stopped at 100 organisms. From September 15 through September 22, ich was counted to a maximum of 200, and after that date all organisms were counted. For gill arches covered with large numbers of ich, the ich organisms were counted in groups of 5 or 10 as an estimate. A clicker counter was used when ich densities were very high.

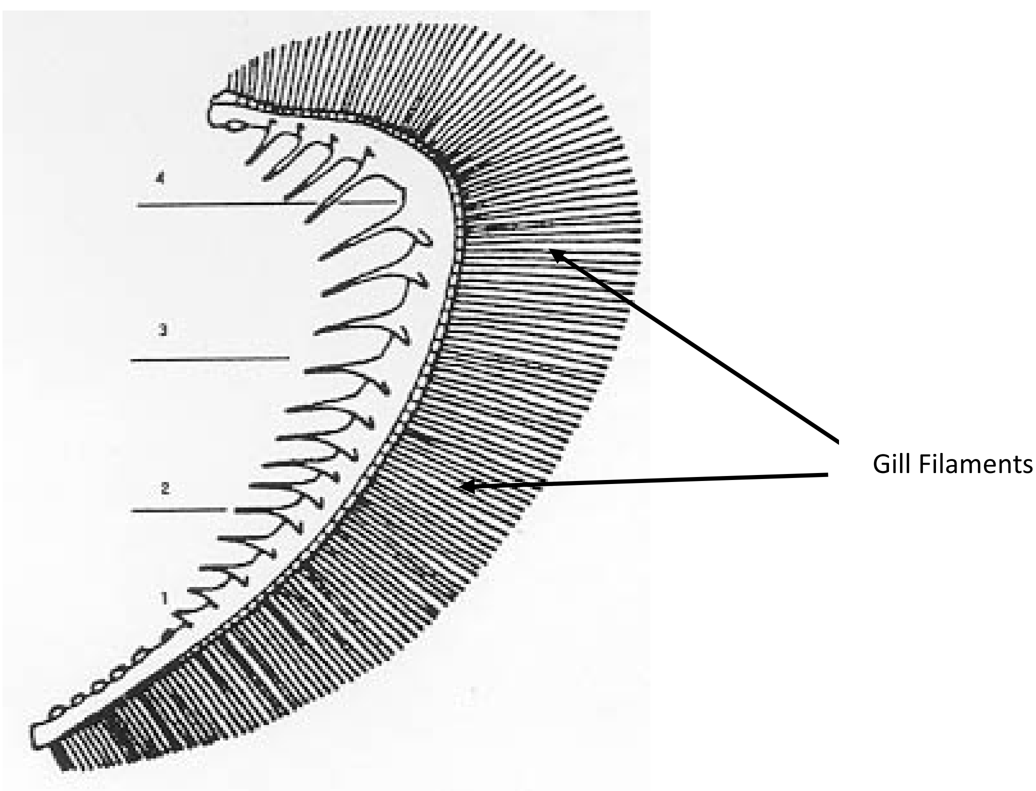


Figure 3: A typical gill arch of an adult salmonid. Long thin lines to the right of the picture are individual gill filaments. The ich organisms were generally found on the surface of these filaments.

[5] 30 ich/gill in 5% or more of fish with a 60 fish desired sample size was a trigger for emergency flow releases (TRRP 2012).

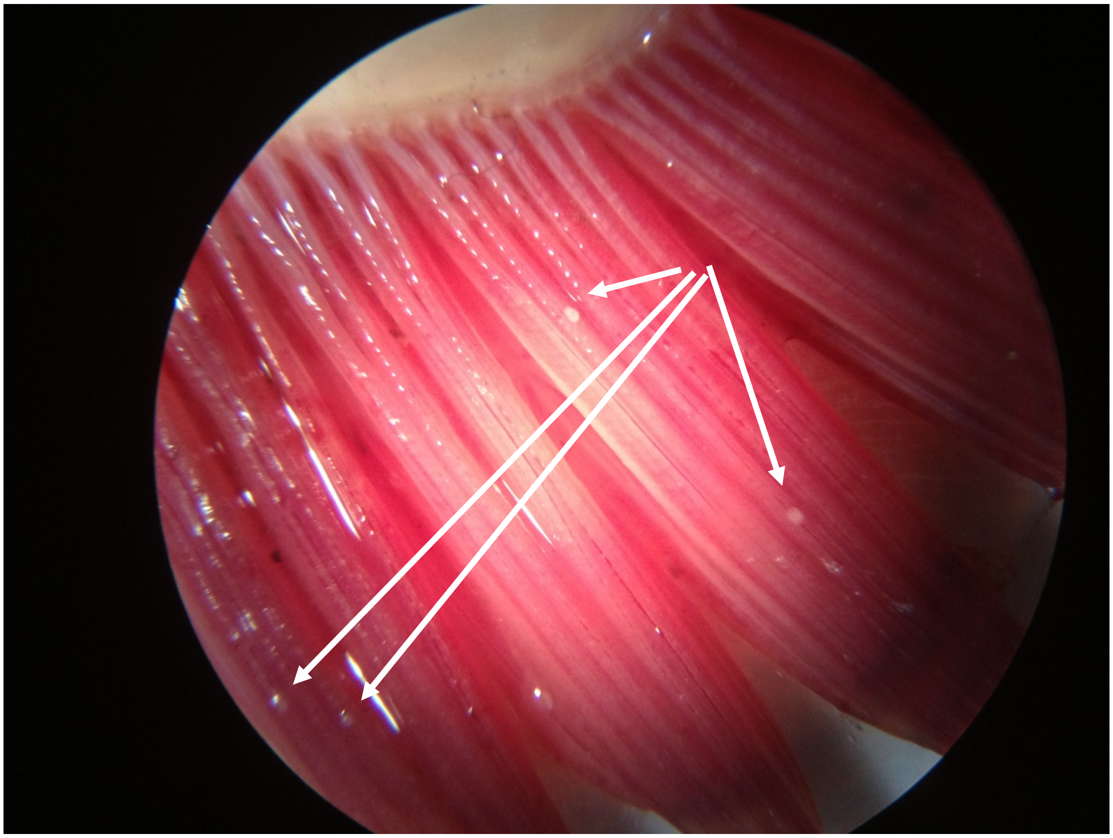


Figure 4: Photograph of ich on Chinook salmon gill filaments from the Klamath River in 2014. Arrows point to individual ich organisms on the surface of the gill. Mature ich organisms are about 0.75 mm long, and are visible to the naked eye.

Field Counts Using Compound Microscope

For wet mounted glass slide samples examined by CNFHC on September 15 and October 1 (Appendix 2), five to ten filaments were dissected from a sample gill arch, placed onto a standard microscope slide, and covered with a second slide, and chamber flooded with a phosphate buffered saline solution. The filament surface was examined for trophonts with a brightfield microscope using a 4 -10x objective. If no trophonts were observed, the slide was flipped over and re-examined. This procedure was repeated on a second group of filaments and the number of trophonts observed and filaments examined was recorded. Ich organisms were counted by CNFHC until it was apparent that the threshold of 30 ich/gill would or would not be exceeded. These results are presented in a separate report by USFWS (Appendices 2 and 3).

Organisms identified by YTFP personnel as ich in the dissecting scope were verified by CNFHC staff on September 15 to ensure there were no issues with false identification by YTFP staff of other organisms as ich (e.g., metacercaria or other) in the field. YTFP field identification of ich was confirmed as accurate.

Hatchery Samples

On October 14, 2014 and on several occasions afterwards, YTFP personnel traveled to Iron Gate Hatchery and to Trinity Hatchery to inspect fish gills at the spawning facilities for ich using the dissecting microscope technique described above. Workers clipped gills and placed them into Ziploc bags for immediate counting on site by YTFP investigators using the dissecting microscope technique described above. Pressure washers were used by hatchery staff on the gills of certain fish, and those gills were not inspected for ich.

Additional gill samples were inspected by CDFW staff at Iron Gate Hatchery and Trinity River Hatchery, and are presented in Appendix 3.

Shasta Fish Racks

An additional two fish from the Shasta River fish counting facility were also examined for ich on October 21, 2014. The fish were moribund, but not dead when they were collected from the fish weir. The gills were inspected for ich using the dissecting microscope technique described above. Because the timing was similar to the hatchery sampling, these results are included with the hatchery results.

Examination of Other Environmental Data

River Flow

Information on river flows was collected from USGS websites. Daily average flows were used.

Water Temperature

Water temperatures were obtained from USFWS, and YTFP unpublished data.

Pre-spawn Mortality

Pre-spawn mortality data was provided by USFWS and California Department of Fish and Wildlife (CDFW).

Fall Chinook Run Size

Fall Chinook escapement to the river mouth estimates from 1978 – 2014 were taken from Megatable (CDFW 2014). The pre-season run size projection of adult fall Chinook to the river mouth was obtained Preseason Report III Council Adopted Management Measures and Environmental Assessment Part 3 for 2014 Ocean Salmon Fishery Regulations (PFMC 2014).

Blue Creek Thermal Refugia Adult Abundance

YTFP conducted snorkel surveys throughout the summer period (July-September) to obtain estimates of adult salmonid abundance at this thermal refugia[6](Figure 5). Refugia abundance in 2014 was compared to previous years including 2002, the year of the Klamath River fish kill event. To obtain an index of adult abundance in a given year, only the maximum count for each summer period was used for analysis.

[6] A thermal refuge is an area of colder water that fish hold in when mainstem Klamath River temperatures exceed approximately 22°C (Strange 2010, YTFP 2011).

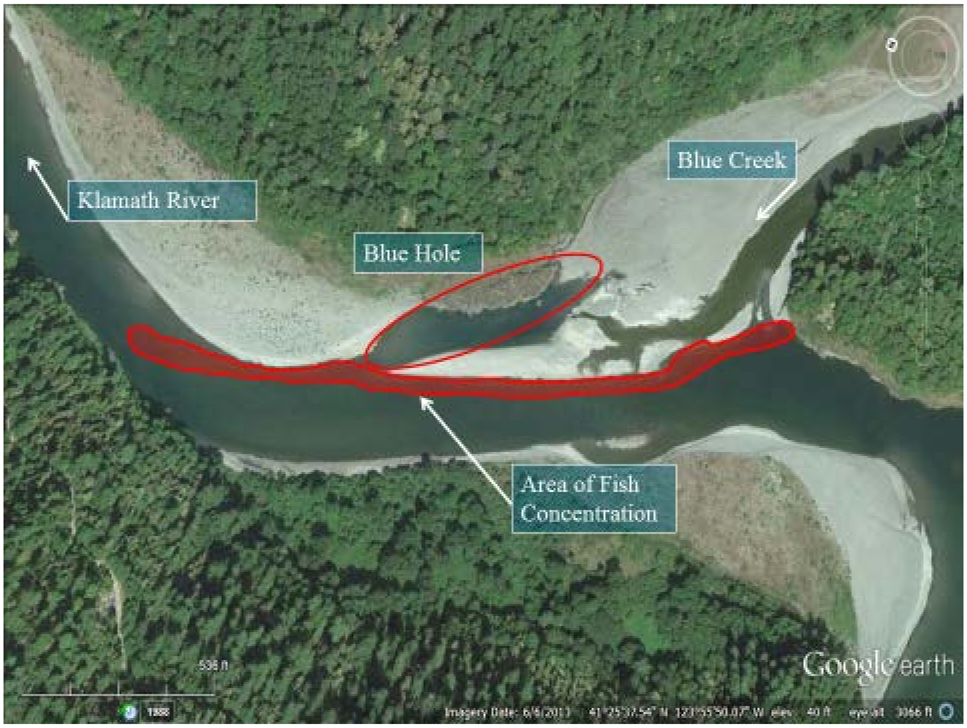


Figure 5: Blue Creek thermal refuge area. This site is located approximately 15 river miles from the mouth. "Blue Hole" is a backwater area fed by subsurface cold water from Blue Creek that holds a high number of adult and juvenile salmonids in certain years. Blue hole is not a feature of the Blue Creek thermal refuge area every year, but was present in 2002, 2003 2013 and 2014.

Data Analysis

For ich severity, a non-parametric local linear smoothing was applied to the data using statistical software R (function sm.regression from sm library =4) (Bowman and Azzalini 1997). For probability of infection, a local binomial regression was performed using R software with the routine sm.binomial from the kernel smoothing library (h=7) (Bowman and Azzalini 1997) (Peter Baker pers. comm. 2015).

Results

Fish Capture

From July 17, through November 13, 2014, YTFP examined the gills from 398 Chinook salmon from the Klamath and Trinity Rivers (Table 1). This does not include the 26 fish captured near Blue Creek on September 15 for CNFHC analysis because they were examined with different techniques. The average fork length of the fish captured was 77 cm, with a maximum length of 105 cm and a minimum of 38 cm.

Table 1: Sampling summary for Klamath and Trinity Rivers in September and October, 2014. YTFP examined 398 fish July through November, and USFWS examined 26 fish on 9/26/14.

Dates	Location	Sample Size	Method	Analysis
7/17-9/12	Lower Klamath River, primarily Blue Creek and Tectah sites	162	Various, mostly gillnet	Examination of gills and ich count with dissecting microscope
9/13-10/8	Lower Klamath and Trinity	146	Gillnet	Examination of gills and ich count with dissecting microscope
9/15	Lower Klamath River	26	Gillnet	USFWS examination for ich with compound microscope
10/2	Klamath River just above Trinity River confluence and Ishi Pishi Falls	10	Gillnet, dipnet	USFWS examination for ich with compound microscope (these fish included in the 9/13-10/8 total because they were also examined with dissecting scope)
10/21-11/13	Iron Gate and Trinity Hatchery	90	hatchery take	Examination of gills and ich count with dissecting microscope

[NOTE: Total sampled is 424 due to the additional 26 fish that USFWS sampled on 9/15/14].

