Survey of Systemic Bacteria Load and External Parasites in 1+ Blue & Tui Chubs Collected in Upper Klamath Lake and Clear Lake: Early Spring and Late Summer 2016.

Case Numbers: 16-060, 16-066, 16-108, 16-111

Principal Investigator(s): Ron Stone & J. Scott Foott

Sample Date(s): 4/28/2016, 5/5/2016, 8/3/2016, 8/10/2016

Estimated funds expended: $6000

Summary:

Aeromonas spp. bacteria were isolated from kidney tissue each of the four sampling efforts. A *Myxobolus* spp. cyst was also observed histologically that may have posed some health impact due to cartilage dysplasia and its effect on gill structure. It does not however appear that external parasitic or bacterial infections were significant health impairments for fish sampled from Upper Klamath Lake and Clear Lake during spring and summer conditions 2016. It is possible the 1+ age fish examined for this effort were demonstrating innate resistance having survived one full year in their lake habitat. Because of this, 1+ chubs may not be an adequate surrogate for 0+ endangered suckers. Due to their size this type of bacterial examination effort cannot be performed on 0+ suckers. No evidence of viral infection was observed by cell culture on EPC or CHSE cell lines.

Objective:

The primary purpose of this pilot effort is to; 1) evaluate prevalence of bacterial and parasite composition and 2) determine if bacterial and parasite infections contribute to lack of 0+ sucker recruitment. Evaluations will be made early and late summer (pre and post algal bloom). Sample locations are; Upper Klamath Lake, Or (UKL) and Clear Lake, Ca. Both Blue Chub *(Gila coerulea)* and Tui Chub *(Siphateles bicolor)* were selected as surrogate species for the federally listed endangered suckers. Fish samples will be obtained from Clear Lake within one week of fish being sampled in UKL. Target sample numbers are approximately 320 total fish (4 sample efforts, 60 fish each).

Methods:

Bacteriology: Kidney samples were aseptically removed from 30 fish, placed into sterile 2.0mL bullets tubes and transported cold back to the Ca-NV FHC laboratory. Samples were weighted, diluted 1:9 (10x) in sterile HBSS and placed into a bullet blender for tissue disruption. 100ul of supernatant was spread onto Brain Heart Infusion Agar (BHIA). Samples were incubated for

48hrs at 22°C, colonies tabulated, and the data recorded. Preliminary bio-chemical tests were conducted on 1-3 individual colonies from each BHIA plate consisting of ≥10 isolated colonies to screen for gram-negative, oxidase-positive, non-spore-forming, rod-shaped, motile bacteria indicative of *Aeromonas spp.* Small subsets of bacterial colonies were run through the Biolog microbial identification and characterization software program in attempt to identify bacterial isolates.

Ampicillin Dextrin Agar (ADA, Hardy Diagnostics, CRTIERION™ Cat.no. C7581and addition of 10mg of sterile Ampicillin Cat.no. SR136E) was also used and evaluated on August samples only. ADA is a selective media used in the isolation, differentiation and quantitative enumeration of *Aeromonas* spp. bacteria. 100ul of supernatant was spread onto ADA, incubated for 48hrs at 22°C, colonies tabulated, and the data recorded. *Aeromonas* spp. grown on ADA agar yield faintly yellow to yellowish-green colonies. Biochemical tests were performed on a subset of isolates to confirm they were gram-negative, oxidase-positive, non-spore-forming, rod-shaped, motile bacteria and were presumptively categorized as *Aeromonas spp.*

Virology: A portion of kidney was aseptically removed from 60 fish, pooled (5p) and screened for viral agents on EPC and CHSE24 cell lines. Initial screening was conducted over a 21d time period and cell lines were observed regularly for any cytopathogenic effect. Methods used in The National Wild Fish Health Survey to collect, process and test fish tissues are standardized. Detailed procedures and laboratory protocols can be found in The National Wild Fish Health Survey Procedures Manual.

Histology: Entire head samples from 20 fish (with the exception of UKL in August, n=30) were held in Davidson's fixative for 24hrs, 2 gill filaments dissected and decalcified in Rapid Bone Decalcifier for 2hrs, processed for 5µm paraffin sections, and stained with hematoxylin and eosin.

