

FY2004 Report:

Blood cell and plasma chemistry profile of adult Snortnose Sucker (*Chasmistes brevirostris*) during a blue-green algal bloom: USFWS – USGS cooperative live cage study in Upper Klamath Lake, July 2004.



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Background- In support of the United States Geological Survey (**USGS**) monitoring program for both endangered suckers and water quality in Upper Klamath Lake, the California – Nevada Fish Health Center (**FHC**) conducted a cooperative study on physiological responses of adult suckers to extreme lake alkalinity (pH > 9.5) during an algal bloom. Blue green algal (*Aphanizomenon flos-aquae*) blooms during the summer are often associated with high pH (> 9.5), low dissolved oxygen levels (<2ppm after bloom crash), and elevated ammonia concentrations in the lake. These periods have been associated with fish kills in the recent past. This work was partially funded by the US Bureau of Reclamation (interagency agreement 04AA204033).

Methods

In late June 2004, water quality monitoring by USGS determined that lake pH was markedly elevated in the northern portion of Upper Klamath Lake due to the initial algal bloom of the summer. Two sites with Hydrolab DataSondes™ (GP5 and GP12) were selected for live cage deployment based on their initial difference in pH (Figure 1). Cages were held at the same depth and within 75 m of the dataSondes.

Adult Shortnose suckers (**SNS**), *Chasmistes brevirostris*, were captured alive by trammel nets. The nets were fished for 4 hours on the night of 29 June near the Odessa creek region of Pelican Bay. Nets were examined at one hour intervals and captured fish were transported to the exposure sites and placed into live cages (1m lengths of 0.5 m diameter PVC pipe enclosed with 5 mm mesh) suspended 1 m off the bottom by a float and anchor system. On 2 July and 8 July, fish were sampled at both sites and live specimens released back into the lake. Scales and otoliths were obtained from mortalities. An average time between live cage retrieval and blood collection was 5 minutes and ranged from 3 – 7 minutes. Fish were lightly anesthetized with MS-222, measured for fork length, the species and sex recorded, examined for external abnormalities (gill rot, hemorrhage, sores, copepod and leech infection), a 10 µL bacterial loop rubbed against the first gill filament and the mucus placed into a 40µL pH 6.0 Phosphate buffer vial for lysozyme activity assay, a non-lethal gill lamellae clip (< 10 mg) placed into a 100µL of SEI buffer vial for gill ATPase assay, and a blood sample collected from the caudal vessels with a 1cc heparinized syringe. The blood sample was processed immediately by removing 20µL samples from the syringe with a sterile pipette to perform the following:

1. inoculate a slant tube of Brain Heart Infusion agar for bacterial isolation,
2. prepare a blood smear for differential leukocyte counts (absolute methanol fixed and later stained with a Diff-Quick™ kit),
3. load a microhematocrit tube for determining hematocrit (STATSPIN centrifuge)
4. load a 380 µL tube of Rees-Ecker fixative (20x dilution) for total white cell counts by hemocytometer,
5. hemoglobin assay (frozen on dry ice).

The remaining blood was centrifuged for 3 – 5 minutes and 2 aliquots of plasma frozen on dry ice for clinical chemistry.