On July 24, 2014, a photograph was taken of a gill arch on a pink salmon captured at Pecwan Creek refugia that appeared to show ich, although it was unconfirmed by slide imprint and subsequent microscopic evaluation. On August 21, 25 and 27, and on September 10, 2014, spots were seen on an adult Chinook salmon and slide imprints was taken. When the slide imprints were submitted to CNFHC for confirmation of ich on September 12, 2014, ich was confirmed from the 8/21, 8/27 and one of the 9/10/14 fish. It is possible that the other fish sampled on 9/10/14 also had ich, but were not captured on the slide imprint. As a result this information, YTFP increased sampling effort, and on September 13, 2014, detected ich on the gills of 7 of 9 Chinook salmon examined that were captured near Tectah Creek (rkm 36). USFWS fish health experts were contacted, and YTFP captured 26 fish on September 15, 2014 for examination by USFWS staff from CNFHC. CNFHC found ich on 11 of 26 fish examined, with 6 of the 11 having more than 30 ich per gill arch. The complete results of this examination are presented by USFWS in a separate report (Foote 2014a, Appendix 2).

After September 13, ich levels increased significantly and rapidly (Figure 6, Figure 7). By the end of September, infection rates were approaching 100% and many fish had over 600 organisms per gill. However, a field examination by USFWS of fresh caught Chinook salmon in the Klamath River near Weitchpec on October 2, 2014, showed no hyperplasia (tissue swelling) in the gill tissue (Scott Foott, pers. comm. 10/6/2014 Appendix 2).

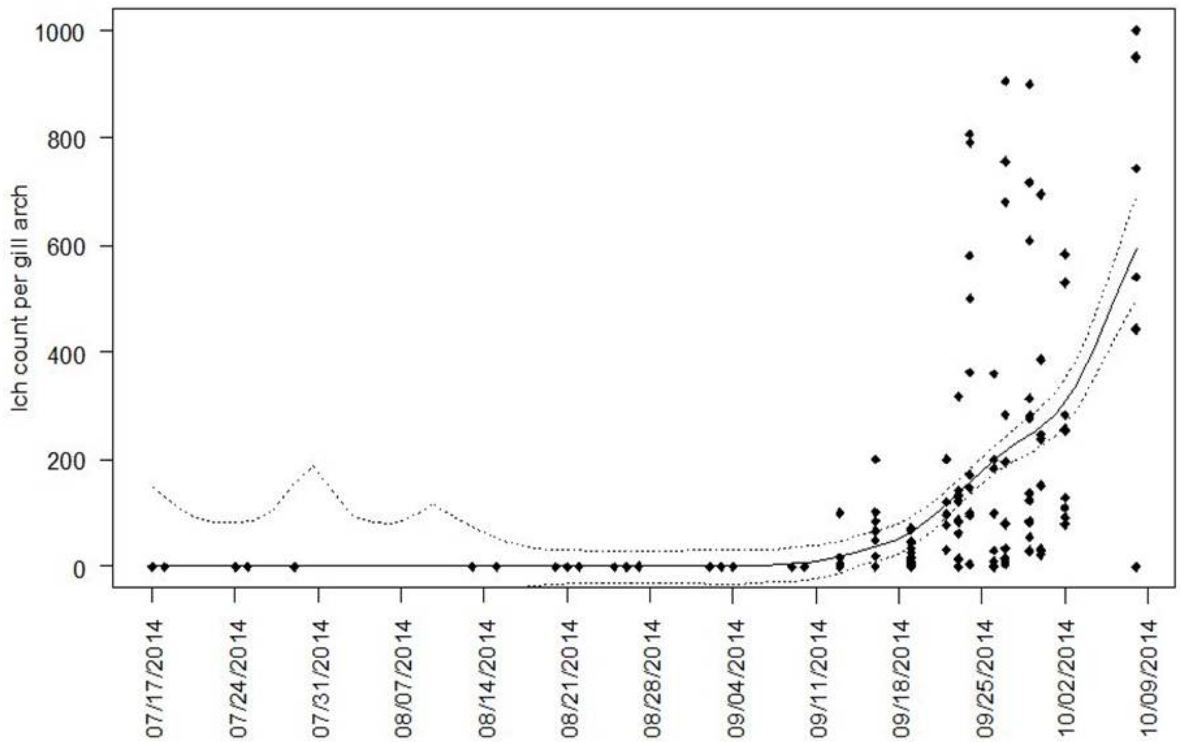


Figure 6: Maximum Ich count between left and right gill arch during the 2014 Ich sampling period (a surrogate for infection severity) fit with non-parametric model curves using local linear smoothing. Hoopa Valley and Karuk data not presented in this graph. Each data point represents at least one individual fish (some data points overlap each other). Counts on 9/13/14 were stopped at 100 organisms, and between 9/14 and 9/22 were stopped at 200 organisms. After 9/22 all ich were counted. Dotted lines represent one standard deviation. Hatchery results are not included in this data set.

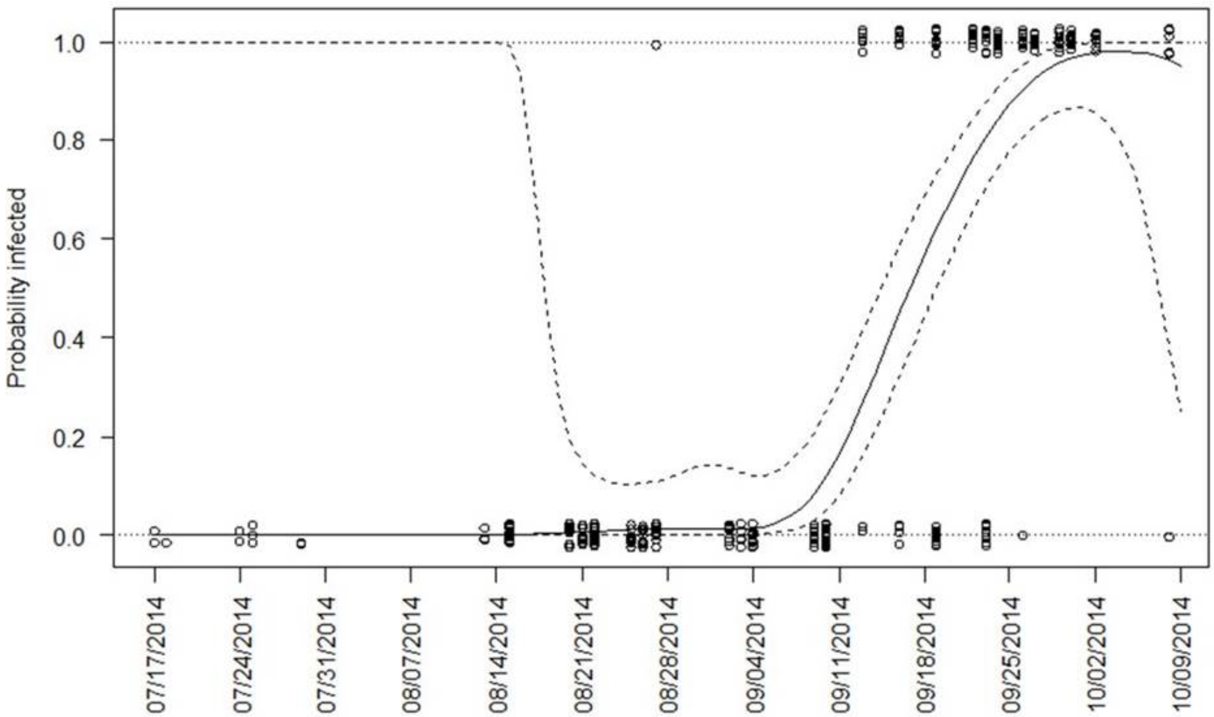


Figure 7: The probability that an individual Chinook salmon would be infected with Ich as a function of time, fitted in R with the routine sm.binomial from the kernel smoothing library (h=7)(Bowman 1997). The dotted lines are two standard deviations from the modeled value. Hatchery results are not included in this figure.

As noted above, although three fish appeared to have had very low levels of ich prior to mid-September, the infection began to increase rapidly on or around September 13, 2014 (Figure 6, Figure 7). Once ich was confirmed as present, the sampling design had two objectives: 1) determine the geographic extent of the ich outbreak, and 2) determine the change over time of the severity of ich infection in adult migrating salmonids.

Table 2 shows summarized results for ich sampling done on the Yurok Reservation between September 13 and October 8, 2014. Ich numbers are presented as categories due to the inherent varibility in surface counts of ich on these gills, which resulted from:

1. The ich organisms were mobile, and could sometimes be observed moving as the counts progressed;
2. Some ich organisms appeared to slough off in the Ziploc bags;
3. There was wide variation in ich counts between left and right gills indicating that ich was not distributed uniformly over all surfaces of a given fish.

Misidentification of ich organisms did not appear to be a factor, as USFWS twice confirmed that the organisms observed through the dissecting scope were in fact ich. Metacercaria had been identified as a possible source of misidentification, but investigators were able to determine the difference between ich and metacercaria by visual inspection as confirmed by CNFHC experts.

When ich counts were obtained from both gills the maximum count per gill arch was presented.

Ich counts remained relatively low until September 13, 2014, and then climbed rapidly. By the end of September, very few fish had ich below detection threshold, and after 9/28, only 12.5% of fish sampled had less than 30 organisms per gill and 47% of fish showed infection rates of over 200 ich/gill arch.

Table 2: Ich counts at all locations on Yurok Reservation by date. Results from Hoopa Valley and Karuk Tribe are presented separately. Prior to 9/23 ich counts were stopped at 200/gill.

	Ich/gill arch category					
Date Range	0	1-30	31-200	201-600	601-1000	Sample size (fish)
9/13-9/16	6	7	6	0	0	19
9/19-9/27	21	18	34	7	5	85
9/29-10/2	0	4	13	11	4	32
10/8	1	0	0	2	3	6

Table 3: Ich counts at all locations on Yurok Reservation by date presented as percentages rather than raw numbers. Data is same as used for Table 2.

	Ich/gill arch category					
Date Range	0	1-30	31-200	201-600	601-1000	Sample size
9/13-9/16	32%	37%	32%	0%	0%	19
9/19-9/27	25%	21%	40%	8%	6%	85
9/29-10/2	0%	13%	41%	34%	13%	32
10/8	17%	0%	0%	33%	50%	6

Table 4: Ich counts per gill at Blue Creek/Tectah Creek (rkm 26-33) as grouped by date of sample. Blue and Tectah Creek are approximately 6 km apart, so they are grouped together for analysis. If both gills were counted, the maximum was used.

	Ich/gill arch category					
Date Range	0	1-30	31-200	201-600	601-1000	Sample size
13-Sep to 18-Sep	2	6	1	0	0	9
19-Sep	6	7	4	0	0	17
23-Sep	8	0	5	0	0	13
27-Sep	0	4	3	1	3	11
8-Oct	1	0	0	2	2	5

Table 5: Ich counts per gill in the Klamath River at Weitchpec just above the confluence with the Trinity River grouped by week of sample. If both gills were counted, the maximum was used.

	Ich/gill arch category					
Date Range	0	1-30	31-200	201-600	601-1000	Total fish sampled
16-Sep	4	1	5	0	0	10
22-Sep to 24-Sep	0	1	13	4	2	20
29-Sep to 02-Oct	0	1	10	8	3	22

Table 6: Ich counts per gill in the Trinity River at Weitchpec just above the confluence with the Klamath River grouped by date of sample. If both gills were counted, the maximum was used.

	Ich/gill category					
Date	0	1-30	31-200	201-600	601-1000	Total fish sampled
9/23/2014	3	2	3	1	0	9
9/30/2014	0	3	3	3	1	10
10/8/2014	0	0	0	0	1	1

Sampling at Iron Gate and Trinity Hatchery

A total of 88 fish were sampled from Iron Gate and Trinity Hatcheries. In contrast to the high levels of ich per gill arch observed on the Yurok Reservation on the lower Klamath River, hatchery fish only showed light infections of ich (Table 7).

Table 7: Ich counts per gill at Iron Gate and Trinity Hatcheries. Only one gill per fish was inspected.

Location	Date	Sample size	No Ich	proportion with no ich	Max Ich
IG Hatchery	10/14/2014	24	13	54%	8
IG Hatchery	10/21/2014	24	13	54%	6
IG Hatchery	10/29/2014	20	15	75%	3
TR Hatchery	11/13/2014	20	11	55%	3
Totals		88	52	59%	

Ich Sampling from Hoopa Valley Tribe

The Hoopa Valley Fisheries Department sampled a total of 32 fish and inspected their gills for ich using the same methods described above with the dissecting microscope (Table 8). 23 adult salmonids were sampled with a gillnet near Campbell Creek on the upstream end of the Hoopa Valley Reservation, and nine additional coho salmon were obtained from tribal members who were harvesting fish from the river at unknown locations on the reservation.

Table 8: Results of ich monitoring of the Hoopa Valley Tribal Fisheries Department. Data courtesy Hoopa Valley Tribe.

Species	Date	Location	Sample Size	Infected	Severe Infection (greater than 30/arch max)	Percent infected	Percent severe infection	Percent infected with severe
CH	9/18/2014	Campbell Creek confluence	12	12	5	100%	42%	42%
SH	9/18/2014	Campbell Creek confluence	3	1	0	33%	0%	0%
CH	9/29/2014 - 10/2	Red Rock	6	6	4	100%	67%	67%
SH	9/29/2014 - 10/2	Red Rock	2	2	0	100%	0%	0%
Coho	9/29/2014 - 10/2	Red Rock	9	9	5	100%	56%	56%

River Flows

Beginning on August 23, the Bureau of Reclamation released additional flows from Lewiston Dam on the Trinity River[7] (Figure 8, Figure 9). As these flows ended on September 13, additional cultural flows were released from Iron Gate Dam on the Klamath River, reaching the lower Klamath River on September 14. As the Klamath River cultural flows subsided, emergency flow was then released from Lewiston Dam in response to rising ich levels in the lower Klamath. Continued high ich levels in early October led to additional flow releases from Iron Gate Dam. In mid to late October, a series of rain events raised flows in the lower Klamath River to above 10,000 cubic feet per second (cfs).

[7] Lewiston Dam is a re-regulation dam below Trinity Dam and Reservoir on the upper Trinity River.

