U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2014 Technical Report:
Juvenile Lost River Sucker sentinel survival in Upper Klamath Lake mesocosm netpens (June - September 2014).

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Summary - Survival and tissue responses were evaluated in 5 sentinel groups of Lost River suckers (*Deltistes luxatus***)** from brood years 2012 (BY12), 2013 (BY13) and 3 sentinel groups of fry (brood year 2014 = BY14) exposed at 3 locations (North mid-lake, mouth of Williamson R. and Harriman Springs) in Upper Klamath Lake 17June – 2September 2014. Replicates were sampled every 14 days post-exposure (dpe) for BY12 and BY13, and every 7 and 14dpe for BY14 from hoop net pens that spanned the entire water column. There were two periods of extreme hypoxic conditions in the North Lake associated with 100% mortality (1-15July; minimum 0.05mg/L oxygen and 29July-19August; minimum 0.57mg/L oxygen). Mortality of BY12 and BY13 sentinels was low at the North lake site for the other 3 exposures. Water quality at the Williamson R. and Harriman Springs sites was considered benign during all exposures. The undiagnosed mortality of BY12 and 13 sentinels was approximately 2x higher at Williamson R. than Harriman Springs. Interlamellar hyperplasia of the gill and *Ichthyophthirius multifiliis* infection was common at both the Williamson R. and Harriman Springs sites. These conditions were found in both high and low mortality sentinel groups. Fry (BY14) had high mortality at all sites until the last exposure (19August – 2September) when they were 30 mm in length. Long-term exposure group (78d) mortality for BY12 and BY13 was 90% and 47% at Williamson R., 77% and 73% (36dpe) at North Lake, and 77% and 60% at Harriman Springs, respectively. There was 100% mortality in all long term (50dpe) BY14 groups. BY13 survivor hematocrit, plasma protein, plasma sodium, and histological tissue assessment did not provide diagnostic evidence of cohort mortality and were largely normal. Small fry were far less likely to survive exposure than older suckers.

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Introduction

Lost River Suckers (LRS, *Deltistes luxatus*), a native species of Upper Klamath Lake (UKL) in Southern Oregon, were listed as endangered in 1988. Little to no recruitment of the Upper Klamath Lake population has occurred for over a decade (Burdick et al. 2008). Factors contributing to Lost River Sucker (LRS) decline include over-harvest, habitat loss, negative interaction with non-native fishes, sporadic survival of 0+ suckers, and episodic mass mortality of adults (Markle and Cooperman 2002, Terwillieger et al. 2003, Rasmussen 2011). This fish inhabits the eutrophic waters of Upper Klamath Lake in Southern Oregon that supports massive summer blooms of the cyanobacterium *Aphanizomenon flos-aquae*. These blooms are associated with chronic high alkalinity (pH> 9.5) as well as transient periods of anoxia (dissolved oxygen < 2.0 ppm) and elevated ammonia (Wood et al. 1996). Our data from several studies and other workers suggest that recruitment failure of 0+fry in Upper Klamath Lake is indirectly tied to adverse water quality (Martin and Saiki. 1999, Meyer and Hansen. 2002.). Sentinel fry studies in 2011- 2013 have observed highly variable mortality rates that are often but not always associated with hypoxia (Foott et al. 2012, 2013, 2014).

Specific objectives of this study were:

1. Expand on early rearing techniques. Attempt spawning of Shortnose Sucker (*Chasmistes brevirostris*) adults held at the lower Williamson R. weir.

2. Expand the number of sites and sentinel age classes. Determine temporal survival of sentinel fry, yearlings, and 2 year olds in the North region of Upper Klamath Lake, Williamson River mouth, and Harriman Springs mouth during the summer. The latter 2 sites are expected to have better summer water quality and could be used for in-situ rearing.

3. Perform hematological and histological assessment of yearling LRS sentinel survivors to diagnose whether specific pathologic conditions were associated with cohort mortality.

4. Monitor temperature, ammonia, dissolved oxygen, and pH of water at the three sites in order to observe relationships to survival.

5. Perform critical minima challenges for dissolved oxygen for the 3 age classes under defined pH and temperature conditions (results presented in separate report).

Methods

Shortnose Sucker spawn - Between 17April and 19May 2014, 60 adult SNS were examined for gamete release on 5 separate occasions (17April, 23April, 6May, 15May, and 19May). The adults were captured at the USGS Williamson R. weir and held in 4x4ft (1.8m^3) net pens. Fish were added to the cages over a 2 week period in April, however, we did not track residence time for each animal.

Fish rearing BY14 – On 17 April 2014, gametes were obtained from 4 male and 2 female Lost River suckers captured by USGS at Sucker Springs, Upper Klamath Lake OR. Fish were immediately released after a small portion of gametes were collected (~148g wet weight of eggs and 4 mL of semen per male). We estimate 40,700 eggs were collected based on an egg count estimator of 275 eggs/g (pers. comm. S. Vanderkooi, USGS). This egg collection represents a small percentage of the 50,000 – 100,000 eggs per female reported for 500 – 750mm FL Lost River sucker female (Buettner and Scoppettone 1990). Gametes from 2 females were expressed directly into a plastic Tupperware container with semen from 2 males immediately added to the same container. Gametes were gently mixed with enough lake water to cover the eggs for 10min, excess fluid poured off, the container re-filled with lake water and then placed into an ice chest for transport to the CA-NV Fish Health Center. Upon arrival at the wetlab, lake water was poured off and eggs disinfected in a 100mg/L iodophor solution for 5min. This process was repeated once more to ensure adequate surface disinfection of fertilized eggs. Virological assay of ovarian and seminal fluid did not detect virus (USFWS and AFS-FHS 2007).

Eggs were incubated in multiple upwelling cylinders to minimize clumping and fungus (12.7cm PVC with 250µm mesh screen bottom). Eggs were held at 12.9 - 13.4˚C until larvae were actively swimming and observed feeding. There was no manipulation of eggs or detection of fungus until 23April, 6 days post-spawn. On 24April, upwellers received a single formalin treatment at 100 mg/L (2 ml formalin: 2,000 ml water for 10min). Due to premature hatch, no further treatments were administered and all visible larvae were transferred to 2.8L rearing baskets within a 40L aquarium. On 25April approximately 6,939 eggs with visible fungus were removed from the upwellers. Estimation was done by weighing multiple masses of eggs and fungus that were first placed on a paper towel to absorb excess water; eggs were counted out of the masses to find the weight per eggs with fungus. Total mass of eggs and fungus was found and then divided by the average weight per egg with fungus to give an estimation total of egg loss. By 30April, approximately 17,800 more eggs were estimated lost due to fungus and 100% of larvae had hatched and were actively swimming. We estimate that the population experienced a 60.5% loss from egg to 20mm fry as final larvae count was 16,072. Loss was higher than the 2013 egg to 20mm fry rearing (28% loss, Foott et al. 2014).

All larvae were subsequently reared in 2.8L baskets (16.5cm x 12.7cm x 13.3cm frames covered by a 500µm mesh bag) suspended within 40L aquaria. Newly hatched artemia nauplii were fed daily starting on 30April, however, no feeding response was observed until 13May. At 15 days post-hatch (dph), larvae were transferred from rearing baskets

to 40L aquaria where they were fed a mixture of artemia nauplii and decapsulated artemia cysts. On 19May (25dph) a larval diet (Otohime™ A diet; granule size 250µm, manufactured by Marubeni Nisshin Feed Co., Ltd, Tokyo, Japan, and purchased directly from Reed Mariculture, Inc. Campbell, CA, 95008, USA) was introduced . Feed size was increased to a combination B1 (~250-360µm) and B2 (360-650µm) on 20May as it was decided that A-diet was too small. Feed size was then increased every 9 to 18 days depending on feeding response and CYCLOP-EEZE® was mixed in to supplement slower developing fish.

Fry were transported to the lake on 15July within plastic bags containing 2 - 2.5L of water adjusted to pH 9.0 at ambient lake temperature and filled with oxygen. Bags were then placed inside coolers to minimize movement and temperature change. Our intent was to provide some acclimation to lake conditions during the 3h transport to the lake. No virus was detected in a 60 fry sample performed in July prior to the first movement to Upper Klamath Lake (USFWS and AFS-FHS 2007).

Fish rearing BY13 and BY12 – A portion of the BY13 and BY12 LRS population were reared over the winter and used as yearlings and two year old sentinel fish in 2014. Fish were held in separate 900L troughs on ambient water at a flow rate of 18.9 – 26.5L/min and fed a dry pelleted diet (Otohime). Feed size ranged from 1.7mm – 2.3mm over the holding period. The first transport of BY13 and By12 suckers to the lake was in plastic bags containing 2 - 2.5L of water adjusted to pH 9.0 at ambient lake temperature and filled with O2. The remaining exposures of BY13 and BY12 fish were transported in a fish haul tank (pH 9.0).

Exposures – Five exposures were conducted between 17June and 2September at three sites for the BY13 and BY12 groups and three exposures between 15July and 2September at three sites for the BY14. The sites were 1) North mid-lake near Modoc point, 2) Williamson R. (at the mouth), and 3) Harriman Springs (at the mouth) (Figure 1).

Each short term (7 and 14d) net pen consisted of an outer and inner hoop net. Outer hoop dimensions were 3.66m high x 0.6m diameter enclosed with 5mm UV treated netting. The approximate volume was 1.03m^3. Each hoop net was anchored to the lake bottom and two buoys attached by D-rings at the water's surface to ensure each net pen maintained its vertical integrity. Each buoy was given a unique number to identify sampling groups. Smaller inserts were placed into each net pen where fish were held during each exposure period. The smaller inner hoop nets were weighted in order to insure contact with the lake bottom. A polyethylene jug was attached at the top to maintain vertical integrity. This allowed fish to be sampled by removing the insert while the outer net pen remained in its original location. A new insert could then be added to the net pens along with new sentinel fish for the following exposure. Overall dimensions of the inner hoop nets were 3.66m high x 0.5m diameter enclosed with 1.6mm netting. The approximate volume was 0.72m^3.

Long term cages for BY12 and BY13 were constructed of a vinyl-coated polyester mesh with rectangular holes measuring approximately 1.6mm by 0.8mm. The 3m deep mesh was supported by a floating, square PVC pipe frame measuring 1.2m across. The

floating frame and mesh extended 0.3m above the water surface to prevent fish from jumping out. The top of the floating frame was covered with a removable 12.7mm square plastic mesh to prevent predators from entering the cage. The approximate volume of the long term cage was 4.32m^3. Two corners of the floating frame were attached to buoy lines, which were in turn anchored in the sediments providing contact with the lake bottom. A second PVC pipe frame measuring 1.2m across, and weighted internally with metal rods, was attached to the cage mesh 0.3m from the bottom of the cage. Ropes connecting the corners of the bottom and floating frames allowed for removal of the entire cage from the water. Two corners of the bottom frame were attached to the buoy lines using carabineers, which allowed the bottom frame to slide up and down the buoy lines during sampling without requiring removal of the buoy lines and associated anchors. No hard structures connected the top and bottom frames, allowing the cages to flex in the wind and waves.

Net pens containing BY13 and BY12 LRS were sampled every 14 days. BY14 fry were sampled every 7 and 14 days at each of the three exposure sites. The number of live and dead fish were recorded from each net pen, live fish anesthetized in MS222, a subset of 2 BY13 fish dissected for TEM liver archive (Karnovsky fixative for 6h and stored at 4°C in cacodylate buffer), 3-5 BY13 had a blood sample taken for hematocrit measurement as well as plasma sample, Three to five fish (all year classes) were fixed in Davidson's fixative for histological examination, and the remaining survivors frozen for whole body triglyceride.

Water Quality –Water quality measurements (temperature (˚C), dissolved oxygen (mg/L), and pH) were logged hourly with YSI data sondes suspended approximately 1.5m below the water's surface from a buoy located in the center of the net array. The number of readings taken by each datasonde ranged from 335-338 with 1 reading of 505 (exposure 4). Ammonia (Hach low range kit, DR800 colorimeter) values were obtained from a composite water column sample (5.1cm PVC pipe with stopcock 0.5m above bottom) to calculate unionized ammonia (calculation used datasonde temperature and pH data for the sample time). This spot measurement occurred prior to fish sampling.

Figure 1. Map of the study area in Upper Klamath Lake, Oregon, showing the Northern lake, Williamson River, and Harriman exposure sites.

Histology - Histological samples were held in Davidson's fixative for 48h, processed for 5µm paraffin sections, and stained with hematoxylin and eosin. Sagittal sections were made of smaller fish with tissues examined including skin, muscle, gill, thymus, olfactory pit, eye, brain, liver, intestine, adipose tissue, acinar cells, kidney, and the peritoneal

cavity. Fish larger than 70mm were dissected and specific organs (kidney, gastrointestinal tract, gill, and liver) processed for histology. Abnormalities were rated on an ordinal 0, 1, 2, 3 scale based on distribution (none, focal, multifocal, and diffuse).

Sentinel total protein, plasma sodium, and hematocrit – The caudal peduncle was severed and blood collected in heprinized microhematocrit tube, centrifuged (HemaStat II, Separation Technologies), hematocrit recorded and the buffy coat of white blood cells scored (not observed, scant, defined band), plasma was frozen on dry ice and later stored at -70°C until assayed for total protein (1µL sample in BCA protein assay kit, Pierce) and sodium (5µL plasma diluted in 10mL distilled water assayed by a Jenway flame photometer).

