FY 2000 Investigational Report:

Health Assessment of VAMP Release Groups – 2000

Merced
2000
fall chinook

Ken Nichols*, Rick Harmon and J. Scott Foott
US Fish and Wildlife Service
CA-NV Fish Health Center
24411 Coleman Fish Hatchery Rd.
Anderson, CA 96007
(530) 365-4271 Fax: (530) 365-7150

September 2000

* Kenneth_Nichols@fws.gov

Health Assessment of VAMP Release Groups – 2000

Ken Nichols, Rick Harmon and J. Scott Foott
US Fish and Wildlife Service, CA-NV Fish Health Center

Summary

All release groups appeared healthy with no significant abnormalities. No viral or bacterial pathogens were detected. Early infections of the PKX parasite were detected in 2 fish by histology. Stress treatments demonstrated healthy energy reserves and plasma ion levels in all groups examined.

Methods

Merced River Fish Facility (MRFF) Fall-run chinook salmon were sampled on 5 dates at 3 release locations. Sample dates were 04/18/00 at Durham Ferry (DF1), 04/19/00 at Mossdale Crossing (MC), 04/22/00 at Jersey Point (JP1), 04/29/00 at Durham Ferry (DF2) and 05/02/00 at Jersey Point (JP2). The sampled fish were cohorts of marked chinook released at the same locations 24 hours earlier. Forty-two fish were sampled at each site except for DF1 where only 12 fish were available due to a live box failure. The fish were removed from the live box in groups, euthanized by an overdose of tricain methane sulfonate, measured, scored for organosomatic analysis and tissue samples were collected for pathogen and physiological assays.

Organosomatic analysis included length, weight and observation of abnormalities. Blood samples were centrifuged for hematocrit and leukocrit measurements and plasma collection. Condition factor ($K = weight/length^3 * 10^5$) was calculated for each fish. Kidney tissue was assayed for bacterial pathogens. Internal organs were examined by histology for parasites and abnormalities. Samples of gill tissue were assayed for Gill Na^+, K^+-ATPase (ATPase) activity levels as an indicator of saltwater pre-adaptation (McCormick and Bern 1989).

The ability to adapt to stress was assessed by looking at plasma glucose and chloride levels in stressed and unstressed treatments. The unstressed treatment fish were removed from the live box as quickly as possible and immediately euthanized as above. The stressed treatment consisted of holding the fish out of the water for 30 seconds, allowing them to recover for 45 minutes and sampled as above.

On 04/13/00, 60 fish were randomly sampled from the entire hatchery population at MRFF. Sampling of these fish included organosomatic analysis, ATPase, histology, bacteriology, and virology. No stress physiology work was done at MRFF. Statistical analysis was done using SigmaStat for Windows.

Results and Discussion

Pathogens

No clinical signs of disease were noted in any of the groups examined. No bacterial or viral pathogens were found at MRFF or in the release groups. ELISA assay for *Renibacterium salmoninarum* (the causative agent for BKD) found no significant differences between release groups. ELISA OD values ranged from 0.065 to 0.115 indicating low *Rs* antigen levels.

Histological Examinations

No significant abnormality was observed in sections of intestine, pyloric caeca, pancreatic tissue (acinar cells), liver, kidney, or gill from the 45 fish sampled for histology (MRFF= 12, DF1= 11, MC = 5, JP1 = 6, DF2 = 6, and JP2 = 6). Early stage Proliferative Kidney Disease was observed in one MC fish and another from the JP2 sample group. This condition was characterized by low numbers of the PKX trophozoite parasite in the kidney blood sinuses without associated lesions. Eosinophilic granular cells (EGCs) were quite prominent in the lamina propria layer of the intestine and pyloric caeca from approximately half of each sample group. These immunodefensive cells are found in many organs, particularly those in direct contact with the environment such as gill, skin, and digestive tract. They are often associated

with parasitic infections and contain both peroxidase and lysozyme (Seinbjornsson et al 1996, Sire & Vernierl 1995). Earlier assumptions that EGCs acted as mast cells have been found to be incorrect as histamine is not present (Sire & Vernierl 1995). While not unusual to see in adult chinook, we have not observed such high numbers of intestinal EGCs in juvenile chinook from the Sacramento and Klamath R. systems. No lesions or parasites were associated with the EGCs found in the MRFF fish. It is possible that genetic differences may play a role in the different presentation of EGCs in San Joaquin chinook.