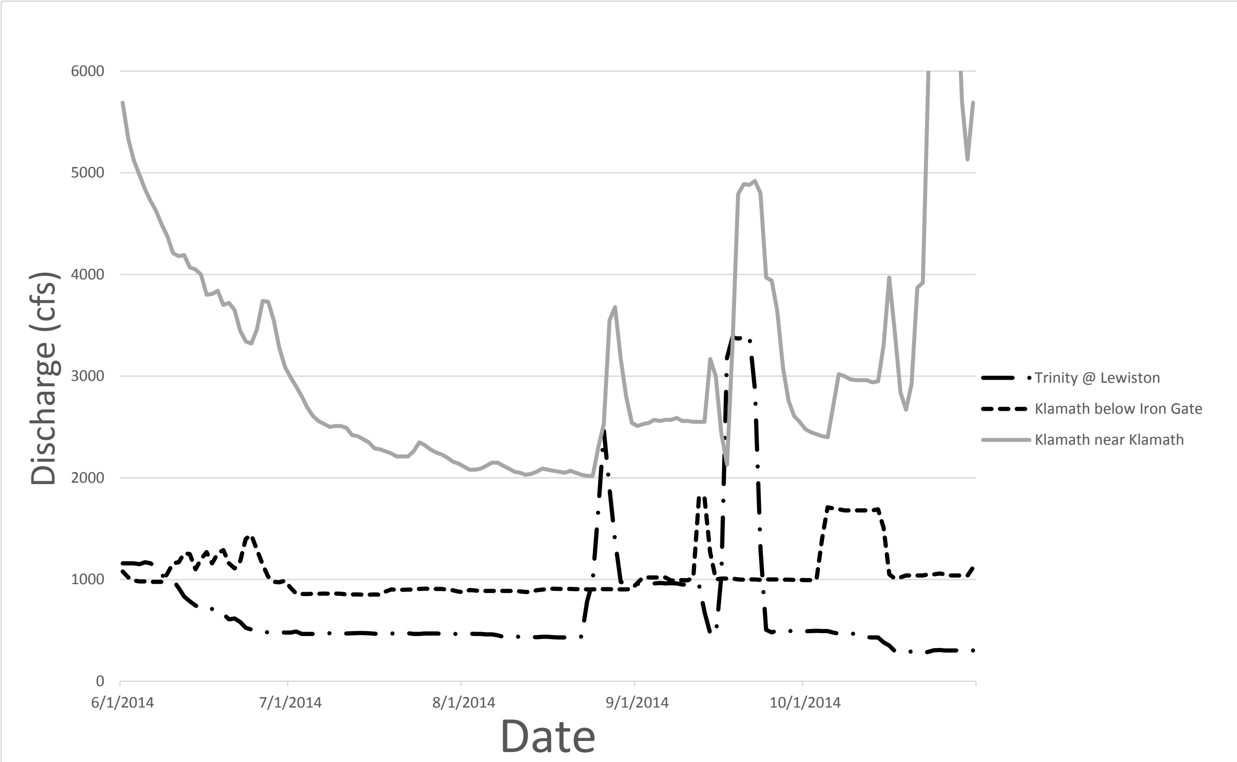


Figure 8: Daily average river flows for the Trinity River at Lewiston (Trinity River mile 110), Iron Gate Dam on the Klamath River (river mile 190), and the Klamath River near Klamath (KNK) (river mile 8). The KNK gage is most representative of the flow conditions present in the ich outbreak zone in the lower Klamath River.

....

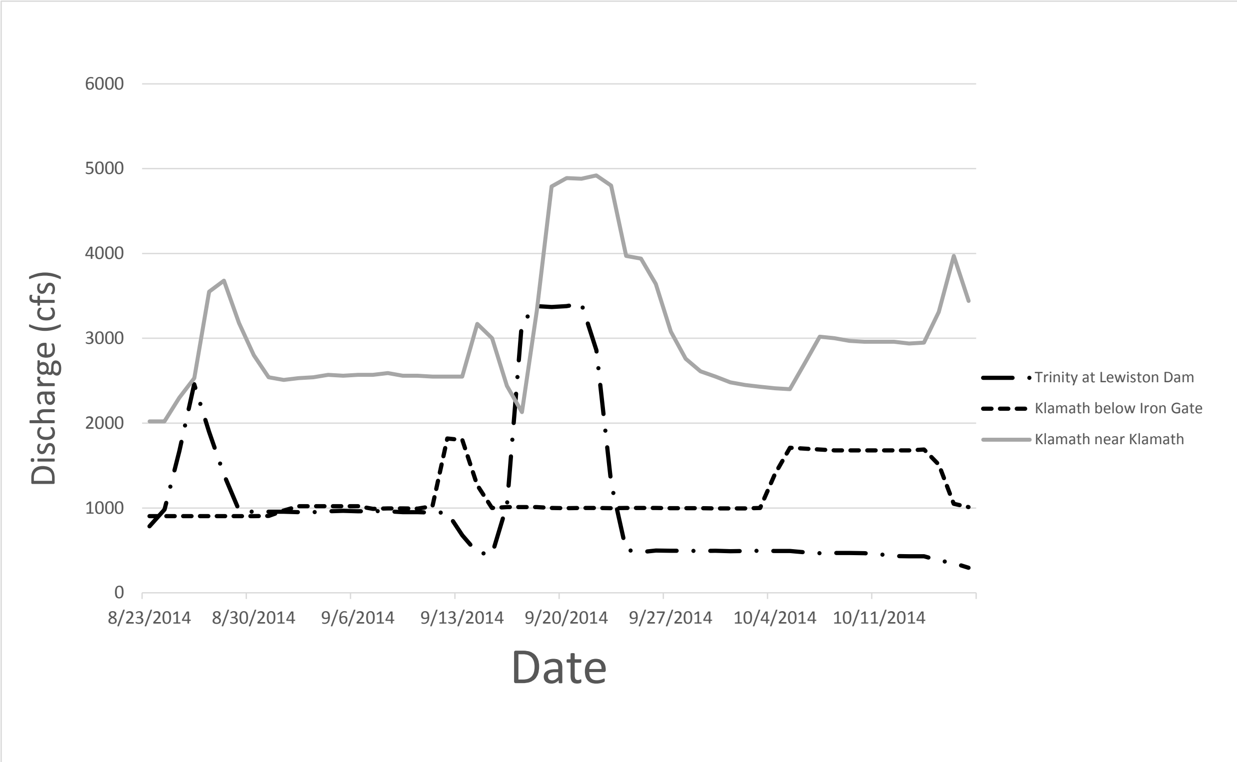


Figure 9: Flows on the Klamath and Trinity Rivers during the implementation of preventative and emergency flow releases from Lewiston Dam on the Trinity River and Iron Gate Dam on the Klamath River. This is the same data as Figure 8, but a show a narrower range of dates to enhance detail. The flow increase at the Klamath near Klamath gage in mid-October was from a precipitation event.

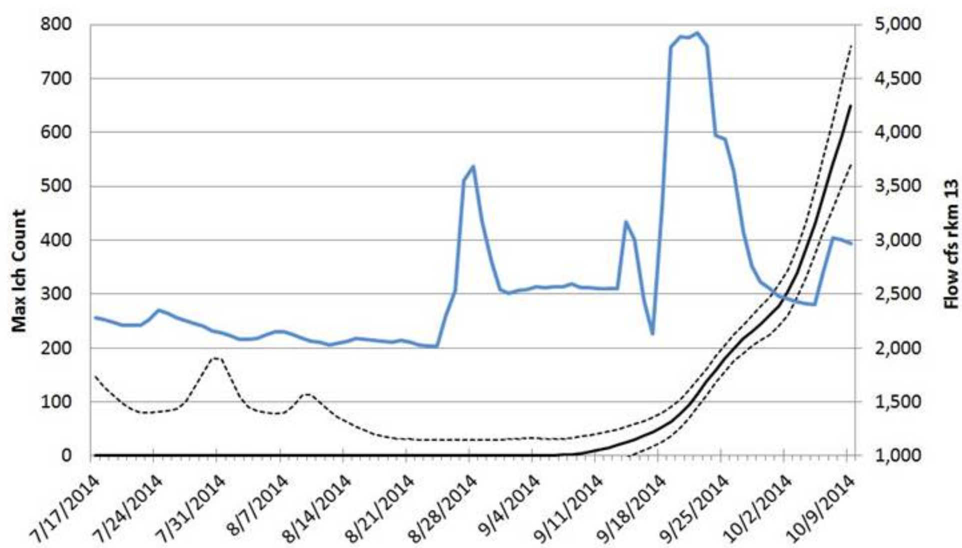


Figure 10: Infection severity curve (from Figure 6) compared to flow in the lower Klamath River at the KNK gage (rkm 13).

Water Temperatures

Water temperatures were relatively high in the Klamath River mainstem throughout the summer months, not falling below 22°C until cool water arrived from releases from Lewiston Dam on August 27. After that, the increased flow combined with other factors, such as declining sun angle, decreased day length, and increased topographic shading, lowered water temperatures below 22°C for the remainder of the summer and fall migration period. Figure 11 shows water temperature at three locations in the lower Klamath and Blue Creek. Data is from Yurok Tribal Fisheries Program Hobo© temperature loggers, and from the USGS gage at the Klamath near Klamath river gauge.

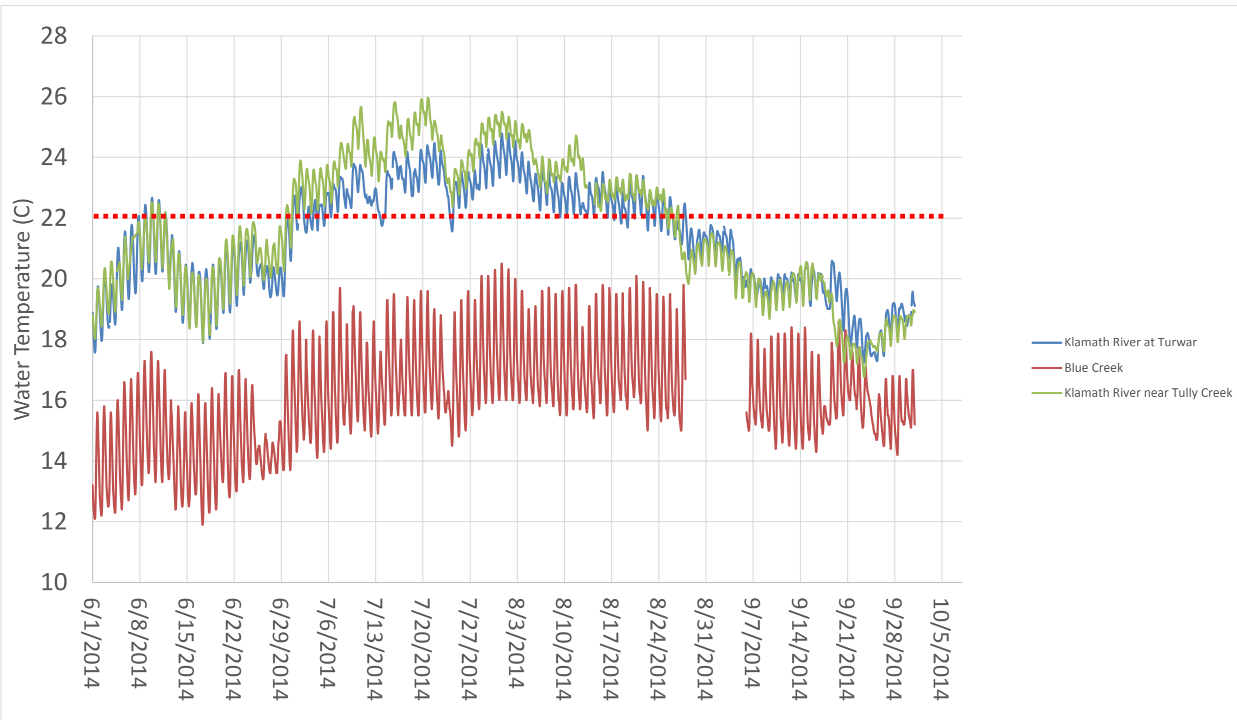


Figure 11: Water temperatures at two locations on the Klamath River mainstem (Tully Creek at rkm 56, and Terwer at rkm 10). Also shown is the water temperature of Blue Creek, which is the largest thermal refuge area in the lower Klamath and the location of the majority of holding adult fish when mainstem temperatures are over approximately 22°C (horizontal line). (unpublished data YTFP, obtained with calibrated Hobo® tidbit thermistors).

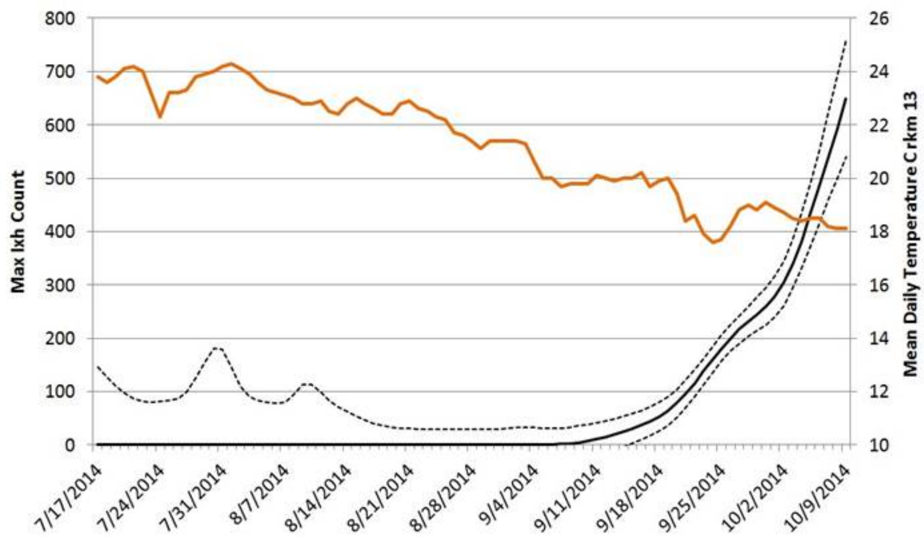


Figure 12: Infection severity curve (see Figure 6) compared to daily average water temperature at the KNK gage. Clearly visible is the large temperature drop on September 18 due to the large pulse flow from the Trinity River arriving at the KNK gage.