Whole body triglyceride - Standard length (mm) and weight (g) were recorded for each frozen fish and its condition factor calculated ($KSL = WT/ SL^3 x 10^5$). Konig and Borcherding (2012) report freezing has less effect on morphometrics than preservation in ethanol or formalin. BY13 fish were cut into multiple pieces and placed in two to four 55mL tubes due to size. Cold distilled water was added to the 55mL tubes at a 1:1 ratio containing each fish and blended for 60 – 90s with a Biospec M133 homogenizer. BY14 fish were placed into 35mL tubes; due to the small size of the fish and a minimum of 1mL of cold distilled water was added. Dilution ratios for fry ranged from 1:3.8 to 1:39.5 dependent upon fish weight and processed as above. Tissue triglyceride content (mg TG/g tissue) was assayed by a modification of Weber et al. (2003). Absolute isopropanol was added (5x dilution w/v for BY13 and 2x dilution w/v for BY14 fish) to an aliquot of homogenate, mixed at room temperature for 20min, centrifuged at 3220xg for 5min, and replicate 10µL samples of the 10x diluted supernatant (BY13) and between a 8x -79x diluted supernatant (BY14) used in an enzyme assay for triglyceride (Pointe Scientific triglyceride GPO kit).

Laboratory data statistical analysis - Analysis was performed with SigmaPlot 12 software on raw data. Normality was tested by the Shapiro-Wilk method at the P= 0.05 level. One-way Analysis of Variance (ANOVA) or T-test (data with normal distribution, reported with F or t value) or Kruskal-Wallace ANOVA or Mann-Whitney Rank Sum (nonparametric analysis) with subsequent multiple comparison procedure (Dunns method, alpha < 0.05) was used to compare groups.

Permits- Permits for this project are as follows: sub-permit FWSKFFWO-8 (USFWS Klamath Falls Office) under the USFWS Region 8 regional blanket permit (TE-108507), Section 10 Federal authorization number TE007907-15 (adult brood stock, TE007907-15), Oregon Scientific Take Permit Number 19294, and California DFG Standard Importation Permit number 2014-3092.

Results

Shortnose Sucker spawn- Viable eggs were not obtained over the 5 week examination period (Appendix 1). Low numbers of over-ripe eggs and small volumes of milt (<1mL) were expelled by the adults (40 females and 20 males) with the exception of one female (PIT309.1C20AE9056) on 6May. This female released 51mL of overripe eggs.

On 15May, a female (PIT 300007742EDDC) released 5mL of eggs that were fertilized with milt but were "bulls eye" within an hour of water hardening and did not produce larvae (Appendix 1 photo). No mortality occurred and all fish were released at the weir site on 19May.Two fin clips were preserved in RNAlater and provided to Klamath FWO for genetic analysis. Over-ripe egg samples collected on 23April and 6May had an average weight of 0.0034 and 0.0043 g. Water temperature at the weir site (USGS data) ranged from 9.5 - 15°C.

Control Fish Culture– During 2014 rearing, there were no significant mortality events in the captive laboratory populations (LRS fry, yearlings, and two year olds) throughout the study period. Fry showed a 1.9-2.2% loss in wetlab during all exposure periods with most deaths being fallout of smaller fry. To examine holding stress and mortality, LRS of all age groups were placed into net pens suspended in a 670L flow-through tank on 25July, fed every other day, and removed on 8August (14d). All fish survived the 2 week holding period. The wetlab populations were sporadically sampled to assess fish condition before transport to Upper Klamath Lake. Condition factors for BY13 and BY 12 were >1.0; however, condition factors for BY 14 were <1.0 and ranged between 0.53 (21July) and 0.90 (20August) (Appendix 2; Table 1).

North Lake specific data

North Lake water quality – High alkalinity (17June-1July maximum pH 10.43 and 1July – 15 July maximum pH 10.59) and episodic hypoxia occurred at the North Lake site in July (Appendix 3, Figure 2). Between 1 – 15July, dissolved oxygen concentrations were less than 0.75 mg/L for 12h, 0.75 – 1.0 mg/L for 4h, and 1.0 – 1.5 mg/L for 6h (Figure 2 and 3). Similarly, the 29July-19August exposure had hypoxic conditions with a minimum of 0.57mg/L. It is anticipated that fish were exposed to prolonged periods of dissolved oxygen levels far below LD_{50} values of 1.58mg/L (Meyer and Hanson 2002 and Saiki et al. 1999). Temperatures in North Lake during 1July – 15July (mean 22.51˚C and maximum 26.07˚C) and 15July – 22July (mean 22.46˚C and maximum 25.95˚C) were elevated but below LD_{50} levels (Appendix 3, Figure 3-1, Table 3-2). Unionized ammonia (Appendix 3 Table 3-1, UIA) were below the 96h LD_{50} value of 0.5 – 1.1 mg /L (Saiki et al. 1999, Meyer & Hansen 2002). The highest UIA measurement of 0.26 mg/L occurred on 8July. In contrast to 2013, water quality at the North Lake site showed higher temperatures, a wider range of D.O., and greater alkalinity in July.

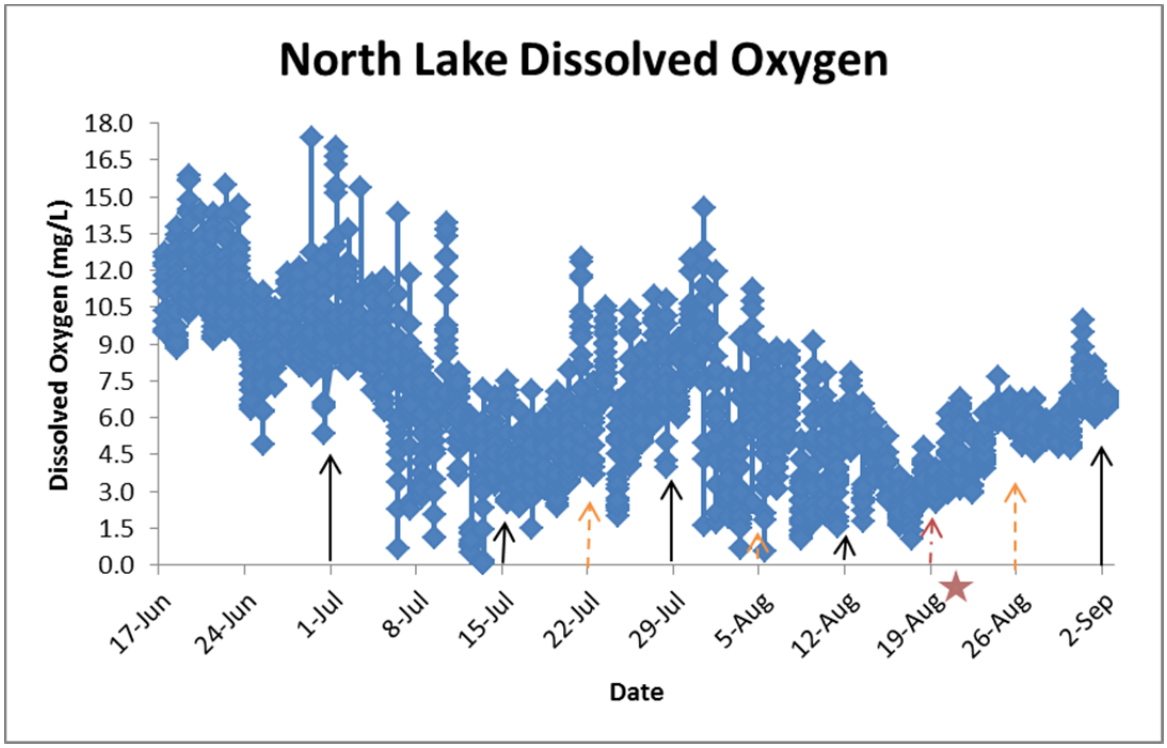


Figure 2. Dissolved Oxygen North Lake site 17 June – 2 September. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

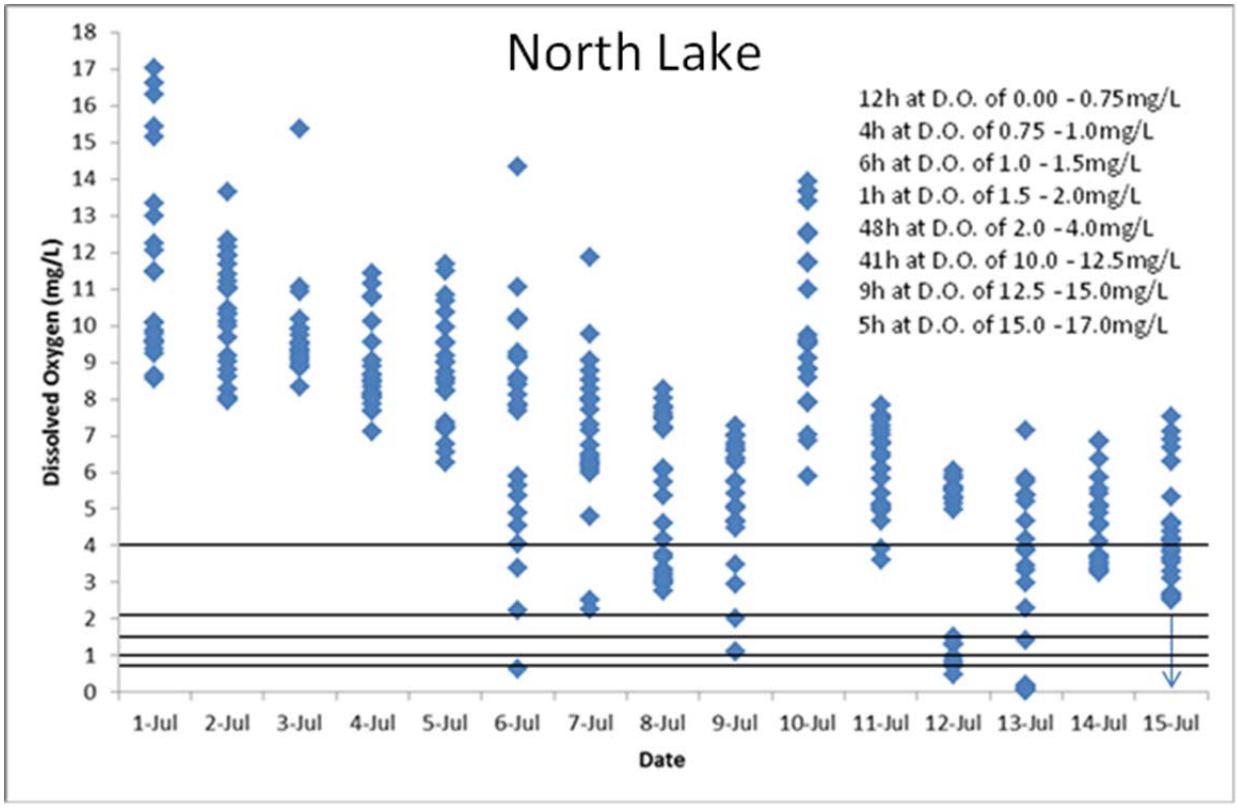


Figure 3. Dissolved oxygen (mg/L) measured North Lake probe from 1-15 July. Lines set at 4, 2, 1.5, 1.0, and 0.75 mg/L. Arrow indicates sample date on which 100% mortality was determined for long term BY12 and BY13 LRS.

Sentinel survival North Lake – Hypoxia was associated with the 2 significant BY12 and BY13 sentinel mortality events during the 01July – 15July and 29July – 19August exposures. All sentinels, including the long term net pen fish, died in these 2 exposure periods (93% of BY12 during 29July- 19August). Mortality was low (0-27%) during the other exposures for both age groups (Appendix 4, Table 4-1). The long term sentinel cages were restocked on 29July for a 36 d exposure (sampled 02September). While high mortality (77% and 73%, respectively) occurred to these BY12 and BY13 sentinels, the survivors managed to live through the 29July-19August hypoxic events. The percent mortality for BY14 fry sentinels was variable (15 – 90%) and did not show any temporal trend in their 3 North lake exposures beginning 15July (Figure 4, Appendix 4, Table 4-3). Similar to older fish, no long term sentinel fry survived at this site (15Jul – 2 Sept).

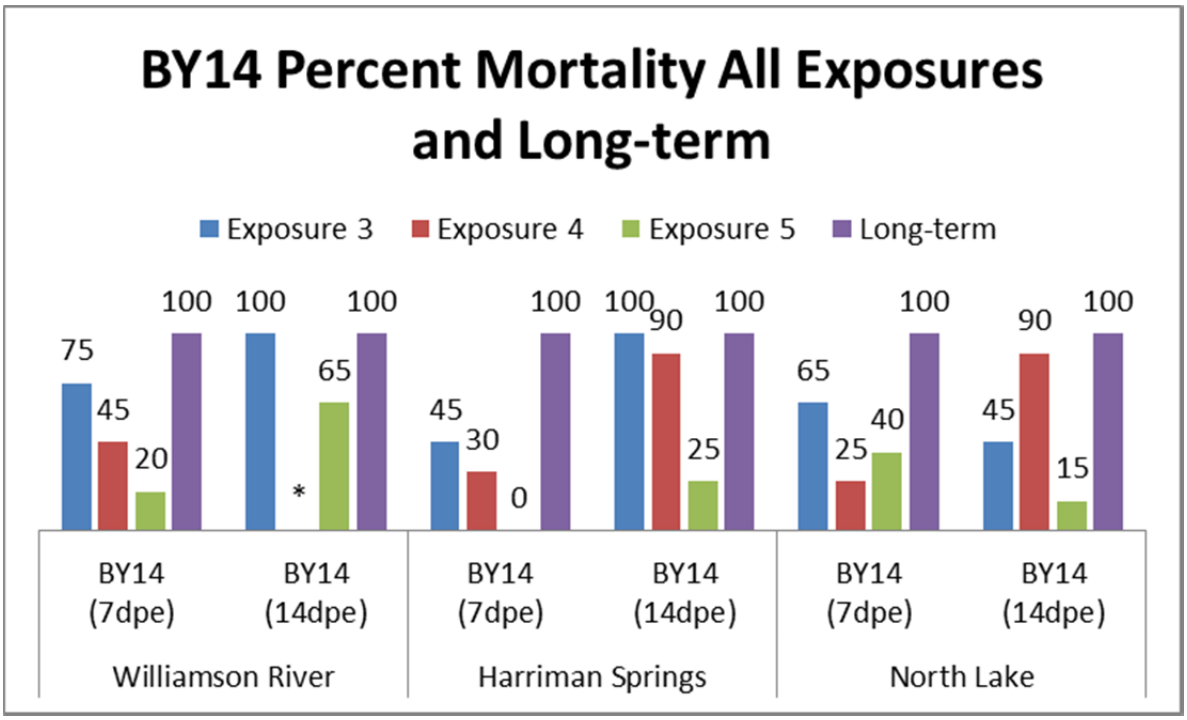


Figure 4. Percent mortality for BY14 all exposures (#3 – 5) and long-term groups at Williamson River, Harriman Springs, and North Lake.

