FY2003 Report:
Health Monitoring of adult Fall-run Chinook Salmon in the lower Klamath River, August – October 2003

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Background: In response to the 2002 Klamath River fish die-off, the Department of Interior submitted a document, *Recommendations for averting another adult salmon die-off*, to Federal Court Judge Oliver Wanger in March 2003. An epizootic of the external parasite *Ichthyophthirius multifiliis* (**Ich**) was a significant factor in the 2002 Klamath R. fish die-off. While the parasite is not uncommon in the watershed, the extent of the disease outbreak and associated mortality was unique. The bacterial disease Columnaris also contributed to the 2002 mortality and is endemic to the basin. Massive adult mortality in the watershed has not been linked with Columnaris disease by itself. Increased Trinity River flows were released in August and September 2003 to stimulate up-stream migration of Trinity River Fall-run Chinook from the Klamath River and thereby avoid or reduce any disease-related mortality event.

In July 2003, a technical team developed specific recommendations that included a 17,000 acre-feet emergency release reserve that could be used in September 2003 if an adult salmon epizootic was imminent. An intensive monitoring effort of adult Fall-run Chinook salmon in the lower Klamath River for the presence and severity of infection by *Ichthyophthirius multifiliis* (**Ich**) and *Flavobacterium columnare* (Columnaris disease or gill rot) was proposed by the technical team to serve as a warning system. As part of this effort the USFWS California – Nevada Fish Health Center (**FHC**) provided technical support. This report describes the adult Chinook monitoring effort conducted by biologists with the Yurok Tribal Fisheries (**YTF**), Karuk Tribe Dept of Natural Resources (**KDNR**), and the USFWS Arcata Fish and Wildlife Office (**FWS**).

The specific language submitted to the Trinity Management Council on 9/5/03 for this disease trigger is listed below:

" Disease monitoring of fall-run Chinook salmon, captured in the lower Klamath River during August and September 2003, will be performed to alert managers that an 2002 level Ich epizootic may be imminent. The determination of such an epizootic will be based on the approximate doubling in the incidence (cumulative number of positive cases over a set time period) and severity (number of parasites / gill) of Ich infection over a 5 – 7 day period above an arbitrary "normal" background level. As no actual background data exists, the incidence of infection observed in all fish collected prior to the peak migration (estimated at August 10 – September 10) *or* an arbitrary 10% incidence occurring in a weekly sample of 30 or more fish will be used for the background figure. Confirmation of any significant change to the level of Ich infection will be by a USFWS fish pathologist. Columnaris data will also be collected but is not a "trigger" factor by itself. If the California-Nevada Fish Health Center (USFWS) determines that an Ich epizootic is imminent, Dr. MaryEllen Mueller (USFWS, CNO) will be contacted and asked to relay a request to BOR for an emergency flow response. "

Methods: A workshop on sampling technique and pathogen identification was conducted by the FHC on 8/5 at the Yurok Tribal office at Weitchpec. Biologists with the FWS, KDNR, and YTF were in attendance. The importance of limiting examination to gill filaments from live or fresh caught fish ($\leq$ 1hr post-capture) was stressed to the group as the pale white parasite is much more visible on red gill filaments. In addition, the parasite will rupture after extended storage of the gill on ice. Quality assurance of field observations was addressed by submitting the following fixed samples to the FHC: 1) imprints of suspect parasites, 2) gill filaments for histological examination from every 6th fish in large collection group, and 3) collaborative field observation by FHC staff. Sampling kits containing supplies, protocols, datasheets, and a dissection microscope were distributed to each group by the FWS. The lights on the 30X power dissection microscopes were operated off deep cell batteries and power inverters. Three zones of responsibility were agreed upon by the group: 1) net harvest / sport catch in the lower river above Hwy 101 bridge to Blue creek – shared between YF and FWS, 2) Net harvest in the river between Blue creek and the Confluence of Trinity – YF, and 3) Ishi Pishi Falls tribal harvest – KDNR. The sampling goal was set at $\geq$ 30 fish per week for the peak adult abundance period of late September.