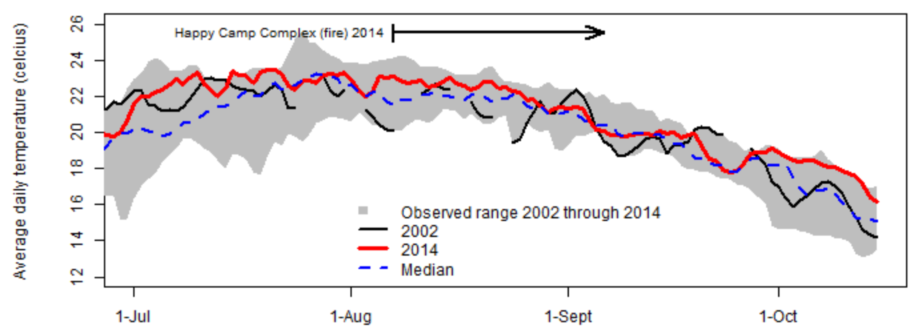


Figure 13: Average and median daily discharge (CFS) and water temperature (°C) in the Klamath River at Klamath (rkm 13.0 and rkm 8.6), CA from July 1 to October 15, 2002 to 2014. Water temperature data are available from July 1, 2003 and from July 19, 2004 through the end of each water year. (Figure from Magneson and Chamberlain 2015).

Prespawn Mortality

In June 2015, NMFS issued a memorandum concerning prespawn mortality of Chinook and coho salmon in the Trinity River (Naman 2015). It stated that:

> *"Coho salmon prespawn mortality in the Trinity River in 2014 was 48%, significantly greater than any other year since 2000 (Figure 1* [Figure 14 in this report]*). Chinook salmon prespawn mortality was 12% in 2014, approximately double the average of 6.2% from 2000 to 2013. Potential causative factors include high water temperatures, poor water quality, or disease infection. High run size of coho salmon and Chinook salmon was unlikely a major factor in coho salmon prespawn mortality because run sizes in previous years have been as high, or higher than those in 2014. Unknown is the extent to which the fish pathogen ich played a role in coho salmon prespawn mortality. However, fall-run Chinook salmon were heavily infected with ich at times in 2014 and did not have as high prespawn mortality as coho salmon in the Trinity River. Also unknown is how the high proportion of coho salmon prespawn mortality in the Trinity River affected the number of coho salmon fry and juveniles produced in 2015.*

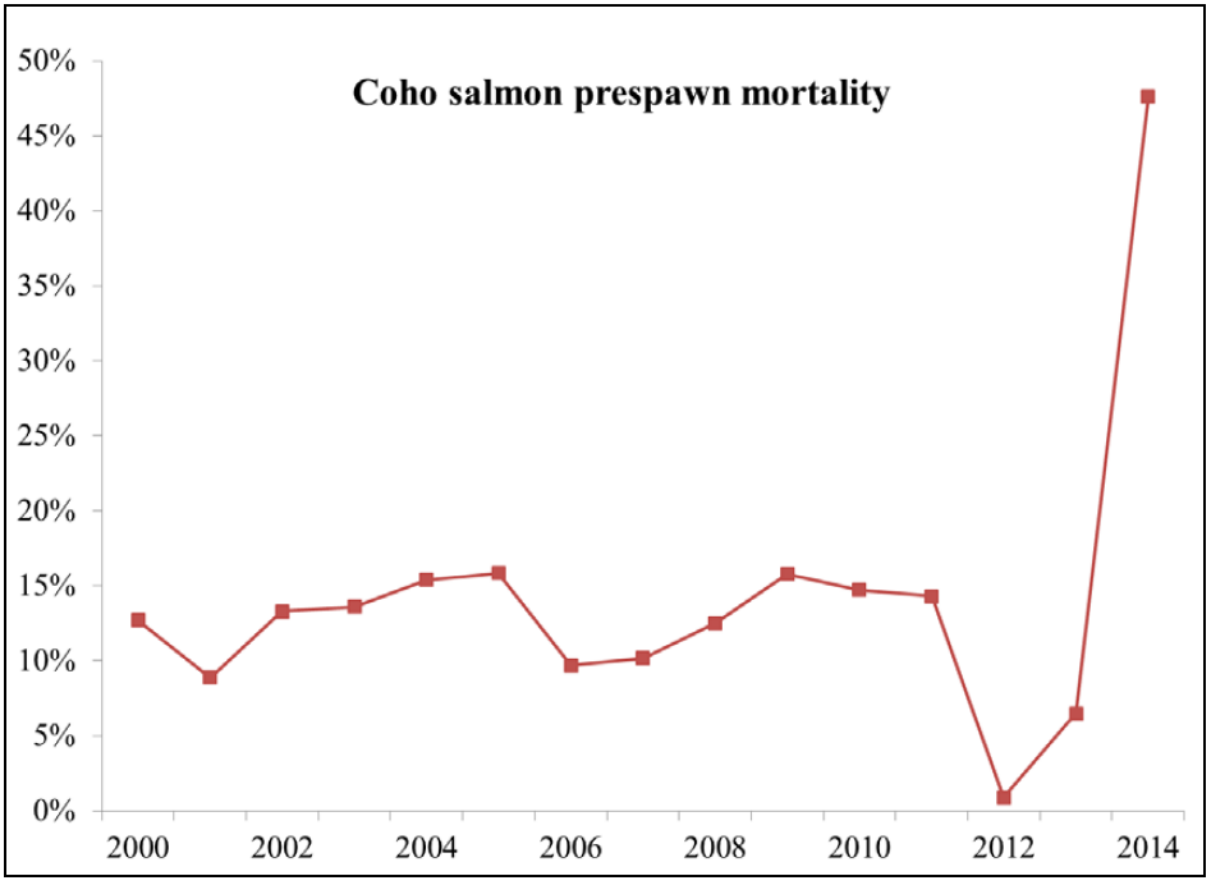


Figure 14: Prespawn mortality since the year 2000 for coho salmon in the Trinity River. Data from CDFW 2014 as cited in NMFS memorandum 6/3/15 (Naman 2015).

Fall Chinook Run Size

Approximately 160,400 adult fall Chinook returned to the Klamath Basin in 2014. This was approximately 1.7 times greater than the 92,800 adult fish that were projected to return preseason, and 43% bigger than the 112,000 average adult run size since 1978, when records were first kept.

Blue Creek Adult Salmon Thermal Refugia Abundance

The maximum counts for adult salmonid abundance observed holding at the Blue Creek thermal refugia area (Figure 5) for each year since 2002 are presented (Table 9). Counts were not done from 2005 through 2011. Salmon and steelhead counts were combined and the maximum observed on any single date is reported. High numbers of adult salmonids occurred in 2002, 2003, 2004 and 2014. Additional flows from the Trinity River were provided during all years except 2002, when the fish kill event happened.

Table 9: Maximum adult salmonid abundance at Blue Creek thermal refugia (includes counts in Blue Hole if applicable). Counts were not performed in 2005-2008, or 2010-2011.

Year	Maximum Adult Count
2002	757
2003	546
2004	1701
2009	0
2012	70
2013	20
2014	3700

Discussion

Drought conditions created favorable conditions for a serious outbreak of ich in 2014, but no large-scale mortality event was observed in the Klamath River. Low flow conditions, high water temperatures in the lower mainstem Klamath River, high numbers of adult salmonids holding in Blue Creek and other thermal refuges earlier in the summer, a larger than predicted fall Chinook salmon run, and the behavioral tendency of fall-run Chinook salmon to delay migration through the lower Klamath River (Strange 2010), all combined to create conditions highly conducive to an ich outbreak. This outbreak reached rapid growth phase in the lower Klamath River in mid-September (Figure 6), but initiated much earlier in mid-summer, with the earliest confirmed observation falling on August 21 (Appendix 1). Although ich was not confirmed by CNFHC staff until mid-September, a photograph of a pink salmon in late July showing probable (but unconfirmed) ich, and a pathology report by CDFW (Kwak 2015) on the Trinity River at the hatchery indicates that ich may have been present much earlier in July.

Although ich numbers observed on the gills of migrating salmon reached higher levels than previously documented in the wild (e.g., Traxler 1998, Maceda-Veiga 2009), the fish did not reach a diseased state (Foott 2015a, 2015b), during sampling observations and, the outbreak did not cause direct mortalities. At very high infection severity, ich causes a disease in fish known as ichthyophthiriosis that is characterized by hyperplasia (swollen inflamed tissue) in the gills and other symptoms that can ultimately cause death by suffocation or secondary infections (Matthews 2005). This was not observed in the lower Klamath River in 2014 (Foott 2015a, 2015b). Two other studies that reported actual numbers of ich on fish captured from a natural setting were from sockeye salmon in the Babine River, BC Canada (Traxler 1998), and from red-tailed barbs in a stream in Spain (Maceda-Veiga et al. 2009), who reported less ich than was seen on Klamath River fish, although as explained below differing methodologies make direct comparisons difficult. For example, Traxler (1998) reported numbers of ich per gill without elaborating on how these counts were obtained. Given that a complete count (pulling filaments apart, counting in between them etc) would take hours per gill arch, it is unknown exactly how the counts in the Traxler study were obtained. Maceda-Veiga et al. (2009) obtained surface counts from the exterior of fish using a dissecting microscope, but did not examine the gills directly.

An outbreak of ich and columnaris on Butte Creek occurred in 2003[8] killing over 11,000 adult salmon in Butte Creek, but the levels of ich were not quantified, and much of the kill was attributed to columnaris rather than ich[9] (Ward et al. 2004, Veek 2003). Because examination of the gills is a lethal sampling method and cannot be done on fish that have been dead for any significant length of time, it is often not feasible to examine fish in larger numbers such as was done for this study because it requires the capture and sacrifice of living fish.

A major information gap regarding ich in the Klamath River is the exact response of the ich to temperature in terms of life cycle span. It is known that higher temperatures consistently accelerates the ich life cycle, enabling the infection to propagate more rapidly, but the exact dynamics of this in the Klamath remain unknown, and evidence is mounting that there are different isolates (i.e. strains) of ich that respond differently to water temperatures (Nigrelli 1976, Aihua and Buchmann 2001, Forwood 2015). Theront production per tomite varies widely relative to temperature and success of the trophont feeding on its host fish (Forwood 2015).

Effects of flow management

An important question to be considered from the events and information gathered in 2014 is whether flow management was effective in preventing a fish kill event in the lower Klamath River such as happened in 2002. The data from 2014 show that even though preventative and emergency flows were provided, ich rose to high levels and yet there was not an observable fish kill in the lower Klamath River. Given the similarities of conditions (absent flow increases) to 2002 when there was a fish kill event, as well as a robust body of scientific evidence linking flow to ich infection severity, it is reasonable to conclude that this outbreak would have resulted in a fish kill if the additional flow releases did not occur in 2014. It should be noted that the first observed ich was August 21, which was before any flow increase had reached the lower Klamath River, indicating that low flow conditions allowed the ich to become established before higher flows were implemented.

This conclusion is supported by numerous scientific studies. For example, Bodensteiner (2000) found that "*Increased water flow successfully controlled ich under both increasing and decreasing thermal regimes in our two laboratory-scale studies.*"

In Ogut et al (2005): "*Mean intensities of the parasite were mainly affected by water temperature. Increased load of grown fish and decreased levels of water due to shortage of rainfalls in August probably enhanced the chance for ichthyophthiriasis outbreaks*."

In Butcher (1947): "*Flowing water is an efficient means of dealing with an epidemic and obviously the most economical. It is doubtful that the running water carries away the encysted forms of the parasite as well as those free-'swimming forms. Comparatively still water is a prerequisite to encystment, however, and by removing the free-swimming forms a flow of water prevents encystment*."

In Hop Wo et al. (2003) from the Nanaimo River: "*The low flow and water levels likely result in delayed fish movement and higher water temperatures which may potentially increase levels of disease and parasites. This is particularly true for the parasite Ich (ichthyophthirius) which matures more rapidly with*

[8] Another, smaller, fish kill event occurred in Butte Creek in 2004.
[9] Columnaris was named as a secondary pathogen in the 2002 Klamath River fish kill event.

higher temperature (Ministry of Environment, Lands and Parks 1993). During particularly low water levels the river flow can be increased with a controlled water release."

In Maceida-Veiga et al. (2009): "*Water quality, suitable water temperature, and low flow may all have contributed to the outbreak* [of ich in Mediterranean streams during a drought]."

The theme of these scientific references is that flow is a contributing factor in determining the severity of a given ich outbreak. It is therefore very likely that increased water flows and associated lowered temperatures reduced the severity of the ich outbreak in 2014 in the lower Klamath River.

In terms of the mechanism for the benefits of increased flows, water velocities and turnover rates have been identified as important factors that impact the probability of infection (Bodensteiner 2000). It is not possible from the data in this report or a review of the literature to verify whether velocities versus turnover rates are the key to reducing infection rates from ich in presence of higher flows (Bodensteiner 2000). However, given that adult salmon tend to have a range of preferred holding velocity no matter what the flow and will change position accordingly to find that velocity (Moyle 2002), I hypothesize that turnover rate is likely a more important factor in the lower Klamath. The issue of velocity versus turnover rate is complex because turnover rate[10] is correlated with velocity, and velocity distribution in systems such as large rivers is very complex. Certainly, the lifecycle of ich (given the very short survival times of theronts if they do not immediately find a fish host) indicate that increased flows that are capable of carrying theronts away from high fish density areas more quickly have a higher likelihood of being effective at reducing an ich outbreak. Trophonts that have left their host to become tomonts also drift for approximately 24 hours (Ewing 1986), and would also be carried (in general) further downstream in higher flows than lower, and a higher percentage could be carried out to sea where they would perish. Also higher flows and velocities would likely interfere with the theront's ability to find and attach to a fish within this narrow timeframe that they can live before finding a host.

The counts that were obtained from the gills of salmon far exceeded any counts from either literature, or from the personal experience of fish pathology experts (Traxler 1998, Maceda-Veiga 2009 Dr. JS Foott pers comm), and yet the fish did not display classic symptoms of ichthyophthiriosis disease. In some ways, the ich infections observed in the Klamath River were somewhat unusual when compared to the literature of ich infestations as described in Matthews et al (2005). Matthews describes ich as always being embedded in the epithelium of the fish, whereas our observations were that a great many if not most of the ich we observed on gill arches were on the surface and did not appear to be embedded. It is possible, however, that trophonts exited the epithelium extremely rapidly upon the death of the host fish we examined.

