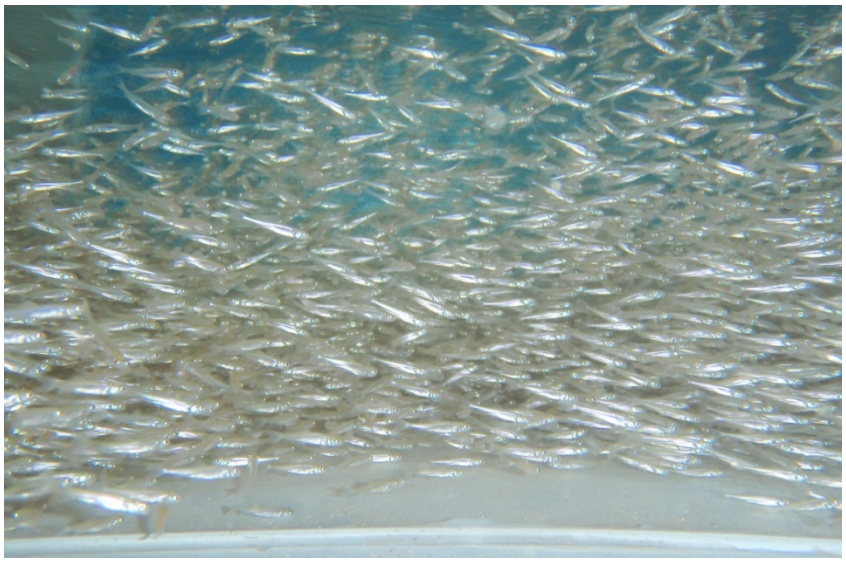
U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2015 Technical Report:

Lost River Sucker Fry Sentinel Survival in Upper Klamath Lake (July - September 2015).



May 2017

Ron Stone[1], Jennifer Jacobs[1], Nicholas A. Som[2], and J. Scott Foott[1**]

[1] U.S. Fish and Wildlife Service
California – Nevada Fish Health Center
24411 Coleman Fish Hatchery Road
Anderson, CA 96007

[2] U.S. Fish and Wildlife Service
Arcata Fish and Wildlife Office
1655 Heindon Road
Arcata, CA 95521

Brock Phillips[3], James Ross[3], Darrin Taylor[3] and Torrey Tyler[3]

[3] Bureau of Reclamation
Klamath Basin Area Office
6600 Washburn Way
Klamath Falls, OR 97603

**direct correspondence

Summary - Survival and tissue responses were evaluated in 10 sentinel groups of Lost River sucker fry (*Deltistes luxatus)* exposed at 4 locations (North Lake, Hank's Marsh, Sucker Springs and South Lake) in Upper Klamath Lake 14July – 22September 2015. Triplicate sentinel net pens were sampled every 7 days. Throughout the study, 18 – 36 mm sentinel fry had high survival after mock transport and 7d confinement in a net pen held within a wetlab tank. A low overall cumulative mortality of 16% was observed amongst all exposure groups (369/2380). Seven of the forty groups had cumulative mortality ≥ 25% with hypoxia associated with one of the affected groups. No parasites were observed in histological sections of sentinel survivors, however, some fry had liver abnormalities suggestive of hepatotoxin exposure. A Bayesian generalized linear mixed effects model indicated that increased temperatures decrease the probability of survival, with each 2.5°C increase in temperature decreasing survival by 47%. Additionally, the model indicated that each day over pH10 decreases survival by 38%. Warm alkaline conditions are typical of Upper Klamath Lake during the summer and are associated with cyanobacteria blooms. The effects of pH and temperature are likely correlative to mortality but not causal.

The correct citation for this report is:
Stone R, J Jacobs, N Som, J Foott, B Phillips, J Ross, D Taylor and T Tyler. 2017. Lost River Sucker Fry Survival in Upper Klamath Lake (July – September 2015). U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Notice The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service.

Introduction

Lost River Suckers (*Deltistes luxatus*), a native species of Upper Klamath Lake 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 their 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 others 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- 2014 have documented 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. Determine weekly survival of sentinel sucker fry held in net pens at 4 locations in Upper Klamath Lake (North Lake, Hanks Marsh, Sucker Springs and South Lake) between July and September 2015.

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

3. Use statistical modeling to ascertain the probability of survival as it pertains to ammonia, temperature and pH during exposure.

4. Perform histological assessment of sentinel survivors to diagnose whether specific pathologic conditions were associated with cohort mortality.

Methods

Fish rearing – On 14April 2015, gametes were obtained from 4 male and 4 female Lost River suckers captured by USGS at Cinder Springs, Upper Klamath Lake OR. Fish were immediately released after a small portion of gametes were collected. An estimated 40,700 eggs were obtained based on an egg estimation 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 a 500 – 750mm FL Lost River sucker female (Buettner and Scoppettone 1990). Eggs were collected in separate cups and placed into individual Tupperware containers. As milt from individual males was obtained it was placed directly onto eggs for fertilization (1:1 male to female ratio). Gametes were gently mixed with enough lake water to cover the eggs for 10min, excess fluid was poured off and re-filled with lake water, aerated with oxygen for 30s, and placed into an ice chest for transport to the CA-NV Fish Health Center's rearing facility. Upon arrival, 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 mini up-weller egg jars (PentairAES, Apopka, FL) at 12 – 13°C and treated with 1300 mg/L formalin to reduce fungus. Larvae were reared in 2.8L baskets (16.5cm x 12.7cm x 13.3cm frames covered by a 500µm mesh bag) suspended within a trough and fed a mixture of 24h artemia and A1 size larval diet (Otohime^{TM} 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) for 2 weeks. Otohime diet size was increased over time. An external protozoan outbreak in May was treated with 100 mg/L formalin and 2% salt baths.

Prior to transport, 12 groups of 20 fish were held in 10L aquariums at 20°C and pH 9.0 (recirculation system) for 24h of acclimation. Fish were transported to the lake within plastic bags containing 1.5L of their aquarium water and filled with oxygen. Bags were placed into ice chests, transported approximately 2h to Weed, CA for exchange with Bureau of Reclamation personnel who transported them an additional 1.5h to the lake. In addition to the sentinels, 3 groups of 20 control fry were handled as above and brought back from Weed to the wetlab. Total transport time was similar to cohorts exposed in the lake. Once returned to the wetlab, control fish were held in 3 separate hoop nets (same as sentinel fish in the lake) housed within a 670L fiberglass tank with flow through ambient water and aeration. Control fish were observed for 7d, mortalities recorded and a subset of survivors sampled for histological examination. No virus was detected from 60 fry sampled in July prior to the first movement to Upper Klamath Lake (USFWS and AFS-FHS 2007). Excess fry were provided to: 1) Dr. Anne Gannam (500 larvae - Abernathy Fish Technology Center), Dr. Josh Rasmussen (300 fry for Lower Klamath NWR pond net pens), and 3) Dr. Barbara Martin (~800 fry, USGS for future mesocosm studies).

Exposures – Ten exposures were conducted between 14July and 22 September at four sites: 1) North Lake, 2) Sucker Springs, 3) Hank's Marsh and, 4) South Lake (Figure 1). Sentinels (n=20/ net pen) were exposed in triplicate net pens at each site for 7d. Each 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 vertical integrity. Each buoy was given a unique number to identify sampling groups. Smaller hoop inserts were placed into each net pen where fish were held during each exposure. The smaller inner hoop nets were weighted in order to insure contact with the lake bottom. The inner nets 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 placed into the outer net along with naïve sentinel fish. Dimensions of the inner hoop nets were 3.66m high x 0.5m diameter enclosed with 1.6mm netting. Approximate volume was 0.72m^3. The number of live and dead fish was recorded for each net pen. Live fish were anesthetized in MS222 and a subset placed into Davidson's fixative for histological examination.

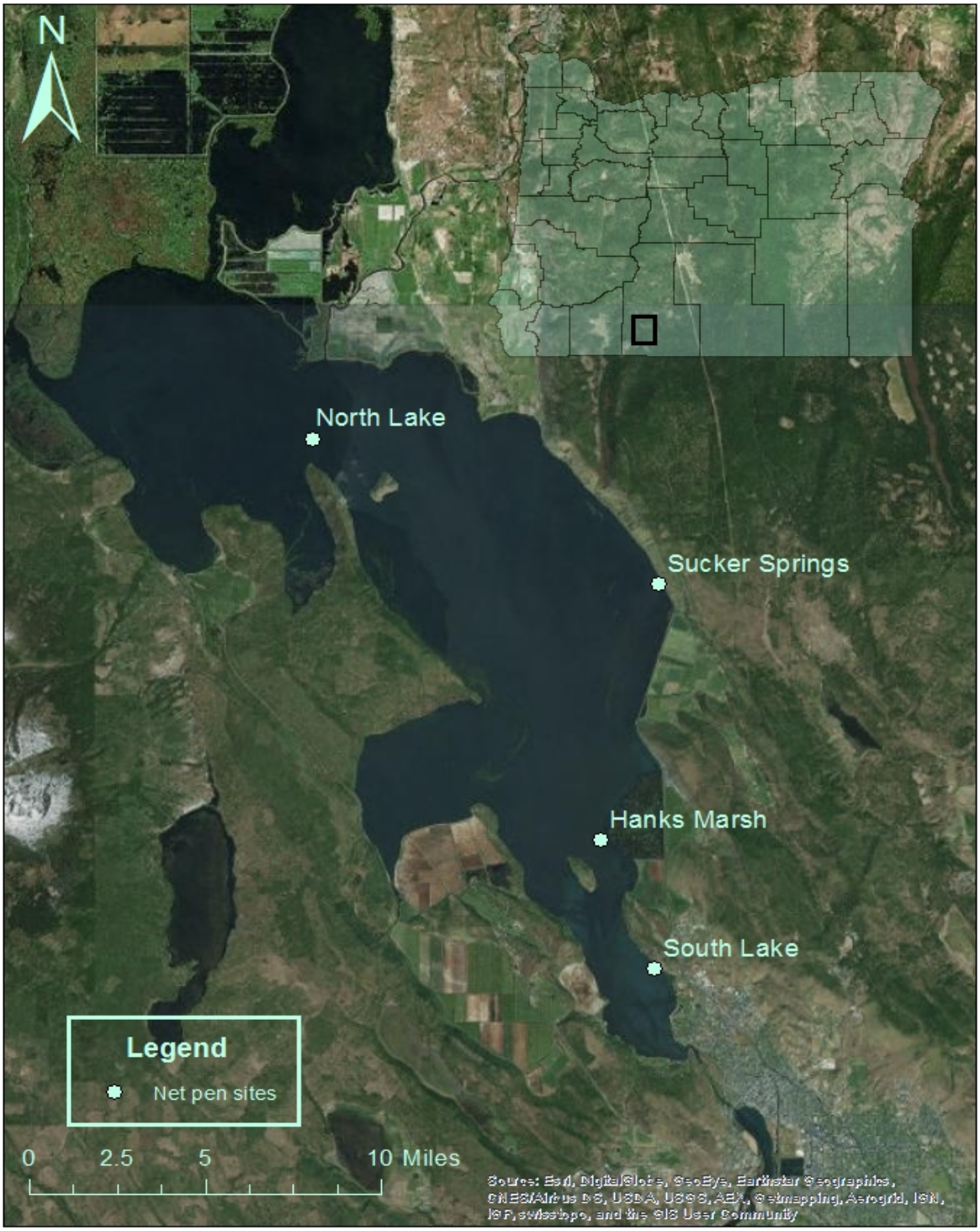


Figure1. Map showing sentinel cage locations in Upper Klamath Lake, Oregon 2015. Site locations are identified as; 1) North Lake, 2) Sucker Springs, 3) Hanks Marsh and, 4) South Lake.

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 at each net location. 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 data sonde temperature and pH data for the sample time). This weekly spot measurement occurred immediately prior to fish sampling.

Histology - Histological samples were held in Davidson's fixative for 48h, a subsample (groups with $\geq$ 25% mortality) processed for 5µm paraffin sections and stained with hematoxylin and eosin. Sagittal sections were cut and examined for gill condition, parasite infection and tissue damage. Abnormalities were rated on an ordinal 0, 1, 2, 3 scale.

