

FY 2001 Investigational Report:
Health Assessment of VAMP Release Groups – 2001



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Summary

Disease and physiological assessment was performed approximately 24 hours post release on juvenile Chinook held at three release sites. No bacterial or viral pathogens were detected in the fish. All groups were infected with the PKX myxosporean parasite (the causative agent of Proliferative Kidney Disease). Infections had progressed to disease in the second set of releases with clinical signs of disease observed in 11% to 22% of those groups. A generally poor response to stress treatments was seen in several groups. Half of the groups failed to mount a hyperglycemic response to stress, and one group demonstrated perilously low blood chloride levels following stress. Partial funding for supplies and lab technician time, totaling \$8000, was provided by the USFWS Stockton Fisheries Resource Office in April 2001 (11230-1933-PY01).

Methods

The California-Nevada Fish Health Center examined 6 release groups of Fall-run Chinook salmon from Merced River Hatchery used in the Vernalis Adaptive Management Plan (VAMP) 2001 smolt survival studies. Three release locations on the San Joaquin River were sampled for two sets of releases between May 1 and May 12 2001 (Table 1). Sampling was performed approximately 24 hours post release on a subsample of salmon smolts held in live cages at the release site.

Fish were removed from the live cage, euthanized by an overdose of tricain methane sulfonate (MS222), measured for fork length, and assessed for external and internal abnormalities. Kidney tissue was assayed for bacterial pathogens, and internal organs were examined by histology for parasites or other abnormalities. Gill tissue was assayed for Na⁺, K⁺-ATPase (ATPase) activity levels as an indication of salt-water preadaptation (McCormick and Bern 1989). Plasma glucose and chloride concentrations were determined from both resting and stressed treatment fish to measure stress tolerance (Sigma Diagnostic kits 315 and 461 respectively). The resting fish were quickly removed from the live cage and immediately euthanized. The stress treatment consisted of holding the fish out of the water for 30 seconds and allowing them to recover for 30 minutes before sampling. On April 9 2001, sixty fish were randomly sampled from the Merced River Hatchery population. These fish were assessed as above with the addition of viral assays on kidney tissue. No stress tolerance work was done on these fish.

Results and Discussion

No bacterial or viral pathogens were detected. Enzyme linked immunosorbent assay (ELISA) for *Renibacterium salmoninarum* (the causative agent of Bacterial Kidney Disease) antigen found no significant difference between release groups. *Rs*-ELISA OD values ranged from 0.066 to 0.087 indicating low antigen levels near or below the negative cutoff.

The incidence of infection for the PKX myxosporean parasite (the causative agent of Proliferative Kidney Disease) in the kidney ranged from 20% in samples taken at Merced River Hatchery to 100% in all of the release groups (Figure 1). Infections had progressed to clinical disease in the second set of releases. Clinical signs of disease were evident during necropsy in 0-3% of the first release groups and 11-22% of the second release groups. Clinical signs of disease included pale gills, swollen kidney, and swollen spleen.

All release groups had similar ATPase activity levels. Levels were significantly higher in all release groups than at the hatchery 3-5 weeks earlier (Figure 2). The increase in ATPase activity demonstrated between the April 9 hatchery sample and release groups (May 1-12) indicated significant smolt development during this period.

Mean resting glucose levels ranged from 53 to 71 mg/dL, which is within the normal range reported for salmonids (Wedemeyer, 1996). Stress treatments induced significant hyperglycemic response in 3 of the 6 release groups (Figure 3). Both Durham Ferry releases and the second Mossdale release failed to show a significant increase in plasma glucose following stress.

Mean resting plasma chloride concentrations ranged from 111 to 127 mEq/L, which is within the normal range reported for salmonids (Wedemeyer, 1996). Ion loss due to stress was significant only in the second Mossdale group with an 8% change (Figure 4). One initial response to stress is an increase in blood pressure and a resulting increase in the perfusion area of the gill lamella. This occurrence results in ion loss due to passive diffusion. Blood electrolyte loss can continue for 4 hours following stress and a total loss of 30% or more can be lethal (McDonald and Milligan 1997). Hypochloremia becomes life threatening in salmonids when levels fall below 90 mEq/L (Wedemeyer et al 1990). Resting chloride levels in the second Mossdale group were in the low-normal range, and were perilously low 30 minutes following stress.

A number of factors may have caused the poor hyperglycemic response observed in several of the release groups. Low liver glycogen is the typical interpretation (Barton et al 1986). Hatchery-reared fish typically have no shortage of glycogen reserves given their high-energy diets. Stress due to transport and holding may have depleted the reserves, but VAMP release fish in 2000 with chronically elevated glucose levels were still able to mount a significant glucose response to stress (Nichols et al 2000). Increases in plasma glucose may have taken longer than the 30 minutes allotted in the stress treatment. While plasma glucose concentrations continue to increase after 30 minutes it is significant that half of release groups were either slow or unable to mobilize energy reserves important for rapid "fight-or-flight" response to stress. Poor stress tolerance is typical of PKX infections (Lom and Dyková 1995). Chronic PKX infection could desensitize the stress response of the fish making them more susceptible to the stress of transport and holding conditions.