It is quite possible that ich has geographic isolates that could consist of locally adapted genotypes that display different effects to their target hosts, different responses to temperature, and different life history strategies (Nigrelli 1976, Aihua and Buchmann 2001, Forwood 2015). Similar to the groundbreaking work done with *Ceratonova shasta* that demonstrated at least four different genotypes each with a preferred host, it is possible that this is a unique strain of ich with its own unique and as of

[10] Turnover rate is reported as a function of inflow and vessel size. For example, a one gallon container experiencing an inflow of five gallons/hour would be said to experience a turnover rate of 5 per hour. In this case, the lower Klamath would be the "vessel" and river flow would be the inflow rate.

yet, unknown characteristics. However, as the fish kill in the Klamath in 2002 showed, ich in the Klamath River is capable of causing very large numbers of salmon mortalities.

Risk Factors for Ich Outbreaks in the Klamath

In order to minimize the risk of a fish kill event, water managers and fish biologists on the Klamath and Trinity Rivers are faced with the task of estimating risk of a fish kill event, given a set of environmental and biological conditions present on the river combined with predictions of water flow, water temperature and run size. The following is a list of factors that can be considered in evaluating the risk of a future ich epizooitic in Klamath and Trinity Rivers. This list was compiled based on a review of the scientific literature, as well as the data from the 2014 ich outbreak.

1. Hydrologic conditions. Scientific evidence and the data from 2002 and 2014 suggests that drought conditions place the river in a state of heightened risk for an ich outbreak. Specifically, mid-August flows in both those years were below 2,800 cfs, while it averaged greater than 2,800 cfs in all the other years;
2. High water temperatures. High water temperatures a) can cause fish to hold at refugia areas such as Blue Creek, b) place stress on holding fish which can compromise their immune systems, and c) speed up the lifecycle of ich, allowing it to increase more rapidly;
3. Large numbers of fish holding in close proximity to each other, such as the thermal refugia at Blue Creek. High water temperatures in certain years can cause large numbers of fish to congregate for extended periods at thermal refugia[11] such as Blue Creek which is the focal location for sampling and is where the first ich was observed in 2014, and again in 2015 (Yurok Tribe unpublished data).
4. Large run size: high abundance of fall-run Chinook in the lower Klamath River increases the density of holding fish in the lower Klamath River which in turn can contribute to a rapid increase in ich;
5. Observed ich in the current water year. If ich is observed at above baseline levels in any given year, it places the fall run under higher risk of a potentially fatal epizootic ich outbreak;
6. Observed ich in the previous water year. Ich was observed in 2003, which was the year after the fish kill event of 2002, and again in 2015, the year after the 2014 outbreak which is the subject of this report. It is probable that an ich outbreak leads to an elevated background level of ich that can carry over to the next year;

All of these risk factors should be considered when evaluating the risk of a future ich outbreak or fish kill event.

The Hatchery Results

At first it was puzzling to encounter fish at Iron Gate and Trinity Hatchery that had migrated through areas where all fish were heavily infected with ich, and in all likelihood were heavily infected themselves, and yet had little to no ich observed on their gills. A likely explanation lies in the fact that for ich counts to remain high as trophonts mature and drop off, there must be a source of theront infection. Apparently there were not enough theronts in the upper Klamath River to re-infect these fish as the trophonts matured, thus ich densities on the gills apparently dropped over time to the levels seen

[11] A thermal refugia is an area of accessible colder water, usually located at a confluence area with a cold water tributary.

in the results. The understanding of this dynamic was hampered by an inability to collect fish for ich counts in the 145 miles of river between Weitchpec and Iron Gate Dam.

Conclusion and Recommendations

As discussed above, it can be difficult to separate mere correlation from causation, especially in a complex system such as a large river system like the Klamath/Trinity. However, in this case, it appears that it is highly likely that increased flows were successful in preventing the high levels of ich observed in Klamath River salmon from reaching lethal levels. Key observations include:

- Ich was first observed in the lower Klamath River prior to increased flows, with a fish from August 21 having confirmed ich;
- Even though an ich outbreak occurred, a fish kill was avoided;
- Ich levels were very high, yet infections generally did not advance to a disease state;
- The outbreak advanced rapidly in mid-September but initiated much earlier in the summer in holding adult salmonids which were under considerable stress due to the low flows and warm water conditions;

Management and research recommendations include:

- Early pulses of colder flows could help clear the river and thermal refuge areas of any infected spring or summer run Chinook salmon and elevated background levels of ich prior to the arrival of the larger fall run in later August and early September;
- Larger pulses should be explored for early September since this appears to be a crucial window for outbreak growth in 2002 and 2014; and any such pulses should last for the entire life span of the ich organism (at least 3-4 days at 21-23°C; longer at lower temperatures) to be maximally effective;
- Preventative high flow releases should persist at least through the first three weeks of September, because in 2002 and 2014 this was the window of ich infection;
- Preventative flow release recommendations should account for the high degree of error inherent in run-size forecasts. In other words, there should be an acknowledgement that run size can be significantly higher than forecast, and thus it may be necessary to implement preventative measures even when smaller run sizes are forecast;
- A high priority should be given to initiation and completion of environmental DNA (e-DNA) studies on the ich so that eDNA water samples can be used to ascertain the risk of a fish kill event in the river rather than sampling adult salmon;
- Other parameters (such as response of Klamath River ich organisms to temperature) should also be investigated with an overall goal of a quantitative ich epidemiology model capable of evaluating existing and expected hydrologic and temperature conditions in the Klamath River.
- In general, it would be advantageous to water and fisheries comanagers to understand as much as possible about the population dynamics of ich in the Klamath River. Further research may reveal more precise or innovative management strategies to control this pathogen.

Literature Cited

Aihua L., Buchmann K. 2001: Temperature and salinity-dependent development of a Nordic strain of Ichthyophthirius multifiliis from rainbow trout. J. Appl. Ichthyology. 17: 273–276.

Belchik, M., Hillemeier, D., and Pierce, R.M. 2004. The Klamath River Fish Kill of 2002; Analysis of Contributing Factors. Yurok Tribal Fisheries Program. 42pp.

Bodensteiner, L.R., Sheehan, R.J., and Wills, P.S. 2000. Flowing water: an effective treatment for Ichthyophthiriasis. J. Aqua. Animal Health 12: 209-219.

Bowman, A W. and Azzalini, A., Applied Smoothing Techniques for Data Analysis, Oxford University Press, New York, 1997.

Dickerson, H.W., Dawe, D.L., 1995. *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders. vol. 1. Protozoan and Metazoan Infections. CAB International, Wallingford, pp. 181–227.

Ewing M.S., Lynn M.E., Ewing S.A. 1986: Critical periods in development of *Ichthyophthirius multifiliis* (Ciliophora) populations. J. Protozool. 33: 388–391.

Foott, J.S. 2002. Pathology report. FHC Case No. 2002-139. USFWS. Anderson, California.

Foott, J.S. 2014a. Final results for 9/15/2014 sampling of adult Chinook from lower Klamath River (rm 16) - Detection of Ichthyophthirius multifiliis. Memorandum. 4pp

Foott, J.S. 2014b. Personal communication email October 6, 2014 re: Adult fish pathology examination on 10/2/2014 in Weitchpec, CA. (Appendix 3).

Forwood, J. M., Harris, J. Landos, M. and Deveney, M., 2015. Life cycle and settlement of an Australian isolate of *Ichthyophthirius multifiliis* Fouquet, 1876 from rainbow trout. Folia Parisitologica. 62: 013.

Guillen, G. 2003. Klamath River Fish Die-off, September 2002: Causative Factors of Mortality. US Fish and Wildlife Service. Report Number AFWO-F-02-03. 128pp.

Hop Wo, N.K., E.W. Carter, D.A. Nagtegaal, and K.E. Jones. 2003. Adult chinook escapement assessment conducted on the Nanaimo River during 2001. Can. Manuscr. Rep. Fish. Aquat. Sci. 2654: 38 p.

Kwak, K. 2015. Fish Pathology Report Trinity River Hatchery, Calif. Dept. of Fish and Wildlife, Rancho Cordova California, October 30, 2015. 2p.

Maceda-Veiga, A., H. Salvadó , D. Vinyoles & A. De Sostoa (2009) Outbreaks of *Ichthyophthirius multifiliis* in Redtail Barbs *Barbus haasi* in a Mediterranean Stream during Drought, Journal of Aquatic Animal Health, 21:3, 189-194.

Magneson, M. D. and C. D. Chamberlain. 2015. The Influence of Lewiston Dam Releases on Water Temperatures of the Trinity River and Lower Klamath River, CA, April to October 2014. U. S. Fish and Wildlife Service, Arcata Fish and Wildlife Office, Arcata Fisheries Data Series Report Number DS 2015-41, Arcata, California.

Matthews R.A. 2005: *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. Adv. Parasitology. 59: 159–241.

McCallum, H.I., 1982 Infection Dynamics of Ichthyophthirius multifiliis. Parisitology 85: 475-488

Moyle, P. B. 2002b. Salmon and Trout, Salmonidae - Chinook Salmon, (Oncorhynchus tshawytscha) in Inland Fishes of California. Los Angeles, California: University of California Press, 251-263

Naman, S. 2015. Untitled Memorandum regarding prespawn mortality in the Trinity River. Data in memorandum from CDFW unnamed source.

Nigrelli R.F., Pokorny K.S., Ruggieri G.D. 1976: Notes on *Ichthyophthirius multifiliis*, a ciliate parasitic on fresh-water fishes, with some remarks on possible physiological races and species. Trans. Am. Microsc. Soc. 95: 607–613.

Strange, J.S. 2003. Adult Chinook Migration in the Klamath River Basin: 2003 Radio Telemetry Study Final Report. Yurok Tribal Fisheries Program; and University of Washington. 73pp. Available at www.yuroktribe.org/departments/fisheries/ reportsandpublications.htm.

Strange, J.S. 2006. Adult Chinook Migration in the Klamath River Basin: 2004 Radio Telemetry Study Final Report. Yurok Tribal Fisheries Program; and University of Washington. 180pp. Available at www.yuroktribe.org/departments/fisheries/

Strange, J.S. 2007a. Adult Chinook Migration in the Klamath River Basin: 2006 Telemetry Study Final Report. Yurok Tribal Fisheries Program; University of Washington; and Hoopa Valley Tribal Fisheries. 87pp. Available at www.yuroktribe.org/departments/fisheries/reportsandpublications.htm.

Strange, J.S. 2007b. Adult Chinook Migration in the Klamath River Basin: 2005 Sonic Telemetry Study Final Report. Yurok Tribal Fisheries Program; University of Washington; and Hoopa Valley Tribal Fisheries. 96pp. Available at www.yuroktribe.org/departments/fisheries/reportsandpublications.htm.

Strange, J.S. 2008. Adult Chinook Salmon Migration in the Klamath River Basin: 2007 Biotelemetry Monitoring Study Final Report. Yurok Tribal Fisheries Program. 60pp. Available at www.yuroktribe.org/departments/fisheries/reportsandpublications.htm.

Strange, J.S. 2010a. Upper thermal limits to migration in adult Chinook salmon: evidence from the Klamath River basin. Trans. Am. Fish. Soc. 139: 1091-1108.

Strange, J.S. 2010b. Summary of Scientific Evidence to Guide Special Flow Releases to Reduce the Risk of Adult Fall Chinook Salmon Mass Disease Mortality in the Lower Klamath River. Yurok Tribal Fisheries Program Technical Report. 17pp. www.yuroktribe.org/departments/fisheries/reportsandpublications.htm.

Sun, H.Y. et al. Endobymbiotic Bacteria in the Parasitic Ciliate *Ichthyophthirius multifiliis*. Applied and Enviornmental Microbiology. 75: 23. Pp. 7445-7452

Trinity River Restoration Program Fall Flows Subgroup 2012. 2012 Fall Flow Release Recommendation. Memorandum to Bureau of Reclamation Area Manager. May 31, 2012.

Turek, S., Rode, M., Cox, B., Heise, G., Sinnen, W., Reese, C., Borok, S., Hampton, M., and Chun, C. 2004. September 2002 Klamath River Fish-Kill: Final Analysis of Contributing Factors and Impacts. California Department of Fish and Game. 183pp.

Ward, P. D., T.R. McReynolds, , C. Garman, 2004. Butte Creek Spring-Run Chinook Salmon, *Oncoryhnchus tshawytscha* Pre-Spawn Mortality Evaluation 2003. California Department of Fish and Game 92pp.

Traxler, G.S., Richard, J., and McDonald, T.E. 1998. Ichthyophthirius multifiliis (Ich) epizootics in spawning sockeye salmon in British Columbia. Can. J. Aqua. Animal Health 10: 143-151.

Veek, T. 2003. Fish Pathologist Report, Calif. Dept. of Fish and Game, Rancho Cordova California, August 4, 2003.1p.

Appendix 1: Complete YTFP 2014 Ich Sampling Results

Table shows all fish collected by YTFP in 2014 as part of the ich monitoring project. "Blue Creek", "Tully Creek" and "Pecwan Creek" all refer to the Klamath River mainstem near these creeks, rather than the creeks themselves. The missing data (8/5-8/7) had no ich detections. CH=Chinook salmon; STH=steelhead; SCKEYE=sockeye salmon; PINK and CHUM are pink and chum salmon respectively. Columnaris is reported on a qualitative scale of 0-4 (not present to severe) and each side recorded separately. Ich counts for the gills are reported separately for left and right side [Ich (L) and Ich (R) respectively]. Max ich is the maximum of (L) and ()R side ich gill counts.