Model analysis - To test if the probability of survival was associated with unionized ammonia (UIA), temperature, and pH, we fit a Bayesian generalized linear mixed effects model. The continuous UIA measure was bracketed into a 3-level categorical explanatory variable where values less than 0.1 were classified as "low", "medium" represented values from 0.1 to less than 0.3, and values at or above 0.3 were classified as "high." In addition to direct temperature effects, the inclusion of temperature in our model captured other variation that can be attributed to temporally-based seasonal differences. To construct our model, we started with a standard binomial logistic regression model, and then extended the linear predictor to include a random effect for sites where exposures were conducted (4 sites), and another random effect to account for variation in each of the exposure net pens. The first random effect captured larger scale variation among the sites, while the latter accounted for smaller scale variation attributable to factors such as net pen orientation, and accounted for potential correlation among individuals in each net pen. Hence, our model was

$$N_{i,j} \sim binomial\left(p_{i,j}, M_{i,j}\right)$$

$$logit\left(p_{i,j}\right) = \beta_0 + \beta_1 * I_{UIA.2} + \beta_2 * I_{UIA.3} + \beta_3 * T_{i,j} + \beta_4 * pH_{i,j} + \alpha_i + \delta_{j(i)};\ \alpha_i \sim N(0, \sigma_s^2);\ \delta_{j(i)} \sim N\left(0, \sigma_p^2\right),$$

where N represents the number of survivors among M placed in each net pen, i indexes site, j indexes net pen, j(i) denotes net pen j nested within site i, the β's are regression coefficients, the I's represent indicator functions for the levels of the UIA covariate, T represents mean temperature over the exposure period, pH represents the number of exposure hours when pH exceeded 10, s denotes a variance component due to sites, p denotes a variance component due to net pen, and α and δ represent the site and net pen level random effects, respectively. We estimated the parameters from our model using Markov Chain – Monte Carlo (**MCMC**) methods implemented with JAGS (Kellner 2014) and R statistical software (R Core Team 2014). For numerical stability, we standardized all continuous explanatory variables via their mean and standard deviation. Lacking direct information regarding the parameter values, we applied non-informative priors. To assess MCMC convergence, we ran 3 simultaneous chains each retaining 3000 posterior distribution samples after a burn-in period and a thinning rate of 150. After convergence, this resulted in 9000 posterior distribution samples for each parameter.

Results

Control Fry – Sentinel fry were considered healthy for each exposure. There was >99% survival (598/600) for transport and 7d holding for control fry in all exposures. A total of 38 control fry, from the 10 exposures, were examined by histology. No abnormalities were observed in the gill or liver. Three fry had a limited number of presumptive calcium phosphate deposits (nephrocalcinosis) within their kidney tubules. Mean standard length ranged from 18.25 to 35.99mm with a mean condition factor of 1.0402 (Table 1).

Table 1. Mean (SE) standard length (SL, mm), weight (g), and condition factor ($KSL = WT/SL^3 \times 10^5$) for wet lab control fish. Measurements for control fish obtained 1d prior to cohort exposure in Upper Klamath Lake 2015.

Date	Group	Exposure No.	SL (mm)	WT (g)	KSL
7/13/15	Wet lab	1	22.02 (0.65)	0.10 (0.01)	0.94 (0.02)
7/20/15	Wet lab	2	18.25 (0.45)	0.05 (0.01)	0.81 (0.05)
7/27/15	Wet lab	3	23.34 (0.97)	0.14 (0.02)	1.05 (0.02)
8/3/15	Wet lab	4	30.29 (1.05)	0.32 (0.03)	1.10 (0.02)
8/10/15	Wet lab	5	26.57 (1.26)	0.21 (0.03)	1.02 (0.04)
8/17/15	Wet lab	6	25.67 (1.18)	0.18 (0..03)	0.94 (0.04)
8/24/15	Wet lab	7	32.02 (0.84)	0.36 (0.03)	1.07 (0.02)
8/31/15	Wet lab	8	35.34 (1.08)	0.53 (0.06)	1.14 (0.03)
9/7/15	Wet lab	9	35.99 (1.17)	0.58 (0.07)	1.19 (0.02)
9/4/15	Wet lab	10	35.54 (1.06)	0.54 (0.06)	1.15 (0.03)

Water quality - From late July through late August, alkaline conditions (pH >10) occurred at all sites indicating cyanobacterial bloom conditions (Appendix 1 – 4). Hypoxic conditions ($\leq$ 1 mg/L dissolved oxygen) were only observed at the North Lake site during the 25August – 1September exposure and was associated with complete mortality of this sentinel group (Appendix 1, Figure 1.2 and 1.4). Maximum unionized ammonia (UIA) at each site ranged from 0.116 to over 0.3 mg/L and occurred at various sample times between 21July and 4August (Appendix 1-4). The highest UIA value was measured at North Lake on 21July however it was not associated with high mortality or prevalent histological tissue abnormalities.

Sentinel Survival – A 16% cumulative mortality (369/2380) was observed amongst the 40 exposure groups. Seven of the forty groups had cumulative mortality greater than 25% (Table 2, Appendix 1 - 4). These elevated mortality groups are discussed below:

Exposure 2; July 21- 28

Elevated cumulative mortality occurred at 3 of the 4 sites; South Lake (SL), Hanks Marsh (HM) and Sucker Springs (SS). Cumulative mortality at each location was 48%, 28% and 33% respectively. Hypoxia was recorded at any location. Sentinel fish exposed at SS were subject to 122h of pH ≥ 10.0 (mean = 10.08) and max temperature of 23.99°C (mean = 22.17°C). UIA readings were 0.00mg/L. At HM, 70h of pH ≥ 10.0 (mean = 9.96) were observed with maximum temperatures of 24.2°C (mean = 21.39°C). UIA readings were 0.016mg/L at input and 0.118mg/L when fish were removed. At SS, fry experienced pH levels ≥ 10.0 for 23 total hours (mean = 9.85) with max temperatures of 26.27°C (mean = 21.43°C). UIA readings were 0.057mg/L at input and 0.128mg/L when fish were removed 7d later. Fish exposed at North Lake (NL) during this time experienced slightly lower cumulative mortality, 22%. No pH readings ≥ 10.0 were recorded (mean pH = 9.61) and maximum temperature was 24.0°C (mean = 21.58°C). UIA at input was over the measurement limit for the HACH HQ40d colorimeter (> 0.305mg/L). Actual UIA reading is not known nor is it known how long UIA remained elevated. Readings at the time of removal were 0.008mg/L (Table 2).

Exposure 4; August 4-11

Elevated cumulative mortality (≥ 25%) was observed only at the NL exposure site. One net pen insert was found unsecured at the cod end making it possible for fish to escape. We cannot confirm that missing fish actually died as a result of their exposure and this net pen was omitted from the mortality results. There were 21h of pH ≥ 10.0 recorded (mean = 9.83) with maximum temperature of 22.96°C (mean = 20.96°C). No hypoxic events were observed. UIA readings were slightly elevated both at input and when fish were removed, 0.095 and 0.096 respectively (Table 2).

Exposure 5; August 11 - 18

SL and HM had cumulative mortality of 38% and 25%, respectively. pH levels ≥ 10.0 were observed at SL for 56h (mean = 9.97) with maximum temperature of 22.79°C (mean = 20.77°C). UIA was 0.033 at input and 0.065 when fish were removed. Fish exposed at HM experienced 47h at pH levels at or above 10.0 (mean = 9.92) with maximum temperature of 22.30°C (mean = 20.14°C). UIA was 0.084 at input and 0.102 when fish were removed. No hypoxic conditions were observed at either site (Table 2).

Exposure 7; August 25 – September 1

There was 100% mortality in all three sentinel cages at the North Lake site. Hypoxic conditions were associated with this event. Minimum dissolved oxygen was 0.63 mg / L for 1h and between 1.5 – 2.0 mg/L for 2h on 28August. Maximum temperature was 22.14°C (mean temp = 19.69°C) with no hours above pH ≥ 10.0 (mean pH = 9.24). UIA was 0.081 at input and 0.056 at 7 d post-exposure (Table 2).

Table 2. Mortality and water quality data for sentinel groups with ≥ 25% mortality. Hypoxic event was defined as ≤ 1 mg/L dissolved oxygen. Unionized ammonia (UIA, mg/L) measured at sentinel input (in) and sample (out). Sites were South Lake (SL), Hanks Marsh (HM), Sucker Springs (SS), and North Lake (NL) during exposures 2, 4, 5, and 7.

Exposure	Site	Exposure Dates	Mean percent mortality	Mortality range	Hours pH >10	Mean °C	Hypoxic event Y/N	UIA in	UIA out
2	SL	July 21 - 28	48	30-60	122	22.17	N		0.00
2	HM	July 21 - 28	28	15-35	70	21.39	N	0.016	0.118
2	SS	July 21 - 28	33	25-45	23	21.43	N	0.057	0.128
2	NL	July 21 - 28	22	10-30	0	21.58	N	>0.3	0.008
4	NL	Aug 4 - 11	28	15 and 25*	21	20.69	N	0.095	0.096
4	SS	Aug 4 - 11	12	5-30	46	20.92	N		
5	SL	Aug 11 - 18	38	30-50	56	20.77	N	0.033	0.065
5	HM	Aug 11 - 18	25	15-35	47	20.14	N	0.084	0.102
7	NL	Aug 25 Sept 1	100	0	1	19.69	Y	0.081	0.056

* One net pen found unsecured and omitted from mortality data set.

Model Analysis- Based on our analysis, there is strong evidence that temperature and pH are associated with the survival of individuals, but not evidence to suggest UIA levels alter survival probabilities. Our analysis suggests the probability that increasing temperatures reduce survival is 97.2%, and the probability that increasing pH10 reduces survival is almost certain at 99.9%. Associated with each 2.5 degree increase in mean temperature over the exposure period, the odds of survival is estimated to

decrease 47% (95% CI: 81% decrease to 2% increase), and for each day over ph10 the odds of survival is estimated to decrease 38% (95% CI: 18% to 57% decrease). The MCMC chains all converged well, with all Rhat values less than 1.1 and no visual evidence of MCMC chain convergence issues. Our model appears to be a good fit to these data, as evidenced by a Bayesian p-value of 0.54 eschewing any goodness of fit concerns.

Diagnostic Histological Examination – Sagittal sections of surviving sentinel fry (n= 90) were examined from net pens which had $\geq$ 25 % mortality during the 7d exposure. No parasites were seen in the histology specimens however several tissue abnormalities were observed in the gill (gill epithelial edema = GED), kidney (hyaline droplets within the tubule epithelium = THD), and liver (continuum of hepatic cell cytoplasm eosinophilic deposits = HED).

Gill lamellar edema was observed in a low prevalence (13 – 25%) of fry from only half of the affected groups and is probably not a significant driver of mortality (Table 3, Figure 2a). This gill abnormality has been seen in previous sentinel exposures and may be due to osmotic imbalance or can be a fixation artifact. The kidney tubule hyaline deposits are presumptive protein filtrate due to diuresis (increased urine production) and represent a non-lethal but detrimental loss of plasma protein to the affected fish (Ferguson 1989). It was observed in 7 of 8 affected groups with prevalence ranging from 8 to 50% (Table 3, Figure 2b). Hyaline deposits have been seen in juvenile Lost River suckers from both laboratory and sentinel studies (Foott et al. 2000, 2009, 2012, 2013, 2014). It is unlikely that this abnormality is strongly associated with sentinel mortality.

The observation of hepatocyte eosinophilic cytoplasmic deposits (HED) likely represents a continuum of apoptotic (cell death) changes that include lysosomal accumulation of protein, mitochondrial swelling, rough endoplasmic reticulum dilation, peroxisome proliferation and cell swelling (Cotran et al. 1989). It was observed in 7 of 8 affected groups with prevalence ranging from 13 to 50% (Table 3, Figure 2d). This liver abnormality has been observed in previous sentinel studies (Foott et al. 2012, 2013, 2014). No relationship was observed between hours at pH >10 and the prevalence of HED in high mortality group survivors (R^2 = 0.194, POI HED = 0.231+(0.00234* hrs < pH10)) or cumulative group mortality and prevalence of HED (R^2 = 0.109, Mort = 0.235+(0.181*POI HED)).