*Denotes the 14dpe sample was not collected as the wrong net was pulled.

<u>Williamson River and Harriman Springs specific data</u>

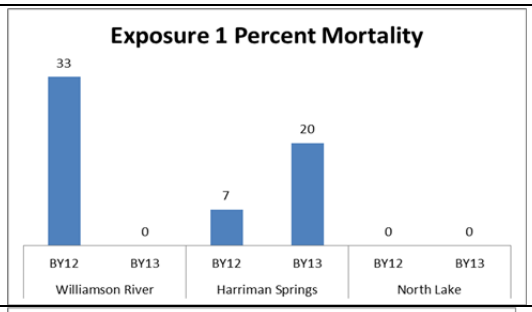
Water quality – Water quality at the Williamson R. mouth (Appendix 3 Figures 3-3 to 3-5, and Table 3-3) and Harriman Springs (Appendix 3 Figures 3-5 to 3-7, and table 3-4) sites were mild in comparison to the North lake site. Williamson R. mouth had UIA ≤ 0.032mg/L (Table 3-3) and Harriman Springs UIA was 0.000mg/L except for one 15July reading that was 0.001 mg/L (Table 3-4). Mean temperatures throughout the exposure periods was 17.3 – 21.03˚C in the Williamson R. mouth with a maximum reading of 26.7˚C. The minimum dissolved oxygen reading at the Williamson R. mouth site was 7.0mg/L with mean reading of 8.98 – 10.21mg/L throughout the exposure periods. Mean pH values for Williamson R. mouth site were 8.60 – 9.29 throughout the exposure periods with a maximum reading of 9.77 during the 17June – 1July and1July – 15July exposure periods.

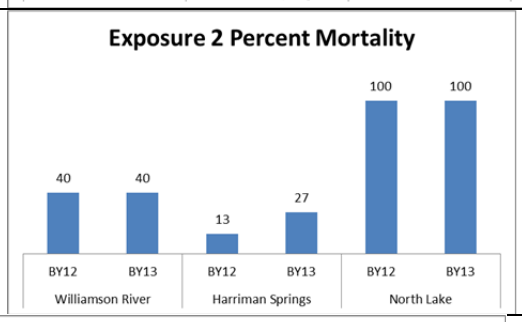
Harriman Springs had the lowest temperature readings with means between 15.89 – 18.62˚C during the exposure periods and a maximum temperature of 22.5˚C. Minor dissolved oxygen swings were observed. Minimum D.O. reading was 4.07mg/L during the 29July – 19August exposure period and a maximum of 15.27mg/L during the 19 August – 2 September exposure. Mean dissolved oxygen readings for Harriman throughout the exposure periods were 8.76 -9.76 mg/L. Harriman Springs had the most benign pH of all three sites during the study period. Mean pH throughout the exposures

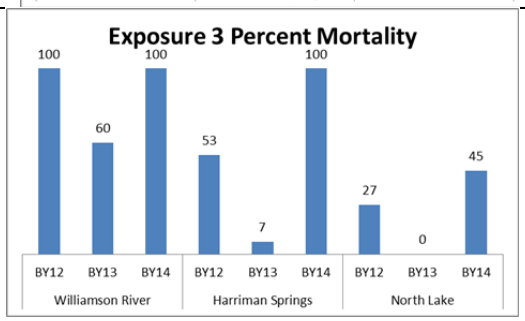
was 7.56 – 8.40 with a maximum pH reading of 9.42 during the 19August – 2September exposure period.

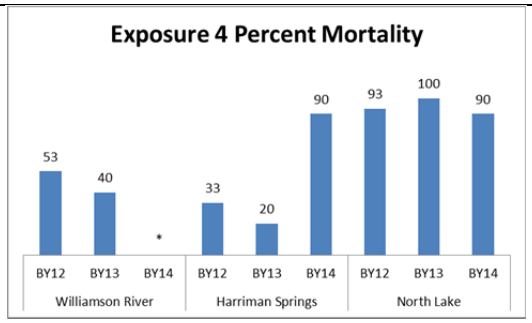
Sentinel survival Williamson River and Harriman Springs – During exposure 3 (15 – 29July), an unexplained high mortality occurred at Williamson R. site with a loss of 100% BY12 and 60%of BY13 (Figure 5). Blood and histological data from survivors of this group were not unique or diagnostic (see below). Mortality of the BY12 and BY13 sentinels ranged from 0 to 53% in the other Williamson R. exposures (Figure 5, Appendix 4). Long-term (78d) BY12 and BY13 sentinels had 90% and 47% mortality (Appendix 4). The BY14 fry had poor survival at the Williamson R. site (100% exposure 3 and 65% in exposure 5). Due to a sample error, the 14 dpe fry were not sampled during exposure 4.

Survival of BY12 and BY13 sentinels tended to be higher at the Harriman Springs site and varied from 0-40% for BY12 and 0-53% for BY13 suckers (Figure 5, Appendix 4). Long-term (78d) BY12 and BY13 sentinels had 77% and 60% mortality at the Harriman Springs site. BY14 fry had poor survival at this site (100% and 90% mortality) until exposure 5 (25% in 19Aug-02Sep).









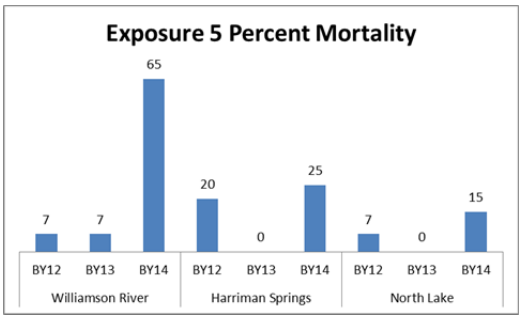


Figure 5. Percent mortality for all age groups held for 14d at the 3 sites for exposures 1-5.

Sentinel triglyceride reserves and condition – Size and triglyceride content (TG) was evaluated in long-term BY13 sentinel groups held for 36d at the North lake and 78d at Williamson R. and Harriman Springs sites (Table 1). Condition factor of North Lake fish was significantly higher than the similar Williamson R. and Harriman Springs fish. North lake BY13 sentinel TG content was significantly higher than both Williamson R. and Harriman Springs fish but similar to wetlab controls (Kruskal-Wallis One Way ANOVA, Table 1). Elevated metabolic demand and/or inadequate diet appeared to influence long-term sentinel energy reserves. Triglyceride content was not analyzed in the BY14 fry as 95% of well-fed wetlab control fry were below the assay detection limit. This is due to small fish size and assay constraint which was also seen in Foott et al. (2013).

Table 1. Mean (SE) standard length (SL,mm), weight (g), condition factor (KSL=WT/ $SL^3 x10^5$), and whole body triglyceride content (mg TG/g fish) of frozen BY13 collected and assayed for triglyceride from wetlab control groups, net pen fish after long term exposures; 78dpe for Williamson R. (WR) and Harriman Springs (HS), and 36dpe for North Lake (NL).

Broodyear 2013						
Date	Group	No.	SL	WT	KSL	TG
2-Jul	Wetlab	3	111 (7.1)	19.3 (2.9)	1.4 (0.05)	19.5 (0.8)
21-Jul	Wetlab	5	124.8 (3.0)	28.1 (1.5)	1.4 (0.05)	19.3 (0.6)
20-Aug	Wetlab	6	145.7 (4.0)	39.6 (3.0)	1.3 (0.02)	17.7 (1.7)
2-Sep	NL 36dpe	5	144.6 (5.8)	45.1 (6.0)	1.5 (0.08)	17.6 (0.5)a
2-Sep	WR 78dpe	7	133.0 (15.5)	31.4 (3.7)	1.3 (0.03)	3.7 (0.9)b
2-Sep	HS 78dpe	6	114.8 (4.8)	18.9 (2.0)	1.2 (0.05)	6.6 (1.5)ab

Sentinel total protein, plasma sodium, and hematocrit – Blood analysis was performed on a select group of BY12 and BY13 wetlab and sentinel cohorts (Table 2). There was no significant difference between lake groups for total protein or sodium (1-way ANOVA, P>0.05). Similarly, only one sample (12July Harriman Spring BY12) had a hematocrit value indicative of anemia (≤ 25%). To identify the effect of extreme capture stress on blood values, five BY12 were stressed by chasing with a net every 5min for 1h and collecting blood for analysis. Hematocrit, plasma protein and sodium were all markedly elevated in the stressed fish (Table 2). Only one sentinel survivor, a BY12 sucker from the 29July-19August hypoxic period, showed such elevated values for all 3 blood parameters (Table 2). No significant difference between BY13 sentinel survivor groups was detected in either plasma protein (Kruskal-Wallis ANOVA on ranks P= 0.28) or sodium (1-ANOVA P= 0.19). If we conservatively set abnormal low thresholds at 2 standard deviations of the BY12 and BY13 wetlab control plasma protein and sodium values (23mg/mL protein and 116mmol/L sodium), a subset of survivors were at or below these low values. Hypoproteinema (≤ 23mg/mL) occurred in 7 of 19 (37%) Williamson R. survivors. Low plasma sodium (≤ 116 mmol/l) occurred in 3 Williamson

R. and 5 Harriman Springs BY13 survivor samples. No obvious relationship between abnormal plasma values of BY13 survivors and BY13 cohort mortality was observed in the data. Survivor BY13 sentinel blood data was not diagnostic for groups with high mortality.

Table 2. Mean (SE) total protein (mg/mL), plasma sodium (mmol/L), and HCT (% of packed red blood cells to total blood volume) of BY12 and BY13 collected from wetlab control groups and groups of sentinels from Odessa, Williamson River, and North Lake net pens.

Broodyear	2012	2013	2012	2013	2013
Location	WL	WL	Stress Test	WL	WL
Date	7/2/2014	7/2/2014	7/2/2014	7/21/2014	8/20/2014
No.	3	3	5	5	2
Total Protein	40.8 (1.9)	34.3 (2.0)	45.0 (2.5)	30.3 (1.5)	32.3 (1.1)
Plasma Sodium	142.7 (9.5)	176.1 (7.6)	209.7 (5.1)	182.4 (12.9)	192.8 (17.4)
HCT%	0.39 (0.02)	0.40 (0.04)	0.48 (0.02)	0.36 (0.02)	0.37 (0.03)
Broodyear	2012	2013			
Location	Odessa	Odessa			
Date	7/1/2014	7/1/2014			
No.	5	5			
Total Protein	25.3 (3.6)	28.1 (1.5)			
Plasma Sodium	126.9 (12.5)	150.1 (9.1)			
HCT%	0.38 (0.04)	0.41 (0.02)			
Broodyear	2013	2013			
Location	WR	Odessa			
Date	7/15/2014	7/15/2014			
No.	5	5			
Total Protein	28.7 (0.5)	32.7 (2.1)			
Plasma Sodium	126.3 (12.0)	154.0 (13.8)			
HCT%	0.38 (0.02)	0.42 (0.01)			
Broodyear	2013	2013	2013		
Location	WR	NL	Odessa		
Date	7/29/2014	7/29/2014	7/29/2014		
No.	4	4	5		
Total Protein	21.3 (2.5)	28.3 (1.3)	31.0 (0.8)		
Plasma Sodium	162.9 (13.0)	182.0 (11.4)	157.3 (12.3)		
HCT%	0.35 (0.04)	0.40 (0.02)	0.40 (0.01)		
Broodyear	2013	2012	2013		
Location	Odessa	NL	WR		
Date	8/19/2014	8/19/2014	8/19/2014		
No.	5	1	5		
Total Protein	26.8 (0.9)	30.8*	24.1 (2.0)		
Plasma Sodium	148.9 (5.6)	187.5*	154.3 (11.7)		
HCT%	0.33 (0.02)	0.49*	0.37 (0.01)		
Broodyear	2013	2013	2013		
Location	Odessa	NL	WR		
Date	9/2/2014	9/2/2014	9/2/2014		
No.	5	5	5		
Total Protein	34.8 (6.7)	25.8 (2.0)	29.2 (2.5)		
Plasma Sodium	152.6 (9.4)	133.3 (8.8)	134.0 (11.5)		
HCT%	0.39 (0.00)	0.37 (0.02)	0.44 (0.03)		

* Denotes no SE as only one fish was sampled, this fish was the only survivor in North Lake during this exposure

Histological observations from all sites- A processing error (elevated wax temperature bath) resulted in brittle sections and altered tissue morphology in the 7 and 14dpe BY14 fry sagittal specimens. This error limits the evaluation of the 51 fry examined to gill macro-parasites (none observed). Kidney, gill, liver, and intestine was examined in 57 surviving BY13 sentinels (Table 3). Due to high mortality, no BY13 sentinel samples were collected from the North Lake in exposure 2 and 4. A single BY12 sucker was sampled at the North lake site on 19August (exposure 4). All tissues were normal including the gill. Hepatocytes, with degenerative eosinophilic nuclei suggestive of coagulative necrosis, were seen in 2September samples from all 3 sites (Figure 5). Proximal tubule epithelium, containing eosinophilic hyaline deposits (Figure 6), were seen in sentinel kidneys from exposures 1 – 4 at various sites (Table 15). Hyaline droplets within kidney tubule epithelial cells are typically excess protein reabsorbed from the filtrate and do not necessarily indicate dysfunction (Daoust and Ferguson 1984). The gill interlamellar cell mass varied markedly in height with extensive outgrowth over the secondary lamellae in variable numbers of fish from each exposure at all sites (Figure 7). A subset of affected fish at the Williamson R. sites also had necrotic foci within the interlamellar cell mass (Figure 7). Severe infections of *Ichthyophthirius multifiliis* (Ich) was observed in fish from both the Williamson R. and Harriman Spring sites during the summer (Figure 8). Such infections were not seen in the long-term cage fish sampled at these sites on 02September. Long-term cage samples also had normal gill structure (no hyperplasia) unlike the 14dpe sentinels sampled on the same date.