Results and discussion

Quality assurance and histological results – Both gill imprints and fixed (Prefer fixative) filaments for histology were collected from a subset of the fish examined in the field for both quality assurance or to confirm a positive field observation. A total of 33 gill filaments were submitted to the FHC for histological examination and 8 sections were observed with Ich trophozoites (8 / 33 = 24%). The majority of samples came from field negative fish, however the correlation of positive histological observations from positive field observation fish was relatively poor (2 of 11 sections = 18%). Encysted metacercaria (presumptive *Nanophyetus salmincola*) was commonly observed in the gill sections. No significant lesion or epidermal hyperplasia was associated with these parasites.

Twenty-six gill imprints were submitted to the FHC, stained with Diff-quick hematological stain, and examined at low power (40X) for trophozoites (Figure 1). Ich was observed in 16 of 26 imprints (62%) with a 62% correlation of field positive (21) to imprint positive (13). This sample type was much more effective for confirmation than histology. Given the high cost of histology (20 – 25 min labor / section + supply costs), future confirmation efforts will center on an imprint of the gill lamellae on which a trophozoites is observed in the field.

On 9/16, I participated in the field collections at Ishi Pishi falls and collaborated the observations of trophozoites by both Toz Soto (Karuk DNR) and Charlie Chamberlain (FWS). *Ceratomyxa shasta* was observed in 1 of 10 lower intestine samples collected for histological examination from these same fish. Cysts also were observed on the body wall and caudal fins of several Chinook salmon. *Henneguya zschokkei* spores were identified within the milky fluid-filled cyst (Fig 2). This myxosporean parasite did not appear to be a health threat to the fish.

Figure 1. Gill imprint containing trophozoites. 25x Diff-quick stain.

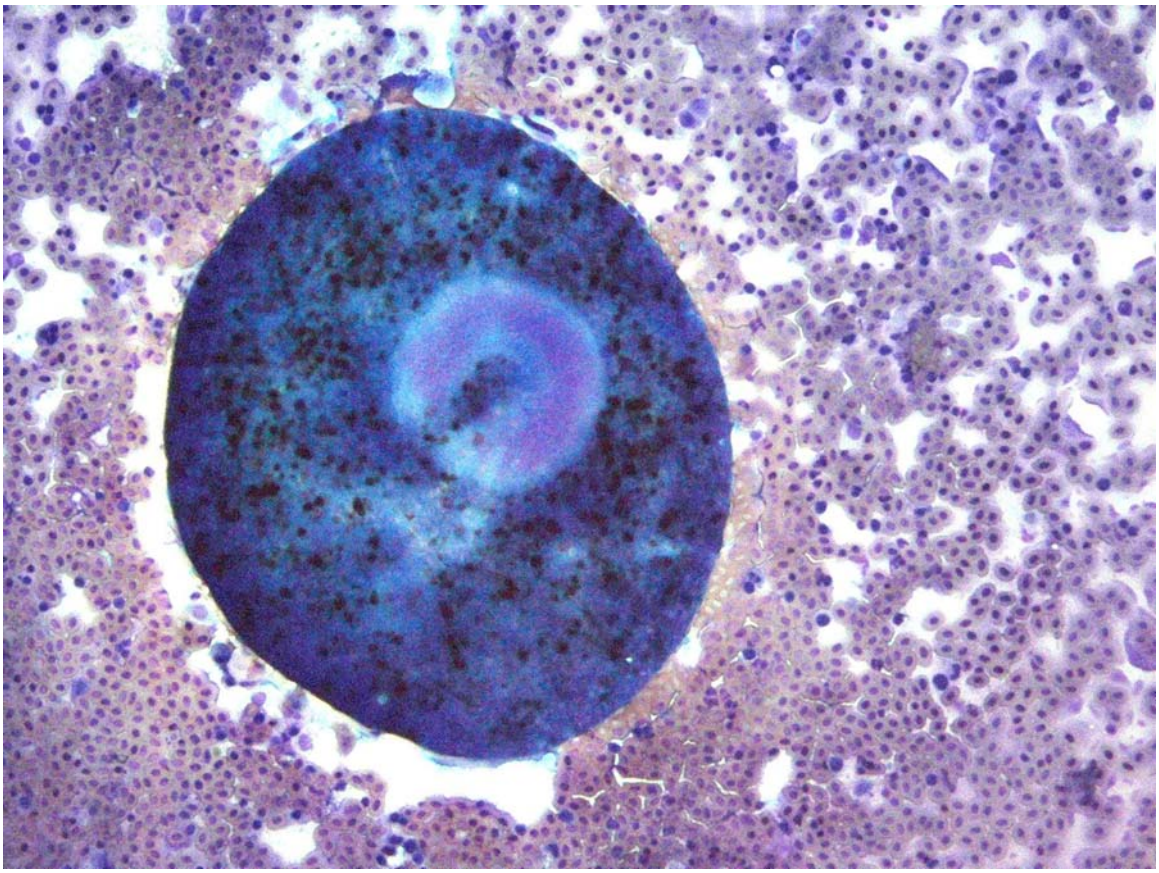
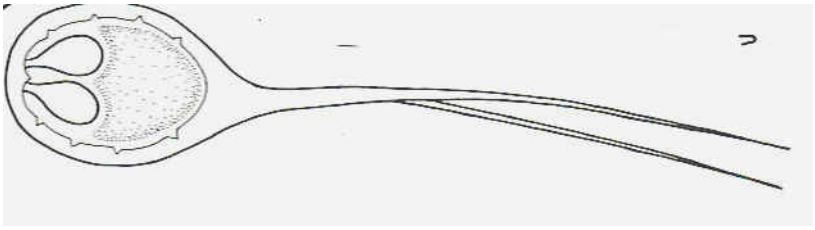