Partners:

Name	Agency	Email	Phone
Jared Bottcher	Bureau of Reclamation	jbottcher@usbr.gov	541-880-2588
Torrey Tyler	Bureau of Reclamation	ttyler@usbr.gov	541-880-2573
Darin Taylor	Bureau of Reclamation	detaylor@usbr.gov	541-8807505
Brock Philips	Bureau of Reclamation	bphilips@usbr.gov	541-880-7500
James Ross	Bureau of Reclamation	jross@usbr.gov	541-880-7500

Results / Discussion:

Statistics – Colony count data was placed into 3 categories; 0 (no growth), 1-300 cfu/g (low) and >300 cfu/g (moderate – high level) based on 300 cfu/g of tissue being the median value of all groups. A statistically significant difference in bacterial prevalence was observed in August between UKL and Clear Lake. Prevalence of infection (POI) in UKL fish exhibiting >300cfu/g was 11/30 (37%) and 2/30 (6%) in Clear Lake (Chi-squared = 9.468 with 2 degrees of freedom. P = 0.009). There was also an observed difference in moderate - high level fish sampled at Clear Lake between May and August (Chi-squared = 6.788 with 2 degrees of freedom, P = 0.034). A greater number of fish with moderate - high level infections (>300 cfu/g) were observed in the May sample (9/30, 30%) when compared to August samples (2/30, 6%) (Table1).

No significant difference was observed between UKL and Clear Lake in the spring (Chi-square = 2.490 with 2 degrees of freedom. P = 0.288). No difference was observed in UKL between the April and August samples (Chi-squared = 4.359 with 2 degrees of freedom, P = 0.113) (Table1).

Table 1. Species sampled, location, time of sample and colony counts on BHI agar (0 growth, 1-300 cfu/g (Low level) of tissue and >300 cfu/g (Moderate – high) of tissue).

Species	Colony Counts on BHIA 0 (no growth)	Colony Counts on BHIA 1-300* cfu/g (Low level)	Colony Counts on BHIA >300* cfu/g (Moderate – High)
Blue & Tui Chub April, UKL	18/30 (60%)	8/30 (27%)	4/30 (13%)
Blue & Tui Chub May, Clear LK	14/30 (47%)	7/30 (23%)	9/30 (30%)
Blue & Tui Chub August, UKL	13/30 (43%)	6/30 (20%)	11/30 (37%)
Blue & Tui Chub August, Clear LK	14/30 (47%)	14/30 (47%)	2/30 (6%)

*300 cfu/g of tissue was the median value of all groups and therefore selected as the cutoff between high and low colony counts.

Bacterial Observations:

- A Mann-Whitney Rank Sum test was performed looking for differences between the number of bacterial isolates grown on BHIA $_{vs.}$ ADA. No statistical difference was observed (P = 0.952). Both media types were effective and produced similar number of bacterial isolates.
- Ampicillin Dextrin Agar appears to be effective for the selective isolation, differentiation, and quantitative enumeration of *Aeromonas* species from fish captured in both UKL and Clear Lake 2016.
- Incubation of bacteria on ADA agar did yield faintly yellow to yellowish-green colonies (*Aeromonas).* Biochemical tests that were performed on a subset of isolated colonies did confirm they were gram-negative, oxidase-positive, non-spore-forming, rod-shaped, motile bacteria presumptively identified as *Aeromonas spp.*
    - Of the colonies grown on ADA in August, 14/18 samples (78%) produced ≥90% yellow only colonies (presumptive *Aeromonas spp*.).

    - Subsets of colonies from both locations in August were selected and ran on Biolog for possible further identification. A short list is provided below:

- *Aeromonas sobria*
- *Aeromonas ichthoismia*
- *Aeromonas hydrophila ss hydrophila* (x3 isolates)
- *Aeromonas schubertii*
- *Aeromonas enteropelogenes*

lower probability identifications

- *Aeromonas jandaei* (x2 isolates)
- *Aeromonas salmonicida*
- *Aeromonas bestiarum*

Histological Observations:

- Intralamellar cell mass (ICLM) has little correlation to presence of parasites
    - more pronounced at distal end of lamellae
    - more pronounced at both locations during spring (good water quality) than August
        - probably a normal state for the population to conserve ions when oxygen demand is minimal = “normal” state
        - At UKL (April), many chubs died in the aerated bucket indicating that respiration was impaired by the level of ICLM during such oxygen demanding conditions.

    - If lamellar structure is to be further evaluated, specimen orientation will need to be standardized.
- Parasites - Higher POI for gill parasites in UKL than Clear lake
    - Few fish with “heavy” infections
    - The *Myxobolus* sp. cysts were the most significant health impact of all parasites observed due to cartilage dysplasia and its effect on gill structure.
    - External parasites, associated with gill, were <u>not a significant health impairment</u> in either the spring or summer in both locations.

