TO: All interested parties
FROM: J. Scott Foott
CA-W FHC, Anderson, CA
SUBJECT: Preliminary results of Spring 1995 Klamath R. chinook smolt study (95-FP-01), Iron Gate Hatchery June release group,

Summary: The Spring 1995 release group experienced a high incidence of severe infection from the intestinal parasite, Ceratomyxa shasta. Their energy reserves were low at the time of release and decline markedly post-release. It appears that 3 distinct outmigrant types were collected during the study; 1) rapid outmigrants with advanced smolt characteristics, 2) weak and sick fish swept downstream, later outmigrating. 3) fish residing in cool water refugia and Chinook captured at the lowest site (Big Bar 81 rkm) tended to be in poor condition compared with fish captured below cool water refugia (especially Red Cap creek). Five weeks post-release, a pulse of relatively healthy smolts were collected at the Big Bar trap. In my opinion, a significant portion of the release group did not survive outmigration due to C. shasta infection.

RESULTS
The CA-NV Fish Health Center, in cooperation with Klamath R. FWO, Coastal Calif. FWO, Iron Gate State Fish Hatchery (IGH), and Mel Willis (CDFG), conducted a health and physiology monitoring program on juvenile chinook released from IGH in mid-June 1995. The November 1995 yearling release will monitored and a final report is scheduled for completion by April 1996. Below are major findings:

Water Conditions- Fish went from 11°C at IGH to 18-22°C in the lower Klamath R. during July. Both Red Cap (85 rkm) and Indian (172 rkm) creeks were cooler than the mainstem and TGH smolts were collected below their mouths - Percent saturation, dissolved oxygen, total dissolved solids and pH were within normal ranges for salmonids and remained relatively constant at all river collection sites. A dissolved oxygen measurement of 3.9 ppm was obtained in the lower raceways at IGH prior to release. This low value indicates insufficient flow for the biomass within the rearing
units. River flow (as measured at Orleans) declined by approximately 50% from the 2nd to the 5th week post-release.

Physiological Data

Liver Glycogen - Total liver polysaccharide (glycogen) in pre-release IGH fish appeared to be low for fed hatchery fish (IGH mean = 2.68 mg/100mg liver compared with 8.95 mg/100 mg liver observed in Coleman National Fish Hatchery chinook fed Biodiet at 4 % BW/day). Glycogen levels dropped 64 - 72 % within 2 weeks after release and continued to decline post-release. Glycogen levels were also low in the 21 June natural chinook sample. While variable, the capture-stress response of plasma glucose elevation was retarded as the liver glycogen reserves were depleted. An IGH chinook sample collected 5 weeks post-release had liver glycogen and stress-glucose levels significantly higher than the previous 2 weekly collection groups.

Hepatosomatic Index (HSI) - The weight of the liver tended to increased during the outmigration period. The cause of this increase is unclear as no abnormality was observed in histological sections of the liver, body weights did not change significantly (condition factor relatively constant in outmigrants) and glycogen levels declined. One possibility is that the hepatocytes take up water as glycogen is depleted.

Visceral Fat and Plasma triglyceride (TRI G) - Pre-release visceral fat ratings were relatively low for fed hatchery fish (all were 1 in a 0,1,2,3 system). Visceral fat declined steadily in outmigrant IGH fish and was absent from natural chinook sampled 21 June. Chinook captured below Red Cap creek maintained visceral fat at a higher rate than fish captured downriver at Big Bar. Circulating lipid stores (plasma triglyceride) rapidly declined after release. Within 2 weeks post-release, TRIG fell 61 - 77 % and by the 3rd week a majority of the fish had levels below the detection limits of the assay. All down-river IGH chinook had TRIG levels below the lowest 25 % value seen in the pre-release sample - The majority of IGH chinook sampled post-release had food in their GI tract with the exception of moribund chinook suffering from severe C. Shasta infection.

Fish size - A large size variation was seen in the IGH release group (mean = 75.1 mm, range = 41-85 mm Fork length), however, only chinook > 85 mm F.L. were captured in the river. It is not clear what happened to the smaller fish, however, there were no health problems specifically identified with the smaller fish. There was no statistically significant
difference in fish size among the outmigrant sample groups. As mentioned above, the condition factor (Wt/length\(^3\)) of the outmigrants was greater than the IGH sample group and relatively constant throughout the study period. The one exception being the IGH fish captured during the 5th week post-release at Big Bar who had similar condition factors as the pre-release sample. Natural chinook collected on 21 June had very low condition factors. It is not clear why the outmigrants maintained their body weight while depleting their energy stores. Perhaps lipid and protein loss in the tissues was replaced by water.