Table 3. Histological observations of BY13 sentinel suckers sampled after 14 d exposures (exposures 1 -5) and long term cages at the Williamson River (WR), North lake (NL), Harriman Springs (HS) sites and from wetlab (WL) controls. The prevalence (no. positive / total) of multifocal hepatocyte coagulated necrosis (HepCGN), hepatocyte eosinophilic cytoplasm inclusions (HepECI), kidney tubule hyaline deposit (Kd THD), gill interlamellar cell mass (Gill ILCM), Gill necrotic lamellar cysts (Gill Ncysts), and gill infection by *Ichthyophthirius multifiliis* (Gill Ich).

Exposure	Site	HepCGN	Hep ECI	Kd THD	Gill ILCM	Gill Ncyst	Gill Ich
E1	WR	0/4	0/4	1/4	0/2	0/2	0/2
17Jun-1Jul	NL	0/4	0/4	**4/4**	1/4	0/4	0/4
	HS	0/4	0/4	0/4	**3/4**	0/4	0/4
	WL	0/2	0/2	0/2	0/2	0/2	0/2
E2	WR	0/4	0/4	**3/4**	**4/4**	**2/4**	**4/4**
1Jul-15Jul	NL	N/A	N/A	N/A	N/A	N/A	N/A
	HS	0/4	0/4	**2/4**	**3/4**	0/4	0/4
E3	WR	0/4	0/4	**2/3**	**4/4**	**4/4**	**3/4**
15Jul-29Jul	NL	0/3	0/3	0/3	**3/3**	0/3	0/3
	HS	0/3	0/3	0/3	**3/3**	0/3	**1/3**
E4	WR	0/4	0/4	**1/3**	**2/4**	**2/4**	**4/4**
29Jul-19Aug	NL	N/A	N/A	N/A	N/A	N/A	N/A
	HS	0/3	0/3	1/4	**4/4**	0/4	**4/4**
E5	WR	**3/3**	0/3	0/3	**3/3**	0/3	0/3
19Aug-2Sep	NL	**2/3**	0/3	0/3	**3/3**	0/3	0/3
	HS	0/3	0/3	0/3	**3/3**	0/3	**3/3**
Long Term							
15Jul-2Sep	WR	0/3	0/3	0/3	0/3	0/3	0/3
29Jul-2Sep	NL	**1/3**	0/3	**1/3**	0/2	0/2	0/2
15Jul-2Sep	HS	**3/3**	1/3	0/3	0/2	0/2	0/2

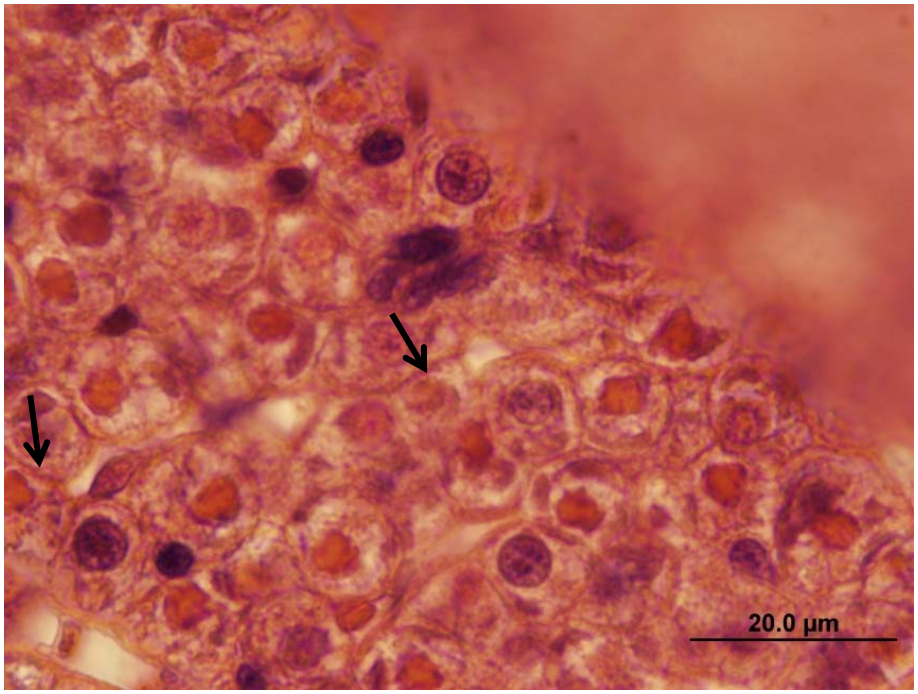


Figure 5. Hepatocytes with degenerative eosinophilic (arrows) nuclei suggestive of coagulative necrosis in a BY13 sucker sample 02September at the North lake site. (1000x magnification).

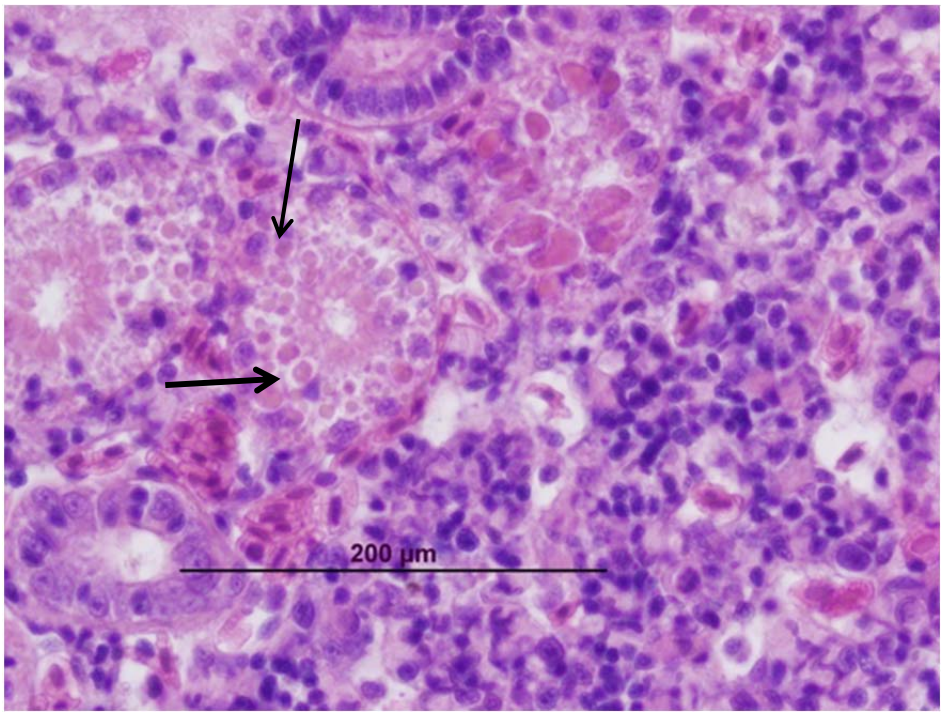


Figure 6. Hyaline deposits in proximal tubules of BY13 fish (19August Harriman Springs, 400x magnification).

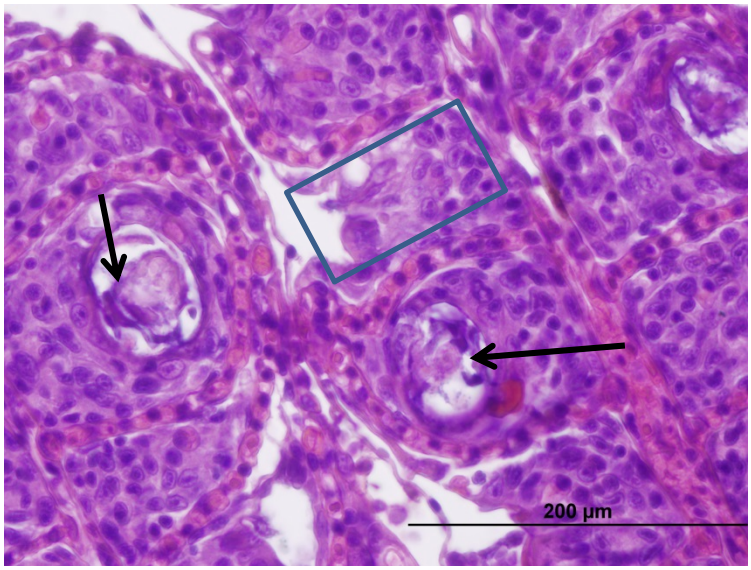


Figure 7. Extensive interlamellar cell mass response (box region) of BY13 fish (15July Williamson R.) with necrotic foci (arrows, 400x magnification).

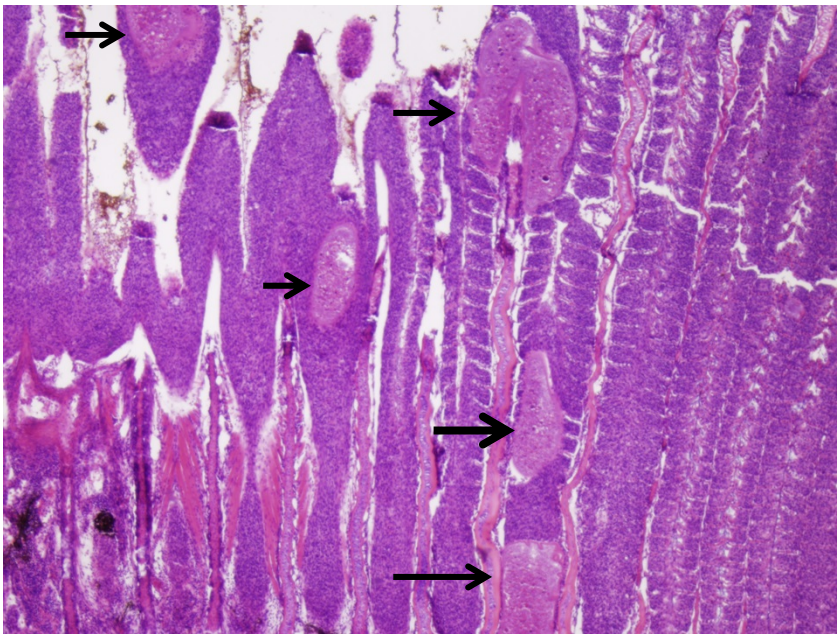


Figure 8. Ich parasites in BY13 gill (15July Williamson R.) with extensive interlamellar cell mass response (40x magnification).

Discussion – No viable eggs were obtained from SNS females held at the Williamson R. weir. Ovulation may occur over a brief period and was missed in our weekly/bi-weekly examinations. Given the capture location, it is likely that the SNS females are not close to ovulation when first captured at the weir. A controlled experiment (temperature and photoperiod) with hormone induction (e.g. HCG injections) is the next logical step to obtain SNS gametes from the Williamson R. population. Another option is to transport SNS from the weir to a fenced reach of their spawning grounds. Larvae or eggs of the segregated population could be collected for propagation.

No significant mortality events were observed in wet lab control fish over all exposure periods. Brood year 2012 and 2013 mortality was less than 1% and BY14 had 1.9 – 2.2% loss with the majority being from fallout (emaciated fish). All three brood years were placed into net pens within wet lab tanks for 14d with 100% survival. No gill or tissue abnormalities were observed in the control histology samples. Sentinel mortality was not likely associated with either poor health of the wet lab populations, initial transport stress, or net pen holding.

Episodic hypoxia was associated with the 2 high mortality events at the North Lake site. During these two hypoxic periods, BY12 and BY13 had ≥ 93% mortality, with 90% loss in the BY14 fry. It is noteworthy that long-term sentinels had lower mortality (73% and 77% mortality over 36d) than their 14d cohorts (≥93% loss) that were placed into the lake at the same time. One difference between the 14d and long-term groups was the 6x larger net pen volume for the long-term sentinels. It is unclear how this factor influenced survival during hypoxia. BY12 and BY13 sentinels had good survival during the other 3 North lake exposures despite high alkalinity (exposure 1 maximum pH 10.43) or temperature (exposure 3 maximum temperature 26°C).

