

## U.S. Fish & Wildlife Service

California Nevada Fish Health Center  
FY1997 Technical Memorandum:

### **Pilot study on physiological effects of simulated planting of Iron Gate SFH juvenile Chinook salmon into summer Klamath River temperatures.**

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**Summary:** An abrupt transfer to 20°C water, simulating a release into high temperature river water, was associated with several physiological impairments (drop in gill Na-K-ATPase activity, increase in globulin plasma proteins, and inflammation of adipose tissue) but did not incur mortality or significantly impair survival in seawater.

**Background:** Iron Gate State Fish Hatchery (IGH) must often release its Chinook smolts into the Klamath River when river temperature is 18°C or greater. Water temperature in the hatchery is typically 4 - 8°C lower than the river. This situation results in an acute temperature change for the release group. This pilot study examined the following responses of smolts moved into a 20°C tank and held for 10d: 1) morphometrics, 2) smolt development, 3) plasma protein profile, and 4) histological changes to tissues. These challenged fish were compared to cohorts held at the ambient mean temperature of 11.7°C.

**Methods:** On 4 June 1997, 45 fall-run Chinook smolts (mean FL 79mm) were moved from raceway C1 into a 20°C, 150L aquarium. The aquarium received a minimum flow of 1 gpm of ambient water, provided with aeration, and contained immersion heaters. A cohort group of 45 smolts were held in an ambient (10 - 12°C) flow-through 155L aquarium. Fish were provided 5-8g of freeze-dried krill every other day to simulate aquatic insect diet. On 12 June, 10 fish from each group were placed into 28 ppt seawater static containers supplied with aeration and held in an ambient (11°C) water bath for 24h. On 13 June, all fish were sampled as follows: 1) Gill tissue (aquaria only) frozen on dry ice in SEI buffer for Na-K-ATPase assay, 2) Organosomatic evaluation performed, 3) plasma collected, 4) tissues fixed for histological evaluation, and 5) 20 fish from each group assayed for bacteria (culture and *R.salmoninarum* ELISA) and viral infection (EPC and CHSE214 cell lines, 5p kidney samples). Temperature was logged hourly with Onset probes. On

14 June, water pH, dissolved oxygen, and ammonia was measured in each aquaria.

**Results:** Water quality at the end of the trial was considered satisfactory: Elevated temperature tank = 5.98 mg/L dissolved oxygen, pH 7.32, total ammonia < 0.01 mg/L and ambient tank = 7.14 mg/L dissolved oxygen, pH 7.15, total ammonia < 0.01 mg/L. Mean temperatures were  $20.4 \pm 0.01^\circ\text{C}$  (18.2 – 22.6°C) in the elevated temperature aquaria and  $11.7 \pm 0.01^\circ\text{C}$  (10.2 – 14.1°C) in the ambient aquaria.

No mortality occurred in either group over the 10d trial. No virus or *R.salmoninarum* antigen was detected. Bacteria were isolated from 4 fish in both temperature groups (*Micrococcus*, *Aeromonas-Pseudomonas*) however no clinical signs of infection were observed in the fish indicating these were asymptomatic infections. No significant lesions or abnormalities were seen in the histological samples from either group (Table 1). Moderate inflammatory changes occurred in the visceral adipose tissue and associated acinar cells of some 20°C fish. These changes are commonly observed in smolts collected from the river.

Table 1. Histological examination. Data reported as number of sections positive / total number of sections (fish) examined.

	Ambient	Elevated
Acinar cells inflammation	0 / 7	1 / 11
Intestine / pyloric ceca abnormalities	0 / 9	0 / 11
Visceral fat inflammation	0 / 9	3 / 11
Liver abnormalities ++	0 / 9	0 / 11
Kidney abnormalities	0 / 10	0 / 10
Heart abnormalities	0 / 7	0 / 8
Gill abnormalities	0 / 9	0 / 10

++ Vacuoles containing glycogen seen in 2 ambient fish and 5 elevated temperature fish.

Fish size and blood cell quantity (hematocrit and leukocrit) were similar between the groups (Table 2). Condition factor was significantly lower in the elevated group (T-test,  $P=0.0546$ ). There was one mortality (elevated temperate fish) in the 24h SW challenge and survivors appeared to tolerate the osmotic change (similar condition factor to FW fish, plasma osmolarity normal) however half of each group had elevated plasma sodium levels (Table 3). Gill Na-K-ATPase levels were on average 2x lower in the elevated

temperature fish (Table 3).

