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California Nevada Fish Health Center
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Juvenile Lost River Sucker survival and energetics in Upper Klamath Lake mesocosm cages: July – December 2011.

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Summary: A captive Lost River sucker (*Deltistes luxatus*) fry population was reared from eggs and later held in 3 m^3 cages at three locations (Goose bay or "delta", north and south Upper Klamath Lake, Oregon) from 20July to 09December 2011. Fry held in delta cages had low survival (≤5% over 21 days) that is likely associated with insufficient feed within mesocosm cages, large diurnal pH fluctuation (~ 2 pH units per day), and hypoxic conditions in late August. Survival in north lake cages was ≥ 48% during July and August and increased in September. Water quality in the lake during the summer was characterized by elevated temperature (mean 22ºC), high pH (mean 9.47 in early August), and normoxic to hyperoxic conditions. No significant infectious disease, lipid peroxidation response in the liver, impaired gill Na-K-ATPase activity, or detection of microcystin LR toxicity was observed in the sentinel fry. There was no definitive diagnosis for source(s) of mortality at either site. Growth and triglyceride content steadily increased in north lake cage fry. By 04October, mean standard length was 69.6 and 73.6 mm in north lake cage groups held for the full 76 d exposure. A condition factor (KSL) of ≥ 1.4 was consistently observed in healthy robust fry held in the north lake cages. Gut contents of fish taken from the cages at the Northern Lake site were almost entirely comprised of midge, predominantly larvae but with an appreciable amount of pupa and adults. In contrast, suckers within the delta cages tended to have consumed a high number of Cladocerans (primarily *Chydorus*) in addition to the midge component. Juveniles held from 04October to 09December had high survival and did not lose weight or body triglyceride content. This initial effort has demonstrated the utility of using captive sucker fry in cages to determine patterns of survival and growth in Upper Klamath Lake.

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Introduction

In 1988, the US Fish and Wildlife Service listed the Upper Klamath basin Lost River Sucker (*Deltistes luxatus*) as an endangered species. 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). Poor water quality has been linked to the adult mortalities (Perkin et al. 2000) and may negatively influence juvenile survival. 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).

Both bacterial and parasitic pathogens have been consistently observed in asymptomatic (apparently normal) juvenile suckers collected in Upper Klamath Lake (Foott and Stone 2005, Foott et al. 2007, Foott et al. 2010). Facultative pathogenic bacteria (*Aeromonas caviae, A. punctata*, and *A. hydrophilia*) have been isolated from the skin and gills of asymptomatic juvenile Lost R. (LRS) suckers in Upper Klamath Lake during August of 2006 (pers. comm. C. Ottinger, USGS Leetown). Synergistic effects of elevated water temperature (rapid microbial multiplication), presence of endemic bacteria and parasites, and stressful water quality (impaired immune functions and insufficient fat reserves for overwinter survival) is a potential avenue of juvenile sucker mortality in Upper Klamath Lake.

Low recruitment success has been observed in skewed age data (Scoppettone and Vinyard 1991). Similarly Janney et al. (2008) report homogeneous size structures in recent years suggestive of poor recruitment. After their first fall, however, young suckers have been rarely observed despite widespread sampling of Upper Klamath Lake with a variety of gears (Buettner and Scoppettone 1990; Burdick et al. 2008; Reiser et al. 2001; Simon et al. 2009). Extremely low catches of age-1 or older adolescent suckers suggests that survival rates of age-0 fish are very poor and that recruitment failure may occur during the first year of life. Burdick et al. (2008) stated that 0+ juveniles catch declines significantly between August and October each year. Several hypotheses concerning the causes of poor age-0 to age-1 survivorship have been suggested including emigration out of the lake (Harris and Markle 1991; Gutermuth et al. 2000) and poor summer water quality conditions (Martin and Saiki 1999).

We focused this pilot study on LRS fry survival and energetics during the late July through October time frame. The study design used captive reared progeny, of Upper Klamath Lake Lost R. sucker adults, that were held in replicate mesocosm cages. Cultured fry provided experimental fish of known age, health, similar size, and energy content. The mesocosm cage represented a link between observational field studies in the lake and controlled laboratory experiments (Sala et al. 2000).

Specific objectives were:

1. Develop techniques to culture LRS fry and rear sentinel fry in mesocosm cages.
2. Determine temporal survival of sentinel fry in the delta and north lake.
3. Monitor parameters of growth, energy reserves, ion regulation, oxidative stress, and perform histological examination of select tissues for abnormalities and pathogen infection in sentinel fry.
4. Monitor temperature, dissolved oxygen, and pH of water at the two sites in order to observe relationships to survival or growth.
5. Document invertebrate composition of both the fry's upper digestive tract (diet) and surrounding environment.

Methods:

Fish rearing – On 21April 2011, gametes were obtained from 4 male and 4 female Lost River suckers captured by USGS at Sucker Springs, Upper Klamath Lake. These fish were released immediately after a small portion of their gametes (~7g wet weight of eggs and 2 mL of semen) were collected. We estimate that approximately 7700 eggs were collected (275 eggs / g x 7g samples x 4 females, pers. comm. S. Vanderkooi, USGS). This egg collection is a small percentage of the 50,000 – 100,000 eggs per female reported for 500 – 750 mm FL female LRS (Buettner and Scoppettone 1990). Once the gametes were all combined, the fertilized eggs were covered with an extender solution (NaCl 5.52g, Glycine 3.75g, Tris 2.42g in 1L water at ambient lake temperature) for 15 minutes. After washing with lake water the gametes were transported to the CA-NV Fish Health Center. Virological assay of ovarian and seminal fluid did not detect virus (USFWS and AFS-FHS 2007). Eggs were incubated in upwelling cylinders (5in PVC with 250µm mesh screen bottom) at 9.5 - 11ºC until larvae were actively swimming and observed feeding. Prior to hatch, it was necessary to treat with 1000 ppm formalin for 20min and pick fungus eggs. Larvae were subsequently reared in 2.8L baskets (6.5" x 5" x 5.25" frames covered by a 500µm mesh bag) suspended within aquaria. Rotifers (*Brachionus plicatilis*) were fed in a solution of Nanno Greenwater Instant Algae (Reed Mariculture®) (Nannochloropsis sp.) to provide visual contrast for the larvae. Newly hatched artemia nauplii were introduced at 20 days post-hatch (dph) and a marine larvae diet (Otohime Marine Weaning Diets, Marubeni Nisshin Feed Co., Ltd, Japan, purchased from Reed Mariculture Inc, Campbell CA) started at

28dph. Pellet size ranged from 250µm - 1800µm over the experiment and 3 feedings were done per day (pellet at estimated 5-6% bodyweight /d). There was an overlap of approximately 4 days for each new diet with the previous diet type. We estimate that the population experienced a 35% loss from egg to 27mm fry and that there was 5000 fry on 20July. No virus was detected in a 60 fry sample performed in July prior to the first movement to Upper Klamath Lake. Fry were transported to the lake within 0.34 cu ft cages held in tanks containing water adjusted to pH 9.0 at ambient lake temperature. Our intent was to provide some acclimation to lake conditions during the 3 h transport to the lake.

Exposures - Five exposures were conducted between 20July and 09December 2011 at 3 sites (Table 1, Figure1). The sites were 1) within Goose Bay referred to as "**Delta",** 2) northern Upper Klamath Lake, and 3) southern Upper Klamath Lake. The southern cage was loaded with 36 juveniles on 04October from cages L7 and L8. Cage groups were identified as either short – term (13 – 21d exposures) or long-term cages (20July – 04Oct at Northern lake site). Mesocosm cages extended the full depth of the water column. On 20July, depth at the northern lake site was approximately 2.8 m and 2.0 m at the delta site. On 04October, the northern lake site was approximately 2.1 m deep. The southern cage site was approximately 1.5 m deep when it was established on 04October, and 1.7 m on 09December.

The cages were constructed of a vinyl-coated polyester mesh with rectangular holes measuring approximately 1/16 inch (1.6 mm) by 1/32 inch (0.8 mm). The mesh was supported by a floating, square PVC pipe frame measuring 4 feet (1.2 m) across (see photograph on title page). The floating frame and mesh extended 1 foot (0.3 m) above the water surface to prevent fish from jumping out. The top of the floating frame was covered with a removable ½ inch (12.7 mm) square plastic mesh to prevent predators from entering the cage. Two corners of the floating frame were attached to the buoy lines, which were in turn anchored in the sediments. To maintain contact with the bottom sediments and allow cage removal, a second PVC pipe frame measuring 4 feet (1.2 m) across, and weighted internally with metal rods, was attached to the cage mesh 1 foot (0.3 m) from the bottom of the cage. Ropes connecting the corners of the bottom and floating frames allowed 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.

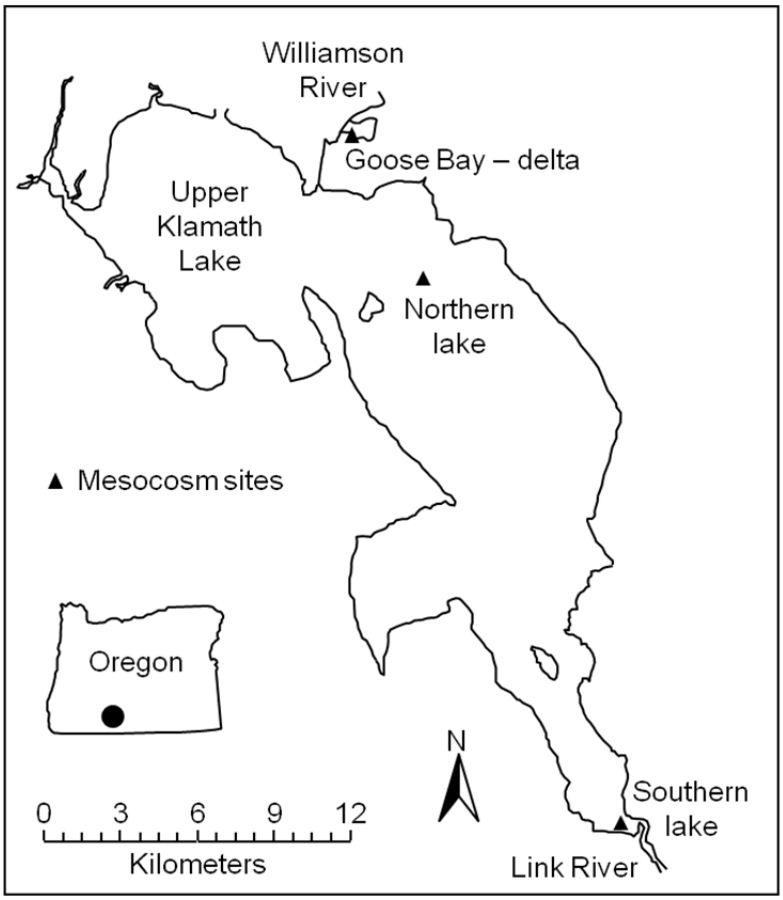

Figure 1.—Map of the study area in Upper Klamath Lake and Williamson River Delta, Oregon, including locations of the three mesocosm sites and the major inflow and outflow.

Table 1. Input of fry and collection of both live and dead fry in mesocosm cages. No delta cage input occurred after 31August.

	20-Jul	10-Aug	10-Aug	10-Aug	31-Aug	31-Aug	31-Aug	21-Sep	21-Sep	21-Sep	4-Oct	4-Oct
Cage	IN	LIVE	DEAD	IN	LIVE	DEAD	IN	LIVE	DEAD	IN	LIVE	DEAD
D1	300	8	3	none	0	0	end	n/a	n/a	end	n/a	n/a
D2	300	31	7	none	1	1	end	n/a	n/a	end	n/a	n/a
D3	300	31	0	none	2	0	end	n/a	n/a	end	n/a	n/a
D4 (st)	100	25	7	100	13	0	end	n/a	n/a	end	n/a	n/a
L5 (st)	100	48	0	100	26	11	none	40	0	200	199	0
L6	300	31	0	none	147**	16	end	n/a	n/a	end	n/a	n/a
L7	300	31	0	none	16	12	none	22	1	none	68	0
L8	300	31	0	none	16	9	none	22	0	none	45	0

** On 8/31 Lake 6 cage was completely sampled.

n/a not applicable as cage was empty and not sampled

Water quality - Water quality measurements of temperature, dissolved oxygen concentration and pH were taken hourly at each site, with monitors exchanged every two weeks throughout the study period of 20July through 09December 2011. Exceptions occurred at the southern lake site from 19 - 31October when data was not recorded due to a programming error, and 15 - 29November when a dissolved oxygen probe failed. Monitors were hung from buoys, resulting in instrument depths of approximately 0.5-1.0 m. Water quality data reported are unedited, and were not corrected for in situ instrument drift or presumed fouling. However, additional monitors were deployed adjacent to one another, providing instrument overlap.

Invertebrate analysis - We used a Wisconsin-style Plankton sampler (180mm diameter) with an anterior reducing cone (130 mm diameter) to collect zooplankton samples weekly beginning 20July through 07September. The bucket or cod of the sampler had a mesh size of 63µm. Samples of zooplankton were taken inside the long-term mesocosms (cages) at each site (Goose Bay (delta) and Northern Lake) and at three random locations nearby the cages at each site for a total of 12 sub-samples each week. In total, 72 samples of zooplankton were collected during 8 weeks. Some weeks had as many as 12 sub-samples, but some had as few as 3 sub-samples because of gear malfunctions or the elimination of within cage samples at the Goose Bay sites by 31August. The sampler was gently lowered to the bottom and allowed to settle for a brief period (approximately 30 sec – 1 min). It was then raised swiftly to the surface, effectively sampling a vertical cylinder of water from the bottom to the surface 130mm in diameter. Samples were stored individually and fixed with 70 % ethanol and a fluorescein dye (Rose Bengal; Fisher Scientific) was added to each sample to facilitate enumeration. Samples were strained through a 63µm sieve (#230), and then added to a known volume of water. The samples were then agitated to uniformly distribute zooplankton throughout the water and a 5ml sub-sample was taken using a Hensen-Stempel Pipette (Wildlife Supply Company). This 5-ml sub-sample was placed into an acrylic Bogorov Counting Chamber (Wildlife Supply Company) with a 5-ml capacity. Zooplankton within these sub-samples was counted once under variable magnification (max 30x) using a binocular dissecting scope. Organisms were generally identified to the lowest feasible taxonomic level, but were eventually binned into Order or Class level groups due to sparse representation in many of the groups. These groupings consisted of members of the 1) Order Cladocera (predominantly *Chydorus* and *Daphnia*), 2) members of the Class Copepoda, and 3) all other invertebrates collected, including Rotifera, Ostracoda, Chironomidae (midge) larvae, and other Dipteran larvae.

The relative abundance of each of these three groups in each sample was calculated and then transformed using the arcsine transformation ($\sin^{-1}\sqrt{\text{relative abundance}}$). These data were then analyzed with a multivariate analysis of covariance (MANCOVA), with the arcsine transformed relative abundance of the

three groups (i.e. Cladocera, Copepoda, and All others) as the response variables. The fixed effects included site (Goose Bay or Northern Lake) and sample type (within a long-term cage [Cage] or in the ambient habitat [Ambient]). The ordinal date when the sample was collected was also included in the model as a covariate. Inclusion of individual nets as random effects nested within site did not appreciably improve the model and so were not included. The analysis was conducted using the *manova* function within Program R version 2.12.2.

We also enumerated the gut contents of juvenile suckers. These fish were collected from within cages at the Goose Bay (n = 15) and Northern Lakes (n = 26) sites, as well as in the ambient environment near the Goose Bay site (n = 8). Collection dates include the 16th, 24th, and 31st of August, and the 8th of September. Fish were anesthetized in a lethal concentration of Tricaine (MS-222, Finquel) and fixed in 70% ethanol. The anterior third of the gut track was removed from each individual as a sub-sample and contents were removed and placed in a Bogorov Counting Chamber for enumeration in the same manner as the zooplankton samples. In contrast to the zooplankton samples, gut contents also included midge pupa and adults, and a low number of amphipods (*Hyalella azteca*). Therefore, the grouping bins were 1) Cladocera, 2) Copepoda, 3) Chironomidae, and 4) all others. Diatoms were also found in gut tracks, predominantly in fish from the cages and ambient environment of Goose Bay; however, these were not included in the calculation of relative abundance of food items because of their extreme small size relative to the other food items taken. The relative abundance gut contents were also arcsine transformed before analysis.