Table 3. Prevalence of specific tissue abnormality (HED = hepatocyte eosinophilic deposits, THD = hyaline droplets within kidney tubule epithelium, and GED = gill epithelial edema) in histological sections of 7d sentinel survivors. Sample groups (n=4 fish/group) were selected for histological examination when their cage mortality was ≥ 25% in one or more triplicate cages. Data includes exposure number, site (SL= south lake, HM= Hanks Marsh, SS = Sucker Springs, and NL = North Lake), mean percent and range of cumulative percent mortality of the 3 cage exposure group, and hours of exposure > pH 10. Bold data indicates mortality ≥ 25%.

Exposure	Site	Dates	Mean % mort	Triplicate cage % mort range	hrs >pH10	HED	THD	GED
2	SL	July21-28	***48***	30-60	122	**50%**	8%	17%
2	HM	July21-28	***28***	15-35	70	**38%**	13%	0%
2	SS	July21-28	***33***	25-45	23	**50%**	**50%**	13%
2	NL	July21-28	22	10-30	0	13%	0%	13%
4	NL	Aug4-11	***32***	15-55	21	25%	**50%**	0%
4	SS	Aug4-11	12	5-30	46	**50%**	**50%**	0%
5	SL	Aug11-18	***38***	30-50	56	**50%**	**33%**	25%
5	HM	Aug11-18	***25***	15-35	47	0%	**50%**	0%

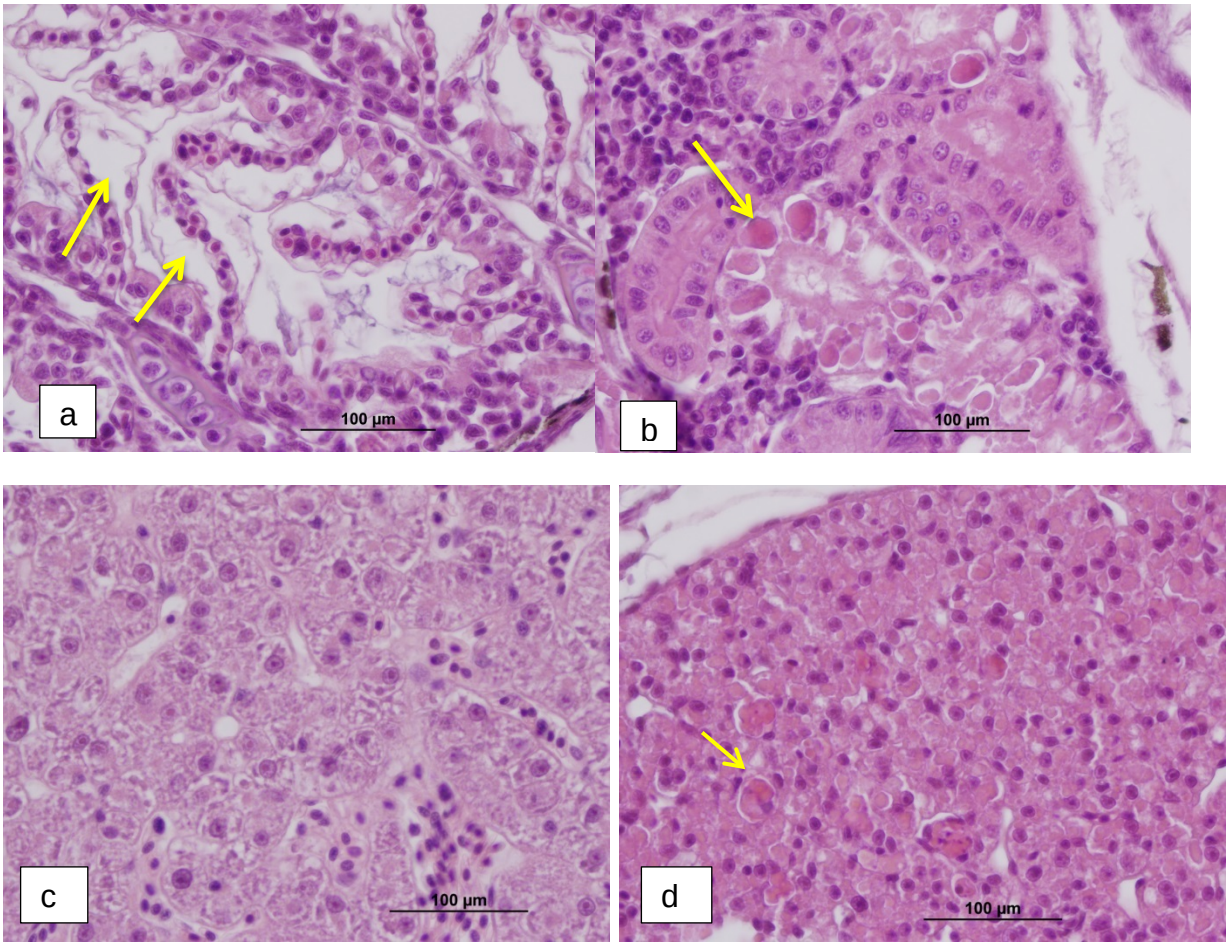


Figure 2. Tissue abnormalities observed in sentinel survivors; a) gill epithelial edema, b) kidney tubule hydropic degeneration or “droplets”, c) normal liver compared to d) hepatocyte with eosinophilic deposits (both cytoplasmic inclusions and apoptotic cells).

Discussion

In the summer of 2015, we employed statistical modeling to determine the probability of Lost River sucker fry survival in Upper Klamath Lake as it pertains to UIA, temperature, dissolved oxygen, and pH during 7d exposures. The selection of these water quality parameters were based on our ability to readily measure them with data sondes or a single colormetric test (UIA). It is an incomplete data set for all abiotic and biotic parameters that affect juvenile sucker survival in Upper Klamath Lake. In particular, exposure to water borne or ingested cyanobacterial was not recorded. Another subjective aspect of the study was our use of 25% cumulative mortality in a 7d exposure group (5 fry in a net pen population of 20) as a threshold for examination by the model and histology. Our intent was to measure acute mortality responses to adverse water quality however, it is likely an underestimate of survival. In 2012, we observed a delayed mortality pattern in 30-35mm sentinel fry held within benthic net pens (Foott et al. 2013). Between 7 and 14d post exposure sentinel groups experienced ≥ 77% mortality with little mortality in the first 7d. In contrast, low mortality was observed in sentinels held at the North Lake site for 14d in 2013. Water quality was judged to be relatively benign that summer.

Our sentinel fry were healthy and resilient to transport stress however they tended to be smaller (means of 18 – 36mm SL) than wild cohorts rearing in the lake. Sentinel mortality was not likely associated with either poor health of this captive population, initial transport stress, or net pen holding. It is likely that these smaller fry were as much or more sensitive to stressors in the lake than larger wild fry in the same locations (Jacobs et al. 2015). The relatively low 16% cumulative mortality of all groups, held at 4 locations in the lake over the 10 week study, suggests that lake conditions were relatively benign in the summer of 2015.

Only one elevated mortality event (North Lake 25August – 1September) could be linked to a documented water quality mortality threshold (hypoxia). In 2014, we observed similar complete sentinel mortality exposed to oxygen levels < 1mg/L (Jacobs et al. 2015). No parasites were observed in survivors of the 2015 elevated mortality groups. This suggests, similar to previous sentinel studies, infectious disease may not have a significant role in short term sentinel mortality (Foott et al. 2013, 2014, Jacobs et al. 2015). Similarly, UIA did not appear to be a strong determinant of acute mortality given the 22% mortality rate of the 21-28July North Lake sentinels exposed to a concentration of over 0.3 mg/L. This value is 3X greater than the other UIA measures during the summer.

Adverse water quality, driven by cyanobacteria, has been identified by other researchers as a significant stressor for Upper Klamath Lake suckers (Martin and Saiki 1999, Wood et al. 1996, Rasmussen 2011). Our model indicated that each day over pH10 decreases survival by 38% and that every 2.5°C increase in temperature decreases survival by 47%. It should be noted that both of these conditions describe the lake during its usual summer bloom cycle and does not demonstrate a direct survival

effect of these parameters. As an example of this assertion, three exposure groups (9 total sentinel cages) with low mortality were exposed to pH ≥ 10.0 for ≥ 25 hours. Cyanobacterial bloom cycles are also likely to expose sucker fry to various toxins. We have observed the presence of microcystin toxin (MC-LRa immunoreaction in intestinal content and epithelium) in previous sentinel studies as well as inhibition of liver protein phosphatase 2a activity (Foott et al. 2012, 2013, 2014). Given this finding, we suggest that juvenile suckers are ingesting the cyanobacterium and thus have a chronic exposure to the toxin(s). *Microcystin aeruginosa*, via its hepatotoxin microcystin-LR, has been identified as human health threat in Upper Klamath Lake by Oregon Department of Agriculture (Gilroy et al. 2000). The dominant blue-green species in the lake, *Aphanizomeno flos-aquae,* is also reported to produce toxins and should be considered a potential factor in fry mortality (Skulberg et al.1984). Abnormal hepatocytes were seen in 50% or less of the survivor livers including low mortality groups (e.g. Sucker Springs 4-11August). The observation of sentinel hepatocytes with abnormal cytoplasmic features (ranging from eosinophilic droplets in the cytoplasm, cell swelling, and vacuolar degeneration to apoptotic cell death) is supportive of our hypothesis of chronic hepatotoxin exposure. Ingestion of microcystin toxin has been shown to induce hepatocyte apoptosis in carp (Fischer and Dietrich 2000). Others have described similar hepatocyte changes in fish exposed to microcystin (Chen et al. 2017, Acuna et al. 2012, and Djediat et al. 2010). Future studies should use transmission electron microscopy to evaluate specific hepatocyte lesions in sucker fry from Upper Klamath Lake.

As reported in earlier sentinel studies, age 0^+ fry mortality in Upper Klamath Lake is likely a result from a suit of stressors that could include non-native fish predation, increased avian predation associated with impaired water quality, chronic hepatotoxin exposure, episodic hypoxia, and infectious disease (bacteria and parasites) that affect the population at different spatial and temporal levels during their first summer.

Roles: Ron Stone, Jennifer Jacobs, and Scott Foott – sentinel culture, histology, data summation, and report. Nicholas Som - survival modeling. Brock Phillips, James Ross, Darrin Taylor and Torrey Tyler – sentinel net pen sampling and water quality data.

Acknowledgements: We thank Oregon Department of Fish and Wildlife, USFWS Klamath Falls office and Region 1 Ecological Services branch, and California Department of Fish and Game for their assistance in obtaining permits for this work. Biologists with the USGS Klamath Falls office assisted us in obtaining gametes. Partial funding was provided by Bureau of Reclamation funding administered by the USFWS Klamath Falls Fish and Wildlife Office (account 4801-1084). Egg and fry culture assistance by Scott Freund.

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 2015-3581.

Reference

Acuna S, D-F Deng, P Lehman, and S Teh. 2012. Sublethal dietary effects of *Microcystis* on Sacramento splittail, *Pogonichthys macrolepidotus*. Aquatic Toxicology 110-111: 1-8.

Buettner, M., and G.G. Scoppettone. 1990. Life history and status of Catostomids in Upper Klamath Lake, Oregon. Report of the Seattle National Fisheries Research Center to the Oregon Department of Fish and Wildlife, Corvallis, Oregon.

Burdick, S.M., A.X. Wilkens and S.P. VanderKooi. 2008. Near-shore and off-shore habitat use by endangered juvenile Lost River and shortnose suckers in Upper Klamath Lake, Oregon: U.S. Geological Survey Open-File Report 2007-1356, 30 p.

Chen L., YF Hu, J. He, J. Chen, JP Giesy,and P. Xie. 2017. Reponses of the proteome and metabolome in livers of Zebrafish exposed chronically to environmentally relevant concentrations of microcystin-LR. Environmental Science and Technology 51(1): 596-607.

Cotran RS, V. Kumar and SL Robbins. 1989. Robbins Pathologic Basis of Disease. 4th ed., WB Saunders Co., Philadelphia.

Djediat C, M Malecot, A de Luze, C Bernard, S Puiseux-Dao, and M Edery. 2010. Localization of microcystin-LR in medaka fish tissues after cyanotoxin gavage. Toxicon 55:531 – 535.

Ferguson HW. 1989. Systemic pathology of fish. Iowa State University Press, Ames Iowa.

Fischer WJ and DR Dietrich. 2000. Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). Toxicology and Applied Pharmacology 164:73 – 81.