Physiological Data

Overall, all groups examined appeared healthy. Gill and eye abnormalities were noted in 4.5% (1 fish each) at JP2. A gill abnormality was noted in 2.7% (1 fish) at MRFF. Condition factors (Table 1) from MRFF and MC samples were significantly higher than DF2, JP1 and JP2 ($P<0.05$, 1-way ANOVA). A decrease in condition factor is normal for fish undergoing smoltification (Wedemeyer 1996), and is within the range we generally see in hatchery fall-run chinook releases (CA-NV Fish Health Center, unpublished data). Mean ATPase activity levels (Table 1) were similar at all locations except JP1 (ANOVA, $P < 0.001$). ATPase activities at JP1 were consistently higher that other locations with 9 of 10 samples above 4.0 compared with 0 or 1 samples in other release groups.

Table 1. Physiological data on the study fish collected prior to release at the hatchery (MRFF) and 24 hour after release from fish held at each release site. Data is given as mean ± SE and sample size.

	MRFF	DF1	MC	JP1	DF2	JP2
Date	4/13/00	4/18/00	4/19/00	4/12/00	4/29/00	5/02/00
FL (mm)	68.3 ± 1.5 n=55	84.4 ± 0.9 n=12	92.9 ± 1.6 n=12	84.8 ± 1.0 n=30	78.9 ± 0.9 n=32	79.1 ± 1.0 n=22
Wt (g)	3.8 ± 0.3 n=31	6.0 ± 0.2 n=12	5.8 ± 0.3 n=20	5.6 ± 0.3 n=20	5.1 ± 0.2 n=20	4.7 ± 0.2 n=22
KFL ($Wt/FL^3 \times 10^5$)	1.04 ± 0.02 n=31	0.99 ± 0.02 n=12	1.05 ± 0.02 n=12	0.93 ± 0.02 n=20	0.97 ± 0.01 n=20	0.94 ± 0.01 n=22
Hematocrit(%)	34.4 ± 1.4 n=12	37.9 ± 1.2 n=12	38.7 ± 0.8 n=20	No Data	36.9 ± 1.5 n=17	37.5 ± 1.5 n=12
Leucocrit (%)	0.33 ± 0.02 n=8	0.71 ± 0.08 n=7	0.56 ± 0.06 n=16	No Data	0.67 ± 0.07 n=16	0.54 ± 0.08 n=8
ATPase (µmoles ATP/mg protein/hr)	2.57 ± 0.36 n=12	2.04 ± 0.30 n=12	2.09 ± 0.27 n=12	4.62 ± 0.47 n=10	1.88 ± 0.19 n=12	1.54 ± 0.21 n=12

Stress Response

There was no significant difference in unstressed plasma chloride levels between release groups (ANOVA, $P = 0.052$). Mean plasma chloride for all groups combined was 121 mEq/l (±2 SE). Plasma chloride levels reported for outmigrating chinook salmon ranged from 99 to 132 mEq/l with minimum values corresponding to peak stress periods (Congleton et al. 2000). Ion loss between the stressed and unstressed treatment groups were significant only at DF2 (t-Test, $P= 0.047$) with a 6.5% decrease in the stressed group (Figure 1). Increased respiration due to stress causes a loss of plasma ions, and ion loss of 30% or greater can lead to delayed mortality (McDonald and Milligan 1997). All fish examined had plasma chloride levels within normal range. Increased salinity at the release site would have reduced ion loss due to stress treatment. No salinity data was taken at the release sites.

Elevated plasma glucose levels after stress were seen in all release groups examined (t-Test, $P <0.05$). Differences between stressed and unstressed treatments ranged from 25% to 108% (Figure 2). Failure to demonstrate hyperglycemia after stress would be an indication of low liver glycogen reserves (Wedemeyer 1996). Unstressed plasma glucose levels were similar at all release sites examined (ANOVA, $P = 0.157$). The mean plasma glucose levels of 166 g/dl

(±6) this year were higher than the 103 g/dl (±2) observed in 1998 MRFF release group (Nichols 1999) and near the values observed in chinook salmon under stress (Barton et al. 1986, Congleton et al. 2000). This may indicate that caged fish were experiencing a prolonged stress response and still had adequate glycogen reserves to mount a hyperglycemic response.

Figure 1. Plasma chloride levels for stressed and unstressed groups at each sample site. Data is given as Mean ± SE. No stressed group was included at DF1 due to small number of fish available at that site. Significant differences ($p<0.05$, t-Test) were found at DF2.