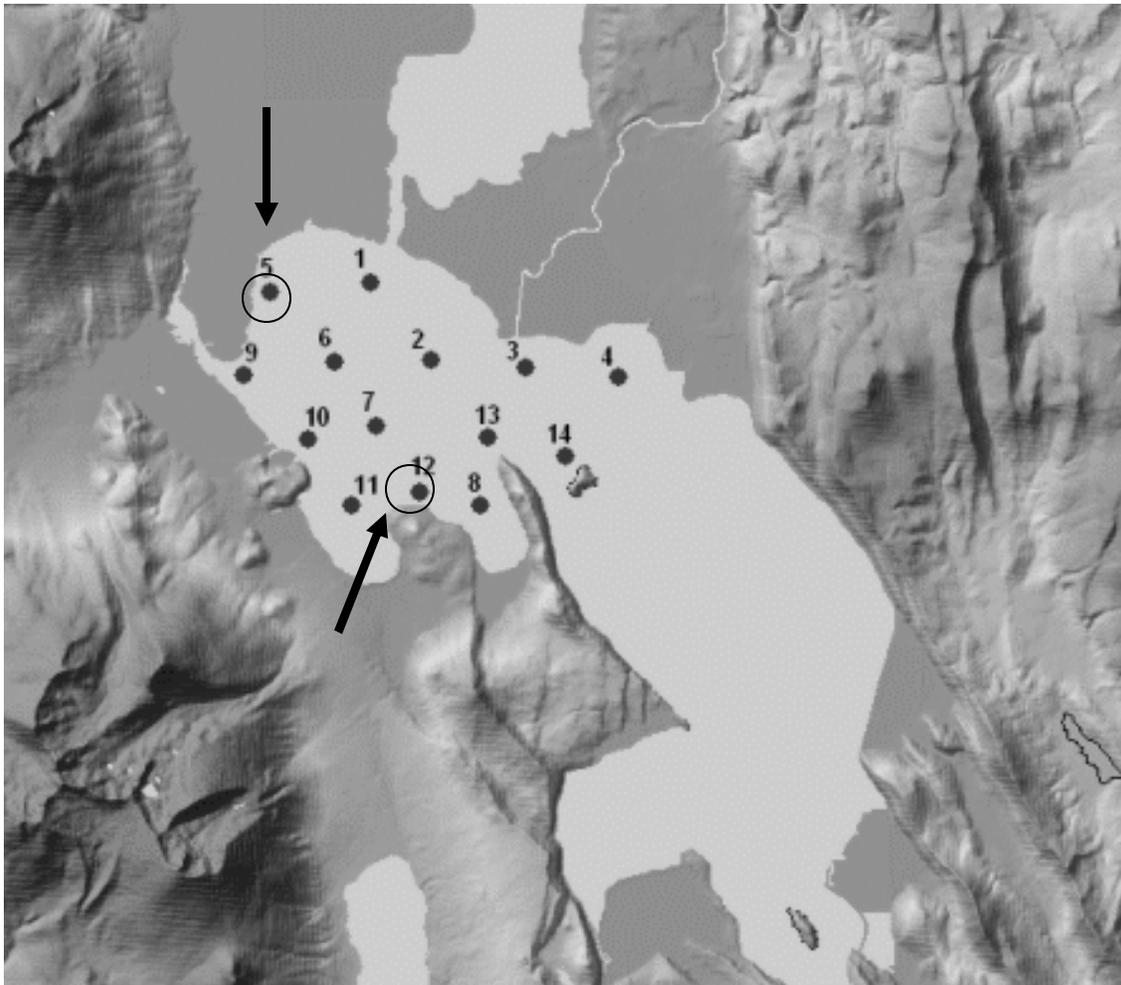
Blood cell assays: A differential leukocyte count was performed at 1000x magnification on the first 100 lymphocytes, thrombocytes, neutrophils, monocytes, and eosinophil / basophils observed on the smear. A Lymphocyte : Granulocyte ratio was calculated by dividing the number of lymphocytes observed by the sum of all neutrophils, eosinophils, and basophils. Total white cell counts (including thrombocytes) were performed with a hemocytometer on a 200X dilution of the sample. Rees-Ecker fixative contains Brilliant cresyl blue dye and differentially stains leukocytes (Lucas and Jamroz 1961). Hemoglobin was assayed by a Raichem (San Diego, CA) kit using the Drabkin method.

Standard microscopic and biochemical tests (such as API-20E) were used to identify isolated bacterial colonies to the genus level.

Plasma chemistry: Plasma samples were stored at -70°C until assayed for lysozyme activity, complement activity, cortisol, calcium, lipoperoxide, chloride, glucose, total protein, electrophoretic protein profile, albumin, and Albumin: Globulin ratio. Raichem kits were used for chloride, glucose, and albumin assays. Total protein concentration was determined by Sigma Chemical Company kit 541-2 and sodium was assayed with a flame photometer. Plasma protein electrophoresis was performed on 1µL samples run on an agarose gel (1M barbital buffer, 90V for 35 min.). The amido black stained gels were scanned and the percent area of each fraction determined with Scanalytics Zero Dscan™ software. Four to five bands were observed on the gels with the farthest migrating band (anodic) defined as “albumin”. Albumin is not found in all teleosts and its transport function can be performed by high density lipoprotein (Smet et al. 1998). No reference to sucker fishes and albumin were obtained in literature searches. The Albumin: Globulin (A/G) ratio was calculated from both the chemical tests for albumin and total protein (Albumin / [total protein – albumin = globulins]) as well as the electrophoretic profile (% OD of 1st band / [all other bands = globulins]). Low A/G ratios can indicate inflammation, kidney dysfunction, and liver disease (Jacobs et al. 1990). Plasma lipid peroxide was assayed with a Kamiya Biochemical company kit (CC-004). Plasma cortisol concentration was assayed with an enzyme-linked immunoassay (Neogen, Lexington, KY) and a 3 order polynomial equation of the standard bound / unbound curve was used to derive the sample values. Plasma was diluted 100X in the kit’s extraction buffer. The lysozyme activity of plasma and mucus (mOD / min/ mL) was determined from 20 µL samples assayed by a turbidimetric method at pH 6.4 (Ellis 1990). Hemolytic activity, of the alternative complement system, against rabbit erythrocytes was assayed by the method of Alcorn et al. (2002). Briefly, a 15µL aliquot of plasma was diluted 15X in buffer (0.1% gelatin, 0.1 M EGTA, 0.1 M MgCl₂ in veronal-buffered saline), and reacted with 1% Rabbit red blood cells for 60 min at 15°C. The hemoglobin content of the reaction well was determined by the absorbance at 540 nm. Percent hemolysis was calculated as follows: $\{(\text{Mean OD sample} - \text{Mean OD sample background}) - (\text{mean OD neg. control} - \text{mean OD Neg. control background}) / (\text{Mean OD 100\% hemolysis control} - \text{mean OD hemolysis background})\} * 100$. Gill Adenosine Triphosphatase activity (ATPase = µmoles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989).

Blood values were compared with samples collected from presumptively healthy adult suckers in April 2003 and May 1997, as well as from suckers sampled in July and August 2003 during several algal bloom cycles (Appendix 1 and 2). These later 2003 samples also included 12 moribund fish (Foott 2004). Plasma chemistry values from carp, tilapia, and catfish were also compared due to the paucity of literature on sucker values (Appendix 3).