Foott JS, K. Nichols, and R Harmon. 2000. FY2000 Investigational Report: Histological and Hematological Evaluation of Juvenile Lost River Suckers Exposed to Sub-lethal Levels of Ammonia, U.S. Fish & Wildlife Service California-Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Foott JS, R. Stone, and R. Fogerty. 2009. FY2006 Technical Report: Effects of simulated algal bloom pH on juvenile Lost River Sucker energetics and growth. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Foott J.S., R. Stone, A. Wilkens, and J. Rasmussen. 2012. Juvenile Lost River Sucker survival and energetics in Upper Klamath Lake mesocosm net pens: July – December 2011. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Foott J.S., R. Stone, and A. Wilkens. 2013. Juvenile Lost River Sucker survival in Upper Klamath Lake mesocosm net pens: July 2012 – March 2013. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Foott J.S., R. Stone, J. Jacobs, and A. Wilkens. 2014. Juvenile Lost River Sucker survival in Upper Klamath Lake mesocosm net pens (July 2013 – October 2013) and laboratory hypoxic challenge. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Gilroy DJ, KW Kauffman, RA Hall, X Huang, and FS Chu. 2000. Assessing potential health risks from microcystin toxins in blue-green algae dietary supplements. Environmental Health Perspectives 108:435 - 439.

Jacobs J, R Stone, and JS Foott. 2015. Juvenile Lost River Sucker survival in Upper Klamath Lake mesocosm net pens (June – September 2014). U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Kellner, Ken. 2014. jagsUI: run JAGS (specifically, libjags) from R; an alternative user interface for rjags. R package version 1.1.

Markle D.F. and M. Cooperman. 2002. Relationship between Lost River and shortnose sucker biology and management of Upper Klamath Lake. Pages 93 – 118 in B Braunworth and T Welch, eds. The 2001 water allocation decisions in the Klamath Basin. Oregon State University Extension Pub., Corvallis OR.

Martin, B.A., and M.K. Saiki. 1999. Effects of ambient water quality on the endangered Lost River sucker in Upper Klamath Lake, Oregon. Transactions of the American Fisheries Society 128:953-967.

Meyer JS and JA Hansen. 2002. Subchronic toxicity of low dissolved oxygen concentrations, elevated pH, and elevated ammonia concentrations to Lost River Suckers. Transactions of the American Fisheries Society 131:656 – 666.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasmussen J.E. 2011. Status of Lost River Sucker and Shortnose Sucker. Western North American Naturalist 71(4):422 -455.

Skulberg, O.M., G.A. Codd, and W.W. Carmichael. 1984. Blue-green algae (cyanobacteria) toxins: water quality and health problems in Europe. AMBIO 13:244 – 247.

Terwillieger M.R. , D.F. Markle, and J. Kann. 2003. Associations between water quality and daily growth of juvenile shortnose and Lost River suckers in Upper Klamath Lake, Oregon. Transactions of the American Fisheries Society 132:691- 708.

USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2007. Standard procedures for aquatic animal health inspections. *In* AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2007 edition. AFS-FHS, Bethesda, Maryland.

Wood T.M., G.J. Fuhrer, and J.L. Morace. 1996. Relation between selected water-quality variables and lake level in Upper Klamath and Agency Lakes, Oregon. USGS Water-resources Investigations Report 96-4079. USGS Information Services, Box 25286, Federal Center, Denver CO 80225.

List of appendices

Appendix 1 North Lake site
Water quality figures (temperature, DO, pH)
Ammonia table
Percent mortality figure

Appendix 2 Sucker Springs site
Water quality figures (temperature, DO, pH)
Ammonia table
Percent mortality figure

Appendix 3 Hanks Marsh site
Water quality figures (temperature, DO, pH)
Ammonia table
Percent mortality figure

Appendix 4 South Lake site
Water quality figures (temperature, DO, pH)
Ammonia table
Percent mortality figure

Appendix 5
Net pen mortality & Water Quality

Appendix 1. North Lake water quality and mortality.

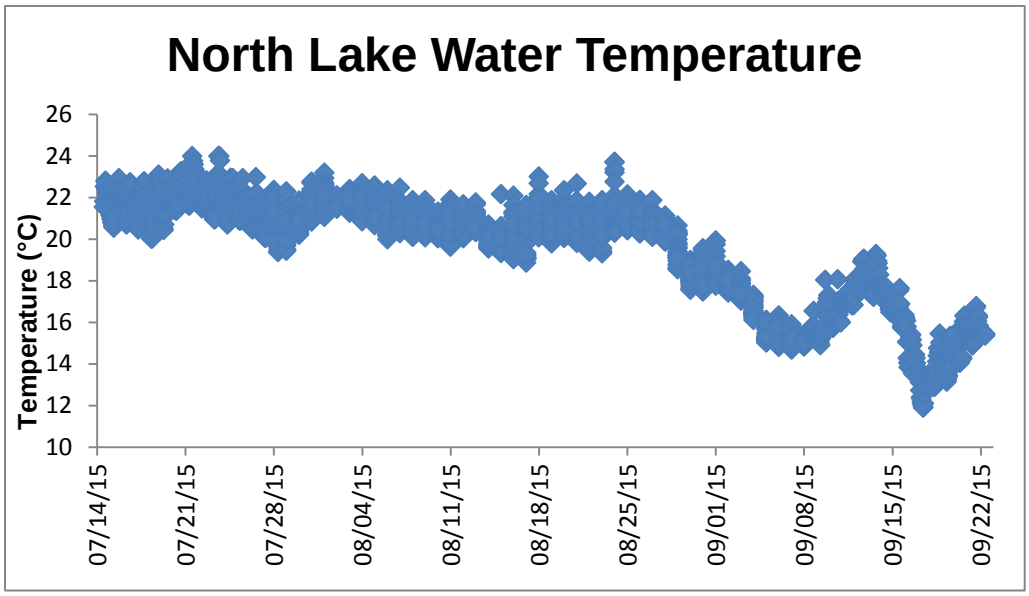


Figure 1-1. Temperature readings obtained at North Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

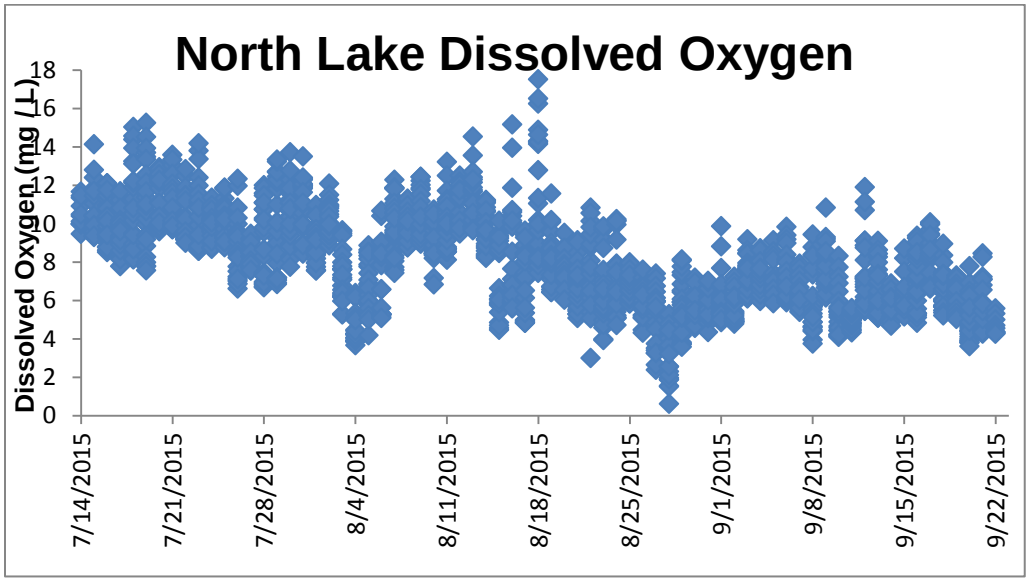


Figure 1-2. Dissolved oxygen readings obtained at North Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

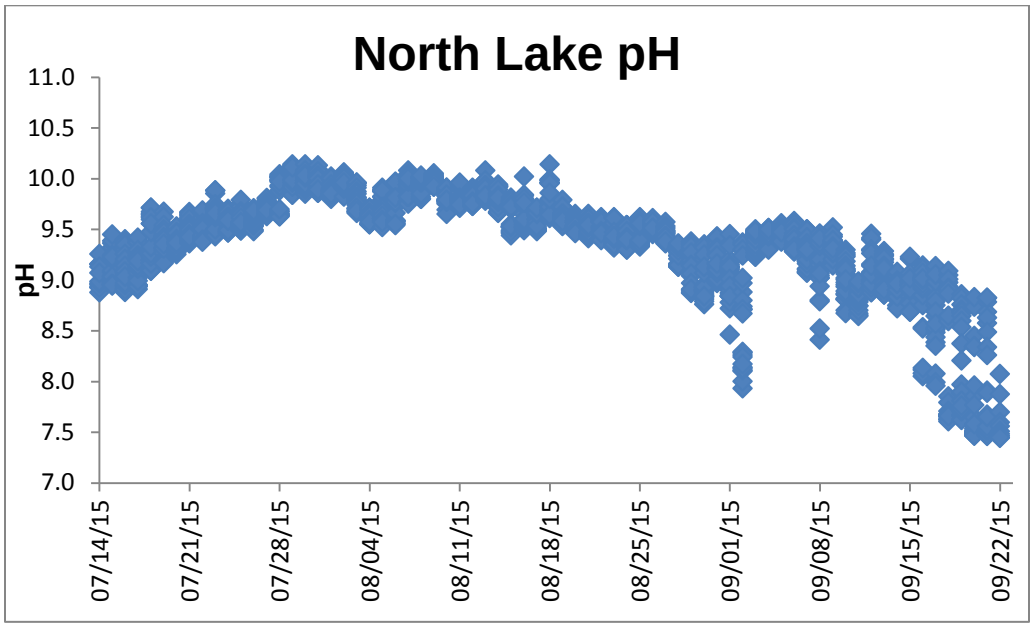


Figure 1-3. pH readings obtained at North Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

Table 1-1. 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. Asterisk (*) indicates composite NH_4^+ reading that reached the high limit detection on the HACH DR850 spectrophotometer used to obtain readings. NH_4^+ was higher than the reported value.

Date	NH_4^+ (mg/L)	Actual Temp (°C)	Actual pH	Temp Used (˚C)	pH Used	% NH3 Used mg/L	**Calculated UIA (mg/L)**
7/14/15	0.21	21.55	8.95	22.00	9.0	31.5%	0.066
7/21/15	0.50*	22.67	9.63	23.00	9.5	60.9%	0.305*
7/28/15	0.01	20.00	9.88	20.00	10.0	79.9%	0.008
8/4/15	0.16	21.85	9.63	22.00	9.5	59.2%	0.095
8/11/15	0.12	20.20	9.80	20.00	10.0	79.9%	0.096
8/18/15	0.18	20.32	9.72	20.00	9.5	55.7%	0.100
8/25/15	0.14	20.64	9.55	21.00	9.5	57.5%	0.081
9/1/15	0.22	18.41	8.72	18.00	9.0	25.5%	0.056
9/8/15	0.05	15.01	9.02	15.00	9.0	21.5%	0.011
9/15/15	0.25	16.73	8.98	17.00	9.0	24.1%	0.060
9/22/15	0.00	15.43	7.45	15.00	7.5	0.859%	0.000