Laboratory analysis

Sample preparation - Standard length and weight were recorded for each frozen fish and its condition factor calculated ($KSL = WT/ SL^3 x 10^5$). Specific growth rate (% bodyweight change / day) was calculated from the mean weight data of all long-term cage fry as: $SGR = 100 x [\log_e(\text{mean } WT_{time2}) - \log_e(\text{mean } WT_{time1})] / \text{days}_{(time2- time1)}$ (Ricker 1979). Cold distilled water was added to a 20 mL tube containing each fish (1:1 w / v) and blended for 30 – 90s with a Biospec M133 homogenizer. Two aliquots (100- 200µl) of the homogenate (**2xWB**) were placed into tared 2mL centrifuge tubes, weighed to the nearest 0.01g to determine tissue weight (homogenate wt. divided by 2), and assayed for triglyceride (immediately) and protein (frozen and later assayed).
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) to an aliquot of homogenate, mixed at room temperature for 20 min, centrifuged at 3220xg for 5 min, and replicate 10 µL samples of the 10x diluted supernatant used in an enzyme assay for triglyceride (Pointe Scientific triglyceride GPO kit). Protein content (mg protein / g tissue) of the homogenate was assayed by a modification of the alkaline digestion method reported by Woo et al. 1978. Briefly, 0.5N NaOH was added to the homogenate (5x dilution w/v), mixed at 45°C for 120 min, centrifuged at 3220xg for 5 min, and replicate 10 µL samples of the 10x

diluted supernatant assayed for total protein by the biuret method (Pierce BCA protein assay kit, Rockford IL). The blank consisted of 1:4 mixture of distilled water and 0.5N NaOH. Albumin diluted in the blank was used as the protein standard.

ATPase - Gill Na-K-ATPase activity of gill tissue was assayed by the enzymatic method of McCormick (1993).

Liver lipid peroxidation - Malondialdehyde (MDA), a lipid peroxidation product, was measured in liver tissue by the method of Almroth et al. (2008) using OxisResearch LPO-586 kit (OxisResearch, 1499 Rollins Road, Burlingame CA 94010). Briefly, a small sample of liver (10 – 20mg) was placed into 0.5mL buffer (PBS, 0.5M butylated hydroxytoluene in acetonitrile), frozen on dry ice, stored at -70ºC, defrosted and rapidly homogenized by sonication (Microson™ Ultrasonic Cell Disruptor), centrifuge (15,000 x g,4ºC,10 min), supernatant reacted with N-methyl-2-phenylindole in acetonitrile and 37% HCl, and its absorbance at 586 nm compared to a MDA standard curve. Lipid peroxidation is a result of oxidative stress on lipid components of the cell membrane and can be induced by xenobiotics (Porter et al 1995). When antioxidant mechanisms are overwhelmed, peroxidation will tend to occur in the fish.

Histology - Histological samples were held in Davidson's fixative or cold zinc formalin (Zfix, Anatech Ltd, Battle Creek MI) for 24h, processed for 5µm paraffin sections, and stained with hematoxylin and eosin. Zinc formalin was chosen to accommodate immunohistochemistry. 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. A subset of zinc formalin fixed specimens were examined for the presence of microcystin-LR antigen in the liver, kidney, and intestine by the immunocytochemistry method of Djediat et al. (2010) using a monoclonal antibody to microcystin-LR (MC10E7, Enzo Life Sciences ALX-804-320) and the universal VECTASTAIN kit (Vector laboratories). Positive (MCLR treated Chinook) and negative (wetlab LRS) control sections were processed with the sample set. The relative quantity of hepatocyte glycogen was rated (0= none to scant, 1 = focal distribution of distinct cytoplasmic droplets, and 2 = multifocal to diffuse distribution of distinct cytoplasmic droplets) from PAS stained sections of zinc formalin fixed livers.

Laboratory data statistical analysis - Analysis was performed with SigmaStat 3.1 software on raw data. Normality was tested by the Kolmogorov – Smirnov method at the P= 0.05 level. One-way ANOVA or T-test (data with normal distribution, reported with F or t value) or Kruskal-Wallace ANOVA or Mann-Whitney U test on ranks (non-parametric analysis) with subsequent multiple comparison procedures (Holm-Sidak or Dunns method respectively, alpha $\leq$ 0.05) was used to compare groups.

Permits- Permits for this project are as follows: sub-permit FWSKFFWO-7 (USFWS Klamath Falls Office) under the USFWS Region 8 regional blanket permit (TE-108507), Section 10 Federal authorization number TE007907-14 (adult brood stock, TE007907-14), Oregon Scientific Take Permit Number 16157 M2, and California DFG Standard Importation Permit number 2011-1623.

Results:

Fish culture and cage evaluation – Fungus infestation of the egg masses required daily removal and formalin baths (1000ppm, 15min). Fry did not appear to fully accept the Otohieme marine larvae diet as their growth was slow after complete weaning from artemia in June. In mid-August, the population experienced a chronic infection and mortality from columnaris and *Ichthyoboda* (costia). The fish were treated with nitrofurazone baths (2-4ppm+1%salt, 1h), formalin baths (30ppm, 1h), chloromine-T baths (20ppm, 1h), and top-coated oxytetracycline feed. Ultraviolet treatment of the water supply may improve this situation in the future. The cages remained anchored in place, showed no signs of vandalism, and no tears were seen. The large cage size made it difficult to bring the cage bottom to the surface and sample all fish.

Sentinel survival- Fry held in the delta incurred a high loss during the initial exposure periods in July and August. Fish were regularly observed to be located in the upper 18 inches of the cages, unless startled. The short-term cage D4 had a 75% loss between 20July and 10August and 87% loss in the subsequent 10 – 31 August period (Table 1 & 2). Similarly, only 8 live fish were recovered from the long-term cage D1 on 10August and none on 31 August. Given that grab samples were taken from long-term cages, we cannot determine loss within the long-term cages D2 and D3 between 20July and 10August. On 31August, only 3 live fish were obtained from cages D2 and D3 (a maximum survival from 10August of <1%). A total of 17 dead fry were observed in the delta cages on the 10August sample without obvious external lesions. No further sentinel introductions to the delta cages were made after 10August.

Sentinels were held in the north lake site from 20July through 04October with 36 juveniles subsequently moved to the south lake site for an additional 67 days of rearing. The short-term cage L5 had a 52% loss by 10August (Table 2 and Figure 2). On 31August, 26 live fish were sub-sampled from L5 and eleven dead fry were observed in the cage. On 21September, the cage was completely sampled and 40 live fish collected. This data indicates that on 31August, a minimum of 66 fish were alive and conversely the cage population had experienced a 34% loss. On 31August, long-term cage L6 was mistaken for a short-term cage and completely sampled of 147 live and 16 dead fry (41% loss to this date). As 31 fry were subsampled from L6 on 10August (300 – 31 = 269), the minimum survival over 10-31August period was 55% (147 live fish / maximum of 269 fish).

Long-term cages L7 and L8 were subsampled through the summer with 38% (L8) and 46% (L7) of their fry accounted for as live fish over the 76 sampling period (20July – 04October). Thirty-five of the 36 juvenile suckers transferred to the south lake cage were sampled as live fish on 09December.

Table 2. Survival of sentinels held in the delta and lake short-term (ST) cages. Data includes dates of exposure and total exposure days. Survival of lake cage 6 is included as the cage was completely sampled on 31August.

Dates	Days	DeltaST	LakeST	Lake6
7/20-8/10	21	25%	48	ND
8/10-8/31	21	13%	66%**	65%**
8/31-9/21	21	ND	ND	ND
9/21-10/4	13	ND	100%	ND

** Represents the minimum survival on this date as a subsequent sampling occurred in these cages.

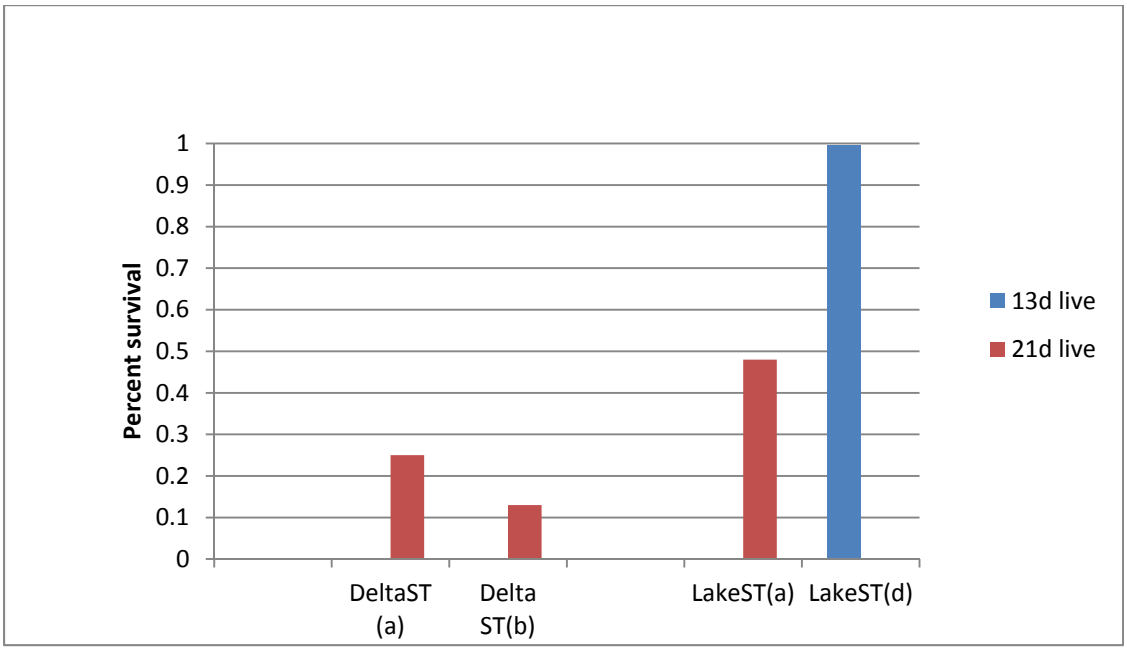

Figure 2. Percent survival of fry held in short-term (ST) delta or lake site cages for 13 or 21 days. Exposure (a) occurred 20July-10August, (b) 10August – 31August, and (d) 21September – 04October.

Water quality - Water temperatures at the northern lake and delta sites showed similar seasonal patterns, and ranged from approximately 12-26°C (Table 3, Figure 3). The delta site averaged 2.7°C cooler than the northern lake site, but temperatures did not reach the median tolerance limit of 30.4-31.8°C identified for juvenile Lost River suckers by Saiki et al. (1999). However, temperatures did reach the low level stress threshold of 25ºC used by Loftus (2001) for testing stress in suckers.

Dissolved oxygen concentrations at the northern lake and delta sites showed distinctly different seasonal patterns (Table 3, Figure 4). Dissolved oxygen at the northern lake site typically ranged from 4-15 mg/L throughout the summer, and only occasionally dipped below the high stress threshold of 4.0 mg/L used by Loftus (2001). However, dissolved oxygen at the delta site was persistently near or below 4.0 mg/L in late August.

Levels of pH at the northern lake site ranged from approximately 8-10, whereas the delta site ranged from approximately 7-9.5 (Table 3, Figure 5). Both sites were typically below the high level stress threshold of 9.75 used by Loftus (2001). Though pH was typically higher at the northern lake site, the delta site exhibited much larger daily swings.

The southern lake site was established in the fall (Water temperatures ranged from approximately 2-14°C (Table 3, Figure 6), dissolved oxygen ranged from approximately 8-14 mg/L, and pH ranged from approximately 7.4-9.

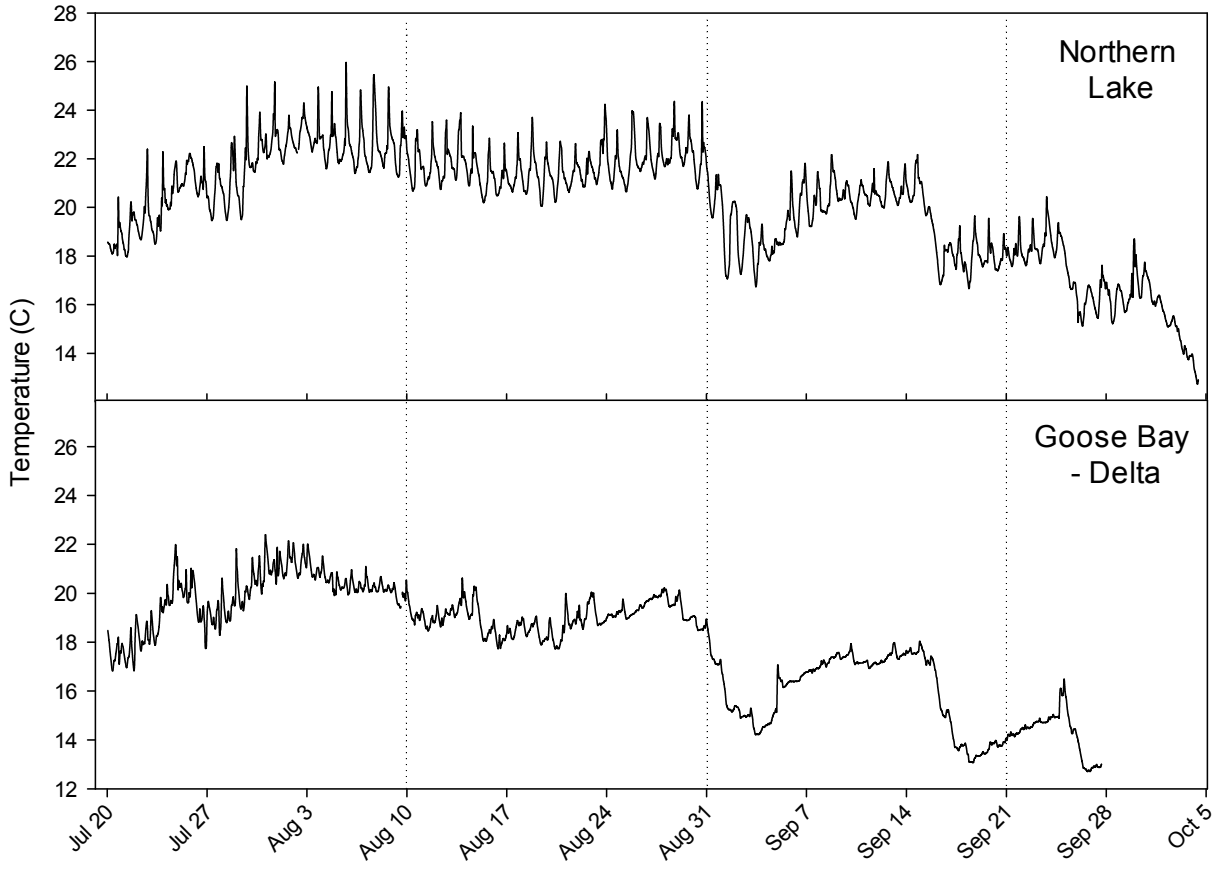

Figure 3.—Water temperatures at the fish cage sites in northern Upper Klamath Lake (top panel) and the Goose Bay portion of the Williamson River Delta (bottom panel) during the summer of 2011. Temperatures were taken hourly using continuous monitors deployed at approximately 0.5-1.0 m depth from a buoy located near the fish cages. Vertical lines indicate when fish were sampled.

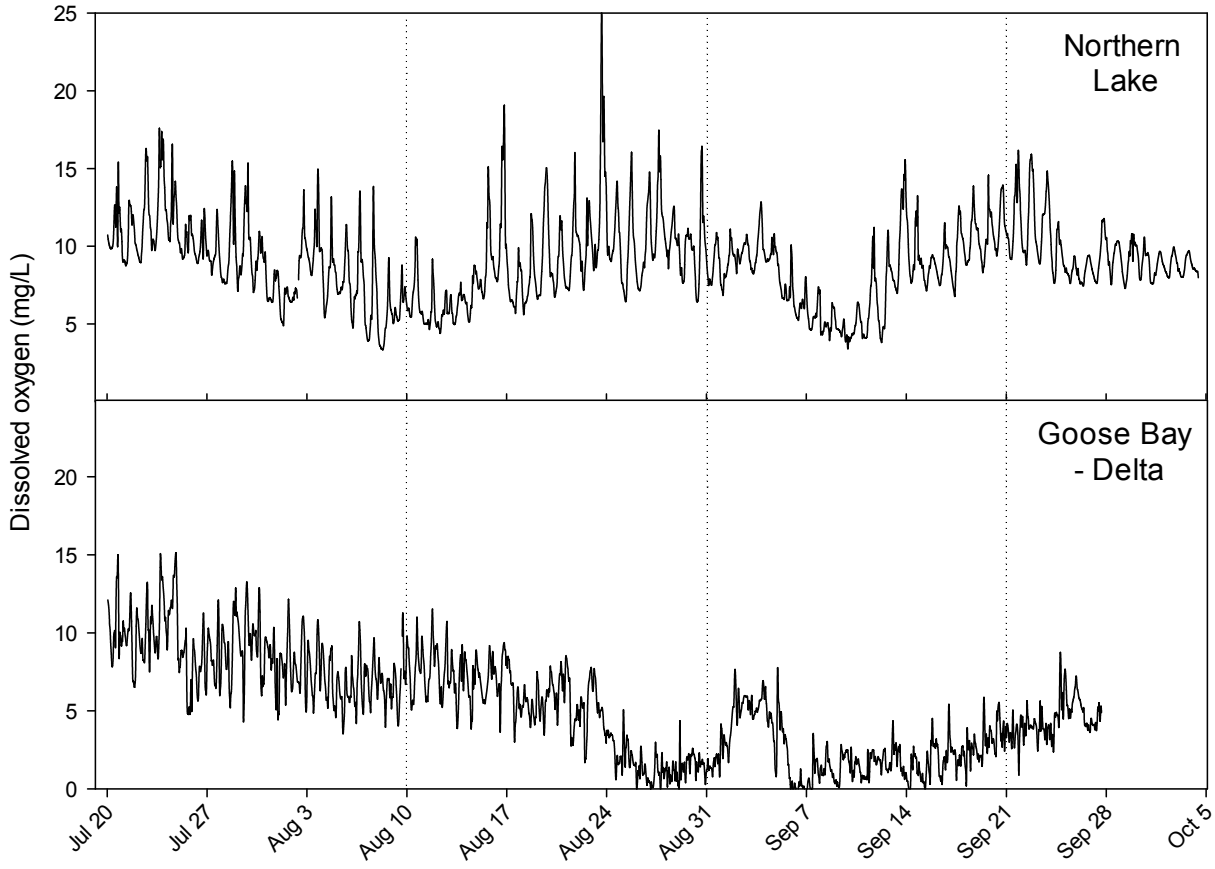

Figure 4.—Dissolved oxygen concentrations at the fish cage sites in northern Upper Klamath Lake (top panel) and the Goose Bay portion of the Williamson River Delta (bottom panel) during the summer of 2011. Dissolved oxygen concentrations were taken hourly using continuous monitors deployed at approximately 0.5-1.0 m depth from a buoy located near the fish cages. Vertical lines indicate when fish were sampled.