Figure 1. Map of exposure sites GP5 and GP12 in north Upper Klamath Lake. Data source http://or.water.usgs.gov/projs_dir/or207/klake_data_2003.html



Results & Discussion

Water quality- Mean daily water temperature between 28 June and 8 July ranged from 20.9 to 23.1°C at GP5 and from 20.3 to 24.9°C at GP12. There was a 2- 3 ° C diurnal fluctuation at both sites (source [http:// or.water.usgs.gov](http://or.water.usgs.gov)). Dissolved oxygen concentration remained above 6 mg /L at both sites during the exposure period. Peak daily DO values reached 12 – 15 mg / L with the percent saturation of oxygen well above 100% at both sites. Both daily mean and maximum percent oxygen saturation was greater at GP5 than GP12 with a peak of 213.5 % on 1 July (Fig. 2). It is possible that these extremely high levels could induce gas bubble trauma or exert additional stress on the fish (Boyd et al. 1994). The influence of algal photosynthesis on water quality is apparent in the similar diurnal trends between pH and oxygen production (Fig. 3). Daily mean pH values ranged between 9.2 – 9.4 at GP12 while GP5 had mean values of between 9.5 – 9.8 (Fig. 3). Maximum pH measurements of 10.06 occurred at GP5 on 1 July. While total ammonia (TAN) was not measured at either site, the alkaline nature of the water would result in between 55.7 – 79.7% of the total ammonia being unionized (Emerson et al. 1975). Bellerud and Saiki (1995) report that unionized ammonia concentrations of > 0.34 mg/L were toxic to SNS and Lost River sucker fry. Given the high percentage of NH₃ at pH 9.8, a TAN of 0.6 mg / L would produce such toxic conditions. Suckers may employ compensatory mechanisms during ammonia stress. Lahontan cutthroat in pH 9.4 water compensate for ammonia excretion by the increase NH₃ partial pressure in the plasma and were not dependent on Na⁺ influx for excretion (McGeer et al. 1994).

A water sample collected at GP5 on 2 July tested at 3.83 mg/L for total hardness (HACH kit and DR850 colorimeter, method 8030, 0 – 4.0 mg/L). This value would appear low and is suspect. Bellerud and Saiki (1995) report that, during the spring, Upper Klamath Lake has a mean water hardness of 34.7 ± 2.4 mg / L with a mean calcium hardness of 18.3 ± 1.8 mg / L. Wedemeyer (1996) states that waters with hardness less than 75 mg/L CaCO₃ are considered as soft waters and provide only limited buffering capacity.

Fish condition- A total of 20 SNS were placed into 10 live cages at each site on 29 June. At 60 hours post-exposure (“3 days”) on 2 July, one dead fish was encountered at GP12 with two GP12 cages lost when they broke loose from their buoys. One cage at GP5 was also lost due to vandalism or a break in a buoy line. At 216 hr (“9 days”) post exposure on 8 July, there were 3 mortalities at both sites. A total of 10 fish (50% of test group) were lost from the study due to pre-sample mortality or loss of cage. The 4 female and 6 male SNS sampled alive were of a similar size range (Table 1). Live cage containment was the likely cause of the eroded fins, hemorrhagic skin around the snout, and some ulcers seen on the ventral and lateral body of the SNS (Table 1). Several ulcers were small and circular suggesting lamprey bites. No *Lernea* or *columnaris* (*Flavobacterium columnare*) lesions were observed on any of the SNS.

Blood cell and bloodborne bacteria - Total erythrocyte concentration, estimated by hematocrit values, were highly variable in the sampled SNS and ranged from 24 – 64% (Table 2). This measurement is affected by sampling stress responses such as splenic contraction, erythrocyte swelling due to hypoxia, or plasma / tissue fluid shift (Houston 1997). Previous sampling of presumptively healthy Upper Klamath Lake adult suckers in the spring of 2003 and juveniles in 1997 had shown a similar range of hematocrit values (Appendix 1 and 2). Hemoglobin concentration showed a positive relationship to hematocrit with the mean corpuscular hemoglobin concentration (average MCHC = 24.1) similar among the fish. These two measurements along with normal erythrocyte morphological seen in the blood smears indicate a normal erythrocyte profile.

Figure 2. Mean and maximum daily percent dissolved oxygen saturation values at GP5 and GP12 during the exposure period.