The apparent differences between release groups appear more site than time (disease status) related. Physiological data while not statistically significant at our sample size suggest trends within sites (Figures 2-4). If it is assumed that rearing and transport conditions were similar for all release groups, the differences were likely due to site conditions. Either the environment at Durham Ferry was less than optimal or the Jersey Point conditions were somehow mitigating for unhealthy fish. These differences between sites may be an artifact of holding conditions. The added stress of a poor

environment would slow recovery, but a factor such as current through the live box, allowing fish light exercise following stress, reduces plasma cortisol concentrations and speeds recovery (McDonald and Milligan 1997). Assuming all the groups would eventually recover stress tolerance may not be an important measure following release due to dissimilar holding conditions. Disease state (such as the 100% PKX infection rate) may be much more important to fish performance. As standardizing the holding conditions would likely not be practical, better information on true differences between groups could be more effectively measured at the hatchery prior to transport.

Table 1. Site and 24-hour post release sample date for the first and second set of VAMP 2001 releases.

Site	First Release	Second Release
Durham Ferry	May 1, 2001	May 8, 2001
Mossdale	May 2, 2001	May 9, 2001
Jersey Point	May 5, 2001	May 12, 2001

Figure 1. Incidence of early stage PKX infection (Early Stage) and Clinical Proliferative Kidney Disease (Clinical) in posterior kidney samples. Early Stage indicates light presence of parasite, but no associated lesion. Clinical indicates presence of parasite with associated lesion likely impairing kidney function.

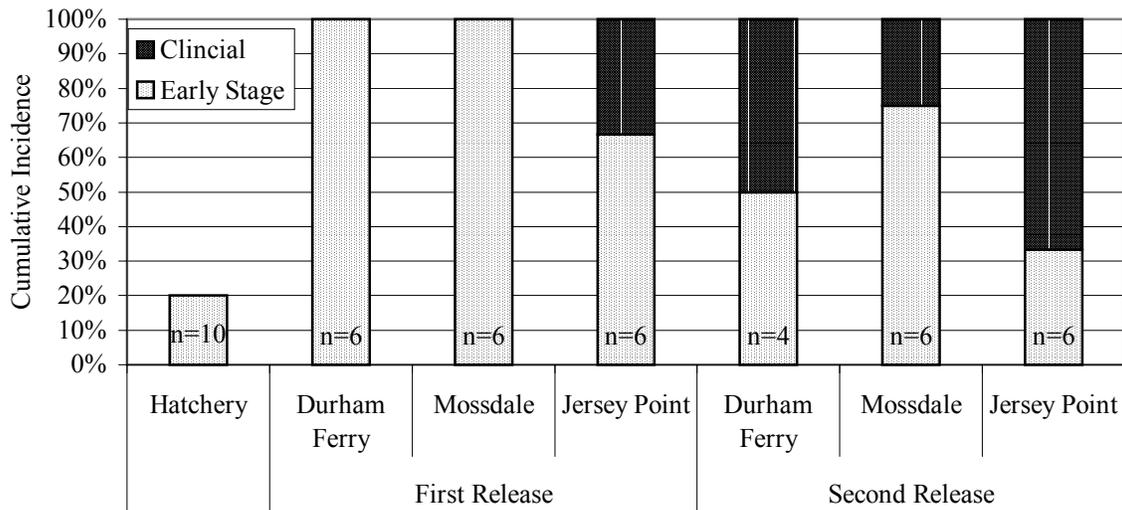
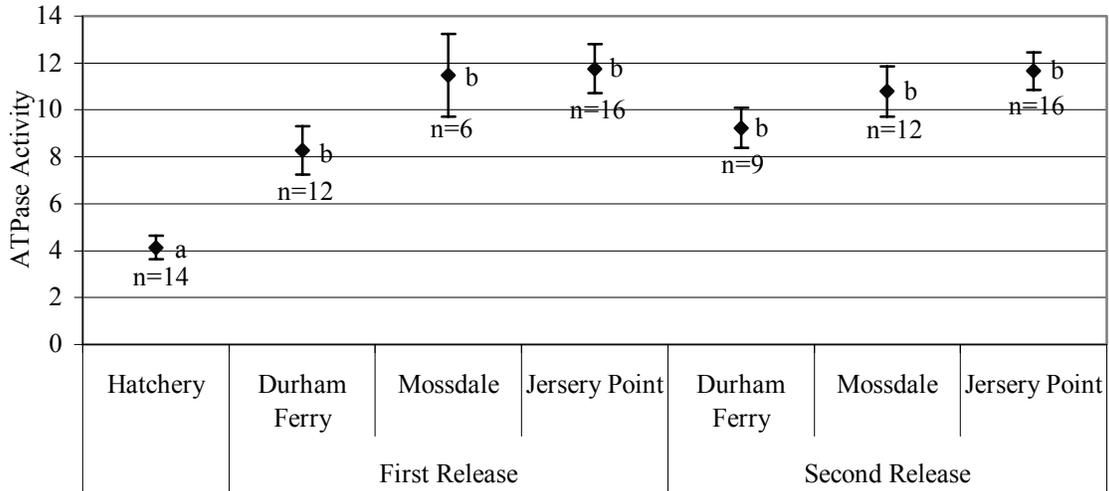
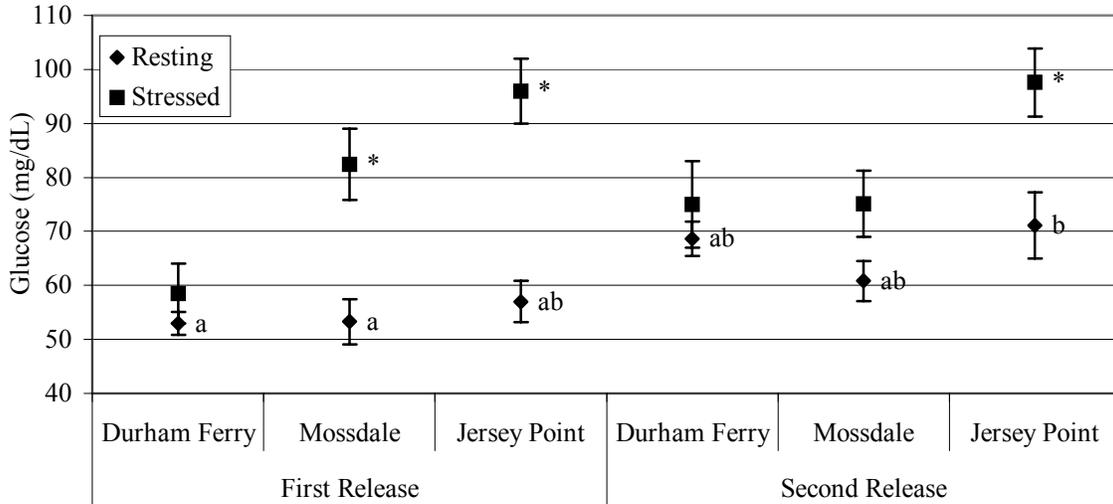