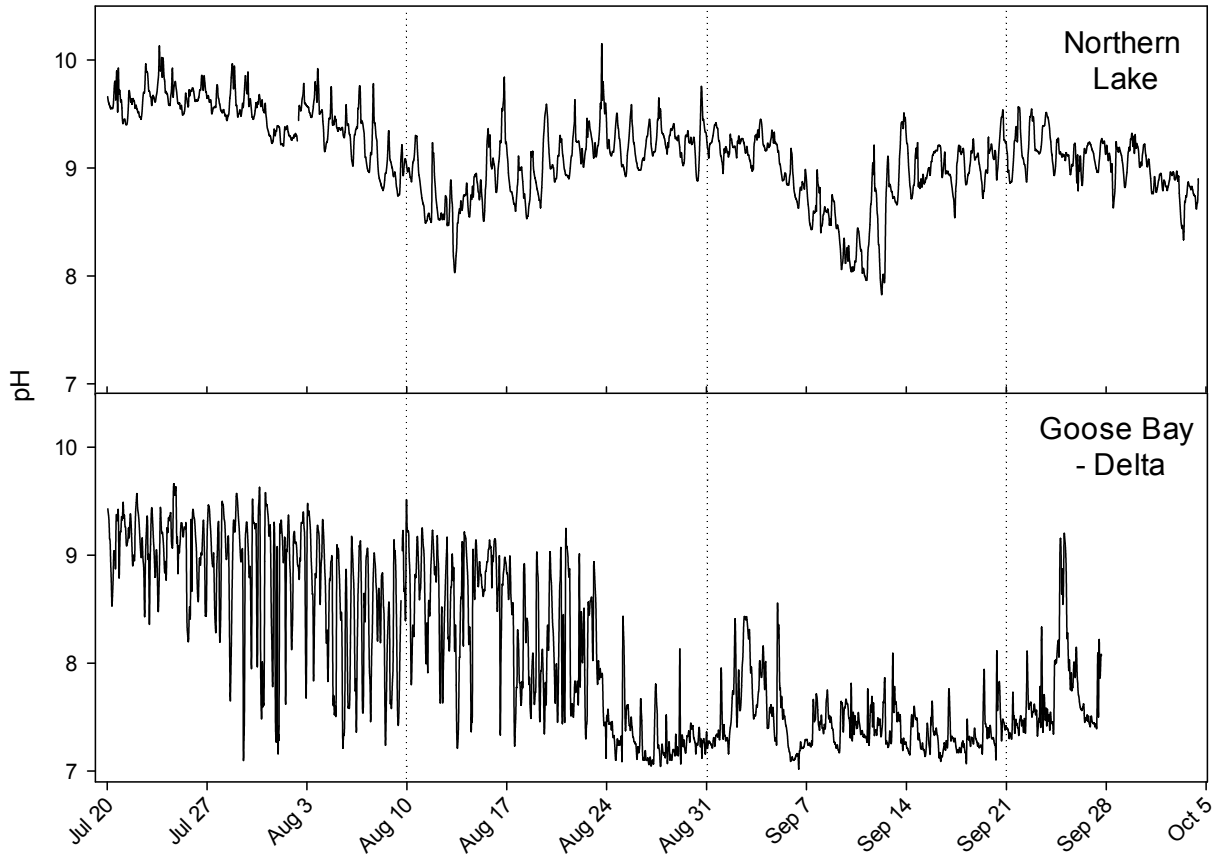

Figure 5.—pH at the fish cage sites in northern Upper Klamath Lake (top panel) and the Goose Bay portion of the Williamson River Delta (bottom panel) during the summer of 2011. pH was taken hourly using continuous monitors deployed at approximately 0.5-1.0 m depth from a buoy located near the fish cages. Vertical lines indicate when fish were sampled.

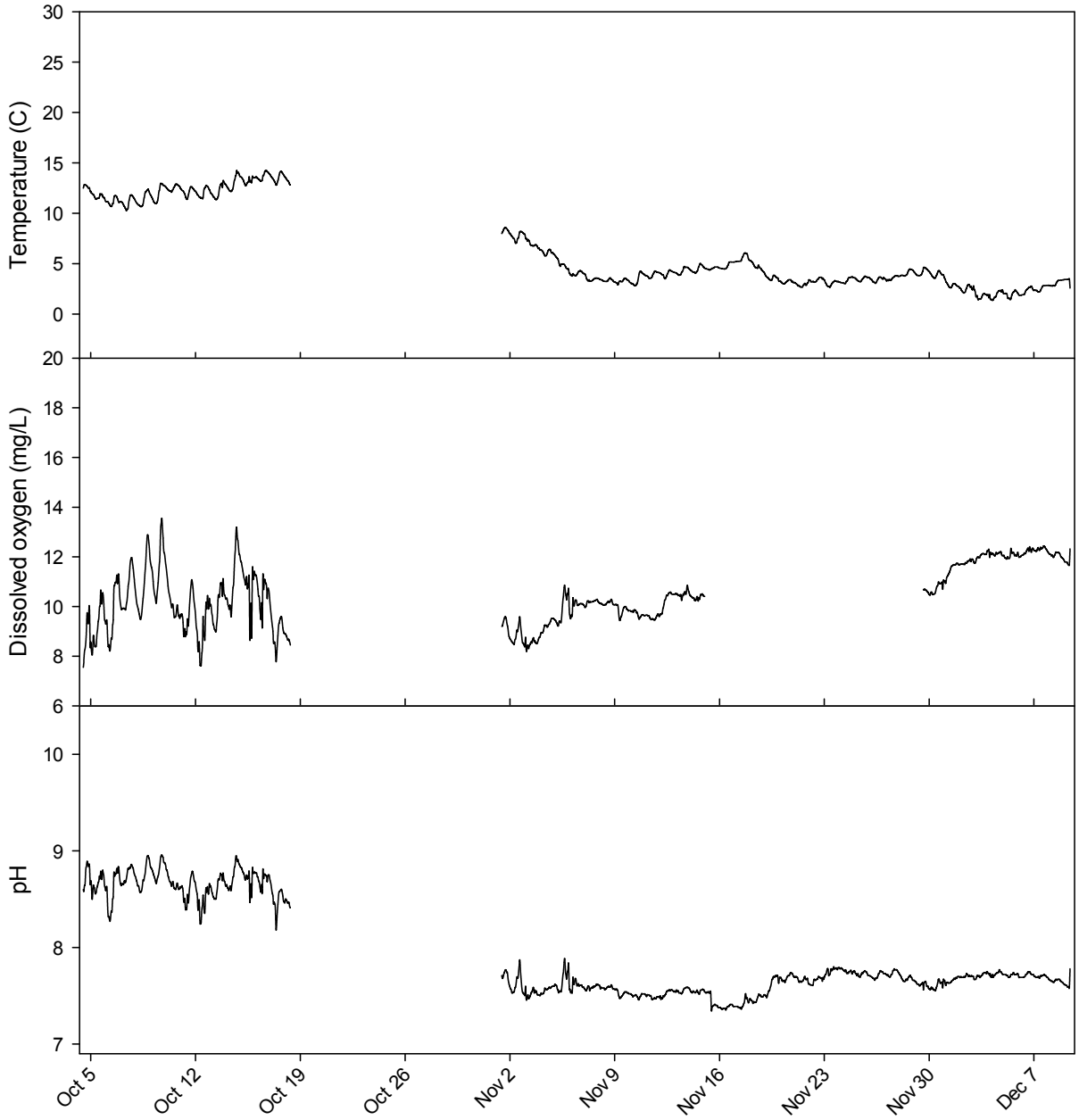

Figure 6.—Water temperature, dissolved oxygen concentration, and pH at the fish cage site in southern Upper Klamath Lake during the fall of 2011. Measurements were taken hourly using continuous monitors deployed at approximately 0.5-1.0 m depth from a buoy located near the fish cage.

Table 3.—Maximum, mean, and minimum measurements of temperature, dissolved oxygen, and pH observed at each site during the five exposure periods of the study. Monitors were hung from buoys, resulting in instrument depths of approximately 0.5-1.0 m. *n* = number of measurements made during each exposure period.

Constituent	Site		Exposure period				
			Jul 20 - Aug 10	Aug 10 - Aug 31	Aug 31 - Sep 21	Sep 21 - Oct 4	Oct 4 - Dec 9
Temper-ature (C)	Northern lake	max	25.9	24.36	22.16	20.39	
		mean	21.50	21.69	19.37	16.64	
		min	17.97	19.57	16.69	12.75	
		n	516	504	504	310	
	Goose Bay - delta	max	22.38	20.57	18.01	16.49	
		mean	19.85	18.95	15.93	14.29	
		min	16.83	17.24	13.07	12.71	
		n	516	504	504	147	
	Southern lake	max					14.26
		mean					6.13
		min					1.35
		n					1245
Dissolved oxygen (mg/L)	Northern lake	max	17.59	25.33	15.56	15.87	
		mean	9.12	9.14	8.31	9.56	
		min	3.35	4.41	3.44	7.29	
		n	516	504	504	310	
	Goose Bay - delta	max	15	11.5	7.66	8.73	
		mean	8.37	4.70	2.44	4.53	
		min	3.58	0.0	0.0	0.88	
		n	516	504	504	147	
	Southern lake	max					13.56
		mean					10.44
		min					7.55
		n					894
pH	Northern lake	max	10.13	10.15	9.52	9.56	
		mean	9.47	9.05	8.89	9.06	
		min	8.74	8.03	7.83	8.34	
		n	516	504	504	310	
	Goose Bay - delta	max	9.66	9.25	8.52	9.16	
		mean	8.80	7.98	7.45	7.75	
		min	7.23	7.05	7.02	7.33	
		n	516	504	504	147	
	Southern lake	max					8.96
		mean					7.90
		min					7.35
		n					1245

Diet composition - Across the three groupings of zooplankton analyzed there was no significant distinction between sampled communities within the cages and the ambient environment ($F_{3,64}$ = 0.7, p = 0.56). An increase in the proportion of the "Other" group in the cage samples throughout the sampling period (Figure 7C) was documented, while proportions of this group declined (Goose Bay) or remained essentially flat (Northern Lake) in the ambient samples. This produced a significant interaction term between ordinal date and sample type ($F_{3,64}$ = 4.0, p = 0.01) in our analysis. As the sampling season began, differences in the relative abundance of both Cladocerans and Copepods between the Northern Lake and Goose Bay sites were markedly different ($F_{3,64}$ = 23.3, p < 0.001). The Northern Lake site tended to have much higher proportions of Cladocera and lower proportions of Copepoda (Figure 7A & B) than the Goose Bay site. However, as the season progressed the relative abundance of each group became more similar between the two sites, until they were essentially the same by the end of our sampling period. These converging trends produced statistically significant interaction term in our model for ordinal date x location ($F_{3,64}$ = 9.5, p < 0.001). Alone, the ordinal date of the collection did not explain sufficient variation to be statistically significant ($F_{3,64}$ = 1.1, p = 0.35); A three way interaction among all three factors (i.e. ordinal date, location and sample type) was not significant ($F_{3,62}$ = 0.8, p = 0.49); nor was the interaction between location and sample type ($F_{3,62}$ =1.6, p = 0.20). Zooplankton data are presented in Appendix 2.

Gut contents were examined on juvenile suckers ranging in size from approximately 24mm standard length (SL) to 80mm SL. Lengths were approximated because caudal peduncles were removed to collect tissue for physiological analyses. Therefore, a simple linear regression was generated to estimate the relationship between the distance from the snout and the dorsal insertion (DI) and SL on fish that were intact (SL = 2.14 x DI – 1.96, R^2 = 0.98, n = 29). This was then used to estimate SL on those fish that had the caudal peduncle removed. Differences in length were significant among sites ($F_{2,42}$ = 8.1, p = 0.001). Goose Bay cages ($\bar{x} = 27.9$ mm SL) were much smaller than Goose Bay ambient captures ($\bar{x} = 41.9$ mm SL, *post hoc* Bonferroni pairwise test p = 0.09) and individuals from the Northern Lake site cages ($\bar{x} = 47.0$ mm SL, p < 0.001). Lengths of Goose Bay ambient fish were not significantly different from those of the Northern Lake cages.

Gut contents of fish taken from the cages at the Northern Lake site were almost entirely comprised of midge, predominantly larvae but with an appreciable amount of pupa and adults (Figure 8). In contrast, suckers within Goose Bay cages tended to have consumed a high number of Cladocerans (primarily *Chydorus*) in addition to the midge component, which was comprised almost entirely of larvae. Juvenile suckers collected in the ambient environment near the Goose Bay cages tended to have a more diverse mix of all groups. This was the only group of fish of the three to have a significant component of Copepods (primarily Cyclopoida). All of these ambient fish also possessed guts literally full

of dark material that had the appearance of muddy substrate. This was not seen in any of the fish from the cages. Diatoms were also more prevalent in fish from Goose Bay (both the caged and ambient fish) than in the Northern Lake site. Because of the zero-inflated nature of the data and the lack of general overlap between gut contents and zooplankton sampled, rigorous statistical analysis was not feasible.

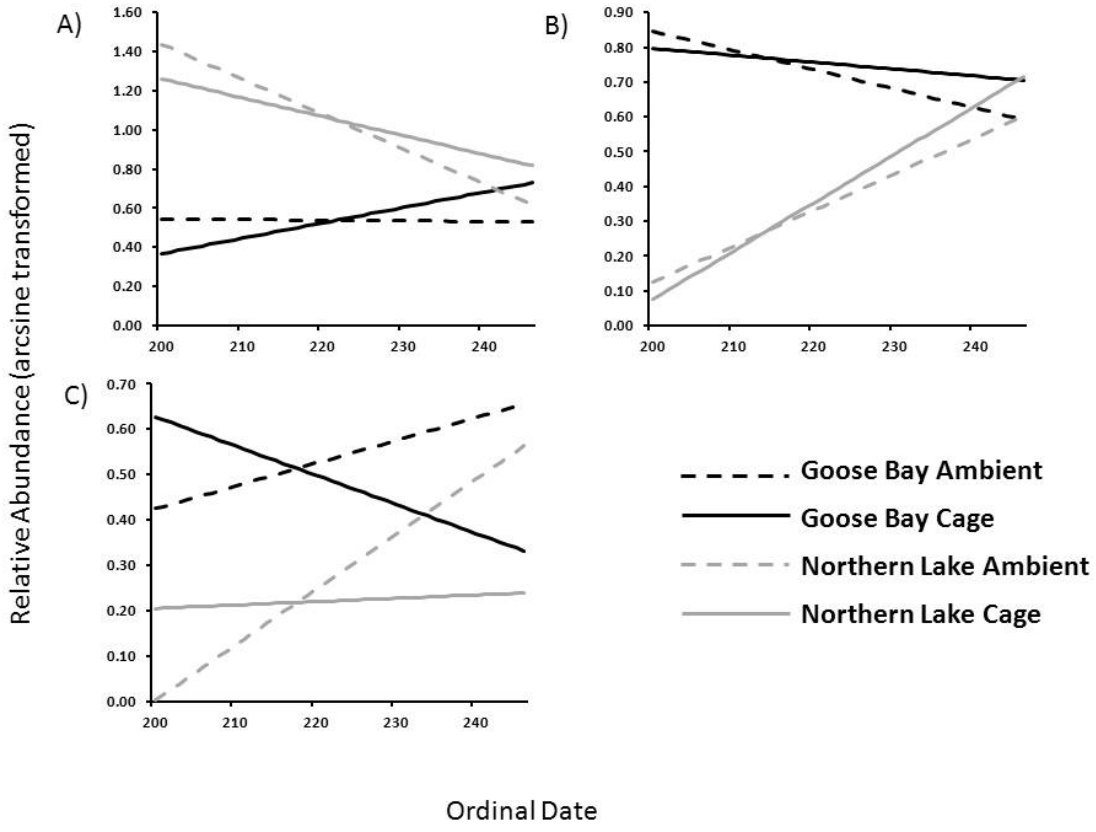

Figure 7. Predicted relative abundances (arcsine transformed) of zooplankton community from a multivariate analysis of covariance for A) Cladocerans, B) Copepods, and C) all other zooplankton and macroinvertebrates at two sites Goose Bay and Northern Lake inside mesocosm cages (cage) and outside (ambient).