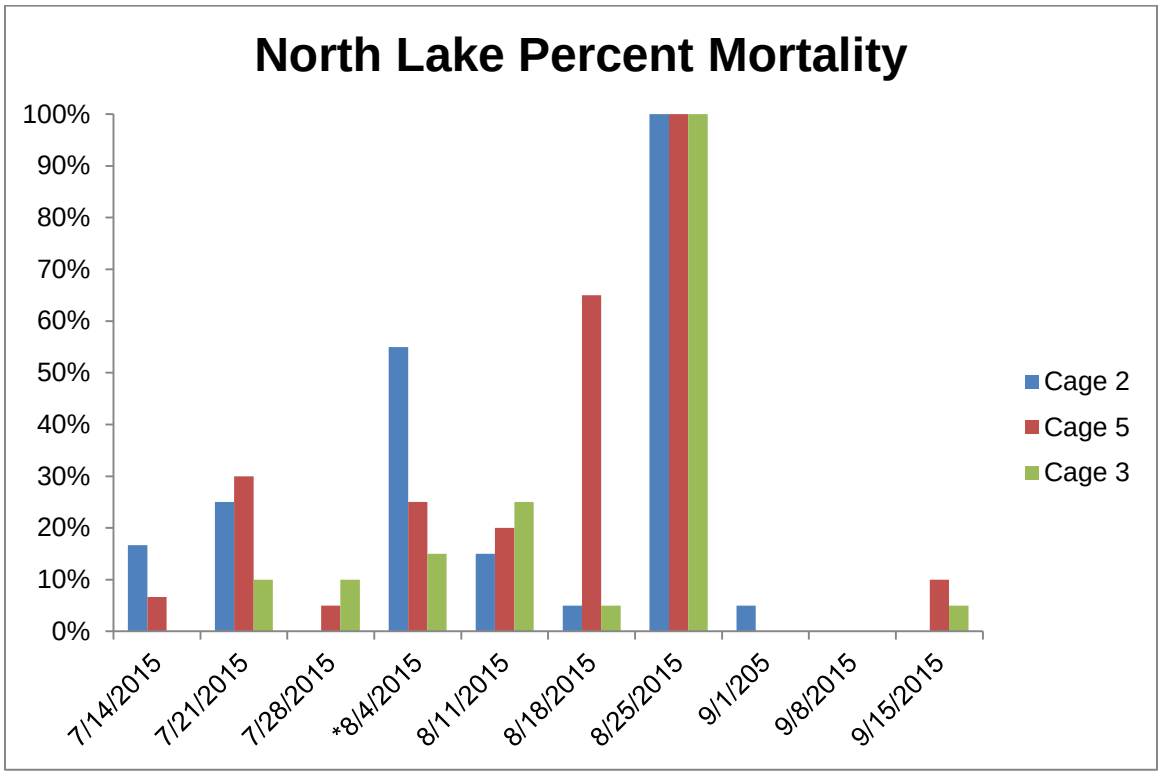


Figure 1-4. Percent mortality for all exposures 14July – 22September 2015 at the North Lake exposure site from net pens sampled at 7d intervals.
* On 4August, one net pen was not secured which invalidates its mortality data.

Appendix 2. Sucker Springs water quality and mortality data.

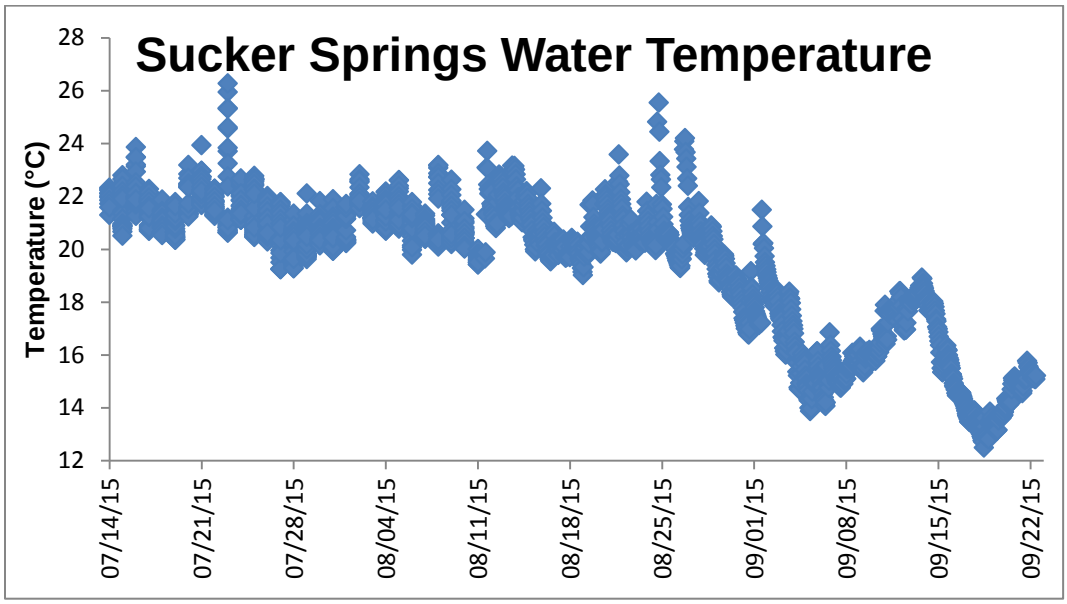


Figure 2 -1. Temperature readings obtained at Sucker Springs site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

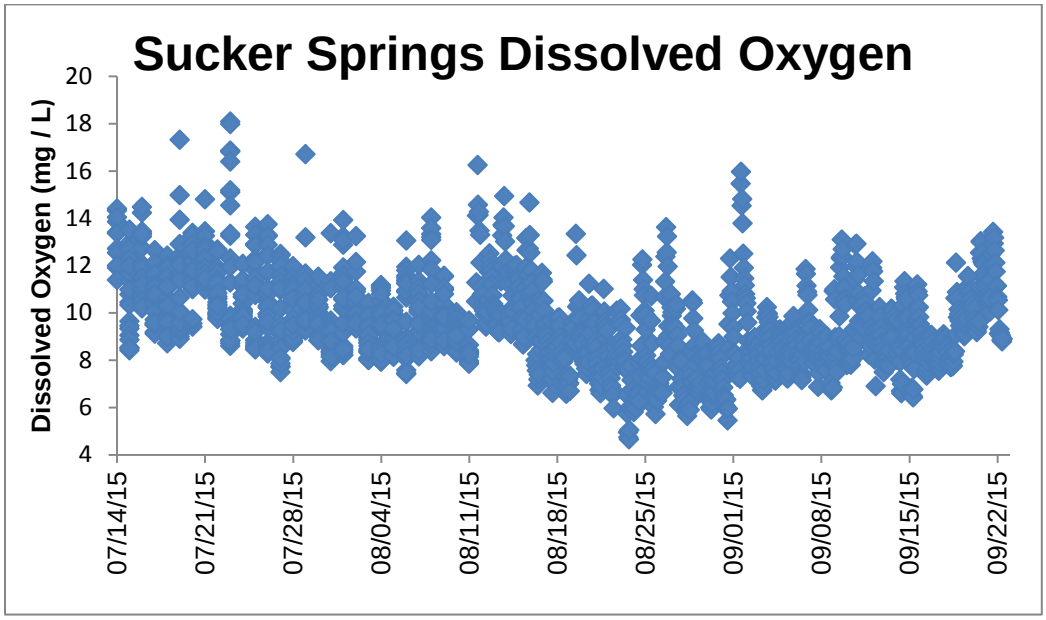


Figure 2-2. Dissolved oxygen readings obtained at Sucker Springs site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

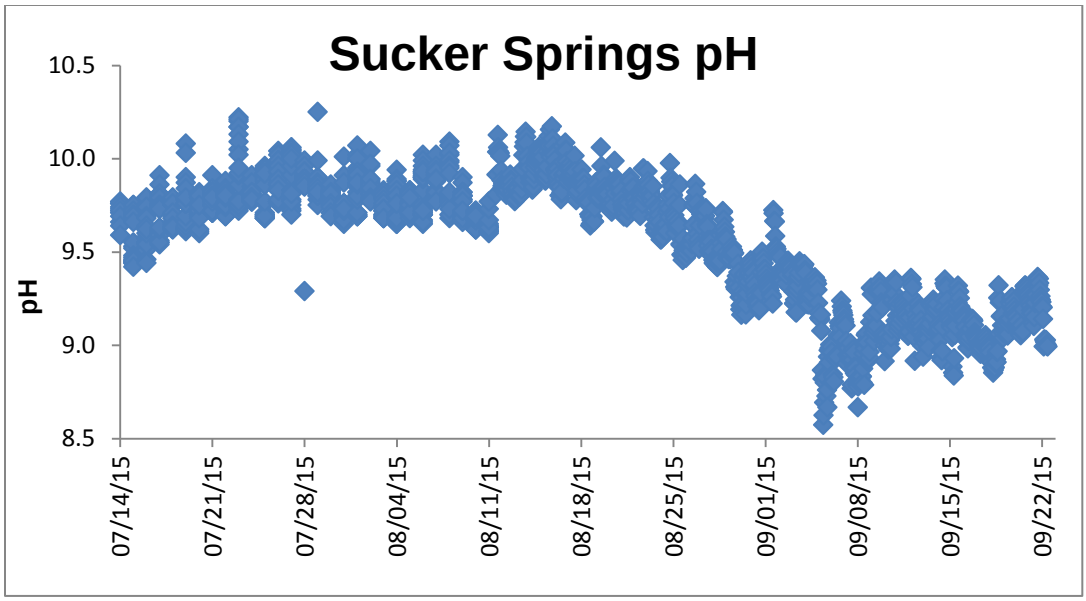


Figure 2-3. pH readings obtained at Sucker Springs site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

Table 2-1. Composite point measurements taken at the **Sucker 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)**
7/14/15	0.04	21.30	9.66	21.00	9.5	57.5%	0.023
7/21/15	0.07	22.08	9.79	22.00	10.0	82.1%	0.057
7/28/15	0.16	20.29	9.86	20.00	10.0	79.9%	0.128
8/4/15	0.24	21.27	9.86	21.00	10.0	81.0%	0.194
8/11/15	0.11	19.65	9.81	20.00	10.0	79.9%	0.088
8/18/15	0.03	20.16	9.76	20.00	10.0	79.9%	0.024
8/25/15	0.02	20.63	9.54	21.00	9.5	57.5%	0.012
9/1/15	0.18	17.22	9.22	17.00	9.0	24.1%	0.043
9/8/15	0.03	16.08	8.95	16.00	9.0	22.8%	0.007
9/15/15	0.07	15.95	9.19	16.00	9.0	22.8%	0.016
9/22/15	0.05	15.22	8.99	15.00	9.0	21.5%	0.011

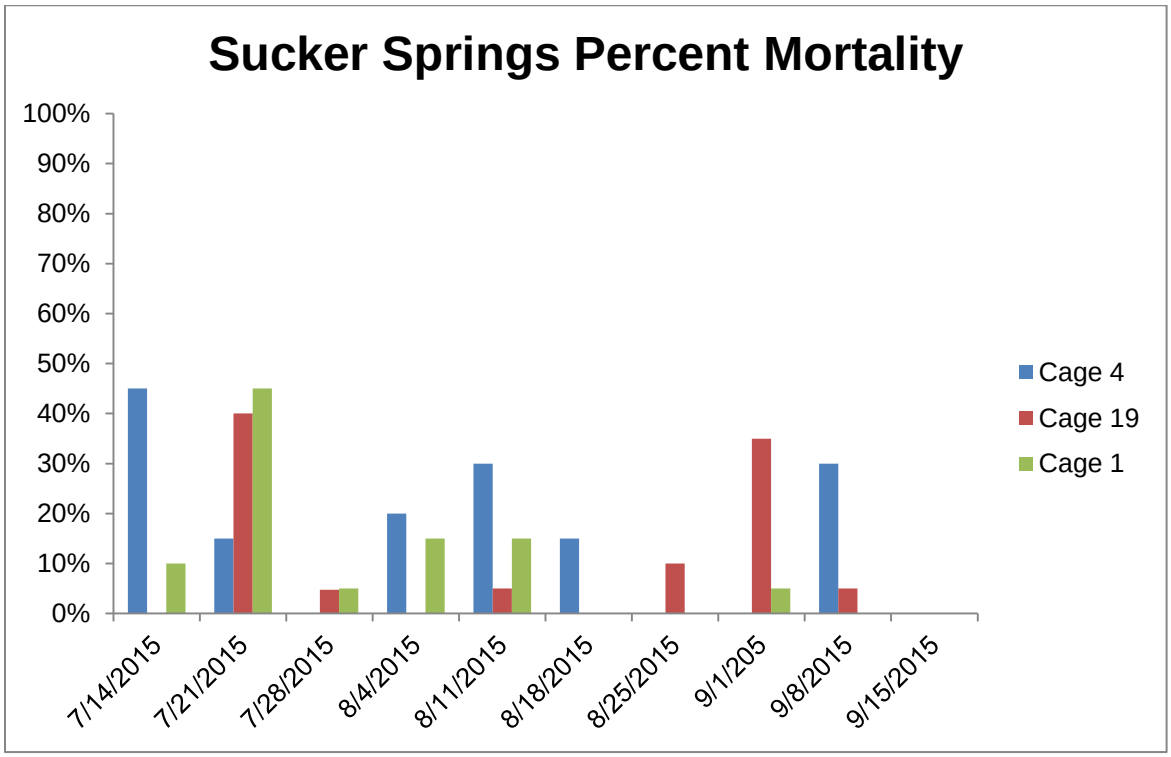


Figure 2-4.. Percent mortality for all exposures 14July – 22September 2015 at the Sucker Springs exposure site from net pens sampled at 7d intervals.