Based on their water quality, the mouth of the Williamson R. and Harriman Springs were evaluated as locations for potential lake rearing. These littoral regions are not optimal rearing habitat for fry or older juveniles based on marginal survival, poor growth, and ectoparasite infections of sentinels held at these sites. Despite benign water quality, 14d survival of older sentinel suckers was variable (0 – 100%) and often low (half of all exposures resulted in ≥ 27% mortality). Over the 5 exposures, cumulative mortality of BY12 and BY13 sentinels was twice as high at Williamson R. as Harriman Springs. Histological examination and blood measurements of 14d survivors were not diagnostic in explaining this difference in mortality. *Ichthyophthirius multifiliis* gill infection was observed in survivors from 4 of 5 exposures at both the Williamson R. and Harriman Spring sites; however, there was no correlation with elevated mortality. Ectoparasite and trematode infection appeared to be more prevalent in the littoral zone of the lake compared to deeper mid-lake. Additionally, low plasma sodium occurred in only a small subset (3 Williamson R. and 5 Harriman Spring) of the BY13 collected for histology. Ion loss is often associated with Ichthyophthiriasis (Tumbol et al. 2001). It is noteworthy, that both *I. multifiliis* infection and gill interlamellar hyperplasia (discussed below) was seen in BY13 survivors from exposure 5 (19Aug-2Sept) but not in the long term survivors sampled the same day. Fish develop immunity to *I. multifiliis* infection and can rid themselves of the parasite over time (Dickerson and Clark 1998). It is possible that the long-term BY13 sentinels, held for 78d at these 2 sites, had developed such

immunity over the summer and recovered from *I. multifiliis* infection. Fat reserves (whole body triglyceride content) of long term BY13 sentinels in the North Lake (36d) were significantly higher than either the Williamson R. or Harriman Spring cohorts (78d). While the North Lake fish were held for half as long, we have observed a similar trend in higher TG values from mid-lake sentinels in 2013 (Foott et al. 2014). This finding suggests inadequate food resources at the littoral sites for these older fish. In summary, we do not recommend that juvenile sucker rearing efforts be conducted at either the Williamson R. mouth or Harriman Springs.

A number of teleost demonstrate the capacity to rapidly remodel gill lamellae in response to external oxygen concentrations and internal oxygen demands (Dhillon et al. 2013, Nilsson et al. 2012, Tzaneva et al. 2011). When oxygen demand is met, a reduction in lamellar surface area minimizes ion loss and increases the barrier to toxins and external pathogens. Conversely if either internal oxygen demand increases or hypoxia occurred, rapid reduction in the interlamellar cell mass (ILCM) would expose more lamellar respiratory surface and enhance respiration. Gill ILCM increase was observed in BY13 sentinels from all exposures and locations. *Ichthyophthirius multifiliis* infection did not appear to be the sole cause of this gill change as North lake sentinels had enlarged ILCM without observed parasite infection. Wetlab controls sampled in June and July did not show this gill morphology. Of particular interest was the difference between exposure 5 (19Aug-2Sep) BY13 sentinels and long-term sentinels. Despite a common exposure experience (normoxic water quality), 14d sentinels had enlarged ILCM while the long-term (36 and 78d exposures sampled on 2Sept) had minor ILCM. We have observed rapid changes (24h) in ILCM during wetlab hypoxic trials with juvenile LRS (pending hypoxic challenge report). ILCM observed in BY13 sentinels held for 14d could be an initial response to lake water quality and/or biological stressors (algae, bacteria, parasites) which changes over time as these fish acclimated to these stressors. The relationship of ILCM to survival is a topic for further research.

Survival of age 0 fry was generally poor throughout the summer regardless of exposure site and appears to be influenced by both fish size and exposure duration. Despite adequate water quality at both the Williamson R. and Harriman Spring sites, fry mortality increased 1.3 - 3x between the 7d and 14d sample with $\geq$ 90% loss. Fry were quite small for the first 2 exposures. Standard length averaged 20 and 23mm with condition factors <1.0 for these 2 exposures. In the last exposure (#5, 19Aug – 2Sep), the 30mm fry mortality dropped to 65% and 25% at Williamson R. and Harriman Springs, respectively. Obvious external parasite infection was not observed in the survivors and we did not have diagnostic data to attribute the high mortalities. It is possible that rapid bacterial infections upon entry to the netpens could contribute to the mortality however daily sampling would have to be conducted to track this potential factor. We speculate that inadequate food resources for smaller fish at these littoral sites could be a factor in the chronic mortality pattern as the North lake sentinel fry tended to have lower 14d mortality (except during the hypoxic event in 29July-13August exposure) with a pattern opposite of the Williamson and Harriman sites (7d mortality > 14d mortality). In 2012, small fry (18 – 22mm) held in benthic cages in the north and south lake experienced a similar mortality pattern with mortality vastly increasing

between 7 and 14d (Foott et al. 2013). Conversely, larger fry (38 – 66mm) had good survival at both the Williamson and mid-lake sites in 2013 (Foott et al. 2014).

In summary, elevated sentinel mortality occurred in both impaired (hypoxic) and benign water quality exposures. Smaller fry (<30mm) have lower survival than larger juveniles. This age group could be particularly sensitive to environmental stressors in the lake. Juvenile rearing near the mouth of the Williamson R. and Harriman Spring is not advised for propagation purposes. Histological and blood evaluation of 14d exposure survivors was not diagnostic for cohort mortality. Our inability to sample moribund fish limits this diagnostic approach. Our working hypothesis is that synergistic effects of adverse water quality, parasitic infections, and ingestion of toxic algae negatively influence predation rates and that specific spatial scale mortality is associated with episodic hypoxia (< 1 mg/L oxygen).

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Appendix 1. Notes on Shortnose adult spawn attempts.

A total of 40 female and 20 male Shortnose suckers (SNS) were obtained and held in 4' x 4' (64ft^3) net pen cages at the lower Williamson River weir (~1mile below the Hwy97 bridge) by USGS, Klamath Fall Field office 17April – 19May 2015. Five attempts were made to obtain gametes from wild caught SNS.

17April. Water temperatures were 11.7˚C, none of the fish examined were gravid and placed back into the holding pens. The second collection was 23April, water temperatures were 9.2˚C. One female expressed approximately 2-3mL of eggs weighing 2.9g, pit tag number 0077431A68. A fin clip was obtained and archived in RNAlater. No gravid males were found and therefore no spawn was conducted. Eggs from the female were counted at the Ca-NV Fish Health Center (Ca-NV FHC) to obtain eggs / mL (see table).

SNS 4/23/14 sample	
Total Weight (g)	2.73
Total # Eggs	801
Weight (g) Per Egg	0.0034

On 23April, attempts by Klamath FWO and USGS to capture SNS by seine or cast net secondary from the Williamson River near Chiloquin were unsuccessful.

On 6May, one female, pit tag number 309.1C20AE9056, gave 51.0g of eggs (~45mL). A fin clip was collected and archived in RNAlater as well as ovarian fluid for viral assay at the Ca-NV FHC (no viral CPE observed). Eggs were divided into two small glass dishes (27.8g and 23.2g respectively). A subsample of eggs was used to obtain average weight of SNS eggs (see table).

SNS 5/6/14 Sample		
	Female 5A	Female 5B
Average Weight (g) of 1 egg	0.0033	0.0044
Average Weight (g) of 10 eggs	0.041	0.046

One male, pit tag number 300.007742730A, was fin clipped and archived in RNAlater.

A fourth attempt to spawn SNS was conducted 15May with water temperatures of 14.9˚C . One female gave 5mL of eggs, pit tag number 300007742EDDC. Eggs appeared soft and over ripe. A fin clip was taken and archived. One male, pit tag number 309.1C20251C07, expressed milt. Gametes were mixed together with enough water to cover eggs for fertilization. Upon introduction of river water, some eggs began to rupture. Gametes were mixed for 10 minutes, water poured off, fresh river water added to the spawning container, and transported back to the Ca-NV FHC aquatic research facility. Eggs appeared swollen.. Eggs were "bulls eyed" 3h later after return to the wetlab. No larvae developed over 12d and the batch was discarded

A final attempt to spawn SNS occurred 19May. All eggs obtained were overripe. All SNS were released from the net pens back into the Williamson River. No mortalities occurred over the holding period.

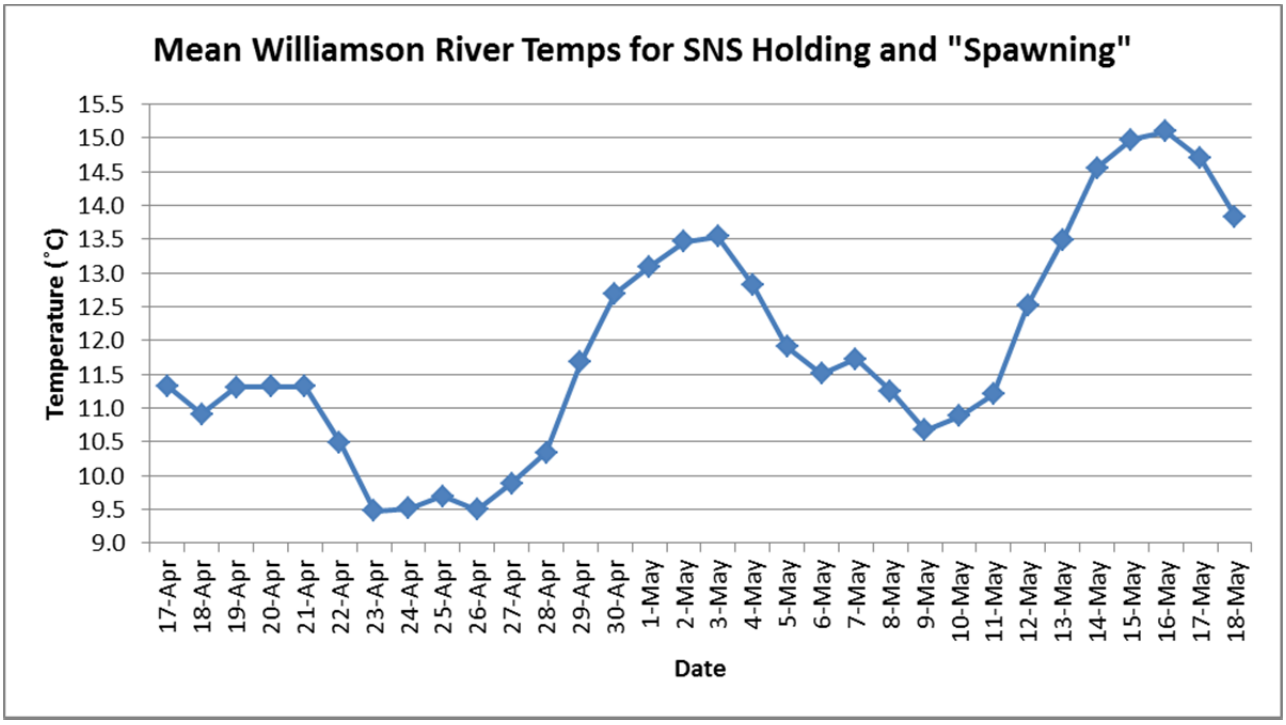

Figure 1-1. Mean daily temperatures on the Williamson River below the Sprague River near Chiloquin, Or during Short nose sucker holding period 14April to 18May 2014.

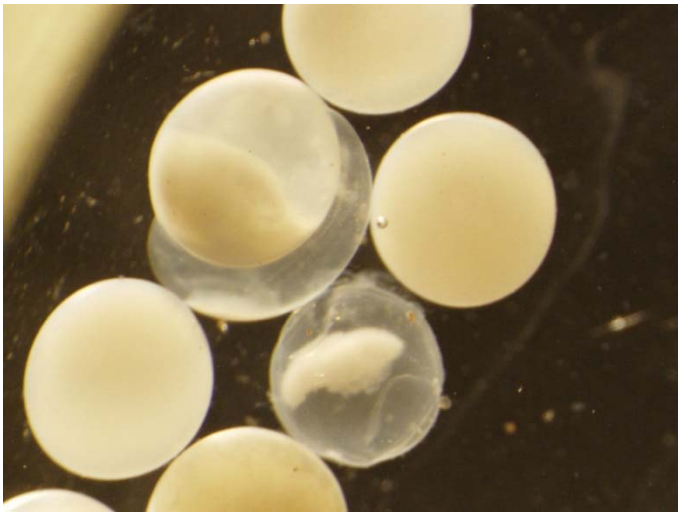

Figure 1-2. Over-ripe eggs “bulls eye” of 15May spawn.

Appendix 2. Morphometrics.

Table 2-1. Mean (SE) standard length (SL, mm), weight (g), condition factor ($KSL=WT/SL^3x10^5$) from wetlab control groups from all broodyears.

Wetlab Controls		
Exp 2 July 2		
Broodyear	2013	2012
No.	3	3
SL	111.0 (7.1)	166.7 (16.9)
WT	19.31 (2.9)	66.6 (16.9)
KSL	1.4 (0.05)	1.4 (0.1)
Exp 3 July 21		
Broodyear	2013	2014
No.	5	5
SL	124.8 (3.0)	19.6 (0.6)
WT	28.1 (1.5)	0.04 (0.1)
KSL	1.4 (0.05)	0.53 (0.03)
Exp 4 July 31		
Broodyear	2014	
No.	15	
SL	19.8 (0.5)	
WT	0.07 (0.01)	
KSL	0.86 (0.07)	
Exp 5 August 20		
Broodyear	2013	2014
No.	6	6
SL	145.7 (4.0)	25.3 (1.9)
WT	39.6 (3.0)	0.17 (0.05)
KSL	1.3 (0.02)	0.90 (0.12)

Table 2-2. Mean (SE) standard length, weight, and condition factors for BY12 from all sites during all exposure periods. Letters for a given exposure parameter indicate statistical significance.