Table 2. Organosomatic data.

	Ambient	Elevated
Fork Length (mm)	79 ( $\pm$ 1)	79 ( $\pm$ 1)
Weight (g)	5.1 ( $\pm$ 0.2)	4.8 ( $\pm$ 0.2)
Condition Factor ( $10^{-5}$ )	0.7746 ( $\pm$ 0.0101)	0.7455 ( $\pm$ 0.0107)**
Visceral Fat Score	1.3	1.1
Hematocrit (%)	42 ( $\pm$ 1)	42 ( $\pm$ 1)
Leukocrit (%)	1.0927 ( $\pm$ 0.1614)	1.0434 ( $\pm$ 0.0875)

\*\* T-test, P= 0.0546.

Table 3. Smolt Development Measurements.

	Ambient	Elevated
Plasma sodium (mmol / L)	165 ( $\pm$ 5) **	167 ( $\pm$ 6)
% Na+ > 170 mmol / L	50 %	50 %
Condition factor ( $10^5$ )	0.757 ( $\pm$ 0.021)	0.721 ( $\pm$ 0.027)
Osmolarity	351 ( $\pm$ 16)	357 ( $\pm$ 21)
Survival	100 %	90 %
ATPase (umoles ADP / mg protein / hr)	11.2 ( $\pm$ 1.4)	6.5 ( $\pm$ 0.7)

\*\* One fish in both groups had a plasma sodium above the analyzer range of 215 mmol/L. Data from these 2 fish not included in calculation of the mean.

Plasma glucose levels were slightly higher in elevated temperature fish (Table 4). The percent lipid values were lower in elevated temperature fish however, calculations were not accurate and data can only be viewed as relative. As moisture content is inverse to lipid and this measurement was similar, it is likely that lipid levels were also similar. Elevated temperature fish had increased globulin protein levels (lower A/G ratio) than ambient cohorts (Table 5, Figure 1).

Table 4. Clinical chemistry data.

	Ambient	Elevated
% lipid - carcass, n= 5	10.8 ( $\pm$ 0.6) **	7.8 ( $\pm$ 0.5)
% moisture - carcass, n=5	80.8 ( $\pm$ 0.3)	81.0 ( $\pm$ 0.4)
Plasma lipase U / L, n= 4	87 ( $\pm$ 9)	NA ++
Plasma Stress Glucose mg / dL, n = 5	77 ( $\pm$ 6)	85 ( $\pm$ 5)

\*\* Mean value significantly greater (T-test, P<0.05).

++ Three of 4 samples below analyzer range (< 10 Units / L), fish 1 = 18 U/L.

Table 5. Plasma protein data from 8 fish of each temperature group. Mean ( $\pm$  SEM) of total protein concentration, Albumin : Globulin ratio (A/G) and the percent area of electrophoretic bands .

	Ambient	Elevated
Total protein (g / dL)	2.5 ( $\pm$ 0.0)	2.4 ( $\pm$ 0.1)
A / G	0.999 ( $\pm$ 0.028) **	0.7760 ( $\pm$ 0.047)
Pre-albumin(s)	33.7 ( $\pm$ 0.9) ++	24.6 ( $\pm$ 2.1)
Albumin	33.1 ( $\pm$ 0.6)	32.7 ( $\pm$ 1.4)
Globulin 1 (alpha)	7.3 ( $\pm$ 0.5)	13.5 ( $\pm$ 1.1) **
Globulin 2	3.8 ( $\pm$ 0.2)	5.2 ( $\pm$ 0.4) **
Globulin 3	5.2 ( $\pm$ 0.2)	9.9 ( $\pm$ 1.0) **
Globulin 4 / 5 (beta)	14.1 ( $\pm$ 0.4) **	9.0 ( $\pm$ 1.2)
Globulin 6/ 7 (gamma)	2.8 ( $\pm$ 0.2)	5.1 ( $\pm$ 0.4) **

\*\* Mean value significantly greater (T-test, P<0.05).

++ Median value significantly greater (Mann-Whitney Rank sum test, P=0.006)

Figure 1. Mean serum electrophoresis profiles.