Figure2. Diagram of *Henneguya zschokkei* spore (Lom and Dykova 1992).



Ich and Columnaris observations - There were no reports of dying adults or observations of fish with obvious gill lesions due to Ich infection during the monitoring period of 8/13 – 10/11/03. This time period encompassed the 9/30 to 10/7 peak run timing in the lower Trinity River (CDFG Willow creek weir catch data). Ich infections were observed in 16% (42 / 268) of the fish examined over the entire sampling period while Columnaris lesions were seen in 11% (29 / 271). While the prevalence of Ich increased over time, the severity (number of trophozoites per gill filament) remained mild. For these reasons, no health emergency was declared in the fall of 2003. The earlier assumption of a "baseline" infection level was incorrect as the prevalence of Ich infection increased over time (Fig. 3). It would appear that early arriving fish become infected (probably from resident fish in the estuary) and horizontally transmit the parasite to subsequent arrivals. As the density of fish increases through the season, the transmission rate may also be enhanced. The mean weekly severity of infection ranged from 0 – 24 trophozoites per gill filament with the maximum rating (> 50) occurring in the 9/10 collection at Ishi Pishi falls (Table 1). The incidence of Columnaris lesions showed no pattern during the monitoring period (Table 1). This disease has been associated with elevated pre-spawn mortality in spring-run adults in the Trinity River.

Figure 3. Incidence of Ich infection on gill arches during weeks of samples > 30 fish.