Broodyear 2012			
Exp 1 (14dpe) June 17 - July 1			
Site	NL	HS	WR
No.	10	9	6
SL	166.9 (4.8)	163.4 (6.2)	164.8 (8.3)
WT	60.3 (4.9)	52.8 (5.6)	54.9 (8.1)
KSL	1.3 (0.02)a	1.2 (0.08)b	1.2 (0.08)b
Exp 2 (14dpe) July 1 - July 15			
Site	HS	WR	NL
No.	8	4	No
SL	161.8 (7.4)	157.0 (9.2)	Fish
WT	53.8 (6.7)	47.2 (7.5)	Samples
KSL	1.2 (0.02)	1.2 (0.04)	Taken
Exp 3 (14dpe) July 15 - July 29			
Site	NL	HS	WR
No.	7	2	No
SL	159.1 (9.8)	157.5 (14.5)	Fish
WT	49.6 (6.6)	43.9 (16.7)	Samples
KSL	1.2 (0.06)	1.1 (0.00)	Taken
Exp 4 (21dpe) July 29 - August 19			
Site	HS	WR	NL
No.	5	2	No
SL	183.0 (7.3)	206.0 (16.0)	Fish
WT	72.3 (12.3)	102.0 (23.7)	Samples
KSL	1.1 (0.1)	1.2 (0.00)	Taken
Exp 5 (14dpe) August 19 - September 2			
Site	NL	HS	WR
No.	11	7	11
SL	204.7 (4.2)	194.7 (6.2)	190.6 (5.1)
WT	110.1 (8.1)	93.3 (8.7)	92.4 (8.9)
KSL	1.3 (0.05)	1.2 (0.03)	1.3 (0.03)
LT (78dpe and 36dpe*) June 17 - Sept 2 and July 29 - Sept 2			
Site	NL*	HS	WR
No.	7	4	2
SL	195.9 (3.5)a	153.0 (10.0)b	165.0 (12)b
WT	93.9 (4.8)a	43.9 (6.8)b	55.6 (11.7)b
KSL	1.2 (0.04)	1.2 (0.07)	1.2 (0.01)

* Denotes North Lake long term exposure was 36d

Table 2-3. Mean (SE) standard length, weight, and condition factors for BY13 from all sites during all exposure periods. Letters for a given exposure parameter indicate statistical significance.

Broodyear 2013			
Exp 1 (14dpe) June 17 - July 1			
Site	NL	HS	WR
No.	10	7	10
SL	102.8 (2.7)b	1.08 (4.9)b	114.4 (2.4)a
WT	15.2 (1.2)	19.5 (2.4)	20.3 (1.4)
KSL	1.4 (0.04)	1.5 (0.03)	1.3 (0.03)
Exp 2 (14dpe) July 1 - July 15			
Site	HS	WR	NL
No.	6	4	No
SL	112.7 (4.1)	109.3 (1.7)	Fish
WT	20.0 (2.0)	16.9 (0.7)	Samples
KSL	1.4 (0.05)	1.3 (0.03)	Taken
Exp 3 (14dpe) July 15 - July 29			
Site	NL	HS	WR
No.	9	9	2
SL	118.6 (5.8)	124.7 (3.2)	131.0 (9.0)
WT	22.6 (3.2)	24.7 (1.6)	30.6 (6.3)
KSL	1.3 (0.03)	1.3 (0.1)	1.3 (0.01)
Exp 4 (21dpe) July 29 - August 19			
Site	HS	WR	NL
No.	7	4	No
SL	117.0 (5.1)	115.3 (6.6)	Fish
WT	21.9 (3.0)	18.0 (2.5)	Samples
KSL	1.3 (0.02)a	1.2 (0.04)b	Taken
Exp 5 (14dpe) August 19 - September 2			
Site	NL	HS	WR
No.	10	10	9
SL	134.6 (5.2)a	108.7 (6.8)b	121.1 (6.2)ab
WT	32.7 (3.9)a	19.6 (3.3)b	26.0 (3.6)ab
KSL	1.3 (0.03)	1.4 (0.05)	1.4 (0.04)
LT (78dpe and 36dpe*) June 17 - Sept 2 and July 29 - Sept 2			
Site	NL	HS	WR
No.	5	6	7
SL	144.6 (5.8)a	114.8 (4.8)b	133.0 (5.8)a
WT	45.1 (13.5)a	18.9 (2.0)b	31.4 (3.7)a
KSL	1.5 (0.03)a	1.2 (0.05)b	1.3 (0.03)b

* Denotes North Lake long term exposure was 36d

Table 2-4. – Mean (SE) standard length, weight, and condition factors for BY14 from all sites during all exposure periods. Letters for a given exposure parameter indicate statistical significance.

Broodyear 2014			
Exp 3 (7dpe) July 15- July 21			
Site	NL	HS	WR
No.	3	7	No
SL	20.3 (0.8)	19.7 (0.9)	Sample
WT	0.059 (0.02)	0.051 (0.01)	Fish
KSL	0.67 (0.14)	0.59 (0.10)	Taken
Exp 4 (7dpe) July 29 - August 5			
Site	NL	HS	WR
No.	10	6	9
SL	23.6 (1.1)	23.8 (1.2)	22.2 (0.9)
WT	0.12 (0.01)	0.15 (0.04)	0.09 (0.02)
KSL	0.93 (0.06)	0.98 (0.11)	0.79 (0.07)
Exp 5 (7dpe) August 19 - August 26			
Site	NL	HS	WR
No.	7	11	15
SL	32.6 (2.0)	27.6 (1.0)	28.7 (1.5)
WT	0.43 (0.08)a	0.24 (0.03)b	0.26 (0.04)b
KSL	1.1 (0.05)	1.1 (0.04)	1.0 (0.05)
Exp 5 (14dpe) August 19 - September 2			
Site	NL	HS	WR
No.	12	10	No
SL	32.1 (1.9)	29.8 (1.8)	Sample
WT	0.47 (0.10)	0.29 (0.04)	Fish
KSL	1.2 (0.07)	1.1 (0.1)	Taken

Appendix 3. Water quality.

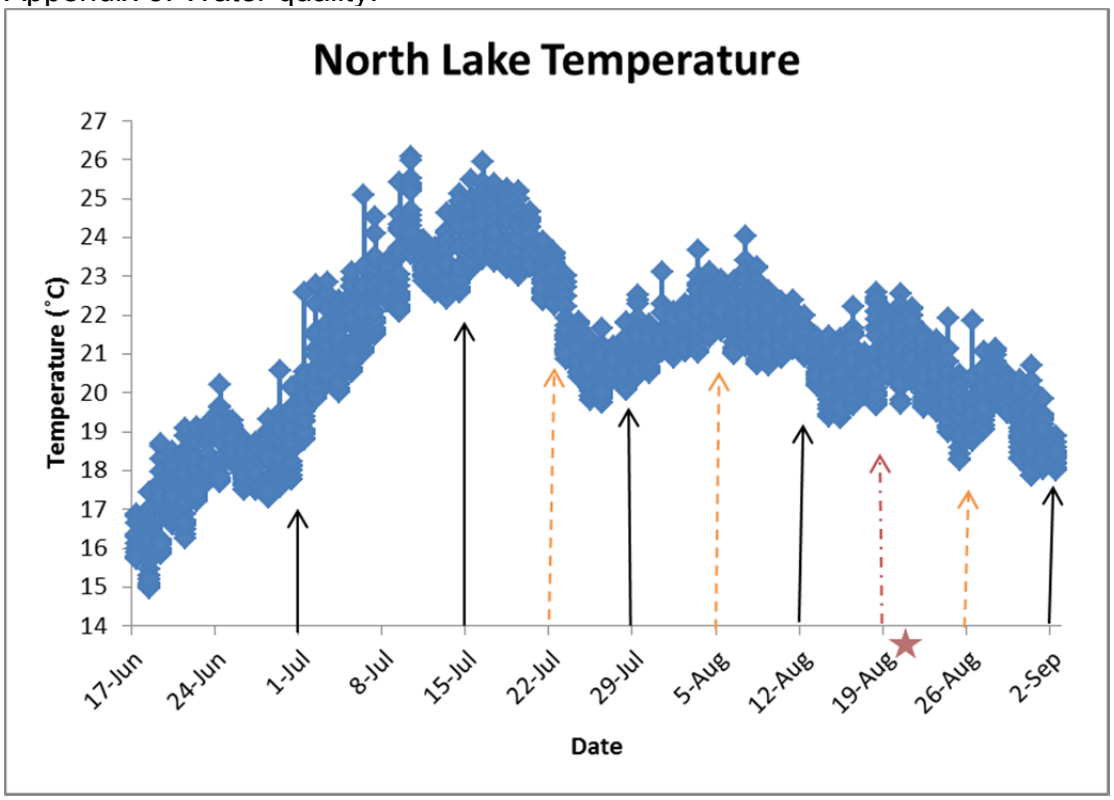

Figure 3-1. Water temperature North Lake site 17 June – 2 September. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

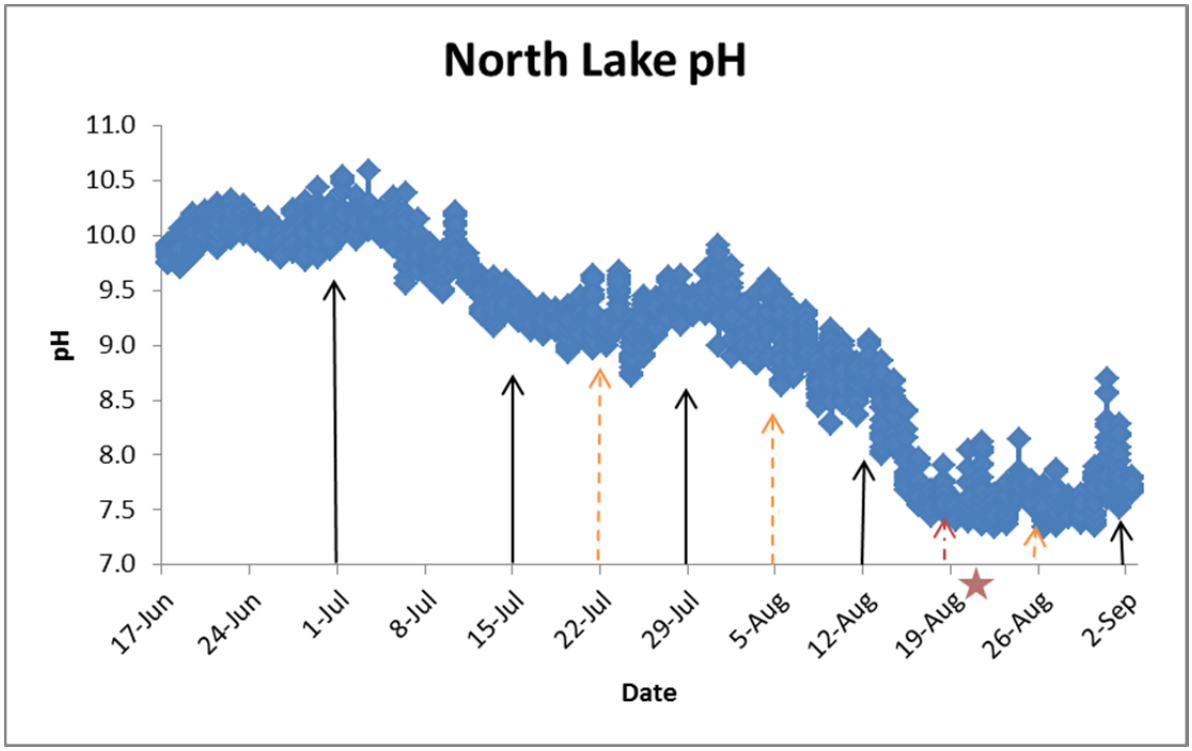

Figure 3-2. pH North Lake site 17June – 2 September. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

Table 3-1. Composite point measurements taken at the **North Lake** exposure site of total ammonia NH_4^+ (mg/L), temperature (°C) and pH as well as temperature (°C), pH and percent unionized ammonia NH_3 in aqueous solution values used to calculate unionized ammonia concentrations.

Date	NH_4^+ (mg/L)	Actual Temp (°C)	Actual pH	Temp Used (˚C)	pH Used	% NH3 Used mg/L	**Calculated UIA (mg/L)**
24-Jun	0.04	17.91	10.08	18	10.0	77.40	**0.031**
1-Jul	0.07	19.99	10.04	20	10.0	79.90	**0.056**
8-Jul	0.43	22.54	9.60	23	9.5	60.90	**0.262**
15-Jul	0.28	23.92	9.48	24	9.5	62.60	**0.175**
22-Jul	0.58	22.97	9.05	23	9.0	33.00	**0.191**
24-Jul	0.19	21.28	9.38	21	9.5	57.50	**0.109**
5-Aug	0.52	22.23	8.65	22	8.5	12.70	**0.066**
13-Aug	0.44	21.16	8.01	21	8.0	4.10	**0.018**
19-Aug	0.32	20.93	7.45	21	7.5	1.33	**0.004**
26-Aug	0.07	19.43	7.52	19	7.5	1.15	**0.001**
2-Sep	0.05	18.46	7.73	18	8.0	3.31	**0.002**

Table 3-2. Maximum, mean, and minimum readings for temperature (˚C), dissolved oxygen (mg/L) and pH obtained from 1.5m below the water *surface* at Harriman Springs, North Lake, and Williamson River exposure sites over an eleven week study period. Standard error ranged from 0.04-0.011 for temperature, 0.03-0.18 for dissolved oxygen and 0.00-0.03 for pH.