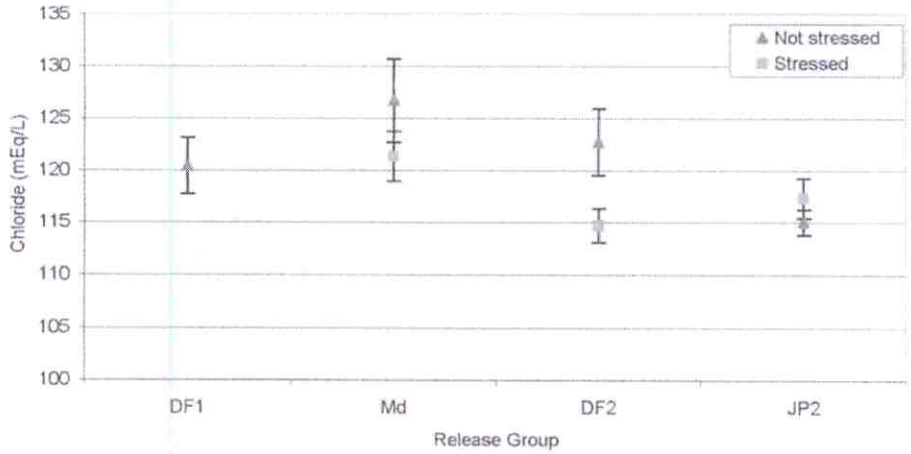
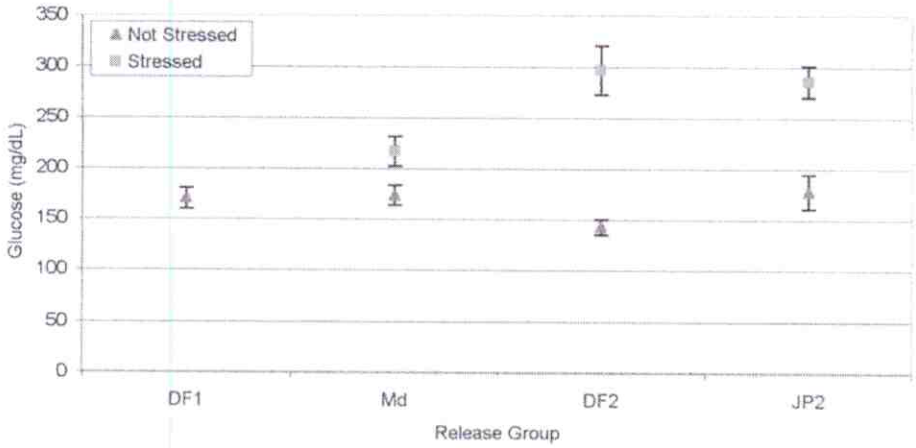


Figure 2. Plasma glucose levels for stressed and unstressed groups at each sample site. Data is displayed as Mean ± SE. No stressed group was included at DF1 due to small number of fish available at that site. Significant differences ($p<0.05$, t-Test) were found at MC, DF2 and JP2.



References

Barton BA, CB Schreck, LA Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 114:245-251.

Congleton JL, WJ LaVoie, CB Schreck, LE Davis. 2000. Stress indices in migrating juvenile chinook salmon and steelhead of wild and hatchery origin before and after barge transportation. Trans. Am Fish. Soc. 129:946-961.

McCormick, SD and HA Bern. 1989. In vitro stimulation of Na^+/K^+ ATPase activity and ouabain binding by cortisol in coho salmon gill. AM. J. Physic. 256: R707-715.

McDonald, G and L Milligan. 1997. Ionic, osmotic and acid-base regulation in stress. Pages 119-144 in GK Iwama, AD Pickering, JP Sumpter, and CB Schreck, editors. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, UK.

Nichols, K. 1999. Health Assessment of Merced River Fish Facility and Feather River Hatchery Juvenile Fall-run Chinook Salmon Released at Mossdale and CWT Fish Recovered at Chipps Island – 1998. IEP Newsletter 12(1): 34-36.

Sire M and J Vernier. 1995. Partial characterization of eosinophilic granule cells (EGCs) and identification of mast cells of the intestinal lamina propria in rainbow trout (Oncorhynchus mykiss). Biochemical and cytochemical study. Biol. Cell 85: 35 -41.

Sveinbjornsson B, R Olsen, and S Paulsen. 1996. Immunocytochemical localization of lysozyme in intestinal eosinophilic granule cells (EGCs) of Atlantic salmon, Salmo salar.

Wedemeyer, G and K Chatterton. 1971. Some Blood Chemistry Values for the Juvenile Coho Salmon (Oncorhynchus Kisutch). J. Fish Res. Bd. Can. 28(4): 606-608.

Wedemeyer, GA. 1996. Physiology of fish in intensive culture systems. Chapman & Hall. New York, NY.