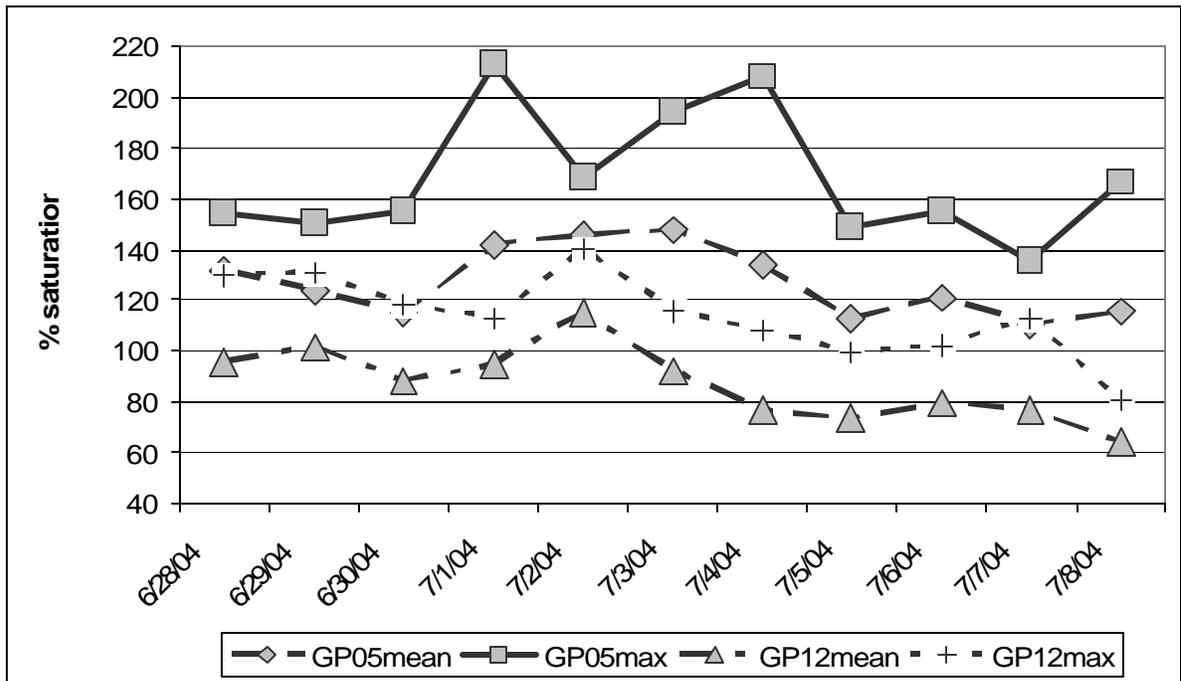


Figure 3. Mean and maximum daily pH values for GP5 and GP12 during the exposure period.

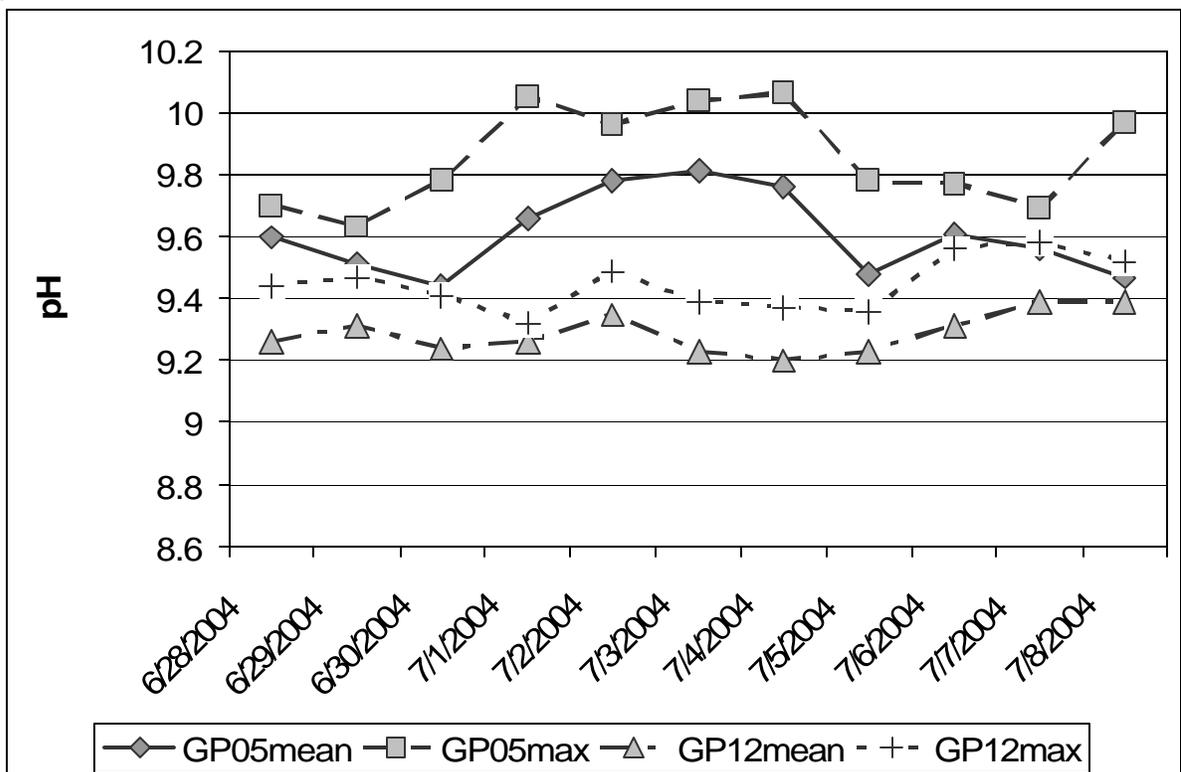


Table 1. Sex and fork length of Snortnose sucker adults. External signs include eroded fins (EF), hemorrhagic snout lesions (HS), and ventral surface ulcer (VU).