Constituent	Site		Exposure period				
			Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
			Jun 17 - Jul 1	Jul 1 - Jul 15	Jul 15 - Jul 22	Jul 29 - Aug 19	Aug 19 - Sep 2
Temperature	Harriman	max	19.13	22.5	21.23	21.74	20.9
		mean	**16.07**	**18.62**	**17.51**	**16.48**	**15.89**
		min	13	16.73	13.21	12.92	11.34
	North Lake	max	20.58	26.07	25.95	24.05	22.57
		mean	**17.88**	**22.51**	**22.46**	**21.34**	**19.9**
		min	14.95	19.3	19.74	19.34	17.87
	Williamson River	max	20.76	26.7	22.71	22.34	19.43
		mean	**17.28**	**21.03**	**19.37**	**17.81**	**16.15**
		min	14.4	18.63	16.23	14.77	14.02
Dissolved oxygen	Harriman	max	12.77	12.96	14.09	14.58	15.27
		mean	**9.76**	**8.76**	**9.1**	**9.37**	**9.69**
		min	5.58	5.67	6.28	4.07	4.53
	North Lake	max	17.41	17.03	12.52	14.53	9.99
		mean	**10.4**	**7**	**5.83**	**5.02**	**5.52**
		min	4.92	0.05	1.49	0.57	2.61
	Williamson River	max	12.76	11.79	10.84	11.3	10.4
		mean	**10.21**	**9.04**	**8.98**	**9.13**	**9.38**
		min	8.58	7.12	7	7.71	8.44
pH	Harriman	max	8.88	8.67	8.71	9.31	9.42
		mean	**7.66**	**7.56**	**7.72**	**8.01**	**8.4**
		min	6.83	6.97	7.08	7	7.09
	North Lake	max	10.43	10.59	9.67	9.91	8.69
		mean	**10.02**	**9.81**	**9.23**	**8.69**	**7.61**
		min	9.71	9.18	8.72	7.44	7.34
	Williamson River	max	9.77	9.77	9.31	9.5	8.96
		mean	**9.29**	**8.97**	**8.79**	**8.6**	**8.8**
		min	8.86	8.39	8.48	7.95	8.57

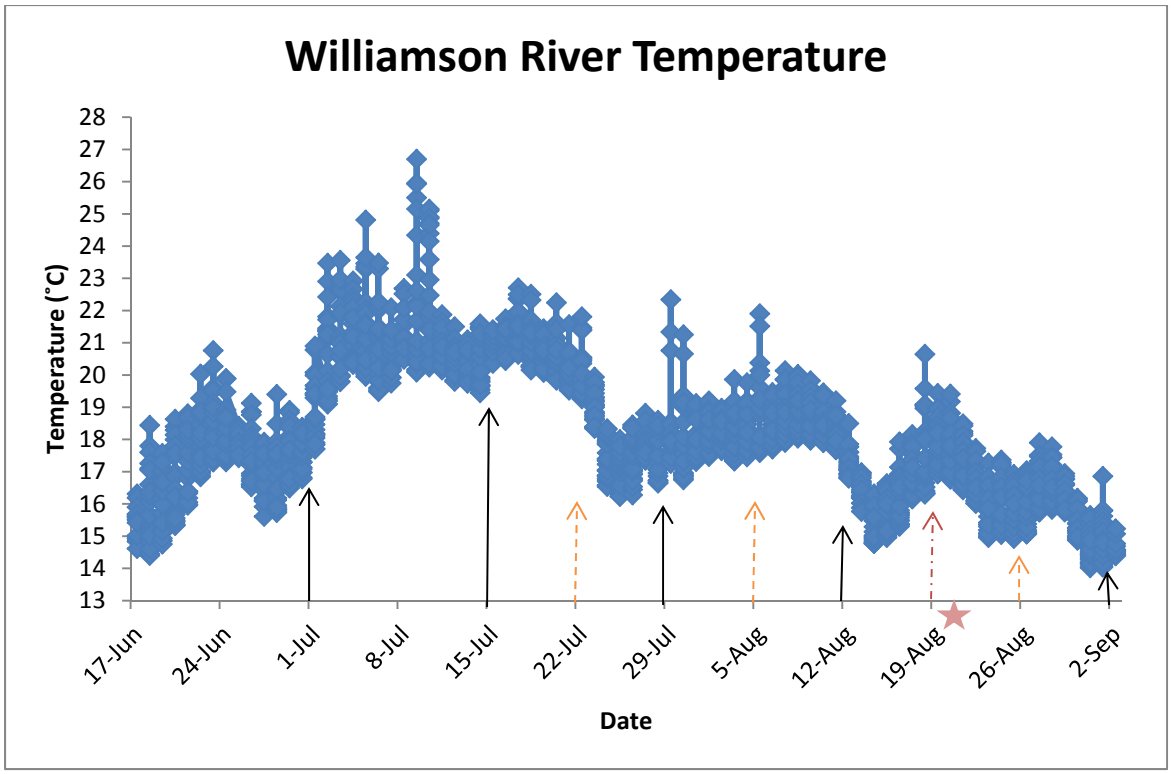

Figure 3-3. Temperature Williamson River site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

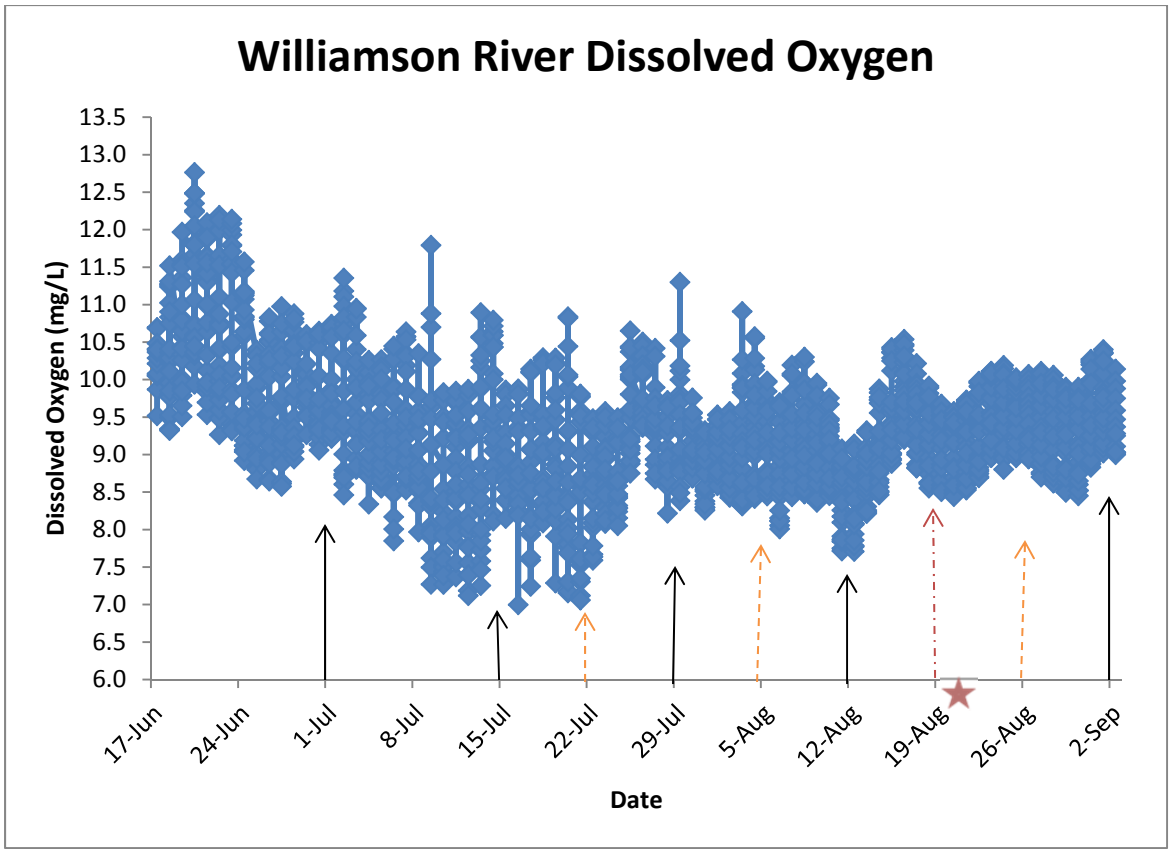

Figure 3-4. Dissolved Oxygen Williamson River site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

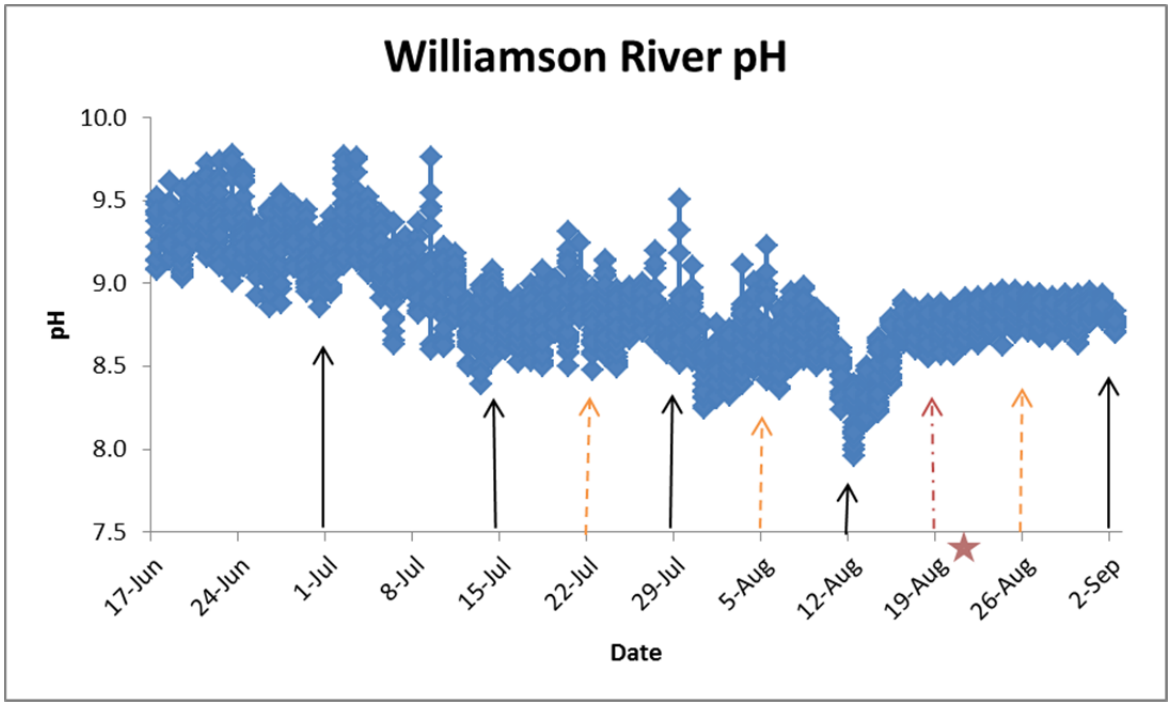

Figure 3-5. pH Williamson River site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

Table 3-3. Composite point measurements taken at the **Williamson River** exposure site of total ammonia NH_4^+ (mg/L), temperature (°C) and pH as well as temperature (°C), pH and percent unionized ammonia NH_3 in aqueous solution values used to calculate unionized ammonia concentrations.

Date	NH_4^+ (mg/L)	Actual Temp (°C)	Actual pH	Temp Used (˚C)	pH Used	% NH3 Used mg/L	**Calculated UIA (mg/L)**
24-Jun	0.03	17.41	9.33	17	9.5	50.20	**0.015**
1-Jul	0.07	17.85	9.16	18	9.0	25.50	**0.018**
8-Jul	0.10	21.73	9.09	22	9.0	31.50	**0.032**
15-Jul	0.10	20.74	8.80	21	9.0	29.90	**0.030**
22-Jul	0.04	19.33	8.65	19	8.5	10.50	**0.004**
24-Jul	0.06	17.45	8.65	17	8.5	9.14	**0.005**
5-Aug	0.16	18.93	8.55	19	8.5	10.50	**0.017**
13-Aug	0.03	16.81	8.37	17	8.5	9.14	**0.003**
19-Aug	na	18.34	8.58	18	8.5	9.78	**na**
26-Aug	0.04	16.93	8.77	17	9.0	24.10	**0.010**
2-Sep	0.07	15.23	8.77	15	9.0	21.50	**0.015**