Appendix 3. Hanks Marsh water quality and mortality data.

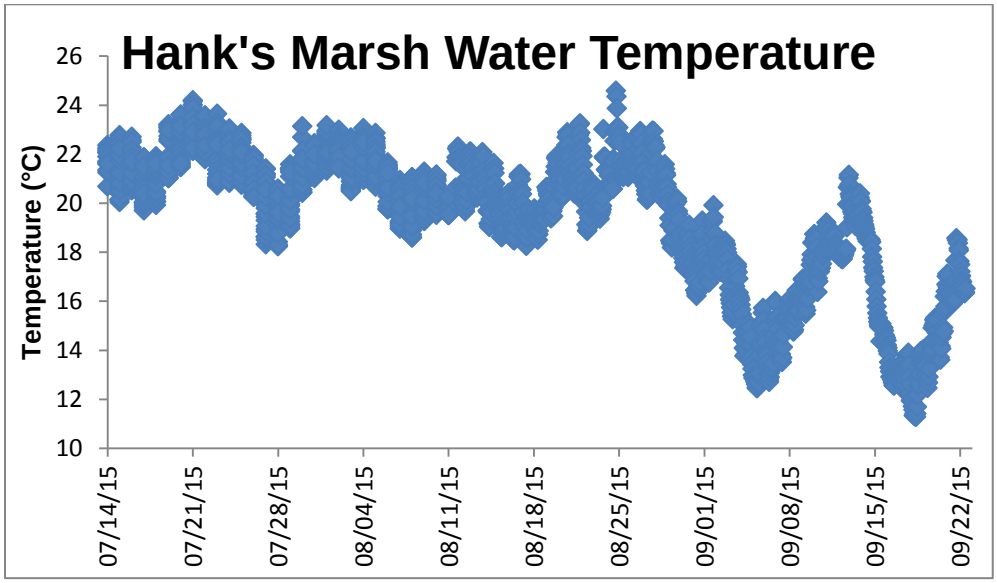


Figure 3-1.. Temperature readings obtained at Hank's March site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

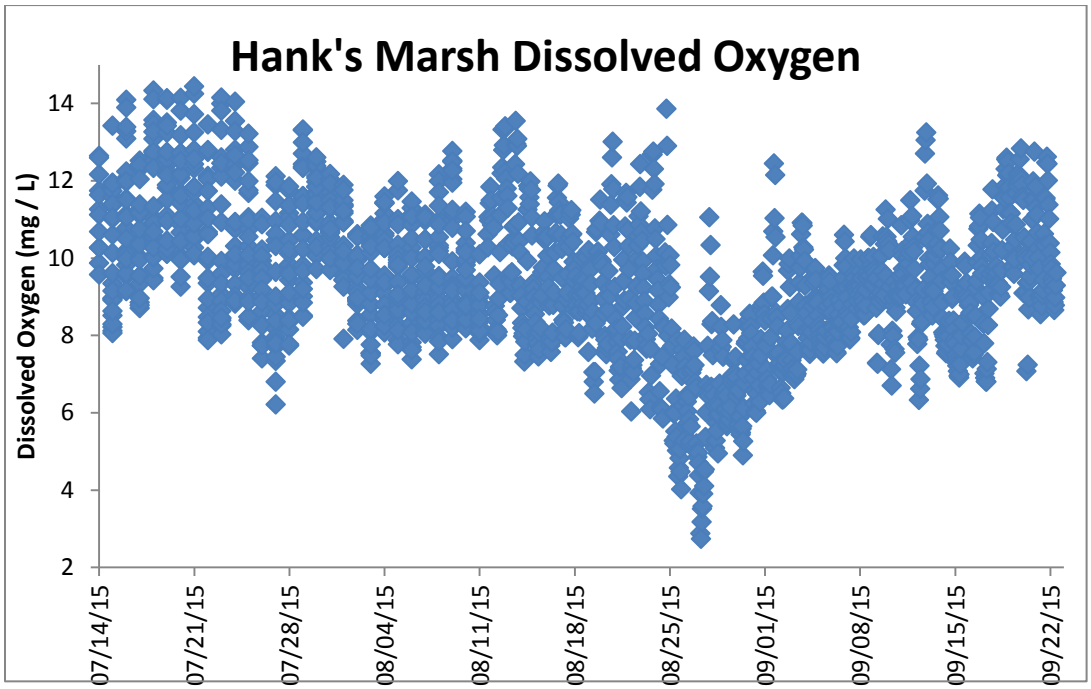


Figure 3-2. Dissolved oxygen readings obtained at Hank's Marsh site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

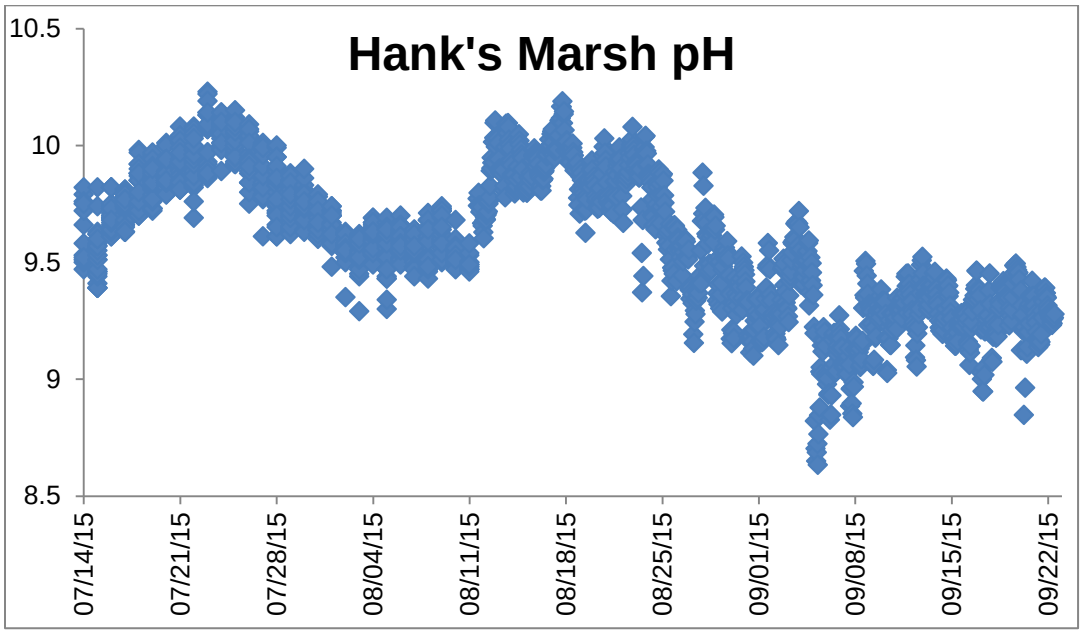


Figure 3-3. pH readings obtained at Hank's Marsh site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

Table 3-1. Composite point measurements taken at the **Hank's Marsh** 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)**
7/14/15	0.01	21.26	9.50	21.00	9.5	57.5%	0.006
7/21/15	0.02	22.24	9.81	22.00	10.0	82.1%	0.016
7/28/15	0.15	19.31	9.86	19.00	10.0	78.7%	0.118
8/4/15	0.04	22.32	9.69	22.00	9.5	59.2%	0.024
8/11/15	0.15	20.27	9.58	20.00	9.5	55.7%	0.084
8/18/15	0.13	19.21	9.96	19.00	10.0	78.7%	0.102
8/25/15	0.17	21.27	9.51	21.00	9.5	57.5%	0.098
9/1/15	0.13	18.58	9.48	19.00	9.5	53.9%	0.070
9/8/15	0.03	15.55	9.15	16.00	9.0	22.8%	0.007
9/15/15	0.07	14.95	9.24	15.00	9.5	46.4%	0.032
9/22/15	0.00	16.52	9.28	17.00	9.5	50.2%	0.000

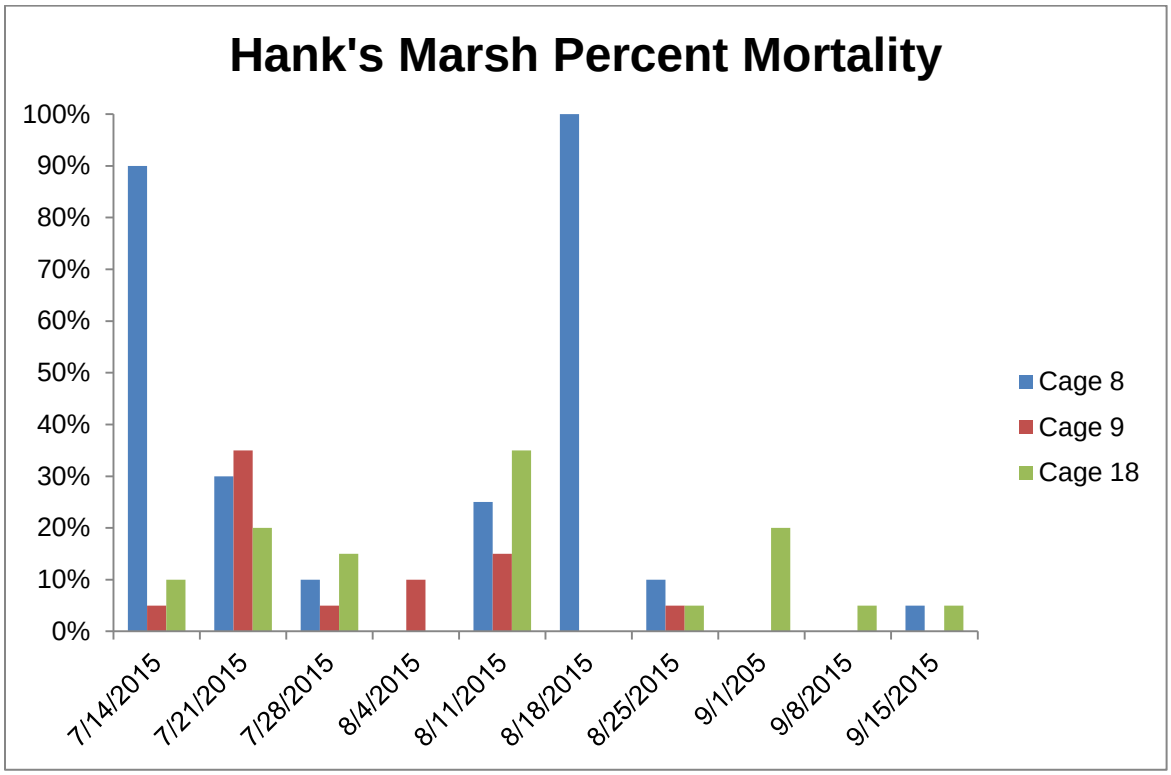


Figure 3-4. Percent mortality for all exposures 14July – 22September 2015 at the Hank's Marsh exposure site from net pens sampled at 7d intervals.

Appendix 4. South Lake water quality and mortality data.