Fish	Site	Date	Sex	FL(cm)	External signs
1	GP5	2Jul	M	43	Normal features
2	GP5	2Jul	M	42	Caudal fin tear
3	GP5	2Jul	M	42	EF,HS,VU
4	GP5	2Jul	F	44	EF,HS,VU
5	GP12	2Jul	F	43	EF,HS,VU
6	GP12	2Jul	M	42	EF,HS,VU
7	GP12	2Jul	M	41	EF,VU
8	GP5	8-Jul	F	48	HS
9	GP5	8-Jul	F	45	HS,VU
10	GP12	8-Jul	M	47	Normal features

Table 2. Blood cell data and bacteria isolates. Data include hematocrit (HCT), hemoglobin concentration (Hb, g/dL), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC/mL, $\times 10^6$), and bacteria isolated from the blood (AP=Aeromonid/Pseudomonid, Ah = *Aeromonas hydrophilia*).

Fish	Site	Date	HCT	Hb	MCHC	$\times 10^6$ WBC/mL	blood bacteria
1	GP5	2Jul	25	6	24	177.5	AP
2	GP5	2Jul	64	10.3	16	175.0	AP
3	GP5	2Jul	36	7.9	22	92.5	NG
4	GP5	2Jul	40	10.2	26	132.5	NG
5	GP12	2Jul	24	5.5	23	55.0	AP
6	GP12	2Jul	35	9	26	40.0	NG
7	GP12	2Jul	Lost	7.5	na	47.5	NG
8	GP5	8-Jul	40	10.1	25	218.8	Ah
9	GP5	8-Jul	28	7.8	28	116.3	Ah
10	GP12	8-Jul	40	10.9	27	226.3	Ah

NG no growth on media

The adult suckers generally appeared to have elevated circulating leukocyte numbers as might be expected in the summer months. Leukocyte numbers ranged from 40 – 226 $\times 10^6$ cells / mL with similar mean and median values of 128 and 124 $\times 10^6$ cells / mL, respectively. Three GP12 fish (#5,6,7) had leukocyte counts lower than 1 standard deviation of the mean and 2 fish collected on 8July (#8 and 10) had values in excess of 1 standard deviation. I have no other sucker data for comparison however sexually mature Chinook salmon can range from 4 – 43 $\times 10^6$ cells / mL (unpublished FHC data on captive Winter-run Chinook leukocyte counts, 2004). I suspect that the measurement is prone to a large degree of laboratory assay variability. Aeromonid bacteria were isolated from the blood of 6 fish (Table 2). The mesophilic motile aeromonad complex (*Aeromonas* – *Pseudomonas* =AP) are common inhabitants of the gastrointestinal tract and skin of freshwater fish. In 2003, these bacteria were isolated in both apparently healthy and moribund adults (Foott 2004).

All fish showed a neutrophilia (elevated) profile in the blood smears with neutrophils comprising 20 – 57 % of all leukocytes (Table 3). These values were similar to adults sampled in July and August 2003 (Appendix 1 and 2). The Lymphocyte- Granulocyte (LG) ratios were < 2 and well below the median 22 recorded in presumptively healthy adults sampled in April 2003. Such a shift can indicate infection, tissue damage, or seasonal blood cell changes (Modra et al 1998). Taken together, these leukocyte data indicate the live cage fish had been stimulated to increase blood cell phagocytic function in response to all or some of the following events: handling and captivity stress, summer water temperatures, or microorganism challenge. Gill sodium-potassium-Adenosine Triphosphotase activities ranged from 0.97 – 2.03 $\mu\text{mole ADP} / \text{mg protein} / \text{hr}$. These values have been observed in Chinook fry and may be relatively normal for freshwater fish (unpublished FHC ATPase calibration data).

Table 3. Differential leukocyte count and gill Na-K-Adenosine Triphosphotase activity. Leukocyte data recorded as percent lymphocyte (L), thrombocyte (T), neutrophil (N), monocyte or eosinophil (ME), and lymphocyte / granulocyte (+monocyte) ratio (L / G). ATPase activity is recorded as $\mu\text{mole ADP} / \text{mg protein} / \text{hr}$.

Fish	Site	date	L	T	N	ME	L / G	ATPase
1	GP5	2Jul	40	9	51	0	0.78	0.97
2	GP5	2Jul	35	46	20	0	1.75	2.03
3	GP5	2Jul	16	26	57	1	0.28	1.84
4	GP5	2Jul	18	46	35	0	0.51	1.13
5	GP12	2Jul	24	49	27	0	0.89	1.72
6	GP12	2Jul						No activity
7	GP12	2Jul	28	53	20	0	1.40	1.74
8	GP5	8-Jul	27	42	31	0	0.87	1.17
9	GP5	8-Jul	18	42	36	4	0.45	1.33
10	GP12	8-Jul	38	35	26	1	1.41	1.99

Plasma samples - All fish showed highly elevated plasma sodium levels that were 59 – 135 % above the normal freshwater fish value of 165 (Appendix 3). These sodium values were also above the mean 126 mmol /L value of the April 2003 adult suckers as well as the May 1997 samples (Appendix 1 and 2). The 1997 and 2003 values were likely lower than normal baseline values due to a capture stress response. Plasma chloride values were 25 – 60% below normal (freshwater fish normal minimum ~100 mEq / L, Appendix 3) in 8 of the 10 suckers. The 3 suckers sampled on 8July had significantly higher chloride values than the 2July group (t-test, 8df, P= 0.027). Exposure site did not affect chloride values in the 2July sample (t-test, 5df, P=0.653). The live cage fish values were similar to suckers sampled during the algal bloom of 2003 (mean ranging from 45 – 71 mEq / L) but lower than the presumptively healthy adults sampled in May 1997 (Appendix 1 and 2).