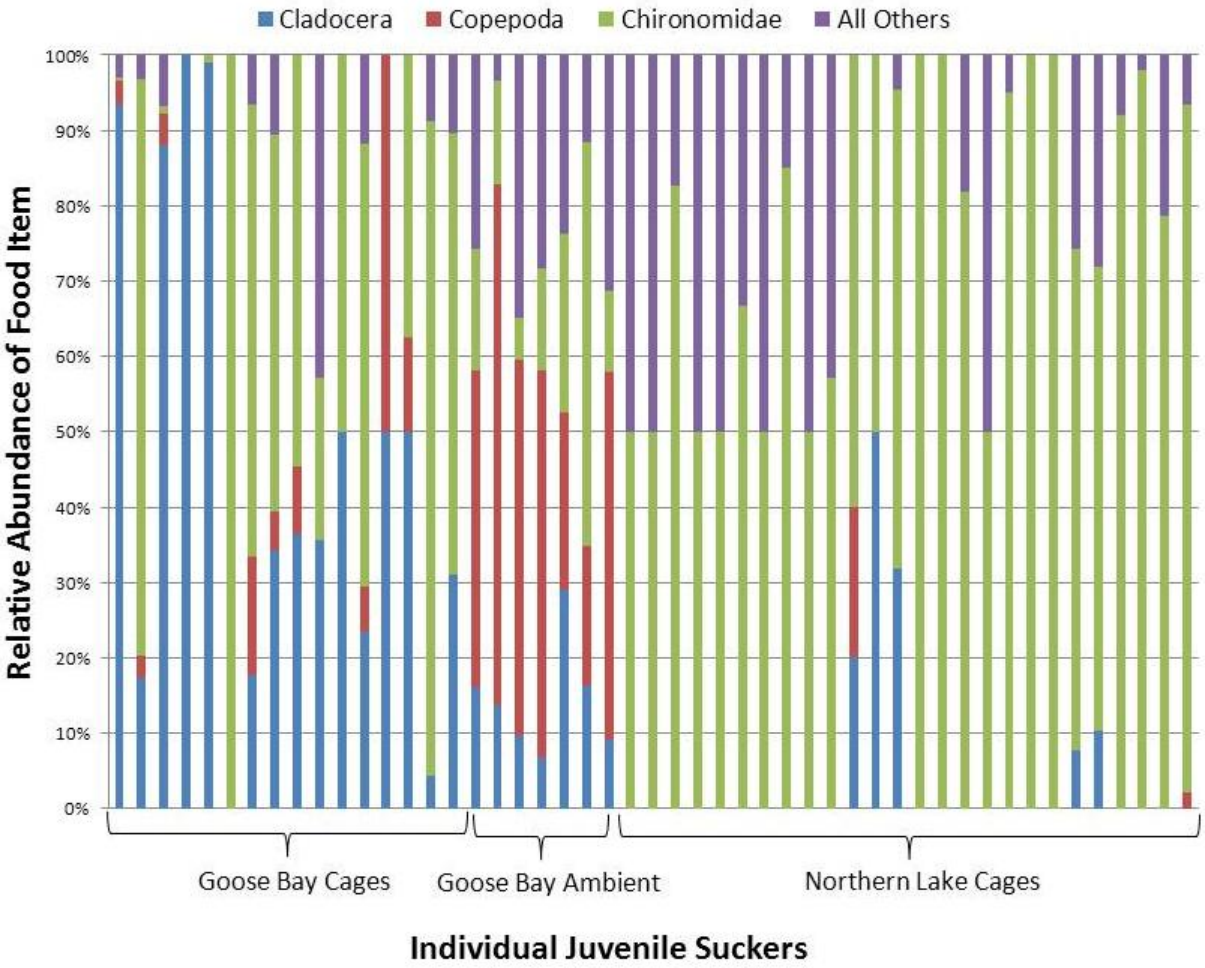

Figure 8 The relative abundance of food items in the gut contents of juvenile Lost River suckers taken from mesocosm cages at Goose Bay and Northern Lake sites and in the ambient environment nearby the Goose Bay site. Items are from a sub-sample of the anterior third of the gut.

Energy and growth- Both delta and lake cage fry showed growth during the summer and fall (Figure 9 and Table 4). Mean standard length increased from 26.3mm of the 20July transport group to 71.6mm for the combined sample of long-term cages L7 and L8 on 04October. No appreciable change in length occurred between 04October and 9December in the south lake cage population. The 31August delta energy sample consisted of 4 survivors(8 total survivors). Consistent weight gain resulted in a similar condition factor of approximately 1.4 for the long-term lake groups with the exception of the 21September sample mean KSL of 1.662 (Figure 10 and Table 4). The 31August delta survivors showed signs of starvation with condition factors less than 1.0. Short-term cage fry gained weight and had increased condition factors from their initial loading values (Figure 11). As seen in the long-term groups, the fish in L5 that were held an additional 21 days (loaded 10August, first sampled on 31 August and again on 21September) maintained a condition factor of approximately 1.4 and were

not significantly different (Mann-Whitney rank sum, P=0.207). Growth was relatively low in August (mean %body weight / d of 2.3 and 3.4%) for long-term lake cage populations and then peaked in September (Figure 12). There was no appreciable growth in the 04October to 09December group. Insufficient food resources appeared to influence the low specific growth rate of the delta cage fish (1.6 and 2.3 %BW/d).

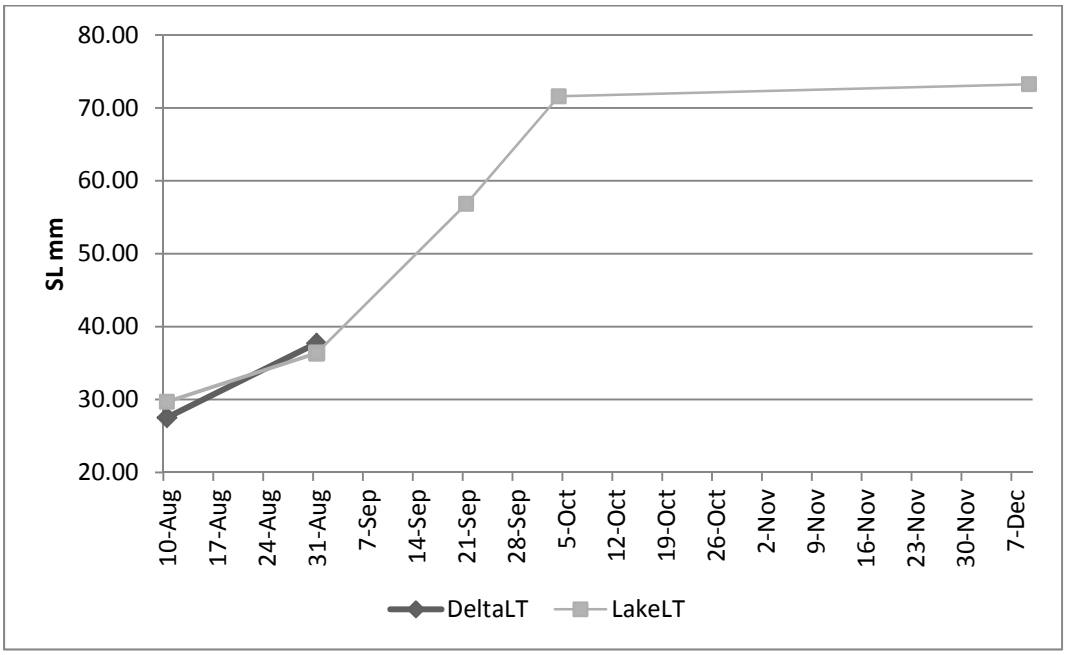

Figure 9. Mean Standard length (mm) of Lost R. sucker fry held in long-term cages within the Delta and North Lake. Fish were sampled between 10August and 9December 2010 (9Dec data from a south lake cage).

Table 4 Standard length(0.1 mm), weight (0.01g), condition factor ($KSL=Wt/SL^3x10^5$),whole body triglyceride and protein content (mg/g fish) of frozen Lost River sucker fry samples from the wetlab population, delta and lake cages. Data reported as mean (SEM).

Sample Group	Sample Date	N	Standard Length	Weight	Condition Factor	Triglyceride	Total Protein
Wetlab	18-Jul	3	NA	NA	NA	14.3 (3.4)	51.2 (5.7)
Delta 1*	10-Aug	6	28.7 (3.2)	0.44 (0.19)	1.485 (0.069)	5.2 (1.6)	26.6 (2.5)
Delta 2*	10-Aug	14	28.7 (0.8)	0.34 (0.03)	1.367 (0.028)	6.0 (1.1)	20.83 (1.4)
Delta 3*	10-Aug	16	26.23 (0.9)	0.26 (0.03)	1.390 (0.066)	4.4 (1.3)	22.8 (1.6)
Delta 4*	10-Aug	9	28.9 (1.7)	0.39 (0.08)	1.460 (0.084)	6.6 (2.3)	25.2 (3.0)
Lake 5	10-Aug	16	35.0 (0.7)	0.64 (0.04)	1.450 (0.020)	6.6 (0.8)	43.3 (1.5)
Lake 6	10-Aug	16	29.8 (0.5)	0.36 (0.02)	1.340 (0.030)	2.8 (0.4)	39.8 (1.6)
Lake 7	10-Aug	16	27.9 (0.4)	0.31 (0.02)	1.410 (0.030)	3.2 (0.3)	36.0 (1.1)
Lake 8	10-Aug	16	31.3 (0.4)	0.42 (0.02)	1.350 (0.030)	3.3 (0.4)	36.6 (1.0)
Wetlab	8-Aug	16	34.5(0.6)	0.56 (0.03)	1.347 (0.077)	14.1 (1.1)	52.0 (1.3)
Delta ST	31-Aug	4	37.7 (1.0)	0.52 (0.06)	0.949 (0.043)	1.7 (0.2)	33.2 (4.1)
Lake 5	31-Aug	16	45.9 (1.7)	1.44 (0.17)	1.407 (0.062)	14.1 (1.4)	46.8 (1.8)
Lake 6	31-Aug	16	39.0 (1.8)	0.96 (0.14)	1.452 (0.036)	8.8 (1.4)	53.8 (4.8)
Lake 7	31-Aug	16	36.4 (1.3)	0.69 (0.07)	1.337 (0.023)	6.1 (1.3)	48.3 (1.0)
Lake 8	31-Aug	17	33.8 (1.9)	0.61 (0.12)	1.337 (0.025)	7.5(1.2)	56.1 (2.0)
wetlab	19-Sep	16	41.4 (0.7)	0.90 (0.06)	1.242 (0.023)	11.3 (0.5)	41.7 (1.2)
Lake 5	21-Sep	16	67.2 (2.4)	4.47 (0.47)	1.380 (0.021)	11.8 (1.0)	36.3 (1.3)
Lake 7	21-Sep	16	60.8 (2.8)	3.50 (0.42)	1.422 (0.012)	10.7 (1.0)	49.1 (2.2)
Lake 8	21-Sep	16	52.8 (3.7)	2.87 (0.56)	1.902 (0.435)	8.6 (1.1)	38.7 (1.5)
Lake 5	4-Oct	16	48.3 (1.1)	1.49 (0.10)	1.302 (0.026)	8.5 (0.5)	30.6 (0.6)
Lake 5	27-Sep	16	44.7 (0.8)	1.00 (0.07)	1.105 (0.041)	13.1 (0.7)	31.6 (0.9)
Lake 7	4-Oct	16	69.6 (3.0)	5.21 (0.65)	1.424 (0.016)	12.1 (0.8)	33.9 (1.0)
Lake 8	4-Oct	16	73.6 (3.1)	5.73 (0.65)	1.320 (0.016)	12.7 (0.8)	32.3 (0.7)
S.Lake**	9-Dec	35	69.0 (2.1)	5.42 (0.43)	1.575 (0.058)	13.9 (0.3)	43.6 (2.0)

- Standard length data of 10Aug delta fry calculated from regression equation for fork length (SL=-3.078+(0.977*FL).

** South lake juveniles from lake 7 and 8 cages moved on 10/4.

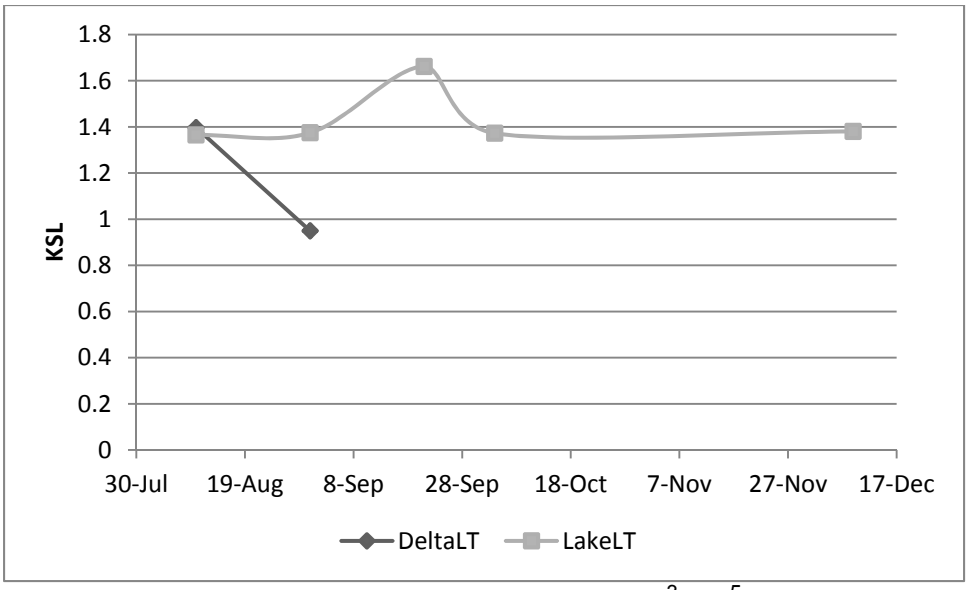

Figure 10. Mean condition factor (KSL= Wt/ SL^3x10^5) of Lost R. sucker fry held in long-term (LT) cages within the Delta and North Lake. Fish were sampled between 10August and 9December 2010 (9Dec data from a south lake cage).

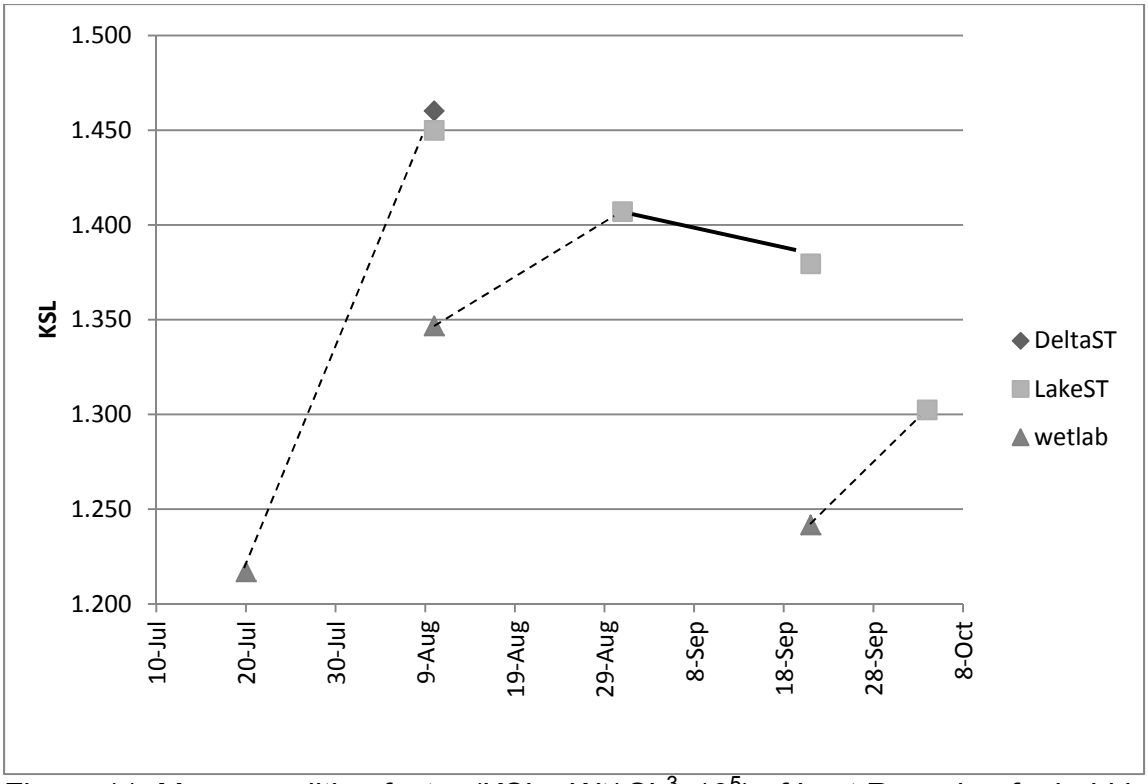

Figure 11. Mean condition factor (KSL= Wt/ SL^3x10^5) of Lost R. sucker fry held in short-term (ST) cages within the Delta and North Lake. Fish were sampled between 10August and 04October. Wetlab data reflects population prior to their movement into cages. Dotted line links wetlab input group with subsequent cage cohorts and solid line links cage L5 fry sampled 31Aug and again on 21Sept.