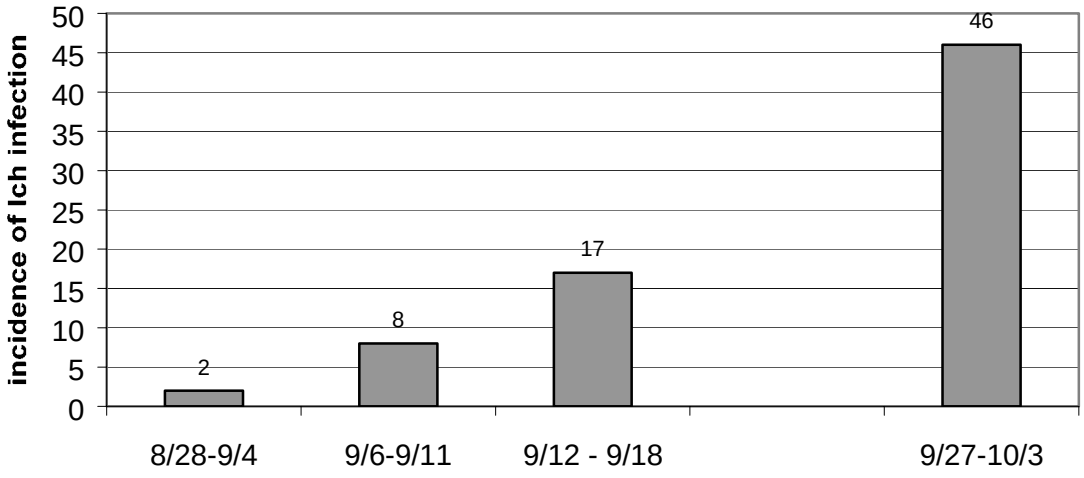


Table 1. Weekly Sample Summary for Ich incidence and severity, and incidence of gill rot (Columnaris lesion).

		Severity rating of positive Ich fish		
Collection week	Ich incidence*	Ich /gill arch average (SD)	Ich /gill arch Min – Max.	Columnaris lesion incidence*
8/10-16	0 / 1 (0%)	na	na	0 / 1 (0%)
8/17-23	0 / 1 (0%)	na	na	1 / 2 (50%)
8/24 –27	0 / 11 (0%)	na	na	1 / 11 (9%)
8/28 – 9/4	1 / 41 (2%)	1	na	13 / 41 (32%)
9/6 – 9/11	7 / 88 (8%)	24 (28)	3 – (>50)	10 / 90 (11%)
9/12 –18	12 / 69 (17%)	2 (1)	1 - 4	1 / 69 (1%)
9/19 – 26	0 / 10 (0%	na	na	0 / 10 (0%
9/27– 10/3	22 / 47 (46%)	9 (8)	1 - 13	3 / 47 (6%)

- sample numbers may not equal as Columnaris is diagnosed by gross examination only
- na not applicable as no positive samples that week

Future monitoring recommendations: Adult pre-spawn mortality has been occasionally reported in the Trinity and Klamath Rivers over the last decade. Columnaris is often associated with moribund adults in the summer (spring-run) and fall. Other potential factors that could contribute to pre-spawn mortality include: a) severe infestation of external parasites such as Ich that lead to respiratory and osmoregulatory distress, b) external damage associated with lamprey bites, net and hook trauma, c) intestinal hemorrhaging due to infection by *Ceratomyxa shasta*, and d) exhaustion of energy reserves due to migration delays in warm waters and / or angling stress.

It would be beneficial to managers to have the following data available on Spring and Fall run Chinook salmon to interpret fish die-off events:

1. Incidence of Columnaris lesions in net harvest fish (estuary, lower Klamath, and Trinity R. Hoopa fishery)
   a. Observation of body or gill lesion noted by harvest biologists
   b. Data analyzed for spatial (general area of lower Klamath and Trinity River) and temporal patterns of disease. This information could link disease incidence with river flow and migration rate (***does delayed migration increase the incidence of Columnaris?***) as well as water temperature thresholds.
2. Incidence and relative severity of Ich infection from subsamples of net harvest Chinook (estuary, lower Klamath, and Trinity R. Hoopa fishery)
   a. Use 10x hand lens with battery operated light on gill filament (no dissection) to identify and count Ich off fresh caught fish
   b. Confirm observation with imprints (unique numbered slides) that are later submitted to FHC

3. Incidence of clinical ceratomyxosis (observed hemorrhagic vent or lower intestine) in net harvest fish (estuary, lower Klamath, and Trinity R. Hoopa fishery)
   a. Confirm observation with imprint of swab sample from intestine (no dissection required).
4. Diagnostic response kits containing supplies for bacteriological, blood, and histological samples would be kept by the basin's agencies to provide for a quick response to observed mortality. Training would be provided on a bi-annual basis by the FWS.

Acknowledgements:
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Yurok Fisheries:	R Benson	FWS	C. Chamberlain	Karuk DNR	T Soto
	S Naman		J Sartori		
	M Hiner		M Cunanan		
	D Jordon				
	R Jackson				
	R Ray				
	J Holt				
	T Thompson				

Reference:

Lom J and I Dykova. 1992. Protozoan parasites of fishes. Elsevier New York.