Calcium concentrations ranged from 7 – 15 mg / dL and were within normal ranges for freshwater fish (Appendix 3). No site or date trend was detected by t-test statistical tests (P< 0.05). Chronic stress was evident in the high plasma glucose and cortisol values seen in 9 of 10 suckers (Table 4). Triglyceride averaged 54 mg/ dL and was similar among all 10 fish. The range of triglyceride values was similar to those observed in suckers sampled in May1997 (Appendix 1). Lipid peroxide (LPO) values were highly variable among the live cage fish and could have been influenced by the 5 week sample storage time (LPO assay

kit instructions state that 2 week storage was maximum for plasma samples). Three suckers (#3, 7, and 9) had values 2 – 10X higher than the other fish and could have been experiencing oxidative stress. (Table 4). Palace et al. (1998) reported that adult lake trout livers had LPO values below 25 nmol / g and the LPO assay manufacturer states that human plasma should range from 0 - 1.3 ng/mL. Lipid peroxides are produced by auto-oxidation of unsaturated fatty acids and are quite unstable in plasma.

Table 4. Plasma chemistry values for adult SNS exposed at GP5 and GP12 sites. Data reported for sodium (Na , mEq/L), chloride (Cl, mEq/L), calcium (Ca, mg/dL), glucose (Glu, mg/dL), triglyceride (TG, mg/dL), lipid peroxide (LPO, nmol/L), and cortisol (ng / mL).

Fish	Site	date	Na	Cl	Ca	Glu	TG	LPO	CRT
1	GP5	2Jul	262	80	12	86	51	4.3	138
2	GP5	2Jul	260	82	12	128	70	1.0	199
3	GP5	2Jul	230	40	9	154	44	40.1	251
4	GP5	2Jul	254	69	10	130	51	2.8	130
5	GP12	2Jul	263	56	15	45	63	0	563
6	GP12	2Jul	230	54	9	153	79	17.5	312
7	GP12	2Jul	236	75	9	134	29	34.8	318
8	GP5	8-Jul	323	121	11	102	59	0	106
9	GP5	8-Jul	253	74	8	154	46	63.9	6
10	GP12	8-Jul	242	101	7	78	50	7.8	177

Water quality did not appear to have an obvious effect on plasma lysozyme levels. Plasma lysozyme activity ranged from 89 – 760 mOD / min / mL while gill mucus yielded much more variable results (Table 5). The lack of correlation between plasma and mucus activity may be related to the influence of contaminating water on gill mucus collection. Despite the 8.5X difference in the minimum and maximum value, no obvious site or date trend was observed in the data. Plasma samples from suckers collected in late July 2003 had activities that ranged from 3800 – 33,000 mOD/ min / mL (unpublished data due to the large number of samples with no measurable activities). Some of these 2003 fish were moribund and had Columnaris lesions. Healthy juvenile Chinook salmon have been shown to have 2 – 4x higher plasma lysozyme activities (Foott et al. 2004). Lysozyme is an important component of the innate immunity of fish with its ability to cleave bacterial cell wall peptidoglycan. It is produced by both macrophages and neutrophils in fish, and increased serum activity has been associated with activation of these phagocytes by infection (Paulsen et al. 2003). Schrock et al. (2001) reported detecting lysozyme activity in skin mucus of juvenile salmonids. Their activity data was 10-fold less than that reported for salmonid kidneys containing phagocytic cells (Lie et al. 1989). Adverse water quality or severe handling stress is reported to induce a reduction in plasma lysozyme activity in trout (Mock and Peters 1990). Alternative complement activity (expressed as % hemolysis of rabbit erythrocytes or ACH50) was high in 9 of 10 fish (Table 5). No site or date trend was obvious in the data. The ACH50 value is calculated as the quality of plasma which will lyse 50% of standardized erythrocyte suspension (Yano 1992).