Date	Sample #	Location	Species	Length(cm)	Sex	Ad clip	Columnaris (L)	Columnaris (R)	Ich (left)	Ich (rt)	Max Ich
7/17/2014	1	Blue Creek	CH	86	M	N	3	3	0	0	0
7/17/2014	2	Blue Creek	CH	82	F	N	0	0	0	0	0
7/18/2014	3	Tully Creek	CH	78	M	N	1	3	0	0	0
7/24/2014	4	Pecwan Creek	PINK	58	F	N	2	0	0	0	0
7/24/2014	5	Tully Creek	CH	77	F	N	0	0	0	0	0
7/25/2014	6	Blue Creek	CH	73	M	N	2	1	0	0	0
7/25/2014	7	Blue Creek	CH	88	M	N	2	3	0	0	0
7/25/2014	8	Blue Creek	CH	74	M	N	0	0	0	0	0
7/25/2014	9	Blue Creek	CHUM	9999	M	N	0	0	0	0	0
7/29/2014	10	Pecwan Creek	CH	82	F	N	0	0	0	0	0
7/29/2014	11	Pecwan Creek	SCKEYE	62	M	N	0	0	0	0	0
8/5/2014	12	Missing Data									
8/5/2014	13	Missing Data									
8/5/2014	14	Missing Data									
8/5/2014	15	Missing Data									
8/5/2014	16	Missing Data									
8/5/2014	17	Missing Data									
8/5/2014	18	Missing Data									
8/6/2014	19	Missing Data									
8/7/2014	20	Missing Data									
8/13/2014	21	Blue Creek	CH	73	M	N	0.5	0	0	0	0
8/13/2014	22	Blue Creek	CH	82	F	N	0	0	0	0	0

8/13/2014	23	Blue Creek	CH	90	M	N	0	0	0	0	0
8/15/2014	24	Blue Creek	CH	74	F	N	0	0	0	0	0
8/15/2014	25	Blue Creek	CH	84	M	N	1	0	0	0	0
8/15/2014	26	Blue Creek	CH	84	F	N	0	0	0	0	0
8/15/2014	27	Blue Creek	CH	84	F	N	0	0	0	0	0
8/15/2014	28	Blue Creek	CH	73	M	N	0	0	0	0	0
8/15/2014	29	Blue Creek	CH	68	M	N	0	0	0	0	0
8/15/2014	30	Blue Creek	CH	95	M	N	0	0	0	0	0
8/15/2014	31	Blue Creek	CH	86	M	N	0	0	0	0	0
8/15/2014	32	Blue Creek	CH	90	M	N	0	0	0	0	0
8/15/2014	33	Blue Creek	CH	83	F	N	0	0	0	0	0
8/15/2014	34.1	Blue Creek	SH	47	F	N	1	1	0	0	0
8/15/2014	34.2	Blue Creek	CH	75	F	N	0	0	0	0	0
8/15/2014	35	Blue Creek	CH	80	F	N	0	0	0	0	0
8/15/2014	36	Blue Creek	CH	87	F	N	0	0	0	0	0
8/15/2014	37	Blue Creek	CH	96	F	N	0	0	0	0	0
8/20/2014	38	Blue Creek	CH	78	F	N	0	0	0	0	0
8/20/2014	39	Blue Creek	CH	78	F	N	0	0	0	0	0
8/20/2014	40	Blue Creek	CH	85	M	N	0	0	0	0	0
8/20/2014	41	Blue Creek	CH	93	M	N	2	2	0	0	0
8/20/2014	42	Blue Creek	CH	80	M	N	0	0	0	0	0
8/20/2014	43	Blue Creek	CH	83	F	N	0	0	0	0	0
8/20/2014	44	Blue Creek	CH	73	F	N	0	1	0	0	0
8/20/2014	45	Blue Creek	CH	82	M	N	0	0	0	0	0
8/20/2014	46	Blue Creek	CH	83	F	N	0	0	0	0	0
8/21/2014	47	Blue Creek	CH	84	F	N	1	0	0	0	0
8/21/2014	48	Blue Creek	CH	78	M	N	0	1	0	0	0
8/21/2014	49	Blue Creek	CH	50	M	N	0	0	0	0	0
8/21/2014	50	Blue Creek	CH	84	F	N	0	0	0	0	0
8/21/2014	51	Blue Creek	CH	78	F	N	0	0	0	0	0
8/21/2014	52	Blue Creek	CH	76	M	N	1	0	0	0	2
8/21/2014	53	Blue Creek	CH	87	M	N	0	0	0	0	0

8/21/2014	54	Blue Creek	CH	82	F	N	1	0	0	0	0
8/21/2014	55	Blue Creek	CH	75	F	N	0	3	0	0	0
8/21/2014	56	Blue Creek	CH	78	F	N	0	0	0	0	0
8/22/2014	57	Blue Creek	CH	92	M	N	1	1	0	0	0
8/22/2014	58	Blue Creek	CH	56	F	N	0	4	0	0	0
8/22/2014	59	Blue Creek	CH	83	F	N	4	4	0	0	0
8/22/2014	60	Blue Creek	CH	94	M	N	0	1	0	0	0
8/22/2014	61	Blue Creek	CH	92	M	N	1	0	0	0	0
8/22/2014	62	Blue Creek	CH	76	F	N	0	0	0	0	0
8/22/2014	63	Blue Creek	CH	81	F	N	1	0	0	0	0
8/22/2014	64	Blue Creek	CH	84	M	N	0	1	0	0	0
8/22/2014	65	Blue Creek	CH	83	F	N	0	1	0	0	0
8/22/2014	66	Blue Creek	CH	78	F	N	0	0	0	0	0
8/22/2014	67	Blue Creek	CH	91	M	N	1	2	0	0	0
8/22/2014	68	Blue Creek	CH	91	M	N	0	0	0	0	0
8/22/2014	69	Blue Creek	CH	78	M	N	0	0	0	0	0
8/22/2014	70	Blue Creek	CH	78	F	N	0	0	0	0	0
8/25/2014	71	Blue Creek	CH	90	M	N	0	0	0	0	0
8/25/2014	72	Blue Creek	CH	88	MF	N	0	0	0	0	0
8/25/2014	73	Blue Creek	CH	88	M	N	0	1	0	0	0
8/25/2014	74	Blue Creek	CH	88	F	N	0	0	0	0	0
8/25/2014	75	Blue Creek	CH	82	M	N	0	0	0	0	0
8/25/2014	76	Blue Creek	CH	91	F	N	0	0	0	0	0
8/25/2014	77	Blue Creek	CH	81	M	N	0	0	0	0	0
8/25/2014	78	Blue Creek	CH	90	F	N	0	0	0	0	0
8/25/2014	79	Blue Creek	CH	86	F	N	0	0	0	0	0
8/25/2014	80	Blue Creek	CH	80	F	N	1	0	0	0	0
8/25/2014	81	Blue Creek	CH	54	M	N	0	0	0	0	0
8/26/2014	82	Blue Creek	CH	87	F	N	0	0	0	0	0
8/26/2014	83	Blue Creek	CH	80	M	N	1	0	0	0	0
8/26/2014	84	Blue Creek	CH	82	M	N	0	0	0	0	0
8/26/2014	85	Blue Creek	CH	87	M	N	3	3	0	0	0

8/26/2014	86	Blue Creek	CH	75	M	N	1	0	0	0	0
8/26/2014	87	Blue Creek	CH	88	F	N	1	3	0	0	0
8/26/2014	88	Blue Creek	CH	71	F	N	0	1	0	0	0
8/26/2014	89	Blue Creek	CH	81	F	N	0	3	0	0	0
8/26/2014	90	Blue Creek	CH	74	M	N	0	0	0	0	0
8/26/2014	91	Blue Creek	CH	65	M	N	0	1	0	0	0
8/26/2014	92	Blue Creek	CH	49	F	N	0	0	0	0	0
8/27/2014	93	Blue Creek	CH	71	F	N	0	1	0	0	0
8/27/2014	94	Blue Creek	CH	83	F	N	2	0	0	0	0
8/27/2014	95	Blue Creek	CH	78	F	N	0	0	0	0	0
8/27/2014	96	Blue Creek	CH	86	F	N	1	0	0	0	0
8/27/2014	97	Blue Creek	CH	95	M	N	1	0	2	0	2
8/27/2014	98	Blue Creek	CH	95	M	N	1	1	0	0	0
8/27/2014	99	Blue Creek	CH	80	M	N	1	0.5	0	0	0
8/27/2014	100	Blue Creek	CH	82	F	N	0	0	0	0	0
8/27/2014	101	Blue Creek	CH	91	M	N	0.5	2	0	0	0
8/27/2014	102	Blue Creek	CH	92	M	N	1	3	0	0	0
8/27/2014	103	Blue Creek	CH	90	M	N	2	0	0	0	0
9/2/2014	104	Blue Creek	CH	87	F	N	0	0	0	0	0
9/2/2014	105	Blue Creek	CH	98	M	N	1	1	0	0	0
9/2/2014	106	Blue Creek	CH	75	M	N	1	0	0	0	0
9/2/2014	107	Blue Creek	CH	80	F	N	0	0	0	0	0
9/2/2014	108	Blue Creek	CH	65	F	Y	1	0	0	0	0
9/2/2014	109	Blue Creek	CH	78	M	Y	0	0	0	0	0
9/2/2014	110	Blue Creek	CH	75	M	N	0	0	0	0	0
9/2/2014	111	Blue Creek	CH	77	F	N	0	0	0	0	0
9/2/2014	112	Blue Creek	CH	83	F	N	0	0	0	0	0
9/2/2014	113	Blue Creek	CH	81	F	N	2	2	0	0	0
9/2/2014	114	Blue Creek	CH	87	F	N	3	4	0	0	0
9/3/2014	115	Below Blue Hole	SH	38	M	Y	0	0	0	0	0
9/3/2014	116	Below Blue Hole	CH	90	F	N	0	0	0	0	0
9/3/2014	117	Below Blue Hole	CH	81	M	N	0	0	0	0	0

9/3/2014	118	Below Blue Hole	CH	78	M	N	0	0	0	0	0
9/3/2014	119	Below Blue Hole	CH	80	M	N	0	0	0	0	0
9/3/2014	120	Below Blue Hole	CH	80	F	N	0	0	0	0	0
9/4/2014	121	Tectah	CH	60	M	N	0	0	0	0	0
9/4/2014	122	Tectah	CH	87	F	N	0	0	0	0	0
9/4/2014	123	Tectah	CH	70	F	N	0	0	0	0	0
9/4/2014	124	Tectah	CH	83	F	N	0	0	0	0	0
9/4/2014	125	Tectah	CH	77	F	N	0	0	0	0	0
9/4/2014	126	Tectah	CH	78	F	N	0	0	0	0	0
9/4/2014	127	Tectah	CH	80	F	N	0	0	0	0	0
9/4/2014	128	Tectah	CH	83	F	N	0	0	0	0	0
9/4/2014	129	Tectah	CH	86	F	N	0	0	0	0	0
9/4/2014	130	Tectah	CH	80	M	N	0	1	0	0	0
9/4/2014	131	Tectah	CH	83	M	N	0	0.5	0	0	0
9/4/2014	132	Tectah	CH	90	F	N	0	0	0	0	0
9/4/2014	133	Tectah	CH	82	F	N	0	0	0	0	0
9/4/2014	134	Tectah	CH	87	F	N	0	0	0	0	0
9/9/2014	135	Tectah	CH	80	M	N	0	0	0	0	0
9/9/2014	136	Tectah	CH	85	F	N	0	0	0	0	0
9/9/2014	137	Tectah	CH	75	M	N	0.5	2	0	0	0
9/9/2014	138	Tectah	CH	83	F	N	0	0	0	0	0
9/9/2014	139	Tectah	CH	78	F	N	0	0	0	0	0
9/9/2014	140	Tectah	CH	76	F	N	0	0	0	0	0
9/9/2014	141	Tectah	CH	74	F	N	0	0	0	0	0
9/9/2014	142	Tectah	CH	97	M	N	2	0	0	0	0
9/9/2014	143	Tectah	CH	87	F	N	0	0	0	0	0
9/9/2014	144	Tectah	CH	95	M	N	1	0	0	0	0
9/9/2014	145	Tectah	CH	85	M	N	0	1	0	0	0
9/9/2014	146	Tectah	CH	72	M	Y	0	0	0	0	0
9/9/2014	147	Tectah	CH	83	M	N	2.5	0	0	0	0
9/10/2014	148	Tectah	CH	75	F	N	0	0	0	0	0
9/10/2014	149	Tectah	CH	86	F	N	0	0	0	0	0

9/10/2014	150	Tectah	CH	75	F	N	0	0	0	0	0
9/10/2014	151	Tectah	CH	64	M	Y	0	0	0	0	0
9/10/2014	152	Tectah	CH	70	M	N	0	0	0	10	10
9/10/2014	153	Tectah	CH	76	M	N	0	0	0	0	0
9/10/2014	154	Tectah	CH	78	M	N	0	0	0	0	0
9/10/2014	155	Tectah	CH	61	M	N	0	0	0	0	0
9/10/2014	156	Tectah	CH	73	M	N	0	0.5	0	0	0
9/10/2014	157	Tectah	CH	76	M	N	0	0	0	0	0
9/10/2014	158	Tectah	CH	80	F	N	1	0.5	0	0	0
9/10/2014	159	Tectah	CH	89	M	N	0	0	0	0	0
9/10/2014	160	Tectah	CH	85	M	Y	0	0	0	0	0
9/10/2014	161	Tectah	CH	86	M	N	0	0	0	0	0
9/10/2014	162	Tectah	CH	72	M	N	0.5	0.5	0	0	0
9/13/2014	163	Tectah	CH	82	m	N	0	0	0	2	2
9/13/2014	164	Tectah	CH	75	m	N	0	0	0	1	1
9/13/2014	165	Tectah	CH	80	f	N	0	0	0	17	17
9/13/2014	166	Tectah	CH	83	m	N	0	0	100	46	100
9/13/2014	167	Tectah	CH	76	m	N	0	0	0	0	0
9/13/2014	168	Tectah	CH	72	f	N	0	0	0	0	0
9/13/2014	169	Tectah	CH	94	f	N	0	1	4	6	6
9/13/2014	170	Tectah	CH	74	f	N	0	0	0	2	2
9/13/2014	171	Tectah	CH	70	m	N	0	0	2	0	2
9/16/2014	172	Weitchpec Klamath	CH	83	f	N	0	0	0	0	0
9/16/2014	173	Weitchpec Klamath	CH	82	f	N	0	0	5	50	50
9/16/2014	174	Weitchpec Klamath	CH	72	f	N	0	0	15	20	20
9/16/2014	175	Weitchpec Klamath	CH	78	m	N	0	0	66	30	66
9/16/2014	176	Weitchpec Klamath	CH	72	m	N	0	0	0	0	0