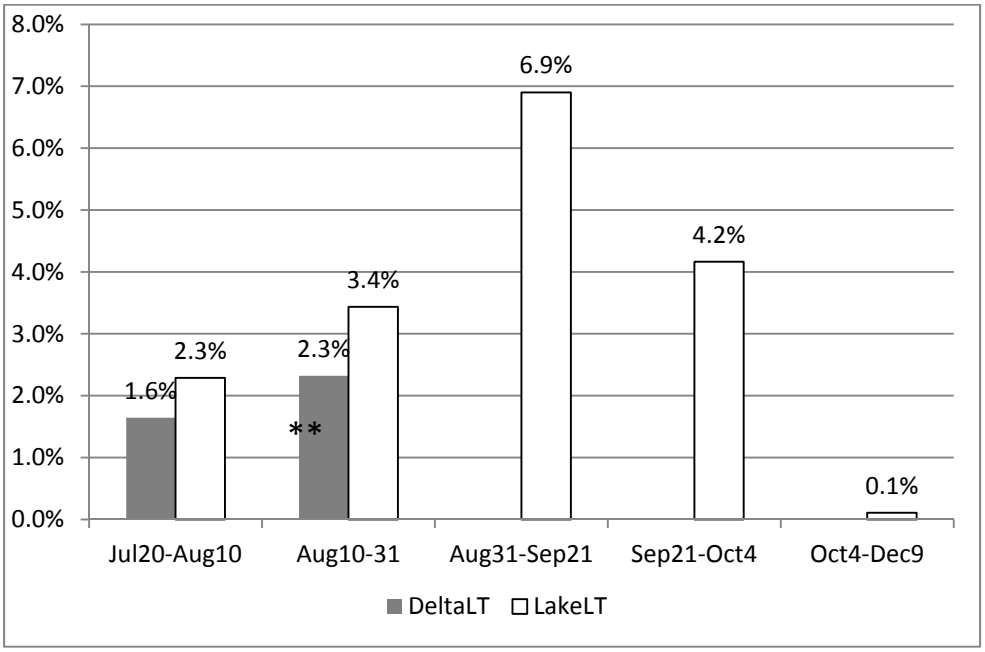

Figure 12. Specific growth rate (% bodyweight change / d) of sucker fry held in the long-term (LT) cages within the delta and the north lake site. Data from mean weight of fry measured during 5 periods during the July 20 to December 9, 2012 experiment. (** Only 4 survivors measured from Delta cages on 8/31).

Whole body triglyceride (TG) gradually increased in the long-term lake cage fish from a mean of 3.1 on 10August to 12.4 mg/g fish on 04October (Figure 13 and Table 4). There was a slight increase (mean 12.4 to 13.9 mg/g fish) between 04October and 09December. Sucker fry held in the delta showed a decline in TG. Fry sampled from short-term cage groups had a variable TG pattern over the experiment (Figure 14). In August, fry maintained a wetlab level of approximately 14 mg/g fish while TG tended to decline during the September exposures. In particular, the 21September wetlab group placed into cage L5 lost approximately 3 mg TG /g fish over the 13d period (significant difference, ANOVA $p<0.01$). There was moderate correlation between KSL and TG for the long-term lake cage fish (27 – 100mm SL, n = 161, TG= - 4.537+(0.211*SL)+(1.771*KSL), r-sq = 0.63). With further field evaluation, this relationship could be extended to similar weight – length data for feral juveniles to detect condition thresholds associated with survival.

Protein content showed a bimodal trend over the experiment with a peak on 31August and 09December (Figure 15). The mean protein value of 33 mg/ g fish for the 04October juveniles was similar to emaciated delta cage survivors sample on 31August Juveniles sampled on 09December had mean protein concentration of 43.6 mg / g fish that represent approximately 10 mg/g fish increase over the 04October sample group. Delta fry were consistently lower than their lake cohorts.

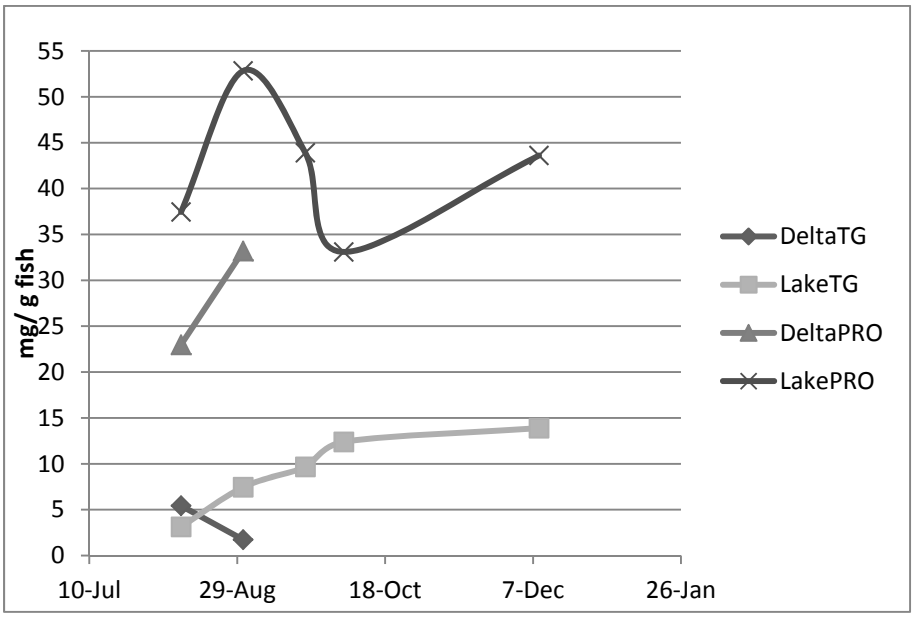

Figure 13. Mean whole body triglyceride (TG) and protein (PRO) of Lost R. sucker fry held in long-term cages within the Delta and North Lake. Fish were sampled between 10August and 9December 2010 (9Dec data from a south lake cage).

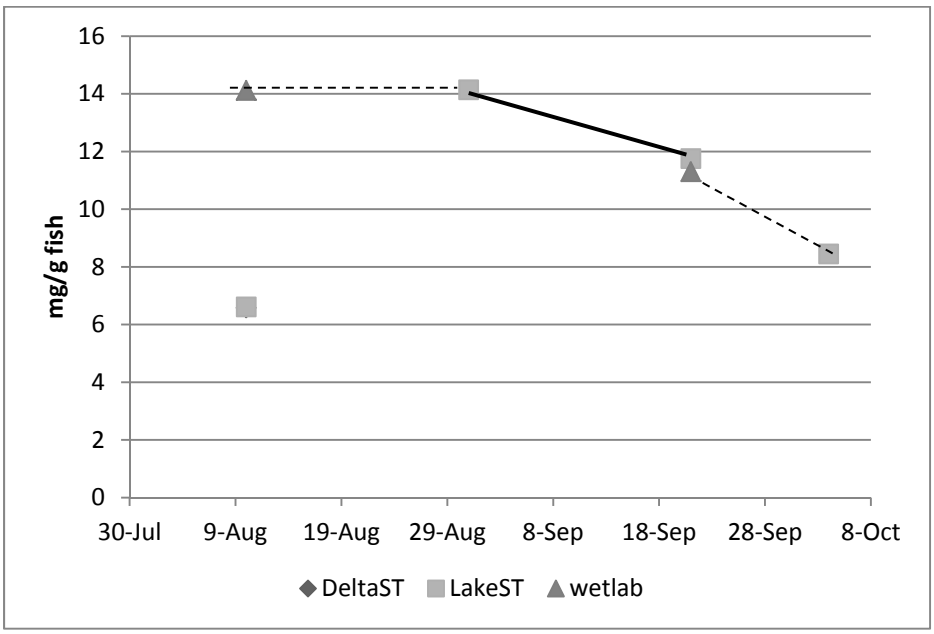

Figure 14. Mean whole body triglyceride (TG. mg/g fish) of Lost R. sucker fry held in short-term (ST) cages within the Delta and North Lake. Fish were sampled between 10August and 04October. Wetlab data reflects population prior to their movement into cages. Dotted line links wetlab input group with subsequent cage cohorts and solid line links cage L5 fry sampled 31Aug and again on 21Sept.

Winter cage – Thirty five of the 36 suckers, moved from the north lake long-term cages 7 and 8 to a south lake cage on 04 October, were sampled alive on 09December 2011. No growth (weight, length, or condition factor) was detected between suckers sampled 04October and their cohorts that reared for 67 days in the south lake (Table 4). As mentioned above, TG was similar to the 04October sample while whole body protein content of 09December fish was approximately 10 mg/ g fish higher than 04October sample group (Kruskal-Wallis ANOVA H=30.3, 2 df, P<0.01). Water quality was considered good during the exposure (pH<9.0, DO > 8.0) with the water temperature ranging between 4 - 13ºC (Figure 6). While standard length varied considerable in the 67 d survival group (50 – 93mm SL), condition factors ranged from 1.24 – 1.58 (Figure 16). Two survivors had low condition factors below 1.00. Low metabolic demand (low water temperature and sessile behavior) and some level of feeding could be factors in the static size and energy content observations. It appears that even relatively small fish can survive up to 67d under winter temperatures if their condition factor is relatively high (KSL > 1.24).

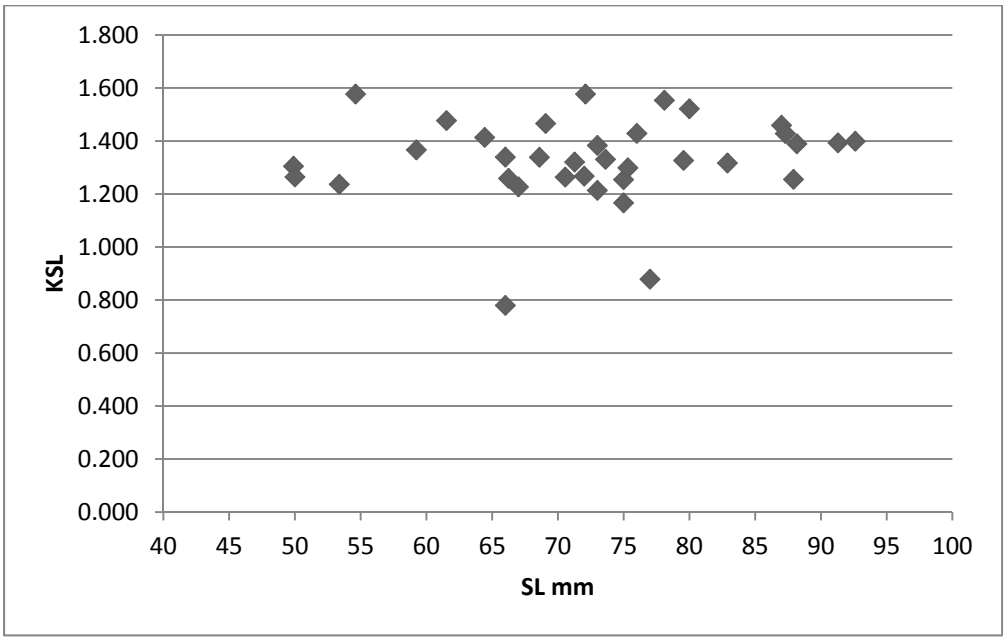

Figure 16. Relationship of standard length (SL, mm) to condition factor (KSL = Wt / $SL^3 x10^5$) of 35 winter-cage survivors.

On 02November, ten juvenile suckers (not identified to species) were collected from the A-canal during a salvage operation. Six of the ten were assayed for triglyceride and protein content as a comparison to the lake cage groups (Table 5). These fish were significantly larger (SL, Wt, and KSL) and had greater energy reserves (TG, Protein) than LRS sampled from lake cages on 04October (ANOVA P< 0.01). It is likely that these salvaged fish were 2 years of age.

Table 5. Mean (SD) standard length (SL), weight (Wt), condition factor (KSL = $Wt/SL^3 x10^5$), whole body triglyceride and protein content (mg / g fish) of 6 juvenile suckers salvaged from A-canal on 02November 2011.

SL (mm)	Wt (g)	KSL	TG	PRO
100 (15)	18.5 (9.2)	1.697 (0.186)	16 (4)	41 (3)

Liver malondialdehyde – The malondialdehyde (MDA) content of livers collected from lake cages on 8/31 and 9/21 were considered to be low (Table 6). We base this assertion on the low sample optically density values (~6.7x less than lowest standard of 1µM MDA) and the marked difference between 31August lake fish and 06September wetlab fry MDA content (Table 6). These wetlab fish had just received a formalin bath followed by a furan bath. We hypothesize that these oxidative stressors induced the higher liver MDA concentrations. In addition, no sign of lipofuscin was observed in adipose tissue of the histological sample set. Lipofuscin is a polymerized residue of oxidized lipid and protein, often associated with age damage but its presence is also a biomarker for oxidative stress (Cotran et al. 1989). We view this peroxidase data as preliminary as it is the first use of the OxisResearch LPO-586 assay kit in our laboratory. Using the same kit Almroth et al. (2008) reported on liver MDA content (nmol MDA/ mg protein) of trout exposed to a polluted river. We are unable to compare our values (standardized by tissue weight) to their data (standardized by protein content of sample). Both starvation and microcystin can produce elevated lipid peroxidation in fish liver (Morales et al. 2004, Jos et al. 2005).

Table 6. Liver lipid peroxidation values (µM malondialdehyde / g liver) for LRS fry collected from Lake cage 6 (42 days of exposure, 8/31/2011), Lake cage 5 (42 days of exposure, 9/21/2011), and the wetlab population on 9/6 and 9/19.

Group	date	no.	mean	SEM
Lake 6	31-Aug	8	9.02	1.30
Wetlab*	6-Sep	5	24.82	4.69
Lake 5	21-Sep	10	7.31	1.75
wetlab**	19-Sep	9	9.30	0.97

* received formalin and furan bath treatment same day of sample

** sampled 3 days after a single formalin bath treatment

Gill Na-K-ATPase - Adenosine Triphosphotase activity ranged from 1.1 – 5.6 µmole ADP/mg protein (Table 7). These values are reflective of salmon parr assayed in our laboratory. The data from the 04October Lake 8 cage is suspect due to unusual protein values (sample protein values of the same group ran on another plate were significantly lower than the suspect cage 8 values, T-test P<0.01). All samples from lake cage 6 collected 10August were lost due to a laboratory mishap. The 04October Lake cage 5 fish, with 42 d exposure, had higher activities (T-test, 22 df, P<0.001) than the Lake cage 7 fish with the full 76d exposure. All other groups of similar collection dates were similar to each other and an obvious inhibition was not observed in gill samples. Zambrano and Canelo (1996) report in-vitro inhibition of carp gill Na-K-ATPase activity by microcystin-LR. Conversely, Malbrouck et al. (2003) describe no such inhibition in goldfish injected with the toxin.

Table 7. Gill Na-K-ATPase activity (µmole ADP/mg protein/hr) for Lost R. sucker fry sampled from the wetlab, delta cages (1 – 4) and north lake cages (5 -8) between 8/8 and 10/4/2011. Data reported as sample number (N), mean, and standard error of the mean (SEM).