Table 5. Plasma enzyme activities for adult SNS exposed at GP5 and GP12 sites. Data reported for complement activity measurements: percent hemolysis of rabbit erythrocyte of a 10x and 20x dilution as well as the alternative complement activity 50% level calculation, plasma (PI lyz) and gill mucus (Mu lyz) lysozyme activity (mOD / min / mL)

Fish	Site	date	10x %H	20x %H	AC50	PI Lyz	Mu Lyz
1	GP5	2Jul	72.6	18.8	10.4	477	nt
2	GP5	2Jul	77.5	45.7	10.2	289	140
3	GP5	2Jul	95.9	38.1	2.6	250	49
4	GP5	2Jul	81.9	57.5	9.5	406	150
5	GP12	2Jul	53.2	26.5	13.7	760	382
6	GP12	2Jul	73.9	41.5	10.5	89	nt
7	GP12	2Jul	98.8	38	6.1	394	235
8	GP5	8-Jul	87.9	34.4	9.5	219	242
9	GP5	8-Jul	68.9	14.5	12.6	169	nt
10	GP12	8-Jul	100	25.5	10.1	271	nt

nt = not detected

Plasma protein values were elevated above presumptively healthy adults sampled in May1997 (Appendix 1). If 3.0 g/dL is set as a normal baseline (Appendix 1 and 3), the live cage suckers had protein values 1.3 – 2.6 X above this value (Table 6). Elevated plasma protein and sodium levels all indicate hemoconcentration with a fluid shift out of the blood. Presumptive albumin was measured by both the Bromcresol Green assay (BCG) and from electrophoretic gels. Bromocresol green can bind alpha proteins as well as albumin and tends to over estimate albumin concentration in humans (Jacobs et al. 1990). Smet et al. (1998) reports that carp do not have albumin per se but use High Density Lipoprotein (HDL) for albumin's transport functions. They report the HDL was the most anodic protein in electrophoresis gels. The A/G ratio ranged from 0.29 to 0.60 by chemical methods and 0.30 to 0.45 by electrophoresis. These A/G values are lower than those observed in presumptively healthy suckers examined in May1997 or April 2003 (Appendix 1 and 2). They are similar to ratios seen in the July 2003 suckers collected during algal bloom cycles and may represent the "normal" elevation in globular proteins that occur in the summer months. Low Albumin : Globulin ratio is an indicator of liver dysfunction and infections in other vertebrates. Given the uncertainty about the presence of albumin in SNS, this immune function parameter may not be useful for critical evaluations.

Table 6. Plasma proteins.

Fish	Site	date	TP	BCG		% IntOD Electrophoresis		
				Alb	A/G	Alb	G2-6	A/G
1	GP5	2Jul	5.9	2.1	0.56	27	73	0.37
2	GP5	2Jul	6.2	2.1	0.49	25	75	0.33
3	GP5	2Jul	5.8	1.7	0.42	24	76	0.32
4	GP5	2Jul	6.0	2.2	0.56	ND	ND	ND
5	GP12	2Jul	5.2	1.7	0.49	ND	ND	ND
6	GP12	2Jul	5.5	1.9	0.52	23	77	0.30
7	GP12	2Jul	4.0	1.5	0.60	31	69	0.45
8	GP5	8-Jul	4.8	1.6	0.52	25	75	0.33
9	GP5	8-Jul	6.6	1.5	0.29	ND	ND	ND
10	GP12	8-Jul	7.7	2.0	0.35	26	74	0.35

Plasma sodium and protein disturbance factors – During stress events, most freshwater fish tend to lose monovalent ions by branchial efflux. This ion efflux occurs due to a combination of increased blood pressure and lamellar perfusion. Divalent ions in the water, such as calcium, reduce this branchial efflux by decreasing gill permeability. The sodium loss in stressed fish rearing in soft-water is magnitudes greater than in hard water. Fish recover their NaCl balance through branchial transport mechanisms (McDonald and Milligan 1997). Acute exposure to alkaline water (pH > 9.5) is reported to inhibit both ammonia excretion and Na⁺ influx in rainbow trout (Wright and Wood, 1985). Unlike the hypernatremia (high sodium) observed in the live cage fish, the previous examples result in reduced plasma sodium concentrations. Sodium and chloride movements are reportedly linked to acid-base regulation due to Na⁺ / H⁺ and Cl⁻ / HCO₃⁻ exchange processes (Wilkie and Wood 1996). The elevated sodium levels measured in the live cage suckers would also suggest a disruption of the fish's acid-base regulation. The mechanism(s) to explain the hemoconcentration trend is unclear and may require consultation with experts in alkaline water and stress fish physiology as well as further experimentation. If confinement stress played a role in this fluid shift, it could confound interpretation of alkalinity effects.

Summary:

- 1) All fish may have experienced a fluid shift from their circulation (as showed by high plasma protein and sodium values). The imbalance would likely impair acid-base balance and gas transport mechanisms.
- 2) The oxygen supersaturation experienced by the fish could induce gas bubble trauma. Total gas saturation would need to be measured before concluding any risk.
- 3) Confinement and handling stress responses were demonstrated in the elevated plasma cortisol and glucose values. Chronic stress could confound interpretation of alkalinity effects.
- 4) Immunosuppression was not apparent in the fish (plasma lysozyme and complement activities, AG ratio, leukocyte profile showing neutrophilia, and total WBC counts indicate a heightened level).