Upper Klamath Lake – south end

	ILCM1	ILCM 2	Lam. Hyperplasia	Trichodina	Monogene	Myxobolus cyst	Amoeba	Metacercaria
April8	**8 (40%)**	**9 (45%)**	8 (40%)	5 (25%)	10 (50%)	4 (20%)	0	3 (15%)
Aug3	8 (27%)	0	0	19 (63%)	12 (40%)	16 (53%)	6 (20%)	2 (7%)

Notes: 08 April, 3 Ich (15%) presumptive epitheliocystis (1 fish). 03 Aug, 17 unidentified encysted parasites (57%). ILCM2 rating is >30% of gill involvement and is considered severe.

Clear lake

	ILCM 1	ILCM2	Lam. Hyperplasia	Trichodina	Monogene	Myxobolus cyst	Notes	Metacercaria
May5	**11** **(55%)**	**4** **(20%)**	12 (60%)	2 (10%)	7 (35%)	0	1 possible glocidia? 1 heavy amoeba infection	0
Aug10	9 (45%)	0	0	3(15%)	7 (35%)	2 (10%)++		1 (5%)

++ presporogonic form within similar cartilagous site (1 fish)

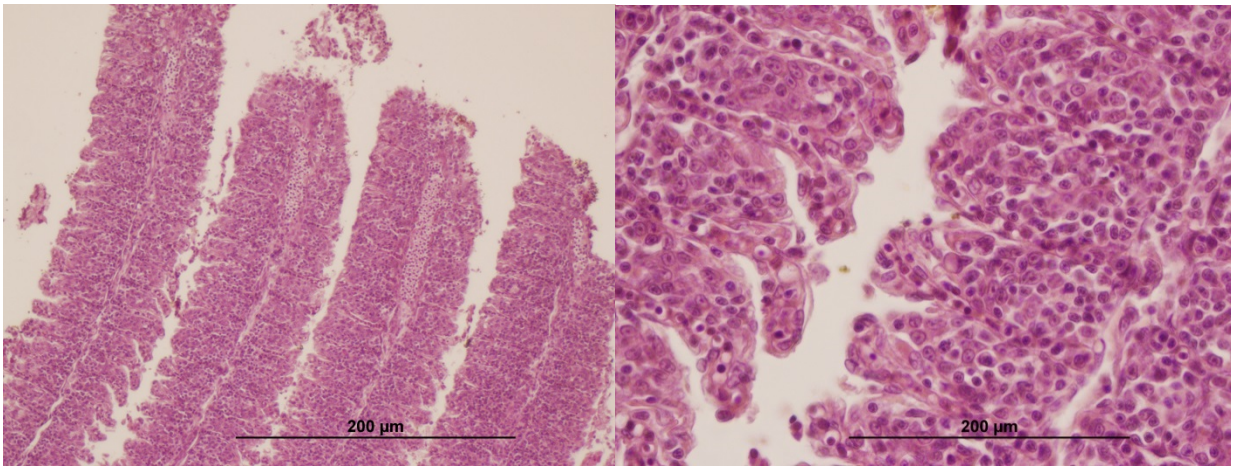

Figure 1. Upper Klamath Lake chub gill severe intra-lamellar cell mass (#2), low and high magnification.

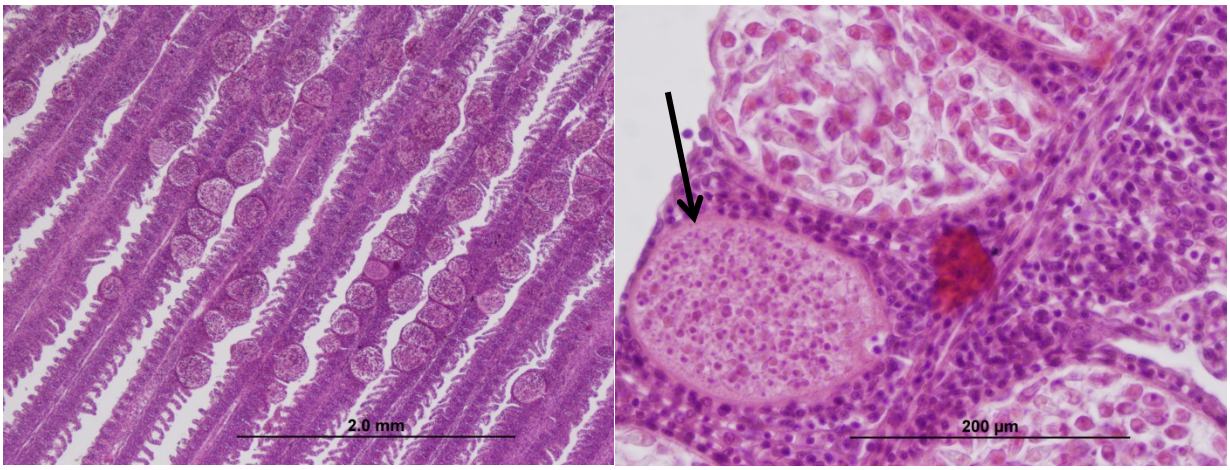

Figure 2. Myxoblus sp. cysts (10x, 40x [arrow Ich trophont]) –April UKL chub.