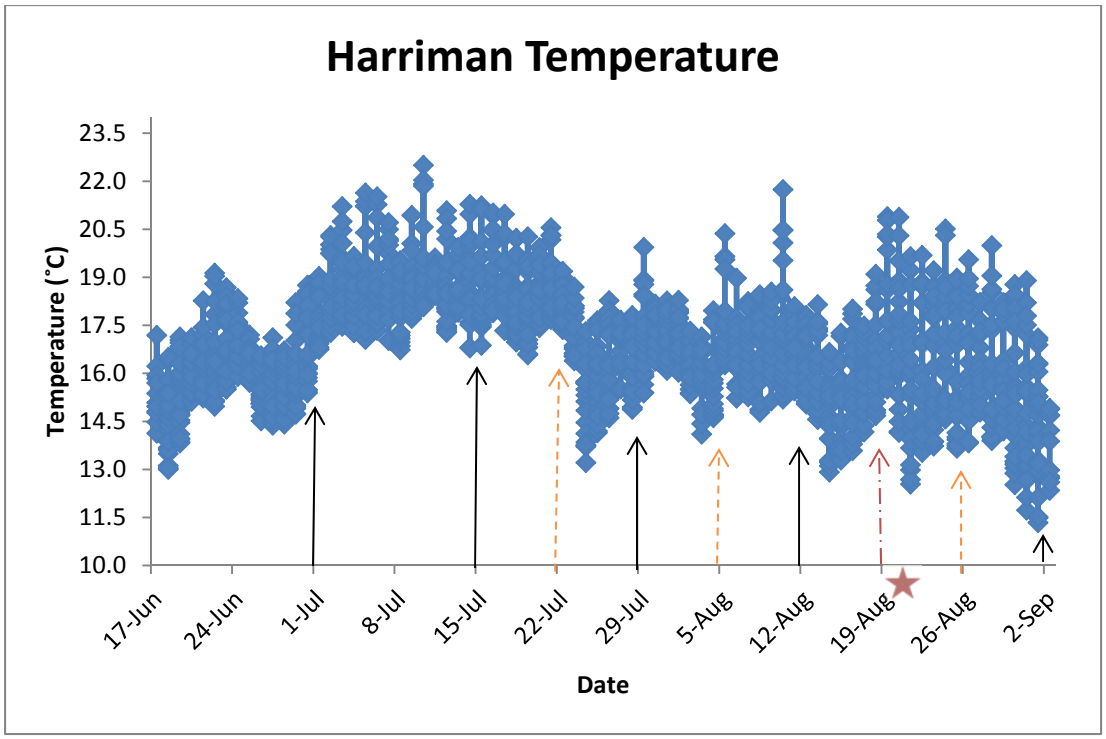

Figure 3-5. Temperature Harriman site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

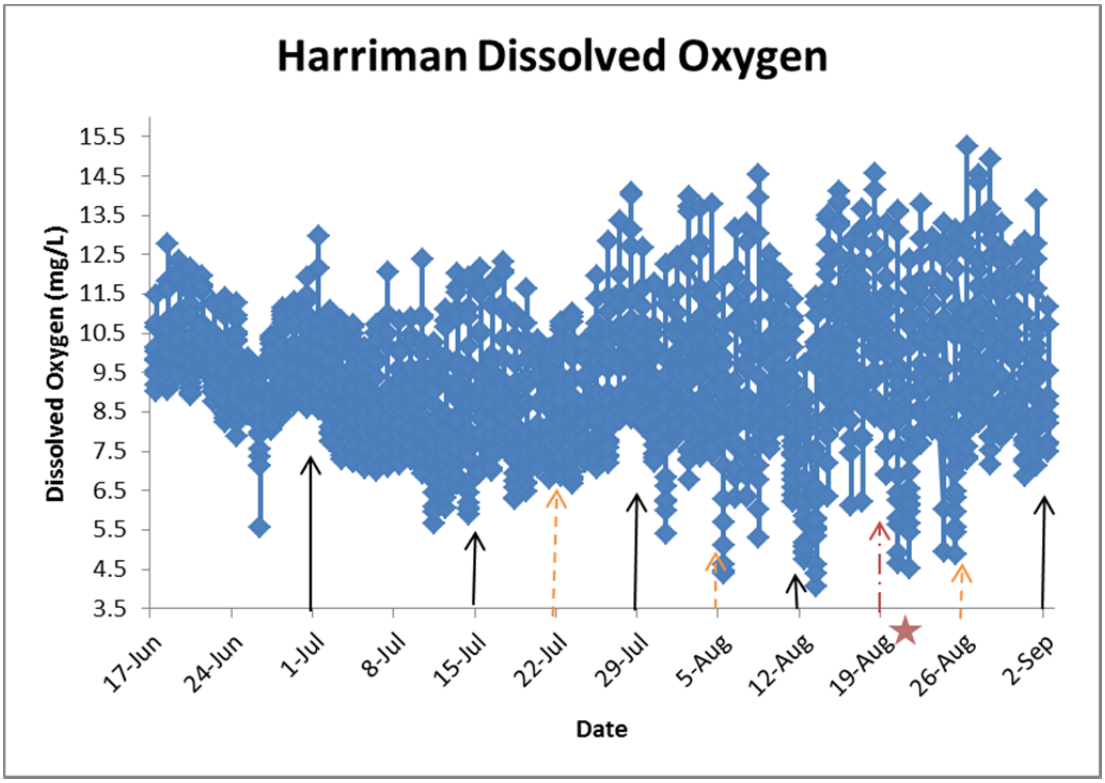

Figure 3-6. Dissolved Oxygen Harriman site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

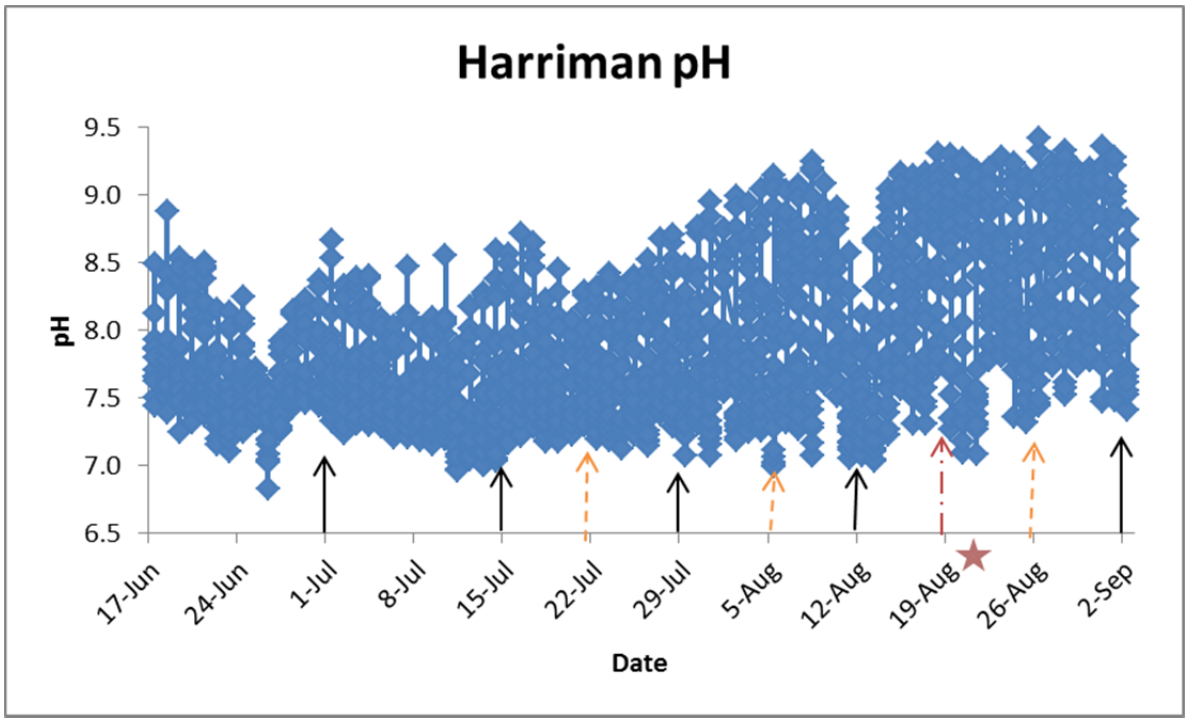

Figure 3-7. pH Harriman site Upper Klamath Lake 17 June – 2 September 2014. Water quality measurements were obtained hourly using continuous monitors deployed at a depth of 1.5m (surface) from a buoy located in the center of the net pen array. Orange (dashed) arrows indicate 7dpe sampling, black (solid) arrows indicate 14dpe sampling, red (dot-dash) arrow and star indicates 21dpe sampling as prior week was missed due to storm.

Table 3-4. Composite point measurements taken at the **Harriman Springs** exposure site of total ammonia NH_4^+ (mg/L), temperature (°C) and pH as well as temperature (°C), pH and percent unionized ammonia NH_3 in aqueous solution values used to calculate unionized ammonia concentrations.

Date	NH_4^+ (mg/L)	Actual Temp (°C)	Actual pH	Temp Used (˚C)	pH Used	% NH3 Used mg/L	**Calculated UIA (mg/L)**
24-Jun	0.03	16.81	7.43	17	7.5	0.996	**0.000**
1-Jul	0.01	16.93	7.44	17	7.5	0.996	**0.000**
8-Jul	0.01	18.82	7.19	19	7.0	0.368	**0.000**
15-Jul	0.03	19.21	7.97	19	8.0	3.560	**0.001**
22-Jul	0.02	18.76	7.46	19	7.5	1.150	**0.000**
24-Jul	0.04	16.62	7.55	17	7.5	0.996	**0.000**
5-Aug	na	17.29	7.45	17	7.5	0.996	**na**
13-Aug	0.02	16.30	7.45	16	7.5	0.925	**0.000**
19-Aug	0.04	15.72	7.35	16	7.5	0.925	**0.000**
26-Aug	0.00	17.23	8.25	17	8.5	9.140	**0.000**
2-Sep	0.01	12.60	7.61	13	7.5	0.738	**0.000**

Appendix 4. Sentinel mortality

Table 4-1. Exposure period, site location, number (n) of mortalities observed per net pen and mean percent mortality for each exposure at Williamson River, North Lake, and Harriman Springs for **BY12.**

Exposure	Site	Mortality Per BY12 Cage (n)	Percent Mortality
Exposure 1 17 Jun – 1 Jul	Williamson River	5	33% (5/15)
	North Lake	0	0% (0/15)
	Harriman	1	7% (1/15)
Exposure 2 1 Jul – 15 Jul	Williamson River	6	40% (6/15)
	North Lake	14	100% (14/14)
	Harriman	2	13% (2/15)
Exposure 3 15 Jul – 29 Jul	Williamson River	15	100% (15/15)
	North Lake	4	27% (4/15)
	Harriman	8	53% (8/15)
Exposure 4 29 Jul – 19 Aug	Williamson River	8	53% (8/15)
	North Lake	14	93% (14/15)
	Harriman	5	33% (5/15)
Exposure 5 19 Aug – 2 Sep	Williamson River	1	7% (1/15)
	North Lake	1	7% (1/15)
	Harriman	3	20% (3/15)
Long Term Exposure 17 Jun – 2 Sep	Williamson River 78 dpe	27	90% (27/30)
	North Lake* 28 dpe (36 dpe)*	20 (30)*	77% (20/26) 100%* (30/30)*
	Harriman 78 dpe	23	77% (23/30)

* Denotes two long-term exposure periods for North Lake as there was 100% mortality of fish at the site. First exposure was 17June – 15July (100% loss) and second exposure was 29July – 2September (77%).

Table 4-2. Exposure period, site location, number (n) of mortalities observed per net pen and mean percent mortality for each exposure at Williamson River, North Lake, and Harriman Springs for **BY13.**

Exposure	Site	Mortality Per BY13 Cage (n)	Percent Mortality
Exposure 1 17 Jun – 1 Jul	Williamson River	0	0% (0/15)
	North Lake	0	0% (0/15)
	Harriman	3	20% (3/15)
Exposure 2 1 Jul – 15 Jul	Williamson River	6	40% (6/15)
	North Lake	15	100% (15/15)
	Harriman	4	27% (4/15)
Exposure 3 15 Jul – 29 Jul	Williamson River	9	60% (9/15)
	North Lake	0	0% (0/15)
	Harriman	1	7% (1/15)
Exposure 4 29 Jul – 19 Aug	Williamson River	6	40% (6/15)
	North Lake	15	100% (15/15)
	Harriman	3	20% (3/15)
Exposure 5 19 Aug – 2 Sep	Williamson River	1	7% (1/15)
	North Lake	0	0% (0/15)
	Harriman	0	0% (0/15)
Long Term Exposure 17 Jun – 2 Sep	Williamson River 78 dpe	14	47% (14/30)
	North Lake* 28 dpe (36 dpe)*	22 (30)*	73% (22/30) 100%* (30/30)*
	Harriman 78 dpe	18	60% (18/30)

* Denotes two long-term exposure periods for North Lake as there was 100% mortality of fish at the site. First exposure was 17June – 15July (100%) second exposure was 29July – 2September (73%).

Table 4-3. Exposure period, site location, number (n) of mortalities observed per net pen and mean percent mortality for each exposure at Williamson River, North Lake, and Harriman Springs for **BY14.**

Exposure	Site	Mortality Per BY14 Cage (n)		Percent Mortality	
		7d	14d	7d	14d
Exposure 3 15 Jul – 29 Jul	Williamson River	15	20	75% (15/20)	100% (20/20)
	North Lake	13	9	65% (13/20)	45% (9/20)
	Harriman	9	20	45% (9/20)	100% (20/20)
Exposure 4 29 Jul – 13 Aug	Williamson River	9	*	45% (9/20)	*
	North Lake	5	18	25% (5/20)	90% (18/20)
	Harriman	6	18	30% (6/20)	90% (18/20)
Exposure 5 19 Aug – 2 Sep	Williamson River	4	13	20% (4/20)	65% (13/20)
	North Lake	8	3	40% (8/20)	15% (3/20)
	Harriman	0	5	0% (0/20)	25% (5/20)
		50d	50d	50d	50d
Long Term Exposure 15 Jul – 2 Sep	Williamson River	50	50	**100% (50/50)**	**100% (50/50)**
	North Lake	50	50	**100% (50/50)**	**100% (50/50)**
	Harriman	50	50	**100% (50/50)**	**100% (50/50)**

* Denotes wrong cage was pulled no information for 14d at Williamson River.