Sample Group	Sample Date	ATPase N	ATPase Mean	ATPase SEM
Wetlab	8-Aug	15	3.29	0.20
Delta 1	10-Aug	5	3.62	0.55
Delta 2	10-Aug	10	3.91	0.39
Delta 3	10-Aug	10	3.17	0.24
Delta 4	10-Aug	10	3.63	0.31
Lake 5	10-Aug	13	3.60	0.22
Lake 7	10-Aug	9	3.82	0.33
Lake 8	10-Aug	10	3.14	0.38
Delta 2	31-Aug	1	4.01	NA
Delta 3	31-Aug	2	4.68	0.35
Delta 4	31-Aug	11	4.04	0.28
Lake 6	31-Aug	11	3.94	0.36
wetlab	19-Sep	10	2.61	0.31
Lake 5	21-Sep	12	3.05	0.33
Lake 5	4-Oct	12	[a] 4.02	0.12
Lake 7	4-Oct	12	[b] 2.26	0.22
Lake 8	4-Oct	10	1.92**	0.25
**	suspect ATPase values			

Histological analysis – A total of 93 fry were examined by histology with 80 of these samples from sentinels held in the delta and lake cages. A majority of the samples were prepared as sagittal sections (shallow and mid-line sections) that allowed for examination of multiple tissues (Table 9). Evaluation of gill, kidney, and liver morphology in zinc formalin fixed tissues was not performed due to fixation artifacts however parasite infection could be detected. The following observations were made on the more limited Davidson' fixative sample set (31August – 04August sample set):

1. focal distribution of gill epithelial cell edema, however, no necrotic changes were seen (Figure 17);
2. large mitochondria-rich "chloride" cells (presumptive identification based on intense eosinophilic cytoplasm);
3. focal distribution of proximal tubule epithelial cells with hydropic droplets (excess protein reabsorbed from filtrate) in 4 fish collected on 27September and 04October (Figure 18); and
4. no necrotic changes in the intestine or liver.

Other histological data on infectious disease from the entire sample set of lake and delta sentinels:

1. no inflammatory response in any tissue indicative of a bacterial infection;
2. high prevalence (16 of 24 fish, 67%) of severe metacercaria infection (black spot) of the muscle and eye of fry sentinels held in the delta (Figure 21a);
3. low prevalence (15%) of asymptomatic *Myxobolus* sp. infection within the kidney tubules of lake sentinels (Figure 21b); and
4. external parasites (*Trichodina* sp. and *Ichthyophthirius multifiliis*) seen on the gill of only 2 delta sentinels (asymptomatic infection).

The degree of hepatocyte vacuolization varied between the sentinel groups (Table 8, Figure 19). Delta sentinels tended to have smaller hepatocytes without vacuoles with lake fish have either a mixed-type or characteristic fatty vacuole (Figure 20a-c). The prevalence of fat vacuoles (#2 rating) was highest in lake sentinels in August with some decline in vacuole content during the 27September and 04October sample. As determined by PAS staining of 12 livers from suckers collected over the experiment at both delta and lake sites, all livers contained scant to moderate quantities of glycogen indicating that vacuoles contained primarily fat (Figure 20d).

Table 8. Tissues examined in the 93 fish histological sample set. Data presented as number of sections containing specific tissue and percentage of specimens with specific tissue.

	no. samples	% of specimens
Olfactory gland	40	43
Skin	65	70
Muscle	65	70
Gill	93	100
Thymus	48	52
Eye	67	72
Brain	58	62
Liver	93	100
Kidney	89	96
Acinar	87	94
Intestine	90	97

Table 9. Prevalence (no. positive/total) of hepatocyte vacuolation rating 0,1, and 2 as well as infections by *Trichodina sp, Ichthyophthirius multifiliis* (Ich), presumptive *Myxobolus sp.* (myxosporean), and metacercaria in fry from wetlab (WL), lake (L), and delta (D) short-term(ST) and long-term(LT) cages .

	VAC 0	VAC 1	VAC 2		Trichodina	Ich	myxosporean	metacercaria
WL 8/9	5	0	0		0/5	0/5	0/5	0/5
D 8/10	16	2	0		0/18	0/18	0/17	12/18
L 8/10	0	13	5		0/18	0/18	0/17	0/18
D-ST 8/31	4	0	0		0/4	1/4	0/4	2/4
D-LT 8/31	2	0	0		1/2	1/2	0/2	2/2
L-ST 8/31	0	1	4		0/5	0/5	3/5	0/5
WL 9/19	3	5	1		0/9	0/9	0/9	0/9
L-5* 9/21	0	7	0		0/7	0/7	2/7	0/7
L-LT 9/21	0	6	0		0/3	0/3	0/3	0/3
L-5* 9/27	2	3	0		0/5	0/5	3/5	0/5
L-ST 10/4	3	7	0		0/10	0/10	0/9	0/10
L-LT 10/4	2	2	0		0/4	0/4	0/4	2/4**
** trematode attached to intestine epithelium								

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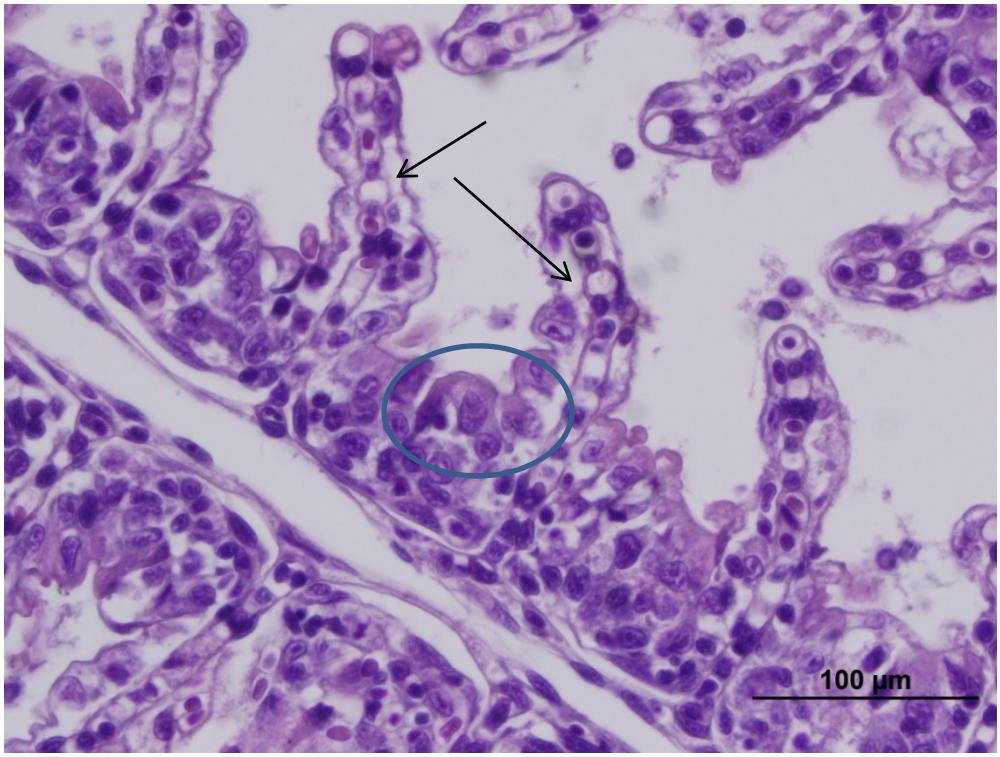

Figure 17 Gill section of 21September Lake 5 cage sucker (Davidson fixative) showing epithelial edema (arrow) and presumptive mitochondria-rich “chloride” cells (circle).

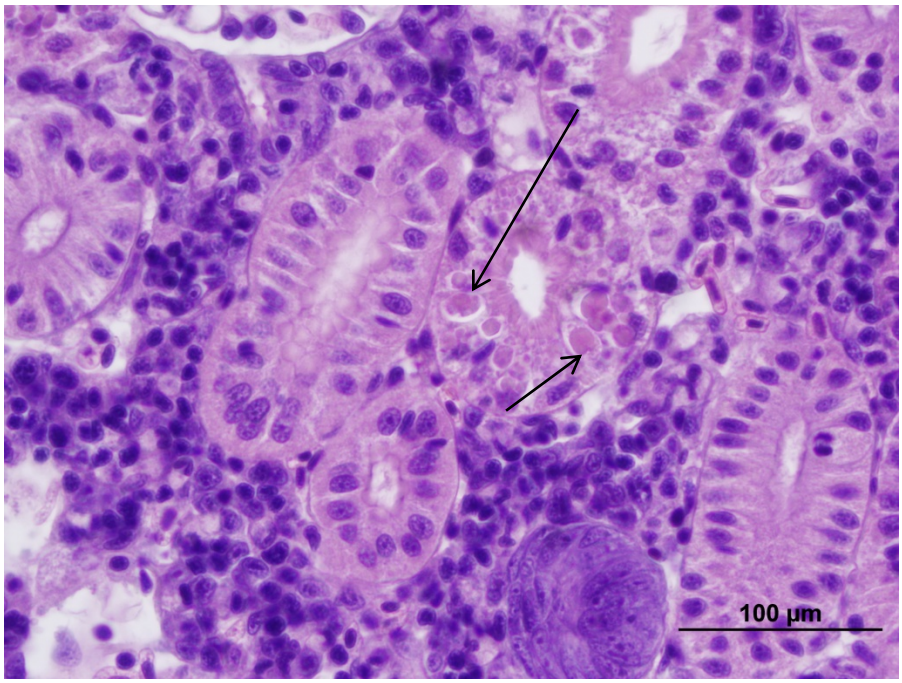

Figure 18. Hydropic droplets within epithelial cell of proximal convoluted tubule of 04October lake sentinel sucker.

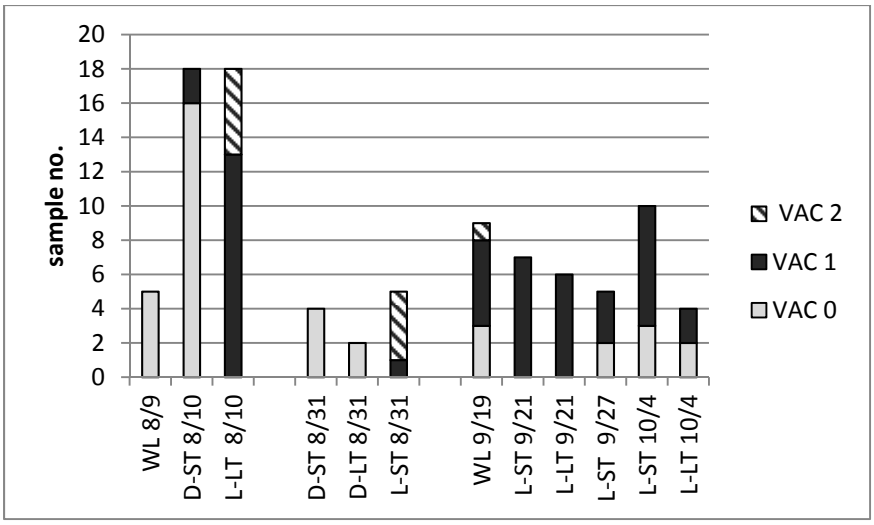

Figure 19. Hepatocyte vacuolization rating (vac 0,1,or 2) of liver sections from fry sampled in the wetlab (WL), long-term (LT) or short-term (ST) cages in the delta (D) or lake (L). Bar graph is of number of sections for each rating.

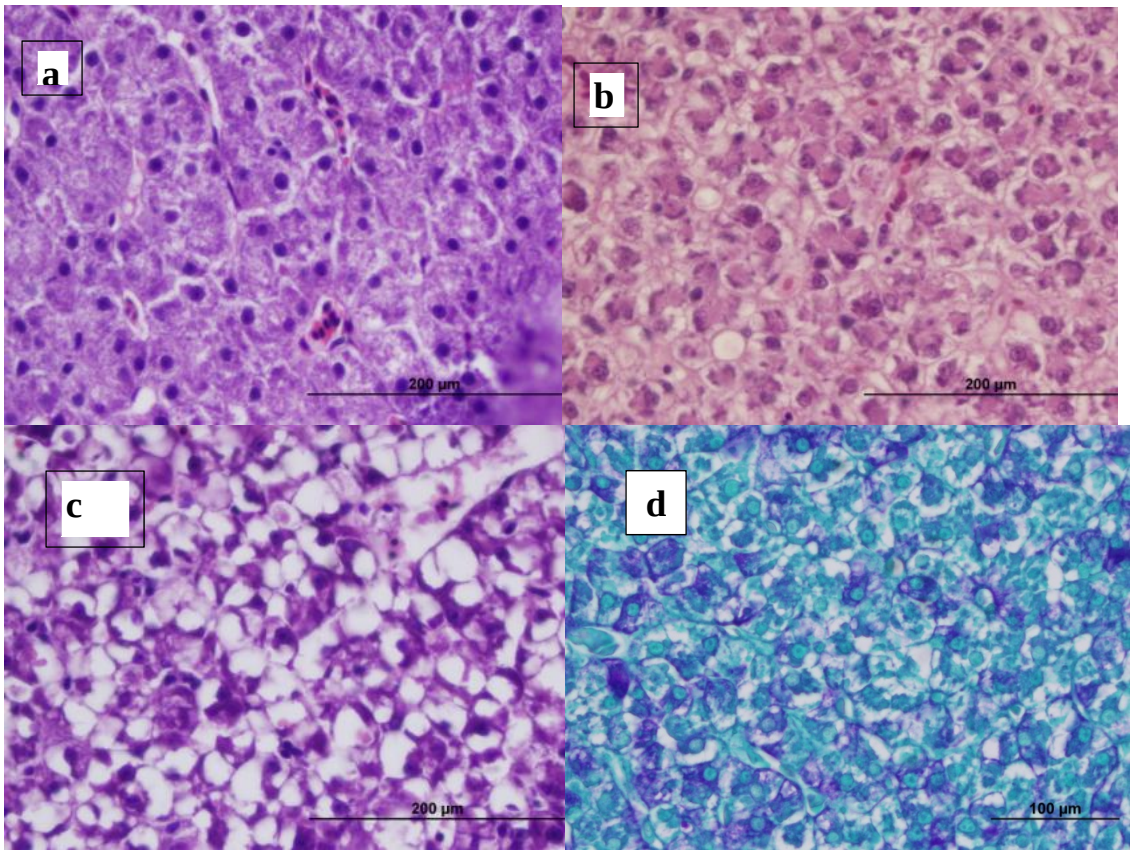

Figure 20. Hepatocyte vacuolization ratings: a) 0 rating – dense basophilic cytoplasm, b) 1 rating – large hepatocytes with cytoplasmic regions of light to no staining with “ragged” vacuoles, c) 2 rating – defined cytoplasmic vacuoles that likely contained fat, and d) PAS stain (glycogen stains purple) of a #1 rating liver.

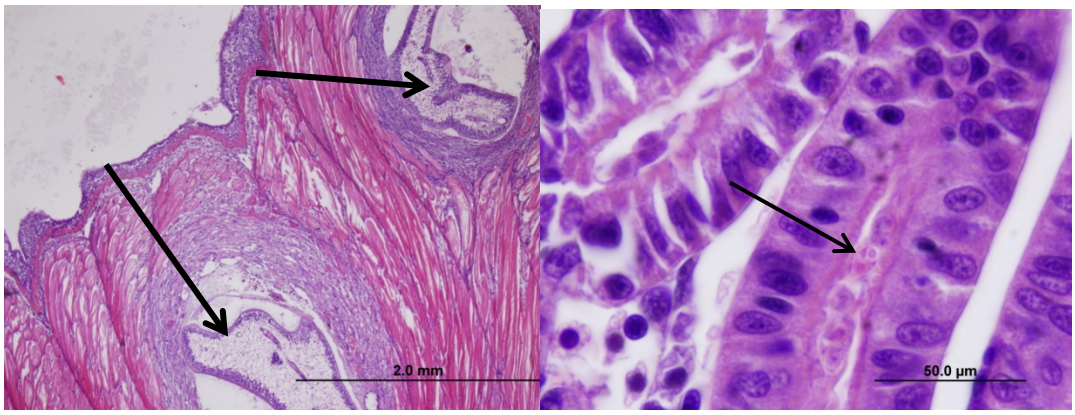

Figure 21. Cross section of metacercaria (blackspot trematode) in muscle of LRS held in delta cages (a). *Myxobolus sp.* spore with polar capsules (arrow), and spore and pre-sporogonic forms in kidney tubule of LRS held in lake.

There was no evidence of microcystin toxicity in the sentinels. A subset of zinc formalin fixed specimens (8) of lake sentinels, collected on 10August, 31August, and 21September, were stained with a monoclonal antibody to microcystin LR. As mentioned above liver necrosis was not observed nor was any MC-LR immunocytochemistry reaction seen in any liver sections. Despite peroxide pre-treatment, non-specific staining was observed in red blood and epidermal "alarm" cells. Interestingly, but not conclusive, MC-LR immunocytochemistry reaction was observed in the brush border of the proximal convoluted tubule cells of all lake sentinels as well as the epithelial cells of the posterior intestine of 3 sentinels (Figures 22 &23). These reactions were not seen in the single wetlab "negative" control section.