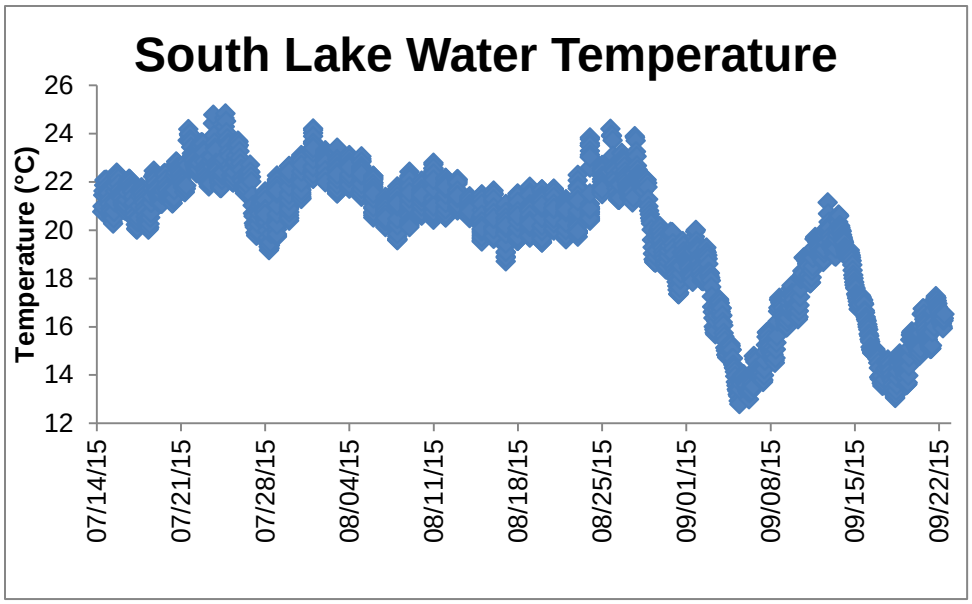


Figure 4-1. Temperature readings obtained at South Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

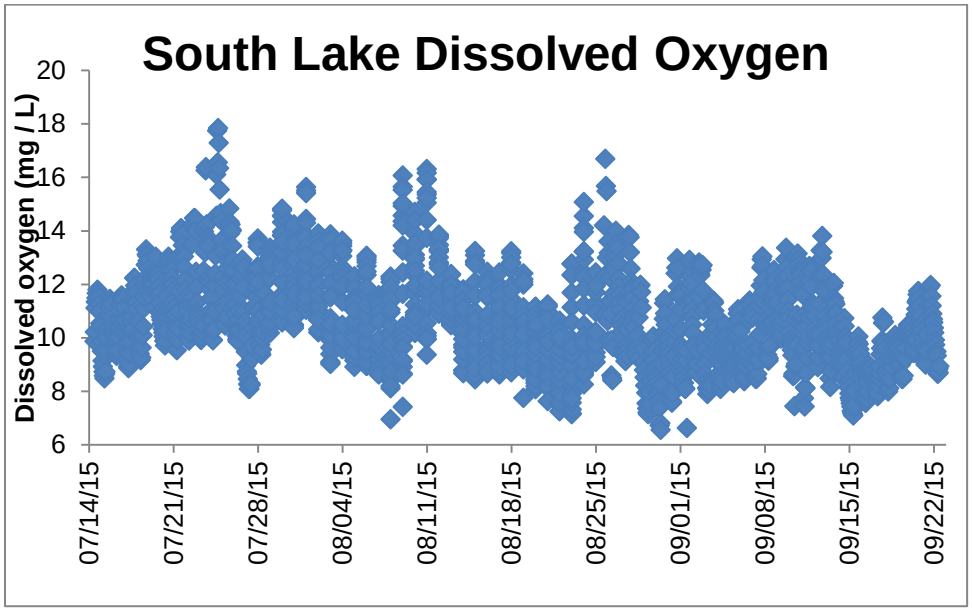


Figure 4-2. Dissolved oxygen readings obtained at South Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

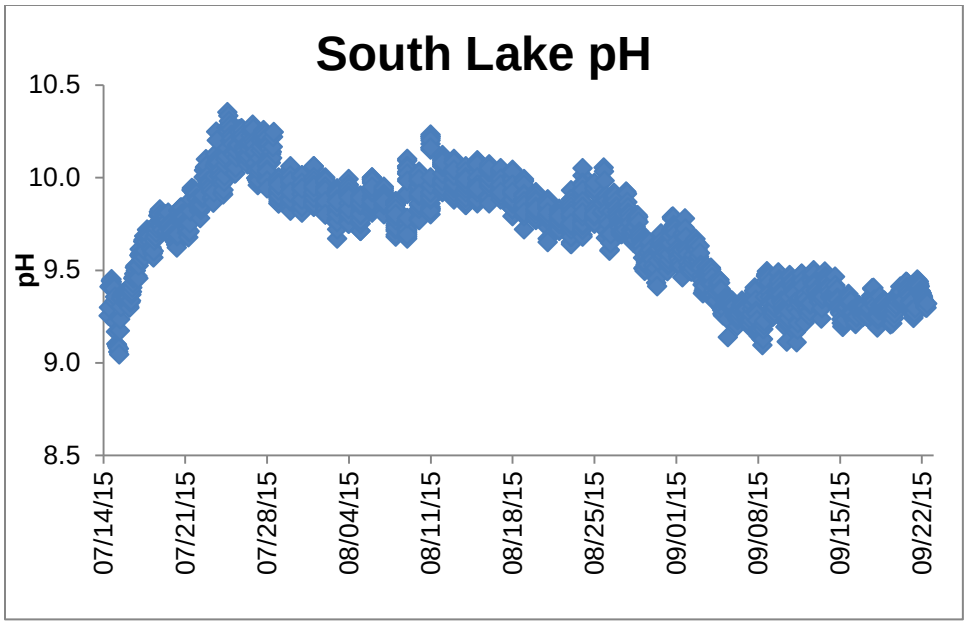


Figure 4-3. pH readings obtained at South Lake site Upper Klamath Lake 14July – 22September 2015. Water quality measurements obtained hourly using continuous monitors deployed 1.5m below the water surface from a buoy located in the center of the net pen array. Dates displayed represent study start date (14July), study end date (22September) and, each 7d exposure sample dates.

Table 4-1. Composite point measurements taken at the **South 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)**
7/14/15	0.00	20.98	9.30	21.00	9.5	57.5%	0.000
7/21/15	0.00	22.26	9.82	22.00	10.0	82.1%	0.000
7/28/15	0.00	21.24	10.00	21.00	10.0	81.0%	0.000
8/4/15	0.14	22.90	9.99	23.00	10.0	83.2%	0.116
8/11/15	0.04	22.22	10.00	22.00	10.0	82.1%	0.033
8/18/15	0.08	20.95	9.98	21.00	10.0	81.0%	0.065
8/25/15	0.12	21.69	9.97	22.00	10.0	82.1%	0.099
9/1/15	0.13	19.36	9.70	19.00	9.5	53.9%	0.070
9/8/15	0.11	18.72	9.22	19.00	9.0	27.0%	0.030
9/15/15	0.09	17.11	9.33	17.00	9.5	50.2%	0.045
9/22/15	0.18	16.51	9.32	17.00	9.5	50.2%	0.090

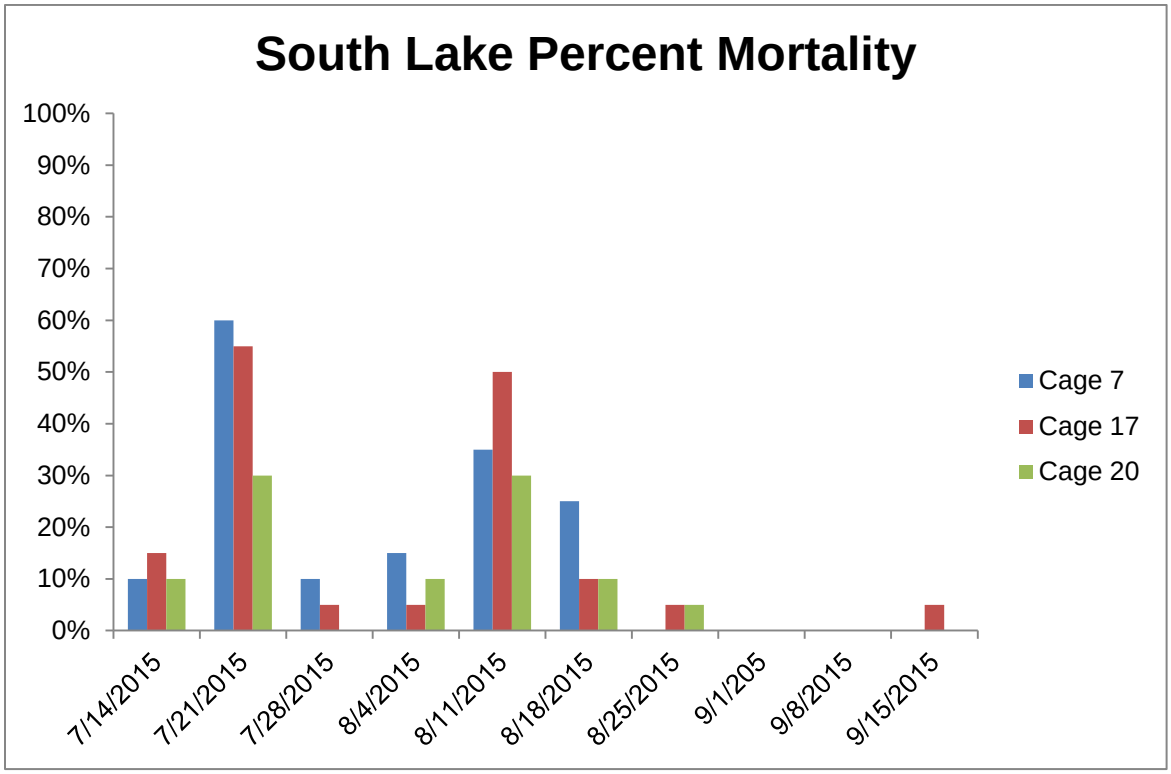


Figure 4-4. Percent mortality for all exposures 14July – 22September 2015 at the South Lake exposure site from net pens sampled at 7d intervals.

Appendix 5 Net pen mortality and water quality. SL=South Lake, HM=Hanks Marsh, SS= Sucker Springs, and NL= North Lake.