9/16/2014	177	Weitchpec Klamath	CH		76	f	N	0	0	102	95	102
9/16/2014	178	Weitchpec Klamath	CH		83	f	N	0	0	30	200	200
9/16/2014	179	Weitchpec Klamath	CH		70	m	N	0	0	0	0	0
9/16/2014	180	Weitchpec Klamath	CH		83	m	N	0	0	0	0	0
9/16/2014	181	Weitchpec Klamath	CH		81	f	N	0	3	85	20	85
9/19/2014	182	Blue	CH		75	m	N	0	0	0	0	0
9/19/2014	183	Tectah	CH		70	f	N	0	0	29	68	68
9/19/2014	184	Tectah	CH		72	f	N	0	0	72	68	72
9/19/2014	185	Tectah	CH		68	f	N	0	0	0	7	7
9/19/2014	186	Tectah	CH		75	m	N	0	0	0	0	0
9/19/2014	187	Tectah	CH		77	m	N	0	0	3	x	3
9/19/2014	188	Tectah	CH		87	m	N	0	0	14	12	14
9/19/2014	189	Tectah	CH		83	m	N	0	0	36	30	36
9/19/2014	190	Tectah	CH		76	f	N	0	0	0	0	0
9/19/2014	191	Tectah	CH		79	f	N	0	0	0	0	0
9/19/2014	192	Blue	CH	x		x	N	0	0	8	26	26
9/19/2014	193	Estuary (old 101 br)	CH		91	x	N	0	0	0	0	0
9/19/2014	194	Estuary (old 101 br)	CH		80	x	N	0	0	12	19	19
9/19/2014	195	Estuary (old 101 br)	CH		78	x	N	0	0	0	x	0
9/19/2014	196	Estuary (old 101 br)	CH		76	x	N	0	0	0	x	0
9/19/2014	197	Tectah	CH		89	m	N	0	0	0	0	0
9/19/2014	198	Tectah	CH		74	f	N	0	0	10	1	10
9/19/2014	199	Tectah	CH		74	m	N	0	0	19	18	19
9/19/2014	200	Tectah	CH		78	m	N	0	0	14	8	14

9/19/2014	201	Tectah	CH	83	f	N	0	0	46	30	46
9/19/2014	202	Tectah	CH	75	m	N	0	0	0	0	0
9/22/2014	203	Weitchpec Klamath	CH	95	m	N	0	0	31	20	31
9/22/2014	204	Weitchpec Klamath	CH	90	m	N	0	2	56	97	97
9/22/2014	205	Weitchpec Klamath	CH	93	f	N	2	0	200	200	200
9/22/2014	206	Weitchpec Klamath	CH	78	f	N	0	0	200	200	200
9/22/2014	207	Weitchpec Klamath	CH	79	f	N	0	0	53	121	121
9/22/2014	208	Weitchpec Klamath	CH	73	f	N	0	0	74	78	78
9/22/2014	209	Weitchpec Klamath	CH	87	f	N	0	0	168	200	200
9/22/2014	210	Weitchpec Klamath	CH	88	m	N	0	2	200	200	200
9/22/2014	211	Weitchpec Klamath	CH	83	m	N	0	0	200	200	200
9/23/2014	212	Weitchpec Trinity	CH	86	f	N	0	0	83	80	83
9/23/2014	213	Weitchpec Trinity	CH	81	m	N	2	0	0	0	0
9/23/2014	214	Weitchpec Trinity	CH	73	m	Y	1	1	x	x	
9/23/2014	215	Weitchpec Trinity	CH	81	m	N	1	0	x	x	
9/23/2014	216	Weitchpec Trinity	CH	98	m	N	2	2	x	x	
9/23/2014	217	Weitchpec Trinity	STH	38	x	Y	0	0	x	x	
9/23/2014	218	Weitchpec Trinity	STH	58	f	N	0	0	0	0	0
9/23/2014	219	Weitchpec Trinity	CH	85	f	Y	1	0	112	122	122
9/23/2014	220	Weitchpec Trinity	CH	83	f	N	0	0	15	11	15
9/23/2014	221	Weitchpec Trinity	CH	70	m	N	0	0	0	0	0
9/23/2014	222	Weitchpec Trinity	CH	96	m	N	0	1	200	317	317
9/23/2014	223	Weitchpec Trinity	CH	74	f	Y	0	0	24	86	86
9/23/2014	224	Weitchpec Trinity	CH	81	m	N	1	0	13	4	13

9/23/2014	225	Tectah/Blue	CH	82	m	Y	0	0	134	60	134
9/23/2014	226	Tectah/Blue	CH	82	f	N	1	1	26	62	62
9/23/2014	227	Tectah/Blue	CH	93	m	N	1	1	142	108	142
9/23/2014	228	Tectah/Blue	CH	72	m	N	0	0	0	0	0
9/23/2014	229	Tectah/Blue	CH	73	m	N	0	0	0	0	0
9/23/2014	230	Tectah/Blue	CH	74	f	N	0	0	0	0	0
9/23/2014	231	Tectah/Blue	CH	87	f	N	0	0	0	0	0
9/23/2014	232	Tectah/Blue	CH	72	f	Y	1	0	0	0	0
9/23/2014	233	Tectah/Blue	CH	88	f	N	1	1	130	113	130
9/23/2014	234	Tectah/Blue	CH	83	m	N	0	0	70	85	85
9/23/2014	235	Tectah/Blue	CH	74	m	N	0	0	0	0	0
9/23/2014	236	Tectah/Blue	CH	80	m	N	0	0	0	0	0
9/23/2014	237	Tectah/Blue	CH	72	m	N	1	0	0	0	0
9/24/2014	238	Weitchpec Klamath	CH	75	m	N	0	0	4	2	4
9/24/2014	239	Weitchpec Klamath	CH	80	f	N	0	0	172	152	172
9/24/2014	240	Weitchpec Klamath	CH	75	m	N	0	0	580	246	580
9/24/2014	241	Weitchpec Klamath	CH	79	m	N	0	0	500	276	500
9/24/2014	242	Weitchpec Klamath	CH	87	f	N	2	2	147	56	147
9/24/2014	243	Weitchpec Klamath	CH	81	f	N	0	1	500	450	500
9/24/2014	244	Weitchpec Klamath	CH	84	f	N	0	0	666	790	790
9/24/2014	245	Weitchpec Klamath	CH	85	f	N	1	2	806	477	806
9/24/2014	246	Weitchpec Klamath	CH	87	m	N	1	0	46	95	95
9/24/2014	247	Weitchpec Klamath	CH	85	f	N	0	1	230	363	363

9/24/2014	248	Weitchpec Klamath	CH		80	m	N	0	0	97	100	100
9/26/2014	249	Tulley Creek	CH	x		f	N	1	2	200	200	200
9/26/2014	250	Tulley Creek	CH	x		f	N	2	1	200	200	200
9/26/2014	251	Tulley Creek	CH	x		m	N	0	0	2	0	2
9/26/2014	252	Tulley Creek	CH	x		m	N	0	1	0	0	0
9/26/2014	253	Tulley Creek	CH	x		m	N	0	1	100	100	100
9/26/2014	254	Tulley Creek	CH	x		f	N	0	0	200	200	200
9/26/2014	255	Tulley Creek	CH	x		f	N	2	3	10	10	10
9/26/2014	256	Tulley Creek	CH	x		m	N	1	1	200	200	200
9/26/2014	257	Tulley Creek	CH	x		m	N	1	0	30	30	30
9/26/2014	258	Tulley Creek	CH	x		f	N	0	0	183	172	183
9/26/2014	259	Tulley Creek	CH	x		m	Y	0	1	233	360	360
9/27/2014	260	Tectah	CH		72	m	N	0	0	160	195	195
9/27/2014	261	Tectah	CH		93	m	N	2	3	15	34	34
9/27/2014	262	Tectah	CH		78	m	N	0	0	215	283	283
9/27/2014	263	Tectah	CH		97	m	N	0	0	504	755	755
9/27/2014	264	Tectah	CH		84	m	Y	0	0	474	905	905
9/27/2014	265	Tectah	CH		86	f	N	0	0	15	11	15
9/27/2014	266	Tectah	CH		87	m	N	1	0	325	680	680
9/27/2014	267	Tectah	CH		75	m	N	0	0	0	4	4
9/27/2014	268	Tectah	CH		84	m	Y	0	0	5	8	8
9/27/2014	269	Tectah	CH		81	m	N	1	0	80	31	80
9/27/2014	270	Tectah	CH		78	m	N	0	0	0	8	8
9/29/2014	271	Weitchpec Klamath	CH		75	f	Y	0	0	152	313	313
9/29/2014	272	Weitchpec Klamath	CH		88	m	N	0	0	246	282	282
9/29/2014	273	Weitchpec Klamath	CH		80	m	N	2	0	717	361	717
9/29/2014	274	Weitchpec Klamath	CH		82	f	N	0	0	510	608	608

9/29/2014	275	Weitchpec Klamath	CH	78	f	N	0	0	29	8	29
9/29/2014	276	Weitchpec Klamath	CH	85	f	N	0	0	277	234	277
9/29/2014	277	Weitchpec Klamath	CH	87	m	N	0	0	115	123	123
9/29/2014	278	Weitchpec Klamath	CH	76	m	N	0	0	39	55	55
9/29/2014	279	Weitchpec Klamath	CH	74	m	N	0	0	395	900	900
9/29/2014	280	Weitchpec Klamath	CH	74	m	N	0	0	52	85	85
9/29/2014	281	Weitchpec Klamath	CH	82	f	N	0	0	82	59	82
9/29/2014	282	Weitchpec Klamath	CH	82	m	N	1	2	101	137	137
9/30/2014	283	Weitchpec Trinity	CH	79	f	N	0	0	278	695	695
9/30/2014	284	Weitchpec Trinity	CH	81	m	N	0	0	386	271	386
9/30/2014	285	Weitchpec Trinity	CH	77	m	N	0	0	246	189	246
9/30/2014	286	Weitchpec Trinity	CH	88	m	N	0	0	28	18	28
9/30/2014	287	Weitchpec Trinity	CH	87	f	N	0	0	179	238	238
9/30/2014	288	Weitchpec Trinity	CH	77	m	N	0	0	22	28	28
9/30/2014	289	Weitchpec Trinity	CH	79	f	N	0	0	28	33	33
9/30/2014	290	Weitchpec Trinity	CH	79	m	N	0	2	55	151	151
9/30/2014	291	Weitchpec Trinity	CH	83	f	N	0	0	19	22	22
9/30/2014	292	Weitchpec Trinity	CH	73	m	N	0	0	150	126	150
10/2/2014	293	Weitchpec Klamath	CH	78	f	N	0	0	530	425	530
10/2/2014	294	Weitchpec Klamath	CH	90	m	N	0	0	129	96	129
10/2/2014	295	Weitchpec Klamath	CH	83	f	N	0	0		257	257
10/2/2014	296	Weitchpec Klamath	CH	80	f	N	0	0		582	582

10/2/2014	297	Weitchpec Klamath	CH		86	f	N	0	0	109		109
10/2/2014	298	Weitchpec Klamath	CH		81	m	N	0	0	283		283
10/2/2014	299	Weitchpec Klamath	CH		77	m	Y	0	0	110		110
10/2/2014	300	Weitchpec Klamath	CH		80	f	Y	0	0	79		79
10/2/2014	301	Weitchpec Klamath	CH		76	f	N	0	0	92		92
10/2/2014	302	Weitchpec Klamath	CH		82	m	N	0	0	254		254
10/8/2014	303	Tectah	CH		75	m	N	0	0	0	0	0
10/8/2014	304	Blue	CH		87	f	N	0	0	950	900	950
10/8/2014	305	Blue	CH		75	m	N	0	0	365	443	443
10/8/2014	306	Blue	CH		79	m	N	0	0	1000		1000
10/8/2014	307	Tectah/Blue	CH		76	f	N	0	0	390	540	540
10/8/2014	308	Weitchpec Trinity	CH		105	m	N	0	0	673	742	742
10/14/2014	309	Iron Gate Hatchery	CH	x		x	x				0	0
10/14/2014	310	Iron Gate Hatchery	CH	x		x	x				0	0
10/14/2014	311	Iron Gate Hatchery	CH	x		x	x				1	1
10/14/2014	312	Iron Gate Hatchery	CH		89	m	x				5	5
10/14/2014	313	Iron Gate Hatchery	CH		48	m	x				0	0
10/14/2014	314	Iron Gate Hatchery	CH		84	m	x				2	2
10/14/2014	315	Iron Gate Hatchery	CH		68	m	x				0	0
10/14/2014	316	Iron Gate Hatchery	CH		72	f	x				1	1
10/14/2014	317	Iron Gate Hatchery	CH		71	m	x				2	2
10/14/2014	318	Iron Gate Hatchery	CH		93	m	x				0	0
10/14/2014	319	Iron Gate Hatchery	CH		77	m	x				2	2
10/14/2014	320	Iron Gate Hatchery	CH		74	m	x				4	4
10/14/2014	321	Iron Gate Hatchery	CH		55	m	x				0	0
10/14/2014	322	Iron Gate Hatchery	CH		89	m	x				6	6
10/14/2014	323	Iron Gate Hatchery	CH		80	m	x				0	0