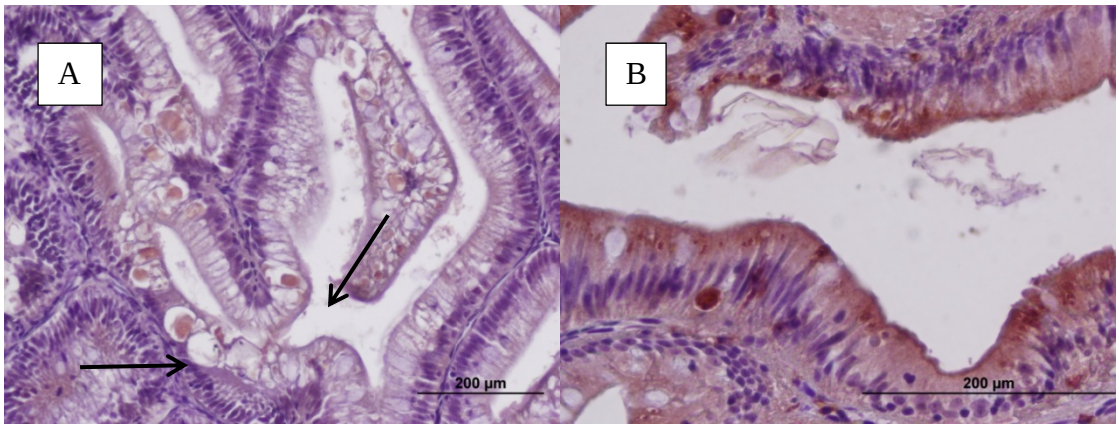

Figure 22 Stain reaction of intestine to monoclonal antibody for MC-LR toxin. A) positive (MC-LR gavage challenged Chinook) control showing red-brown reaction in vacuoles within epithelial cells (arrow), and lake cage sucker (8/10/2011) intestine with red-brown staining limited to the apical region of epithelial cells.

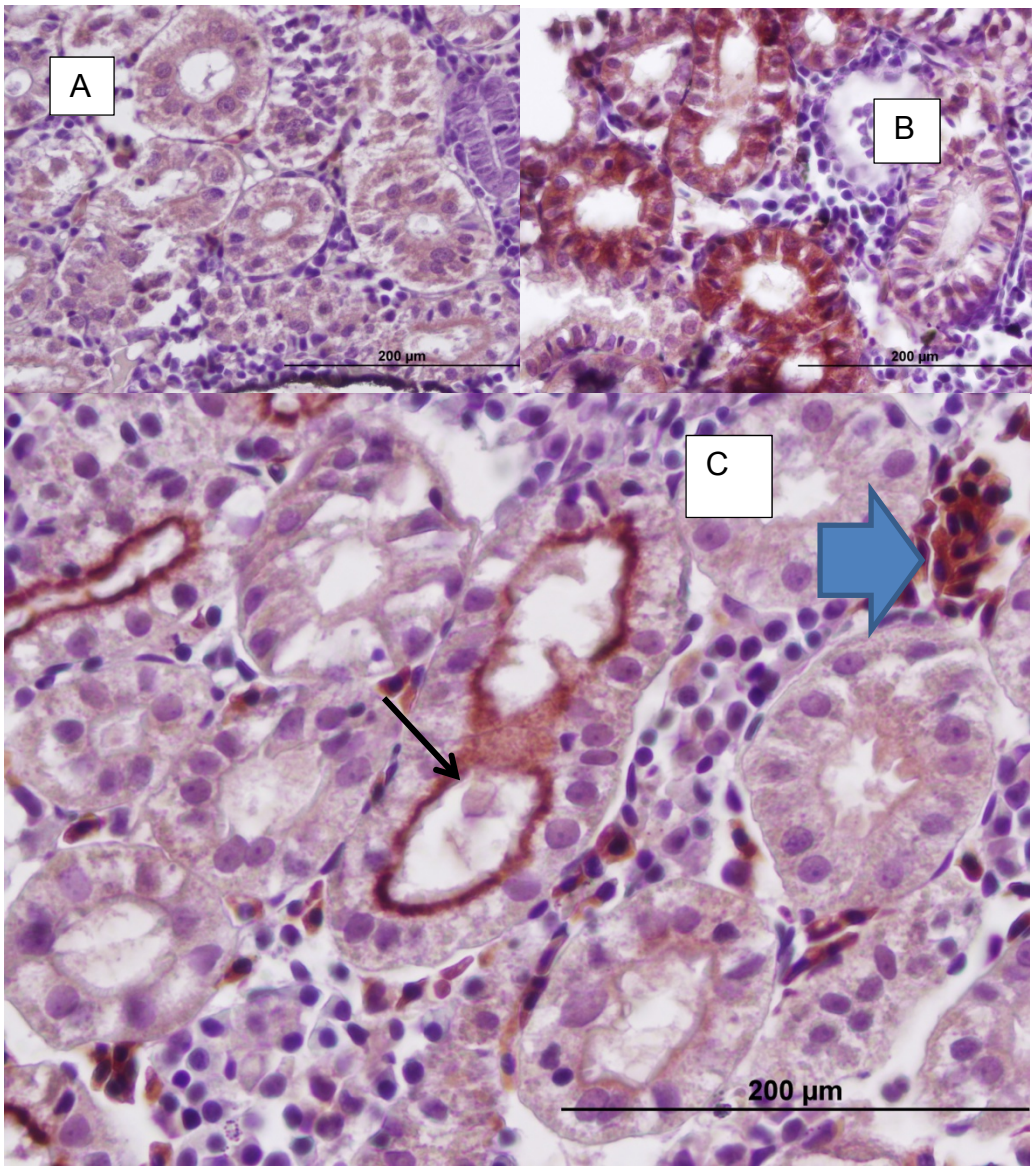

Figure 23. Kidney tubule staining reactions for monoclonal antibody to MC-LR toxin. A) Negative control (wetlab LRS), B) positive (MC-LR gavage challenged Chinook) control showing red-brown reaction in the tubule epithelial cell, and C) Lake cage sucker (8/10/2011) kidney with red-brown staining limited to the proximal tubule brush border (arrow). Note that red blood cells (thick arrow) have a non-specific stain reaction.

Discussion

Lost River sucker fry were reared from fertilized eggs, transported to Upper Klamath Lake, and held in cages with various survival rates. The use of captive sentinel fish provided an experimental population of similar characteristics to evaluate survival and growth. Areas of culture that will require modification in future work include upwelling egg incubators to reduce fungus infection and alternative diet(s) to promote growth. Fry show poor acceptance of the Otohime marine larvae diet. The cause of the late September neural disorder observed in the captive population was not identified however fish moved to the lake on 21September had high survival and did not demonstrate any symptoms (Appendix 1). Immediate mortality upon placement into alkaline lake water did not occur in this study. This observation indicates that the short pH acclimation during transport was sufficient however sentinel group survival needs to be monitored in a shorter time frame to link mortality with handling or transient water quality events. This evaluation will require the use of smaller cages that can be easily emptied by a 2 person crew. Our survival and growth results cannot be directly applied to free-ranging juvenile suckers in the lake or delta. While mesocosm cages provide protection from predators, they can also limit food availability and ability to move from impaired water quality. Comparison of food availability and selection of food items was not feasible in this pilot effort as our zooplankton sampling methods did not effectively capture the organisms that were ultimately selected as food items by the fish. Generally, diet contents coincide with Markle and Clauson (2006). They observed the predominant component of midges taken were larvae and within Cladocera and Copepoda representatives of Chydoridae and Cyclopoida were much more common than other members of these groups.

Delta cage fry – Low invertebrate abundance within delta cages was probably a significant factor in low survival of delta sentinels in August. While size and energy reserves were similar between delta and lake fry samples on 10August, low carcass TG, protein, and condition factor of 31August delta long-term cage survivors indicates starvation during the latter half of the month. Similarly, over 80% of the delta fry liver sections showed little to no hepatocyte vacuolization indicative of marginal fat storage within the liver. Water temperatures of 17 - 22ºC and wide diurnal pH fluctuations of approximately 7.1 to 9.6 would likely exert a strong energy demand on the fry and accelerate starvation. Low dissolved oxygen (<2.0 mg/L) occurred 5 days prior to the 31August sample and may have caused additional mortality. Hypoxic conditions were the likely reason we observed fry near the water surface on 31August. Juvenile Lost River suckers have an LC_{50} for dissolved oxygen concentration of 1.3-2.1 mg/L, whereas LC_{50} values are relatively high for pH (10.3-10.4), un-ionized ammonia (0.5-1.1 mg/L) and water temperature (30.4-31.8°C) (Saiki et al. 1999). The high incidence of metacercarial (black spot) infection in delta sentinels is reflective of the trematode lifecycle that include a snail and bird host. The delta site has large populations of both hosts and thus supports high

parasite infectivity. We cannot ascribe a health effect to metacercarial infections however histological specimens tended to contain multiple metacercaria in relatively small (< 40mm) fry. External parasites were observed in 3 of 24 delta sagittal sections but were not associated with severe lesions. Overt disease was either missed due to the sampling schedule or played only a minor role in the high loss of delta fish. It also possible that predation by Odonate larvae could have occurred as dragonfly nymph exoskeletons were seen within the cages.

Lake cage fry - Survival of lake groups was markedly higher than delta groups however survival of short-term cage fish during the 20July to 10August period was lowest of the lake exposures. Billman and Belk (2009) report similar survival (mean 45.5%) for June sucker fry reared in cages as seen in the August short-term cage groups ($\geq$ 48%) but lower than several later exposure groups. Water quality during August was characterized by elevated (but not lethal) water temperatures (18 - 26ºC), alkaline (8.0 – 10.2 pH, means of 9.5 and 9.0 below LC_{50} values), and hyperoxic with wide (> 5 mg/L range) diurnal swings. Maximum dissolved oxygen values of 17.6 and 25.3 mg/L during the two August exposures are supersaturated for this temperature. It is unclear whether fry rearing in the 3 m deep cages experienced any barometric stress however no clinical signs of supersaturation trauma were seen. Future studies should monitor total gas supersaturation in the cage water column. Loading density appeared to influence growth rate with the 100 fry short-term cage fry growing at higher rates than 300 fry long-term cage cohorts in August. Density effects on survival were not obvious but intuitively, limited food resources in an energy-demanding environment would reduce population survival. Specific growth rate, of surviving fry in long-term cages, increased markedly during September (6.9% BW/d compared to 2.3 and 3.4% during August). In 2009, juvenile suckers were examined from a rotary screw trap in the Link River between mid-July and early September (Foott et al. 2010). These out-migrant juveniles tended to larger than the cultured fry used in our 2011 mesocosm study however both condition factor and whole body triglyceride were similar. Mean TG values in late August – early September 2009 ranged from 7.6 to 15.1 mg/g fish while mean TG in long-term lake juveniles ranged from 6 – 11 mg/g fish during the same period. Condition factor (KSL) for both groups was $\geq$ 1.37 and a value that likely represents a juvenile of good fitness. The moderate correlation (r^2 = 0.63) of KSL with triglyceride reserves suggests that juveniles with a KSL $\geq$ 1.37 have adequate fat. Conversely, a condition factor of $\leq$ 1.0 was associated with low triglyceride content and delta cages with low survival. The high survival and triglyceride content of “winter” cage juveniles suggest that fish of high condition factor would have high overwinter survival. Future studies on overwinter survival could be evaluated from PIT tagged sentinels of various sizes held in benthic cages that would not be affected by ice. The relatively high survival through the summer of cage suckers suggests that predation could be an important factor in low recruitment and warrants attention on avian predation rates for 0+ suckers.

Disease – Neither infectious nor non-infectious disease was observed in the lake cage suckers throughout the experiment. Prevalence of parasite infection (metacercaria or "blackspot" (28%), *Lernaea (*17%), *Trichodina sp.(*63%), and myxozoans(37%)) was markedly greater in the 2009 Link R. out-migrants compared to the lake cage fry (Foott et al. 2010). We speculate that metacercarial and external parasite infections are more likely in the near shore environments as the delta fry had a high prevalence and severity of metacercaria (blackspot) infection and limited external parasite infections. While parasite infection is common in Upper Klamath Lake juvenile fish (Foott and Harmon 1999, Foott and Stone 2005), the majority of these histological specimens do not show severe inflammatory responses suggesting clinical disease. Similarly, we did not detect abnormal liver peroxidation levels indicative of oxidative stress, depressed gill Na-K-ATPase activity, or immunoreactive microcystin LR in the study fish. Vanderkooi et al. (2010) reported that microcystin toxin (MC-LR) was detected in sediment and both gut content and liver tissue of juvenile suckers from Upper Klamath Lake during 2007. They speculated that toxicity could be a juvenile mortality factor. The sampling of survivors at 3 week intervals limits our confidence on excluding disease as a source of juvenile mortality. Future studies need to examine sentinels at shorter time intervals throughout the summer.

In this study we attempted to focus on non-predator mortality factors and observed $\geq$ 48% survival of LRS fry held in north lake cages through the summer. Additionally, these fish had a positive growth rate with adequate energy reserves to survive for 67d under fall and winter conditions. While no definitive conclusions were reached on sources of lake cage sucker mortality, water quality during August is a likely factor and this period could be a critical point for juvenile survival.

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Appendix 1
Exposure period summaries and wetlab population neural episode.

July 20 – August 10 (21d): Fry held in the delta incurred a high loss with short-term cage (D4) having a 75% loss in this time period. A total of 17 mortalities, without obvious external signs of disease, were recovered from the delta cages on 10August. The north lake short-term cage (L5) had 52% mortality in this exposure (Figure 2). Delta fry had a high prevalence of metacercaria infection (blackspot) and little fat within hepatocytes (little to no vacoulation). Initial stocking number appeared to influence growth in both the delta and lake cages. Short-term cages of 100 fry had higher growth rates than cohorts from the 300 fry long-term cages. For example, the mean SL increase of long-term Delta cages (D1-3) was 0.06mm /d compared to cage (D4) growth rate of 0.12mm/d. Whole body TG was similar in both delta cage sample groups (Kruskal-Wallis ANOVA, P=0.494). Similarly, fry in cage (L5) were larger and had higher condition factor and TG than cohorts in (L6 – 8). Mean length increase was 0.16mm/d in long-term cages and 0.41mm/d in L5 (2.6x higher rate). Delta fry were smaller than cohorts in the lake. The short-term cages were significantly different (Mann-Whitney rank sum test, P< 0.05). Whole body triglyceride content of both delta and lake fry dropped ~ 2x (14 to 6.6 mg/ g fish) from the initial loading on 20July to the 10Aug sample despite their increase in length. Apparent food limitations in delta cages was apparent in the high coefficient of variation in SL (17%) and TG (105%) of survivors sampled on 10August . Whole body protein content was lower in the delta fish compared with lake fry (L5 > L6 – 8> D1-4).

Water temperature ranged from 18.0 – 25.9.ºC in the lake and 16.8 – 22.4 ºC in the delta. Algal and macrophyte photosynthesis influenced the diurnal water quality at both sites. Temperatures did not reach the median tolerance limit of 30.4-31.8°C identified for juvenile Lost River suckers by Saiki et al. (1999), but temperatures did reach the low level stress threshold of 25ºC used by Loftus (2001) for testing stress in suckers. Dissolved oxygen ranged from 3.4 – 17.6 mg

/L at the two sites which reflects the hyperoxic conditions of the photosynthesis processes (Figure 4). It is unclear whether the fish experience supersaturation (total gas pressure > 110%). Minium DO concentratins at both sites dipped below the high stress threshold of 4.0 mg/L used Loftus (2001). Diurnal pH ranged from 8.74 – 10.13 in the lake and 7.23 – 9.66 in the delta. Delta fish experienced a daily change of > 2.0 pH units (Fig. 5). High temperatures and wide pH changes were likely energy demanding for the fry during this time period.

August 10 – 31 (21d): Similar to the first exposure, delta fry in the short-term cage incurred a high mortality of 87% in August. Only 4 live fish were collected from long-term cages D2 and D3 (<1% survival from the 10Aug). No live fish were found in D1. In error, the long-term cage (L6) was completely sampled (65% survival for the 42d exposure) while the short-term cage (L5) was only sub-sampled (26 live and 11 dead fry removed). Due to this sampling scheme we can only report the minimum survival of 66% (26 on 10Aug and 40 on 21Sep = 66 of 100 fry) for L5 on 31August. Fry in the lake had positive growth over this period with long-term cage fish having a specific growth rate of 3.4% bodyweight / d. Both triglyceride and protein content increased in long-term lake fry. In particular, this time period had the peak protein values (mean of 53 mg/ g fish) of the experiment which could indicate muscle growth.

Food limitation and cage population again may have influenced growth. The 100 fry short-term cage had a 0.5mm/d increase in length (0.32mm/d for long-term fry) and greater TG content than long-term cohorts (Figure13).. Condition factor did not change from the first exposure. The four survivors collected from the long-term delta cages (D2 and 3) had the lowest TG (mean 1.7 mg/g fish) and KSL (mean 0.95) of the experiment. This data supports the assertion that starvation was a dominant factor in the delta cages. No further fry transfers into delta cages were performed. Histological examination of fry from the short-term cage (D4) again demonstrated a high prevalence of metaceraria infection and little hepatocyte lipid storage. The gill of 2 fry also asymptomatic infections with the ectoparasites *Ichthyophthiruis multifiliis* and *Trichodina sp.* Similar to the first exposure, water quality in the northern lake was characterized by high temperature (mean 21.7ºC, 19.6 – 24.4ºC), alkalinity (mean pH 9.14, pH 8.03 – 10.15), and wide diurnal changes in dissolved oxygen (4.4 – 25.3 mg/L) due to photosynthesis. By the second week of the 3 week exposure, delta fry experienced hypoxic conditions (<2 mg / L dissolved oxygen) with wide diurnal changes in pH (7.05 – 9.25) occurring during the first 2 weeks. During the 31August sample we observed fry on the surface with a dissolved oxygen concentration of 1.7 mg /L at this time.