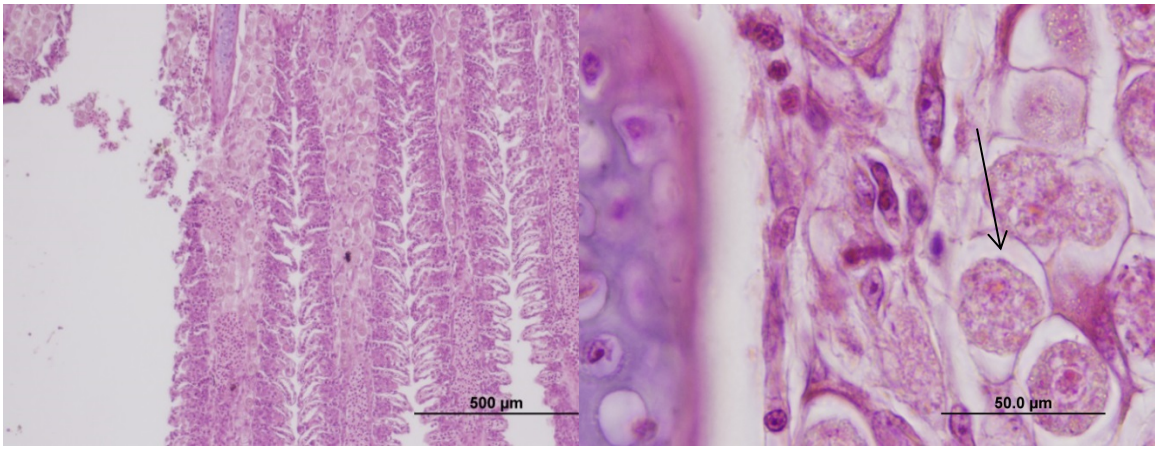

Figure 3. Possible amoeba infection of May5 clear lake chub gill epithelium (left 10x, right 100x).

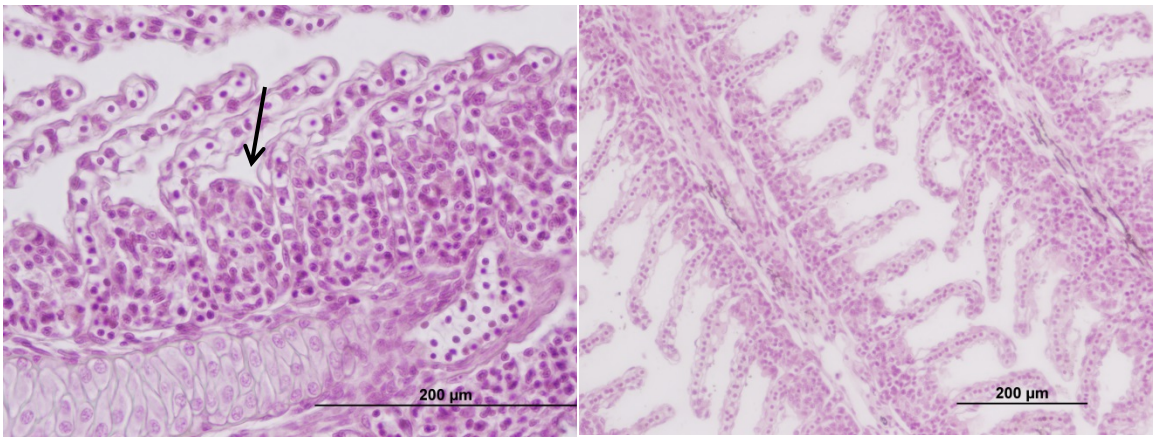

Figure 4. August Clear Lake chub gill – moderate ILCM (#1 rating) distal aspect (left) and no ICLM (right)

Final Report Reference: Survey of Systemic Bacteria Load and External Parasites in 1+ Blue & Tui Chubs Collected in Upper Klamath Lake and Clear Lake: Early Spring and Late Summer 2016, Ron Stone & J.S. Foott, Ca-NV Fish Health Center, USFWS, Anderson Ca, October 7, 2016.