Smolt development Three 24-hr Saltwater Challenges (SW) were conducted with marked IGH fish (1 pre-release, 2 at Big Bar). While there was good survival at IGH, only 33 % of the surviving chinook (mean FL = 83.9 mm) were maintaining normal plasma sodium levels. Although larger (mean FL = 94 mm), there was poor survival (28 % & 6 %) and sodium regulation (40 % & 0 % of survivors maintained normal sodium levels) in IGH chinook challenged at Big Bar 3 and 4 weeks post-release. The highest gill sodium-potassium-Adenosine Triphosphatase (Na-K-ATPase) activities were detected in the lead outmigrant groups captured 2 weeks post-release, The next highest ATPase values were from the natural chinook followed by IGH fish collected prior to their release. IGH chinook captured 3-5 weeks post-release had similar low Na-K-ATPase levels. Na-K-ATPase is an ion transport enzyme system essential for SW adaptation.

Plasma total protein and protein electrophoresis- Plasma total protein levels dropped after release and by 3 weeks post-release all IGH outmigrants had total protein levels below the lowest 25 % value seen in the hatchery sample. Electrophoresis data on the individual plasma protein fractions is still being analyzed, however, C. shasta -infected chinook lost both significant quantity and specific plasma protein fractions.

Leukocrit and hematocrit- Due to high variance, there were no statistically significant differences among the leukocrit values of the sample groups (Lct = % packed white blood cell). The stage of C. shasta infection seemed to influence leukocrit. The mean Lct tended to increase in fish collected 2-3 weeks post-release (early infections) and be the lowest in moribund fish sampled during the 4th week. Hematocrit (Hct = % packed erythrocyte) was significantly higher in pre-release samples than outmigrants. Severe C. shasta infection resulted in anemia.
Ceratomyxa shasta- This myxozoan parasite is endemic to the Klamath river but has not been detected in fish at IGH (1992-1995 surveys). In 1992-1994, only a low incidence of C. shasta infection was seen in outmigrant chinook. By 2 weeks post-release, a 33% incidence rate had occurred in the 1995 outmigrants of which half of the infections were judged severe (attached graph CS#2 = severe infection, CS#1 = parasite present with only moderate lesions). Both incidence and severity of infection tended to increase with time post-release, however, fish captured below the mouth of Red Cap creek were affected to a much lesser degree than other capture groups. With the exception of the relatively healthy 2nd (lead) and 5th week capture groups, most of the outmigrants captured at the Big Bar trap were quite sick from C. shasta infection and may have been swept downriver ("floating dead"). The mortality numbers at the Big Bar trap correspond well with the severity of C. shasta infection, The parasite caused erosion of the intestinal epithelium with contact with the infectious stage between the hatchery (305 rkm) and Indian creek (172 rkm). The natural chinook collected on 21 June had a 21 % incidence of infection with many fish quite sick from the parasite. It appears that infectious stage of C. shasta was quite prevalent this spring and severely impacted the smolt population.

Other parasites- Metacercaria (presumptively Nanophyetus salmincola) were detected in IGH outmigrants by 2 weeks post-release. Both the incidence and severity of infection were considered mild in comparison to the Trinity River. Unlike Trinity R. smolts, metacercaria were often observed in gill tissue rather than the kidney of the IGH outmigrants. Three IGH outmigrants had lamprey wounds. An immature myxozoan parasite, distinct from C. shasta, was commonly seen in the kidneys of outmigrant fish (but not at TGH) and caused inflammation of the glomerulus. There was a high incidence of pancreatic and adipose tissue inflammation. This inflammatory response was not always seen with C. shasta (13 % of IGH sample had this type of lesion) and appears to be independent of the parasite. It is not clear what caused the pancreatitis. Histological samples from the 1993 and 1994 Klamath R. studies did not demonstrate pancreatitis and only show a moderate incidence of inflammation of the adipose tissue.

Renibacterium salmoninarum (RS)- An ELISA procedure was used to test kidney tissue for Rs antigen. RS is the causative
agent of Bacterial Kidney Disease (BKD). While only 3 \% were considered to have active infections, the pre-release group had statistically higher levels of RS antigen than 6 of 9 down-river sample groups. The incidence of antigen (+) fish ranged from 13 - 50 \% in the outgrants and 42\% in the naturals, however, the antigen concentrations were low. It appears that BKD was not a major health concern for this release group.
Incidence of *Ceratomyxa shasta* infection - Spring 1995