August31-September 21(21d): Due to the sampling error on 31August, no survival data was generated for this time period. As mentioned above, 40 live juveniles were sampled from cage L5 on 21September and we collected 22 live

fish from both cage L7 and L8. Juveniles continued to grow and add energy reserves over this period of relatively good water conditions. Whole body TG increased from the 31August level to a mean of 9.7 mg/g fish and condition factor reached a peak for the summer (mean 1.67). Similarly, the peak specific growth rate of 6.9% BW/d occurred in this time period with length increase of 0.97 mm/d. Interestly, whole body protein content decreased from the 31August sample group to a mean of 44 mg/g fish. Water temperature ranged from 16.7 – 22.2ºC and a moderate pH range of 7.83 – 9.52 (mean 8.89). There were relatively wide diurnal changes in dissolved oxygen (4.4 – 15.6 mg/L, mean 8.3) due to photosynthesis.

September 21 – October4 (13d): A neural disorder episode in the wetlab population on 30September (Appendix 1 below) prompted an early sample of the cages on 4October (13d of exposure). There was 100% survival in the short-term cage group that had been loaded on21September however little growth occurred (0.5 mm/d). Whole body TG and protein declined from the wetlab population over the 13d period. Sixty-eight juveniles were obtained from long-term cage L7 and 45 from cage L8. Recovery of live fish over the entire 76 day experiment was 50% in L7 and 41% in L8. Growth continued over this period (1.14 mm/d) but the SGR declined to 4.2% BW/d (Fig.12). Condition factor was similar to previous sample groups and TG increased to a mean of 12.4 mg/g fish. Whole body protein content continued to decline. Water temperature declined from 20 to 12ºC with dissolve oxygen ranging from 7.3 – 15.9 mg/L and pH range of 8.34 – 9.56 (mean 9.06).

October4 – December 9 (67d): On 04October, thirty six juveniles were transferred from the long-term cages at the north lake location to a cage in the south lake (Figure 1). Due to ice buildup, the cage was completely sampled on 09December for survival, gut content analysis, morphometrics, and energy measurements. Water quality was judged to be good but cold (1.4 – 14.3ºC). Thirty five live fish were collected (97% survival over the 67 d period) and included 5 fish $\leq$ 60 mm SL and 2 fish with condition factors < 1.0. No appreciable growth (0.1% BW/d) occurred but condition factor and TG remained at 04October levels. Protein content was similar to the 21September group. The ability to track juveniles of various sizes through the winter would be informative to forecast over-winter survival.

Wetlab population neural episode- On 26September, we observed a presumptive neural disorder (hyper-activity, tetany (Figure 25), twirling behavior) in the main captive population held in one tank. Mortality rose rapidly on 30September (Figure 24.) We performed the following actions:

1. Sampled and destroyed the population on 03October (1 week after first symptoms). A smaller group (~40) that had been held in different tanks was used in an investigative diet trial on the disorder.

2. On 04October, completely sampled all sentinel fry placed into Upper Klamath Lake on 21September (100% survival and showing no symptoms).
3. Assayed fish for virus, bacteria, and parasites.
4. Examine tissues by light and electron microscopy as well as tissue imprints and blood smears.
5. Sampled wild sucker juveniles from Upper Klamath lake and Lake Ewauna on 02November2011 (0 of 20 spleen imprints with characteristic inclusion cell)

No definitive diagnosis was made for the condition. It does not appear that an infectious agent was involved (i.e. no inflammation, viral isolation or parasite detection, occurrence of symptoms in naïve aquarium fish injected with spleens of affected LRS). Fry moved to the lake on 21September were apparently unaffected by the condition as they showed no symptoms and had 100% survival over the 13d exposure. The observation of large mononuclear cells with inclusions (Figure 26a) within the spleen of some affected fish does not appear to be relevant to the disorder. An initial concern was that the inclusions could be rickettsial or viral in nature. This microbial origin was not supported by transmission electron microscopy (TEM), histology, tissue culture assays, or a transmission trial with Plecostomas. Normal granulocytes were observed in the TEM and histological materials (Figure 26b). Due to poor feeding response in August, a new diet was fed in early September. Our current hypothesis is that the condition is related to either nutrient imbalance or toxicity associated with frozen brine shrimp and/or the Nutrifin^{tm} pellet diet. A less likely hypothesis is delayed toxicity from August and early September treatments for columnaris and ichthyobodiasis.

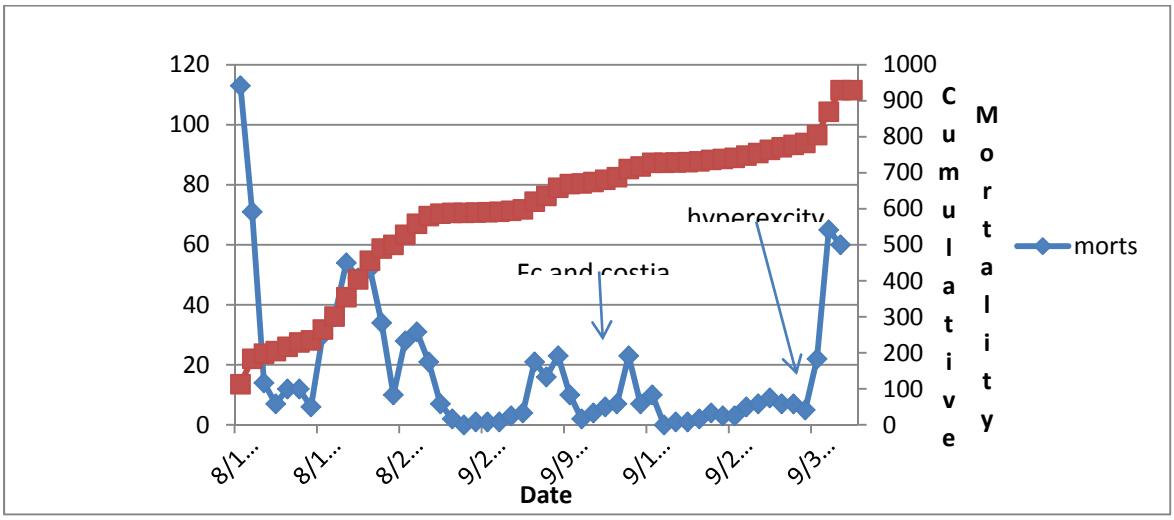

Figure 24. Mortality graph for LRS wetlab population.

Figure 25. Characteristic spasm with lateral flexure “S” shape

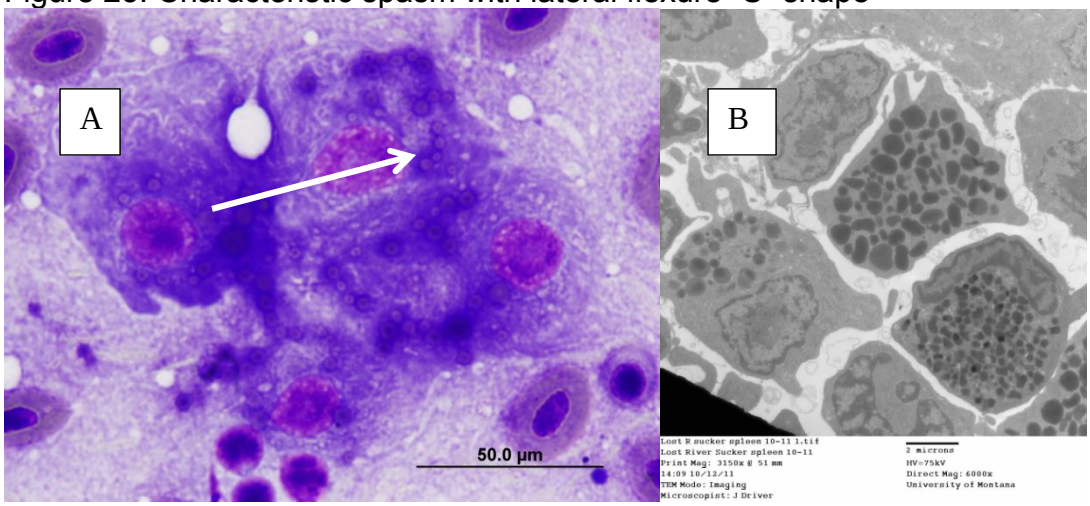

Figure 26. A) Spleen imprint from symptomatic LRS on 9/30 showing large basophilic cell with inclusion (arrow), B) Transmission electron micrograph of spleen cell with inclusions.

Ten sub-adult suckers were obtained from A-canal (02November) and lake Ewauna (01November). Our primary objective was to survey feral suckers for the characteristic monocyte with inclusions seen in the wetlab fry undergoing the neural disorder. No such cells were observed in 20 spleen and kidney imprints. Six suckers from A-canal were assayed for TG and protein. These fish were likely 2 years of age given their large size (mean SL 100.7mm). They had high condition factor (mean 1.697) and triglyceride content (16.4 mg/g fish).

Appendix 2. Counts of zooplankton collected within mesocosms (colorless rows) and random ambient samples nearby the mesocosm set (grey-shaded rows). Samples were collected with a Wisconsin-style Plankton sampler and the entire sample was uniformly distributed in a known volume of water (60 ml) and sub-sampled (5 ml). The number from each taxa in the 5-ml sub-sample is shown here. Samples that were collected but lost due to equipment failure are indicated with empty rows.

Date	Site	ID	Amphipoda	*Daphnia*[1]	Harpacticoida[2]	Ostracoda	Orthocladiinae[4]	Rotifera	*Chydorus*[1]	Cyclopoida[2]	Nauplii[3]	Chironominae[4]	Tanypodinae[4]
20-Jul	GB		0	24	3	28	0	53	0	0	2	2	12
20-Jul	GB		0	87	0	24	0	29	5	7	2	9	27
20-Jul	GB		1	72	7	70	0	7	0	0	6	15	2
26-Jul	GB	1	0	1	3	0	0	31	3	0	0	0	32
26-Jul	GB	2	0	1	2	5	0	61	4	0	0	0	14
26-Jul	GB	3	0	1	0	0	0	5	1	0	0	0	4
26-Jul	GB		0	51	4	21	0	11	3	0	0	2	5
26-Jul	GB		0	26	0	41	4	48	3	2	3	3	57
26-Jul	GB		0	28	3	26	6	72	3	3	0	0	63
26-Jul	NL	6											
26-Jul	NL	7	1	0	31	0	1	1	1	3	0	0	0
26-Jul	NL	8											
26-Jul	NL		1	8	74	6	10	45	3	1	0	0	13
26-Jul	NL		0	0	23	2	2	3	0	0	0	0	0
26-Jul	NL		0	0	23	0	0	2	2	0	0	0	3
4-Aug	GB	1	0	8	13	3	2	26	1	0	0	0	6
4-Aug	GB	2	0	1	11	6	0	22	3	1	2	1	5
4-Aug	GB	3	1	1	1	2	0	2	0	0	0	0	0

[1] Genus of the order Cladocera
[2] Order of the subclass Copepoda
[3] Larvae of the Class Crustacea
[4] Subfamily of the Dipteran family Chirnomidae

Date	Site	ID	Amphipoda	*Chydorus*[1]	*Daphnia*[1]	Cyclopoida[2]	Harpacticoida[2]	Nauplii[3]	Ostracoda	Chironominae[4]	Orthocladiinae[4]	Tanypodinae[4]	Rotifera
4-Aug	GB		0	12	3	9	0	1	0	0	3	10	3
4-Aug	GB		0	45	0	6	0	4	0	0	6	6	10
4-Aug	GB												
4-Aug	NL	6	0	0	3	0	0	0	0	0	0	0	0
4-Aug	NL	7	0	0	42	5	1	0	0	0	0	0	0
4-Aug	NL	8	0	0	2	0	0	0	0	12	1	0	0
4-Aug	NL		0	0	73	3	0	0	0	0	0	0	0
4-Aug	NL		0	0	57	1	1	0	0	0	0	0	0
4-Aug	NL		0	0	94	2	0	0	0	0	0	0	0
12-Aug	GB	1	0	10	15	6	1	25	4	0	0	0	6
12-Aug	GB	2	0	11	2	5	0	8	2	0	2	2	1
12-Aug	GB	3	0	9	0	7	0	18	1	0	0	2	8
12-Aug	GB		0	8	3	0	2	6	0	1	0	0	6
12-Aug	GB		0	0	0	0	0	3	0	0	0	0	0
12-Aug	GB		1	11	1	10	0	21	1	0	0	0	21
12-Aug	NL	6	0	0	8	0	0	0	0	0	0	0	0
12-Aug	NL	7	0	0	40	0	0	0	3	0	0	0	0
12-Aug	NL	8	0	0	2	0	0	0	0	0	0	0	0
12-Aug	NL		0	0	21	0	1	0	0	0	0	0	0
12-Aug	NL		0	0	32	0	1	0	0	0	0	0	0
12-Aug	NL		0	27	0	1	3	0	0	0	0	0	0
16-Aug	GB	1	0	2	0	0	0	13	0	0	0	0	1
16-Aug	GB	2	0	6	3	3	0	19	0	0	0	0	1
16-Aug	GB	3	0	4	3	6	0	14	1	3	0	2	6
16-Aug	GB		0	3	5	4	0	11	7	0	0	0	4
16-Aug	GB		0	7	2	7	0	18	3	0	0	0	19
16-Aug	GB		0	0	3	6	0	12	5	0	0	0	4
16-Aug	NL	6	0	0	20	0	0	19	0	0	0	0	5
16-Aug	NL	7	0	4	42	1	0	0	0	0	0	0	0

Date	Site	ID	Amphipoda	*Chydorus*[1]	*Daphnia*[1]	Cyclopoida[2]	Harpacticoida[2]	Nauplii[3]	Ostracoda	Chironominae[4]	Orthocladiinae[4]	Tanypodinae[4]	Rotifera
16-Aug	NL	8											
16-Aug	NL		0	0	17	0	0	0	0	0	0	0	4
16-Aug	NL		0	0	17	0	2	2	0	0	0	0	1
16-Aug	NL		0	1	22	3	3	7	0	0	0	0	0
24-Aug	GB	1											
24-Aug	GB	2											
24-Aug	GB	3	0	0	13	2	0	6	0	0	0	0	4
24-Aug	GB												
24-Aug	GB												
24-Aug	GB												
24-Aug	NL	6											
24-Aug	NL	7	0	0	0	1	0	4	0	0	0	0	0
24-Aug	NL	8	0	0	25	2	0	6	0	0	0	0	7
24-Aug	NL												
24-Aug	NL		0	0	16	2	0	1	0	0	0	0	2
24-Aug	NL		0	0	2	2	0	6	1	0	0	0	5
31-Aug	GB		2	77	0	64	2	5	6	0	0	0	61
31-Aug	GB		4	26	13	42	0	0	2	0	0	0	49
31-Aug	GB		3	11	0	30	2	35	0	1	1	0	26
31-Aug	NL		2	0	3	2	1	1	1	0	0	0	2
31-Aug	NL		0	0	2	3	0	6	3	0	0	0	0
31-Aug	NL		0	0	9	1	0	0	0	0	0	0	3
31-Aug	NL	7	0	1	5	1	1	1	0	0	0	0	1
31-Aug	NL	8	0	0	6	1	0	2	0	0	0	0	1
7-Sep	GB	1	0	20	16	15	0	6	3	0	0	0	8
7-Sep	GB	2	1	76	61	15	11	4	11	0	0	0	13
7-Sep	GB	3	0	19	6	12	0	40	1	0	0	0	2
7-Sep	GB		0	0	0	0	0	2	1	0	0	0	6
7-Sep	GB		0	82	76	29	0	14	12	0	1	1	23

Date	Site	ID	Amphipoda	*Chydorus*[1]	*Daphnia*[1]	Cyclopoida[2]	Harpacticoida[2]	Nauplii[3]	Ostracoda	Chironominae[4]	Orthocladiinae[4]	Tanypodinae[4]	Rotifera
7-Sep	GB		0	4	1	0	0	1	1	1	0	0	4
7-Sep	NL	6	0	0	4	0	0	1	0	0	0	0	0
7-Sep	NL	7	0	0	3	2	0	4	0	0	0	0	2
7-Sep	NL	8	0	4	1	1	0	5	0	2	1	0	1
7-Sep	NL		0	0	3	1	0	0	1	0	0	0	2
7-Sep	NL		0	5	9	1	1	4	4	0	0	0	2
7-Sep	NL		0	0	8	1	0	14	0	0	0	0	4