10/14/2014	324	Iron Gate Hatchery	CH		67	m	x	0	0
10/14/2014	325	Iron Gate Hatchery	CH		88	m	x	3	3
10/14/2014	326	Iron Gate Hatchery	CH		91	m	x	8	8
10/14/2014	327	Iron Gate Hatchery	CH		73	m	x	0	0
10/14/2014	328	Iron Gate Hatchery	CH		57	m	x	0	0
10/14/2014	329	Iron Gate Hatchery	CH		60	m	x	0	0
10/14/2014	330	Iron Gate Hatchery	CH		59	m	x	0	0
10/14/2014	331	Iron Gate Hatchery	CH		92	m	x	0	0
10/14/2014	332	Iron Gate Hatchery	CH		81	m	x	1	1
10/21/2014	333	Iron Gate Hatchery	CH		66	m	x	0	0
10/21/2014	334	Iron Gate Hatchery	CH		83	m	x	1	1
10/21/2014	335	Iron Gate Hatchery	CH		81	m	x	0	0
10/21/2014	336	Iron Gate Hatchery	CH		49	m	x	3	3
10/21/2014	337	Iron Gate Hatchery	CH		82	m	x	2	2
10/21/2014	338	Iron Gate Hatchery	CH		74	m	x	4	4
10/21/2014	339	Iron Gate Hatchery	CH		66	m	x	0	0
10/21/2014	340	Iron Gate Hatchery	CH		70	m	x	0	0
10/21/2014	341	Iron Gate Hatchery	CH		89	m	x	0	0
10/21/2014	342	Iron Gate Hatchery	CH		75	m	x	1	1
10/21/2014	343	Iron Gate Hatchery	CH		90	m	x	1	1
10/21/2014	344	Iron Gate Hatchery	CH		92	m	x	1	1
10/21/2014	345	Iron Gate Hatchery	CH		66	f	x	6	6
10/21/2014	346	Iron Gate Hatchery	CH		80	f	x	1	1
10/21/2014	347	Iron Gate Hatchery	CH		76	f	x	0	0
10/21/2014	348	Iron Gate Hatchery	CH		75	m	x	0	0
10/21/2014	349	Iron Gate Hatchery	CH		83	m	x	1	1
10/21/2014	350	Iron Gate Hatchery	CH		72	m	x	0	0
10/21/2014	351	Iron Gate Hatchery	CH		86	f	x	0	0
10/21/2014	352	Iron Gate Hatchery	CH	x		f	x	0	0
10/21/2014	353	Iron Gate Hatchery	CH		82	f	x	0	0
10/21/2014	354	Iron Gate Hatchery	CH		74	m	x	0	0
10/21/2014	355	Iron Gate Hatchery	CH		68	m	x	1	1

10/21/2014	356	Iron Gate Hatchery	CH	75	f	x	0	0
10/21/2014	357	Shasta Racks	CH	53	m	x	4	4
10/21/2014	358	Shasta Racks	CH	89	m	x	11	11
10/29/2014	359	Iron Gate Hatchery	CH	74	m	x	1	1
10/29/2014	360	Iron Gate Hatchery	CH	77	m	x	0	0
10/29/2014	361	Iron Gate Hatchery	CH	53	m	x	0	0
10/29/2014	362	Iron Gate Hatchery	CH	68	f	x	0	0
10/29/2014	363	Iron Gate Hatchery	CH	79	m	x	0	0
10/29/2014	364	Iron Gate Hatchery	CH	72	m	x	0	0
10/29/2014	365	Iron Gate Hatchery	CH	70	m	x	0	0
10/29/2014	366	Iron Gate Hatchery	CH	67	m	x	0	0
10/29/2014	367	Iron Gate Hatchery	CH	71	m	x	3	3
10/29/2014	368	Iron Gate Hatchery	CH	66	f	x	0	0
10/29/2014	369	Iron Gate Hatchery	CH	70	m	x	2	2
10/29/2014	370	Iron Gate Hatchery	CH	75	m	x	0	0
10/29/2014	371	Iron Gate Hatchery	CH	69	f	x	0	0
10/29/2014	372	Iron Gate Hatchery	CH	96	m	x	0	0
10/29/2014	373	Iron Gate Hatchery	CH	65	f	x	1	1
10/29/2014	374	Iron Gate Hatchery	CH	69	f	x	0	0
10/29/2014	375	Iron Gate Hatchery	CH	79	f	x	2	2
10/29/2014	376	Iron Gate Hatchery	CH	65	f	x	0	0
10/29/2014	377	Iron Gate Hatchery	CH	73	m	x	0	0
10/29/2014	378	Iron Gate Hatchery	CH	75	f	x	0	0
11/13/2014	379	Trinity Hatchery	CH	68	f	x	1	1
11/13/2014	380	Trinity Hatchery	CH	67	m	x	0	0
11/13/2014	381	Trinity Hatchery	CH	74	f	x	1	1
11/13/2014	382	Trinity Hatchery	CH	58	m	x	0	0
11/13/2014	383	Trinity Hatchery	CH	66	m	x	0	0
11/13/2014	384	Trinity Hatchery	CH	69	f	x	2	2
11/13/2014	385	Trinity Hatchery	CH	60	m	x	0	0
11/13/2014	386	Trinity Hatchery	CH	62	f	x	0	0
11/13/2014	387	Trinity Hatchery	CH	61	f	x	3	3

11/13/2014	388	Trinity Hatchery	CH	69	f	x	1	1
11/13/2014	389	Trinity Hatchery	CH	66	f	x	1	1
11/13/2014	390	Trinity Hatchery	CH	73	f	x	0	0
11/13/2014	391	Trinity Hatchery	CH	67	f	x	1	1
11/13/2014	392	Trinity Hatchery	CH	76	f	x	0	0
11/13/2014	393	Trinity Hatchery	CH	66	f	x	1	1
11/13/2014	394	Trinity Hatchery	CH	68	f	x	0	0
11/13/2014	395	Trinity Hatchery	CH	64	m	x	0	0
11/13/2014	396	Trinity Hatchery	CH	63	f	x	3	3
11/13/2014	397	Trinity Hatchery	CH	68	f	x	0	0
11/13/2014	398	Trinity Hatchery	CH	86	f	x	0	0

Appendix 2: USFWS CNFHC Memorandum on Results of Adult Chinook sampling on the Yurok Reservation on September 15, 2014.

[begin memorandum]

UNITED STATES GOVERNMENT **FISH AND WILDLIFE SERVICE**

Memorandum

TO: Brian Person, USBR DATE: September 30, 2014

Dan Castleberry, USFWS

CC: Nick Hetrick, Darrin Thome & Robert Clarke (USFWS), Mike Belchik & Dave Hillemeier (YTF), Mark Adkison (CDFW)

FROM: J. Scott Foott CA-NV FHC, Anderson, CA

SUBJECT: Final results for 9/15/2014 sampling of adult Chinook from lower Klamath River (rm 16) - Detection of *Ichthyophthirius multifiliis*

On Friday 9/12/2014, Mike Belchik (Yurok Fisheries) contacted the Fish Health Center and requested confirmation of several gill imprint samples. This confirmation of Ich was performed late Friday and additional adult sampling occurred over the weekend. Several suspicious gill samples were observed and a FHC diagnostic trip planned for Monday 9/15/2014. Two Yurok fishery crews and 1 Arcata FWO crew participated in gill net sampling in the vicinity of Blue and Tec Tah Creek (near rm16, water temperature 22°C). A total of 26 adult Chinook were collected and examined by myself for both Ich infection and external clinical signs of disease. All fish were collected from the mainstem river, appeared to actively migrating, and were bright sliver (indicative of relatively recent FW entry).

Ichthyophthirius multifiliis **trophonts of various sizes were observed grossly and by phase microscopy in 11 of 26 fish (42%) with 6 (23%) of the affected fish having greater than 30 parasites per gill arch (heavily infected).** These infections were not associated with overt gill hyperplasia indicating the infection was approximately a week old. Two phone calls were made on site to communicate initial findings (B. Person and N. Hetrick).

Laboratory samples:

1. Gill imprint (fish1) – no Ich observed, note similar negative finding on site
2. Bacterial culture taken from Fish 2 kidney = single colony mixed small GNR and *Bacillus*

   Interpretation : unlikely a systemic bacteremia, no clear relationship to observed red vent (erythemia).

   Histology fish 2 (section 7411):

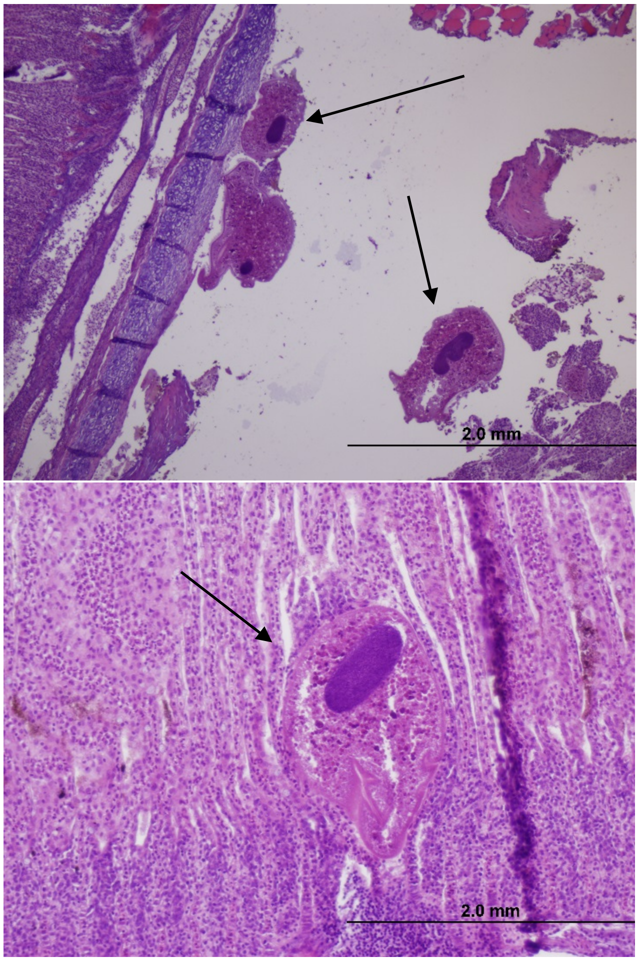
   - Distal intestine cross section- hyperplastic lamina propria, multi-focal sloughing of epithelia, mononuclear cell infiltrate into muscularis
     - No parasite or obvious bacteria foci seen
   - Kidney –interstitial hyperplasia, sinuses with PMNs and cellular debris, no obvious bacteria within inflammatory cells or parasites seen
     - Glomeruli normal, ~10% PCT with eosinophilic precipitate in lumen
   - Gill (section 7412) – Ich on surface without hyperplasia

Interpretation : systemic infection of undiagnosed cause, Ich infection had not progressed to disease state.

3. Histology gill (fish 4,16, 18- sections 7412- 7414)- Ich on surface without hyperplasia

4. Histology distal intestine (fish20, 7415) – normal tissue with a nematode

Summary: The findings indicate that the adult Chinook population, moving through the lower Klamath R. at this, had a high prevalence of Ich infection as well as high parasite loads indicative of the early stages of an epizootic. The fish were **not** in a disease state (moribund).



Ichthyophthirius multifiliis on gills

9/15/2014 datasheet

			9/16 email from Dave H - 3of4 ad-clips were IGH																
			no obvious gillHP seen																
Sampleno	Species	Moribund y/n	Fork length(cm)	Fc lesion	wound	copepod	pet hem	red vent	white spot/gill arch est	4-10x wetmount 5- 15 lamellae	notes	imprints,slide#,tissue	Histo intestine	histo kidney	histo gill	blood tube#	blood smear#	frozen tissue	BHIA bacte
1	CHK	No	85	0	0	0	0	0	not seen	0		gill							
2	CHK	No	86	0	x	0	0	x	not seen	>30 - 13lam				x	x				x
3	CHK	No	83	0	0	0	0	0	not seen	0									
4	CHK	No	72	x	0	0	0	x	not seen	>30 - 10lam, no HP	minor dorsal ifin erosion				x				
5	CHK	No	75	0	0	0	0	0	not seen	0									
6	CHK	No	87	0	0	0	0	0	not seen	0									
7	CHK	No	72	0	0	0	0	0	2spot	0	handlens								
8	CHK	No	76	0	0	0	0	0	not seen	0									
9	CHK	No	73	0	0	0	0	0	not seen	0									
10	CHK	No	69	x	0	0	0	0	1spot	0	minor dorsal ifin erosion								
11	CHK	No	70	0	0	0	0	0	1spot	1ich-6lam									
12	CHK	No	85	0	0	0	0	0	not seen	1ich-12lam									
13	CHK	No	69	0	0	0	0	0	2spot	0									
14	CHK	No	78	0	0	0	0	0	not seen	10ich-10lam **>30**									
15	CHK	No	77	0	0	0	0	0	not seen	0									
16	CHK	No	75	0	0	0	0	0	not seen	30+, 13 on 6 lam >30					x				
17	CHK	No	85	0	x	0	0	0	not seen	20+, 12 on 6 lam >30									
18	CHK	No	68	0	0	0	0	0	not seen	30+ 29/15lam >30					x				
19	CHK	No	69	0	0	0	0	0	not seen	1on 9lam									
20	CHK	No	83	0	0	0	0	x	not seen	0			x						
21	CHK	No	78	0	0	0	0	0	not seen	0									
22	CHK	No	68	0	0	0	0	x	3	0									
23	CHK	No	82	0	0	0	0	0	1	1, 1 on 4lam									
24	CHK	No	68	0	0	0	0	0	1	1, 4on 10lam									
25	CHK	No	84	0	0	0	0	x	not seen	0									
26	CHK	No	72	0	0	0	0	0	not seen	0									
				2	2	0	0	5		11	ICH+								
			POI	8%	8%	0%	0%	19%		42%									
										6	highICH								
										23%									

Appendix 3: Email from Dr. Foott re: Lower Klamath River gill condition sampling on 10/2/2014.

(email slightly re-formatted for clarity)

From: Foott, Scott [mailto:scott_foott@fws.gov]
Sent: Monday, October 06, 2014 3:11 PM
To: Michael Belchik
Cc: Dave Hillemeier; Nick Hetrick; Robert Clarke; Dan Castleberry; Mark Adkison; Wade Sinnen; Knechtle, Morgan@Wildlife; Alex Corum
Subject: Lower KR gill condition 10/2

All,

We finished histological examination of 6 adult chinook gills collected at Weitchpec on 10/2 as well as 4 samples collected at Ishi Pishi Falls by Alex Corum [Karuk Tribe] on the same day.

Only minor to moderate hyperplasia, associated with a few Ich trophophonts, was observed in the sections. Most sections were heavily infected. Two gills had small metacercaria cysts embedded in the lamellar vessels.

Bottom line: fish <u>at this location and time</u> were not in a disease state.
Similarly gill sections taken from lightly infected Shasta R. weir Chinook on 9/25 were normal.

J.Scott Foott, PhD

Project Leader

US Fish and Wildlife Service

California Nevada Fish Health Center

24411 Coleman Hatchery Road

Anderson, CA 96007

phone 530-365-4271

fax 530-365-7150