	IN			OUT										
exposure	Date	SiteID	Net penID	Date	Survivors Count	Dead Count	Temp Max	Temp Mean	hrs pH10+	mean pH	hrs Hypox1	hrs Hypox1.5	UIA in	UIA out
1	7/14/2015	SL	7	7/21/2015	18	2	24.18	21.49	0	9.58	0	0	0	0
1	7/14/2015	SL	17	7/21/2015	17	3	24.18	21.49	0	9.58	0	0	0	0
1	7/14/2015	SL	20	7/21/2015	18	2	24.18	21.49	0	9.58	0	0	0	0
1	7/14/2015	HM	8	7/21/2015	2	18	23.72	21.65	2	9.75	0	0	0.006	0.016
1	7/14/2015	HM	9	7/21/2015	19	1	23.72	21.65	2	9.75	0	0	0.006	0.016
1	7/14/2015	HM	19	7/21/2015	18	2	23.72	21.65	2	9.75	0	0	0.006	0.016
1	7/14/2015	SS	4	7/21/2015	11	9	23.93	21.59	2	9.69	0	0	0.023	0.057
1	7/14/2015	SS	19	7/21/2015	20	0	23.93	21.59	2	9.69	0	0	0.023	0.057
1	7/14/2015	SS	1	7/21/2015	18	2	23.93	21.59	2	9.69	0	0	0.023	0.057
1	7/14/2015	NL	2	7/21/2015	25	5	23.99	21.68	0	9.27	0	0	0.066	0.305
1	7/14/2015	NL	5	7/21/2015	28	2	23.99	21.68	0	9.27	0	0	0.066	0.305
2	7/21/2015	SL	7	7/28/2015	8	12	24.83	22.17	122	10.08	0	0	0	0
2	7/21/2015	SL	17	7/28/2015	9	11	24.83	22.17	122	10.08	0	0	0	0
2	7/21/2015	SL	20	7/28/2015	14	6	24.83	22.17	122	10.08	0	0	0	0
2	7/21/2015	HM	8	7/28/2015	14	6	24.2	21.39	70	9.96	0	0	0.016	0.118
2	7/21/2015	HM	9	7/28/2015	13	7	24.2	21.39	70	9.96	0	0	0.016	0.118
2	7/21/2015	HM	18	7/28/2015	16	4	24.2	21.39	70	9.96	0	0	0.016	0.118
2	7/21/2015	SS	4	7/28/2015	17	3	26.27	21.43	23	9.85	0	0	0.057	0.128
2	7/21/2015	SS	19	7/28/2015	12	8	26.27	21.43	23	9.85	0	0	0.057	0.128
2	7/21/2015	SS	1	7/28/2015	11	9	26.27	21.43	23	9.85	0	0	0.057	0.128
2	7/21/2015	NL	2	7/28/2015	15	5	24	21.58	0	9.61	0	0	0.305	0.008
2	7/21/2015	NL	5	7/28/2015	14	6	24	21.58	0	9.61	0	0	0.305	0.008
2	7/21/2015	NL	3	7/28/2015	18	2	24	21.58	0	9.61	0	0	0.305	0.008
3	7/28/2015	SL	20	8/4/2015	18	2	24.21	22.1	25	9.9	0	0	0	0.116
3	7/28/2015	SL	17	8/4/2015	19	1	24.21	22.1	25	9.9	0	0	0	0.116
3	7/28/2015	SL	7	8/4/2015	20	0	24.21	22.1	25	9.9	0	0	0	0.116
3	7/28/2015	HM	8	8/4/2015	18	2	23.18	21.41	0	9.66	0	0	0.118	0.024
3	7/28/2015	HM	9	8/4/2015	19	1	23.18	21.41	0	9.66	0	0	0.118	0.024
3	7/28/2015	HM	18	8/4/2015	17	3	23.18	21.41	0	9.66	0	0	0.118	0.024
3	7/28/2015	SS	19	8/4/2015	19	1	22.83	20.97	7	9.8	0	0	0.128	0.194
3	7/28/2015	SS	4	8/4/2015	20	0	22.83	20.97	7	9.8	0	0	0.128	0.194
3	7/28/2015	SS	1	8/4/2015	19	1	22.83	20.97	7	9.8	0	0	0.128	0.194
3	7/28/2015	NL	5	8/4/2015	19	1	23.19	21.5	51	9.91	0	0	0.008	0.095
3	7/28/2015	NL	2	8/4/2015	20	0	23.19	21.5	51	9.91	0	0	0.008	0.095
3	7/28/2015	NL	3	8/4/2015	18	2	23.19	21.5	51	9.91	0	0	0.008	0.095
4	8/4/2015	SL	7	8/11/2015	18	2	23.1	21.3	16	9.87	0	0	0.116	0.033
4	8/4/2015	SL	20	8/11/2015	18	2	23.1	21.3	16	9.87	0	0	0.116	0.033
4	8/4/2015	SL	17	8/11/2015	18	2	23.1	21.3	16	9.87	0	0	0.116	0.033
4	8/4/2015	HM	9	8/11/2015	18	2	23.06	20.54	0	9.57	0	0	0.024	0.084
4	8/4/2015	HM	18	8/11/2015	20	0	23.06	20.54	0	9.57	0	0	0.024	0.084
4	8/4/2015	HM	8	8/11/2015	20	0	23.06	20.54	0	9.57	0	0	0.024	0.084
4	8/4/2015	SS	19	8/11/2015	20	0	23.19	20.92	7	9.78	0	0	0.194	0.088
4	8/4/2015	SS	1	8/11/2015	17	3	23.19	20.92	7	9.78	0	0	0.194	0.088
4	8/4/2015	SS	4	8/11/2015	16	4	23.19	20.92	7	9.78	0	0	0.194	0.088
4	8/4/2015	NL	5	8/11/2015	15	5	22.69	20.96	21	9.83	0	0	0.095	0.096
4	8/4/2015	NL	2	8/11/2015	9	11	22.69	20.96	21	9.83	0	0	0.095	0.096
4	8/4/2015	NL	3	8/11/2015	17	3	22.69	20.96	21	9.83	0	0	0.095	0.096
5	8/11/2015	SL	7	8/18/2015	13	7	22.79	20.77	56	9.97	0	0	0.033	0.065
5	8/11/2015	SL	20	8/18/2015	10	10	22.79	20.77	56	9.97	0	0	0.033	0.065
5	8/11/2015	SL	17	8/18/2015	14	6	22.79	20.77	56	9.97	0	0	0.033	0.065
5	8/11/2015	HM	9	8/18/2015	17	3	22.3	20.14	47	9.92	0	0	0.084	0.102
5	8/11/2015	HM	18	8/18/2015	13	7	22.3	20.14	47	9.92	0	0	0.084	0.102
5	8/11/2015	HM	8	8/18/2015	15	5	22.3	20.14	47	9.92	0	0	0.084	0.102
5	8/11/2015	SS	19	8/18/2015	19	1	23.72	21.04	46	9.92	0	0	0.088	0.024
5	8/11/2015	SS	4	8/18/2015	14	6	23.72	21.04	46	9.92	0	0	0.088	0.024
5	8/11/2015	SS	1	8/18/2015	17	3	23.72	21.04	46	9.92	0	0	0.088	0.024
5	8/11/2015	NL	5	8/18/2015	17	3	22.16	20.47	3	9.74	0	0	0.096	0.1
5	8/11/2015	NL	2	8/18/2015	16	4	22.16	20.47	3	9.74	0	0	0.096	0.1
5	8/11/2015	NL	3	8/18/2015	17	3	22.16	20.47	3	9.74	0	0	0.096	0.1

Appendix 5 Net pen mortality and water quality. SL=South Lake, HM=Hanks Marsh, SS= Sucker Springs, and NL= North Lake.

No.	IN			OUT										
exposure	Date	SiteID	Netpen	Date	Survivors.Cc	Dead.Cc	Temp.Ma	Temp.Mea	hrs pH1	mean pH	hrs Hypc	hrs Hypox	UIA in	UIA out
6	8/18/2015	SL	17	8/25/2015	15	5	23.84	20.95	7	9.82	0	0	0.065	0.099
6	8/18/2015	SL	20	8/25/2015	18	2	23.84	20.95	7	9.82	0	0	0.065	0.099
6	8/18/2015	SL	7	8/25/2015	18	2	23.84	20.95	7	9.82	0	0	0.065	0.099
6	8/18/2015	HM	9	8/25/2015	20	0	24.59	20.97	11	9.82	0	0	0.102	0.098
6	8/18/2015	HM	18	8/25/2015	20	0	24.59	20.97	11	9.82	0	0	0.102	0.098
6	8/18/2015	HM	8	8/25/2015	0	20	24.59	20.97	11	9.82	0	0	0.102	0.098
6	8/18/2015	SS	1	8/25/2015	20	0	25.55	20.74	1	9.76	0	0	0.024	0.012
6	8/18/2015	SS	4	8/25/2015	21	-1	25.55	20.74	1	9.76	0	0	0.024	0.012
6	8/18/2015	SS	19	8/25/2015	20	0	25.55	20.71	1	9.76	0	0	0.024	0.012
6	8/18/2015	NL	5	8/25/2015	7	13	23.71	20.84	0	9.52	0	0	0.1	0.081
6	8/18/2015	NL	2	8/25/2015	19	1	23.71	20.84	0	9.52	0	0	0.1	0.081
6	8/18/2015	NL	3	8/25/2015	19	1	23.71	20.84	0	9.52	0	0	0.1	0.081
7	8/25/2015	SL	7	9/1/2015	20	0	24.2	20.47	2	9.68	0	0	0.099	0.07
7	8/25/2015	SL	20	9/1/2015	19	1	24.2	20.47	2	9.68	0	0	0.099	0.07
7	8/25/2015	SL	17	9/1/2015	19	1	24.2	20.47	2	9.68	0	0	0.099	0.07
7	8/25/2015	HM	9	9/1/2015	19	1	22.96	19.67	0	9.42	0	0	0.098	0.07
7	8/25/2015	HM	18	9/1/2015	19	1	22.96	19.67	0	9.42	0	0	0.098	0.07
7	8/25/2015	HM	8	9/1/2015	18	2	22.96	19.67	0	9.42	0	0	0.098	0.07
7	8/25/2015	SS	1	9/1/2015	20	0	24.2	19.43	0	9.46	0	0	0.012	0.043
7	8/25/2015	SS	19	9/1/2015	18	2	24.2	19.43	0	9.46	0	0	0.012	0.043
7	8/25/2015	SS	4	9/1/2015	20	0	24.2	19.43	0	9.46	0	0	0.012	0.043
7	8/25/2015	NL	5	9/1/2015	0	20	22.14	19.69	0	9.24	1	2	0.081	0.056
7	8/25/2015	NL	3	9/1/2015	0	20	22.14	19.69	0	9.24	1	2	0.081	0.056
7	8/25/2015	NL	2	9/1/2015	0	20	22.14	19.69	0	9.24	1	2	0.081	0.056
8	9/1/2015	SL	20	9/8/2015	20	0	20	15.58	0	9.37	0	0	0.07	0.03
8	9/1/2015	SL	7	9/8/2015	20	0	20	15.58	0	9.37	0	0	0.07	0.03
8	9/1/2015	SL	17	9/8/2015	20	0	20	15.58	0	9.37	0	0	0.07	0.03
8	9/1/2015	HM	8	9/8/2015	20	0	19.92	15.38	0	9.17	0	0	0.07	0.007
8	9/1/2015	HM	18	9/8/2015	16	4	19.92	15.38	0	9.17	0	0	0.07	0.007
8	9/1/2015	HM	9	9/8/2015	20	0	19.92	15.38	0	9.17	0	0	0.07	0.007
8	9/1/2015	SS	1	9/8/2015	19	1	20.23	16.14	0	9.09	0	0	0.043	0.007
8	9/1/2015	SS	19	9/8/2015	13	7	20.23	16.14	0	9.09	0	0	0.043	0.007
8	9/1/2015	SS	4	9/8/2015	20	0	20.23	16.14	0	9.09	0	0	0.043	0.007
8	9/1/2015	NL	5	9/8/2015	20	0	19.93	16.42	0	9.16	0	0	0.056	0.011
8	9/1/2015	NL	2	9/8/2015	19	1	19.93	16.42	0	9.16	0	0	0.056	0.011
8	9/1/2015	NL	3	9/8/2015	20	0	19.93	16.42	0	9.16	0	0	0.056	0.011
9	9/8/2015	SL	20	9/15/2015	20	0	21.14	18.32	0	9.29	0	0	0.03	0.045
9	9/8/2015	SL	7	9/15/2015	20	0	21.14	18.32	0	9.29	0	0	0.03	0.045
9	9/8/2015	SL	17	9/15/2015	20	0	21.14	18.32	0	9.29	0	0	0.03	0.045
9	9/8/2015	HM	8	9/15/2015	20	0	21.16	17.92	0	9.24	0	0	0.007	0.032
9	9/8/2015	HM	9	9/15/2015	20	0	21.16	17.92	0	9.24	0	0	0.007	0.032
9	9/8/2015	HM	18	9/15/2015	19	1	21.16	17.92	0	9.24	0	0	0.007	0.032
9	9/8/2015	SS	1	9/15/2015	20	0	18.92	17.05	0	9.13	0	0	0.007	0.016
9	9/8/2015	SS	4	9/15/2015	14	6	18.92	17.05	0	9.13	0	0	0.007	0.016
9	9/8/2015	SS	19	9/15/2015	19	1	18.92	17.05	0	9.13	0	0	0.007	0.016
9	9/8/2015	NL	3	9/15/2015	20	0	19.28	16.97	0	8.99	0	0	0.011	0.06
9	9/8/2015	NL	5	9/15/2015	20	0	19.28	16.97	0	8.99	0	0	0.011	0.06
9	9/8/2015	NL	2	9/15/2015	20	0	19.28	16.97	0	8.99	0	0	0.011	0.06
10	9/15/2015	SL	7	9/22/2015	20	0	17.26	15.14	0	9.26	0	0	0.045	0.09
10	9/15/2015	SL	17	9/22/2015	19	1	17.26	15.14	0	9.26	0	0	0.045	0.09
10	9/15/2015	SL	20	9/22/2015	20	0	17.26	15.14	0	9.26	0	0	0.045	0.09
10	9/15/2015	HM	18	9/22/2015	19	1	18.58	14.27	0	9.22	0	0	0.032	0
10	9/15/2015	HM	9	9/22/2015	20	0	18.58	14.27	0	9.22	0	0	0.032	0
10	9/15/2015	HM	8	9/22/2015	19	1	18.58	14.27	0	9.22	0	0	0.032	0
10	9/15/2015	SS	4	9/22/2015	20	0	16.21	14.24	0	9.11	0	0	0.016	0.011
10	9/15/2015	SS	19	9/22/2015	20	0	16.21	14.24	0	9.11	0	0	0.016	0.011
10	9/15/2015	SS	1	9/22/2015	21	0	16.21	14.24	0	9.11	0	0	0.016	0.011
10	9/15/2015	NL	2	9/22/2015	20	0	16.77	14.5	0	8.21	0	0	0.06	0
10	9/15/2015	NL	5	9/22/2015	18	2	16.77	14.5	0	8.21	0	0	0.06	0
10	9/15/2015	NL	3	9/22/2015	19	1	16.77	14.5	0	8.21	0	0	0.06	0