Figure 2. Gill Na⁺,K⁺-ATPase activity (mmol ADP/mg protein/hr) levels in VAMP 2001 release groups and at Merced River Hatchery 3+ weeks prior to release. Data given as Mean ± SEM and number of samples.



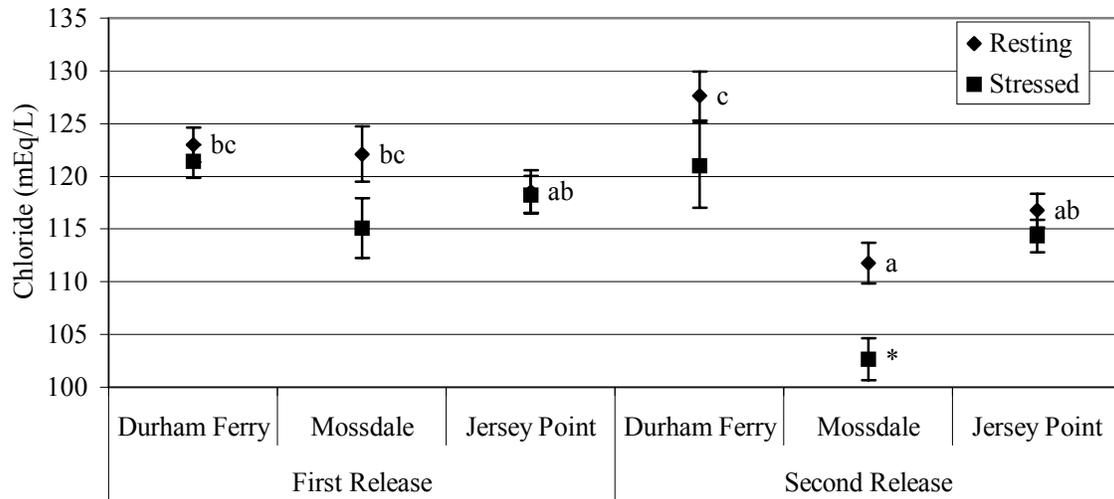
Notes:
a<b (P<0.05, ANOVA)

Figure 3. Resting and stressed plasma glucose concentrations in VAMP 2001 release groups. Data given as Mean ± SE. Sample number is 12 for all groups except first Durham Ferry Resting (n=11).



Notes:
* = difference between Resting and Stressed (P<0.05, t-test)
a<b between Resting levels (P<0.05, ANOVA)

Figure 4. Resting and stressed plasma chloride concentrations in VAMP 2001 release groups. Data given as Mean \pm SE. Sample number = 12 for all groups except first Durham Ferry Resting (n=11).



Notes:

* = difference between Resting and Stressed ($P < 0.05$, t-test)

a < b < c between Resting levels ($P < 0.05$, ANOVA)

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