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Appendix 1

Blood and plasma parameters of Upper Klamath Lake suckers sampled in May (7 adults and 6 juveniles that were presumptively healthy) and September (during fish die-off) 1997. Most chemistries run on Kodak DT60 analyzer. Albumin: Globulin ratio derived by both chemical (BCG reagent) and electrophoretic method (SEP). The number of samples out of analyzer range (OR) is reported as fraction. **ASSUME ALL VALUES INFLUENCED BY EXTENSIVE HANDLING STRESS.**

	<u>Mean</u>	<u>Max.</u>	<u>Min.</u>	<u>OR</u>	
Sodium. (mmol/L)	134	141	125	1 / 6	
Chloride (mmol/L)	107	132	91	0 / 6	
Magnesium (mg/dL)	3.5	4.3	2.6	0 / 6	
Potassium (mmol / L)	2.4*	3.0	1.5*	2 / 6	*1 above 1 below analyzer range
Protein (g/dL)	2.7	5.1	1.2	0 / 6	
Albumin (g/dL)	1.4	1.9	1.1	1 / 6	
A / G (chemical)	0.68	0.92	0.55	2 / 6	
A / G**(SEP)	0.23	0.40	0.14	0 / 6	
Triglyceride (mg/dL)	70	114	33	1 / 6	
Glucose (mg / dL)	146	201	121	0 / 6	
Osmolarity (mmol/kg) 278	294	255	0 / 6		
Alkaline Phosphatase (u/L)	41	57	20	0 / 6	
Hematocrit (%)	44	49	36	0 / 7	

** Smet et al. 1998 = carp do not have albumin per se but use HDL. They report the HDL was the most anodic protein in electrophoresis gels. This broad immune function parameter may not be useful for some fish species.

Juvenile (1-2yr Hatchery) – Sampled 5/97

Osmolarity (mmol/kg) 248	288	281	0 / 6	
	(assume 280 – 290 is “normal” for stressed, healthy sucker)			
Protein (g/dL)	3.0	3.8	2.8	0 / 2
A / G**(SEP)	0.97	1.28	0.67	0 / 2
Hematocrit (%)	45	51	34	0 / 4

September 1997 adults (5 of 7 adults with columnaris lesions, all with Lernaea)

Whole blood shipped on ice, plasma separated in 24hrs.

A / G**(SEP)	0.15	0.24	0.10	0 / 6
Protein (g/dL)	1.9	3.0	1.1	0 / 7
Osmolarity (mmol/kg) 236	257	211	0 / 7	
Hematocrit (%)	34	53	11	0 / 7

Appendix 2. 2003 data (Foott, 2004)

Table 3. Mean percentage (std dev.) differential leukocyte counts.

L:G ratio	Lymphocyte	Thrombocyte	Neutrophil	Eosinophil	Monocyte	
08April n= 20	69 (19)	21 (11)	6 (7)	0.5 (1.1)	0.1 (0.3)	22 (24)
16July n= 18	42 (14)	32 (11)	22 (10)	0.4 (0.8)	0.3 (0.6)	3 (2)
30July n = 10	60 (14)	23 (10)	16 (7)	0.5 (1)	0	4 (2)
15August n = 7	82 (9)	13 (8)	6(3)	0.3 (0.8)	0	16 (6)
Moribund 8/15 – 9/5 n= 12	44 (22)	23 (11)	25(29)	2 (7)	6 (10)	7 (10)

Table 4

Mean (Std. deviation) concentrations and values of plasma total protein, albumin, Albumin / globulin ratio (A /G) , glucose, choride, and sodium. Number (n) of samples reported for each group. Statistical differences, among sample groups for any particular sample date, are indicated by different letters (ANOVA, P< 0.05).

Sample Date	Total Protein (g / dL)	Albumin (g / dL)	A / G	Glucose (g / dL)	Chloride mEq / L	sodium mmol/L
08April n = 19	4.1 (1.4) a	1.5 (0.4) n = 19	0.73 (0.39) a n = 19	140 (43) a n=19	103 (8) a n = 19	126 (13) n = 19
16July n = 20	4.0 (1.0) a	1.2 (0.3) n = 20	0.57 (0.67) b n = 20	136 (58) a n= 20	65 (14) b n = 20	ND
30July n = 9	3.2 (1.5) a	1.0 (0.1) n = 8	0.78 (0.51) ab n = 8	79 (24) b n = 8	60 (17) b n = 8	ND
15August n = 10	5.3 (1.5) b	ND	ND	88 (33) b n = 10	71 (17) b n = 10	ND
Moribund 8/15 – 9/5 n = 13	1.8 (1.3) c	1.8 (4.4) n = 13	0.59 (0.36) ab n = 13	81 (54) b n = 10	45 (32) b n = 10	ND

Appendix 3

Plasma chemistry values for farm-reared carp, tilapia, and channel catfish held in freshwater.

- 1 Chen et al. 2003
- 2 NBS information bulletin 56, 1995
- 3 Palackova et al. 1994.
- 4 Svobodova et al.1994.
- 5 Wedemeyer 1994

	Tilapia-1	Tilapia-2	Carp-3	Carp - 4	Catfish-5	Range*
Na mmol/L	159-164	135.4				135 – 164
Cl mEq/L	120-136		138		132	120 -138
Ca ²⁺ mg/dL	16 -18	7.8	18.8		13.5	8 – 19
Protein	3.7-4.3	2.9	2.2	2.5 – 3.7	2.2	2 -4
A / G	0.48-.051		0.64	1.9		0.48 -1.9
Hb			4.8	5.9 – 8.3	4 – 8	4 – 8
MCHC			0.22			0.22
WBC / L			72.3	0.73 - 52		0.73 -72
Triglyceride		125.3				125

* values rounded to nearest 1.0