

Hatchery Reform Project Progress Report

1. **Project Title:** Nature versus Nurture: Do hatchery practices impair brain development and compromise fitness of salmon?
2. **Agency:** NMFS
3. **HSRG Funding Amount:** \$30K

4. **Project Status:**

Previous studies have shown differences in brain allometries between hatchery and wild trout. However these differences could not be attributed to genetic or environmental factors. The goal of this project is to determine if rearing environment affects brain development in juvenile steelhead. Our research plan was to first develop and validate methods to quantify the rate of neurogenesis in juvenile salmon. And secondly to use these methods to determine if rearing environment affects the rate of neurogenesis in the brain of juvenile salmon. The method for estimating the rate of neurogenesis involved injecting fish with bromodeoxyuridine (BrDU), which is incorporated into newly synthesized DNA, and at a later time point collect brain tissue and use immunocytochemistry to quantify the amount of BrDU incorporated into specific brain areas. We also planned to compare this method to simple measurements of dimensions and volumes of specific brain areas. Therefore, the first step was to establish and validate methods for estimating brain volumes using histology and image analysis. Therefore, the first step was to collect brain samples from juvenile fish, and begin processing samples for brain volume measurement.s.

We had the opportunity to collect brain samples from juvenile steel head which have been reared on three different environments as part of another study by B. Berejikian (Northwest Fisheries Science Center, Seattle). The advantage of collecting these samples was that Dr. Berejikian and colleagues are evaluating behavior in the experimental fish and it may be possible to link any brain size differences to behavioral differences. In that study, Skookumchuch steelhead were reared in one of three environments from first feeding (March 2001) through September 2001: 1) conventional, 2) enriched and 3) natural. The conventional environment consisted of 4 ft diameter fiberglass tanks. The enriched environment consisted of 4 ft diameter fiberglass tanks containing camouflage cover, and defoliated pine trees. Fish were fed under water, not from the surface as with the conventional environment. The natural environment was a natural stream with no supplemental food. Fish fed only on natural diets. All fish were reared in fresh water at the University of Washington, Big Beef Creek Field Station near Seabeck, Wa. Water temperature in the tanks was relatively constant

The experimental rearing environments are shown in Figure 1-3 below.



Figure 1. Conventional rearing environment



Figure 2. Enriched rearing environment



Figure 3. Natural rearing environment

At the termination of the rearing period, 20 fish per treatment (10 per replicate tank) were euthanized with an overdose of buffered MS222. Body lengths and weights were recorded, and whole body photographs were taken for future measurements of eye diameter. Finally, whole bodies were fixed in 10% buffered formalin for histological analyses of brain volumes.

Examples of fish reared on the three environments are shown below in figures 4-6.



Figure 4. Fish reared in conventional rearing environment.



Figure 5. Fish reared in enriched rearing environment.



Figure 6. Fish reared in natural stream environment.

In addition to coloration differences, the fish reared in the natural stream environment were significantly smaller than those in the conventional or enriched environments (Figs. 7-8). However, condition factor of fish reared on the enriched environment was significantly lower than fish reared on either the natural or conventional environments (Fig. 9).

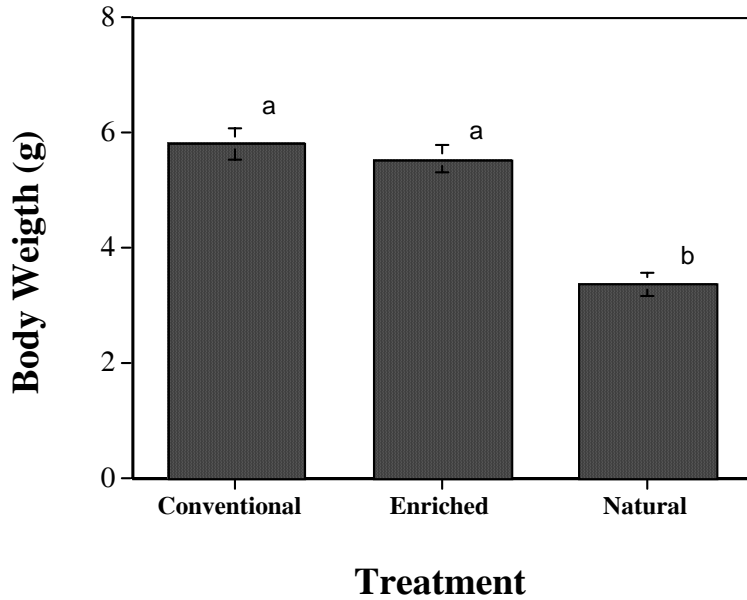


Figure 7. Body weights of steelhead reared on three rearing environments. Data are mean +/- SEM, n= 20 fish/treatment. Significant differences are indicated ($p < 0.05$).

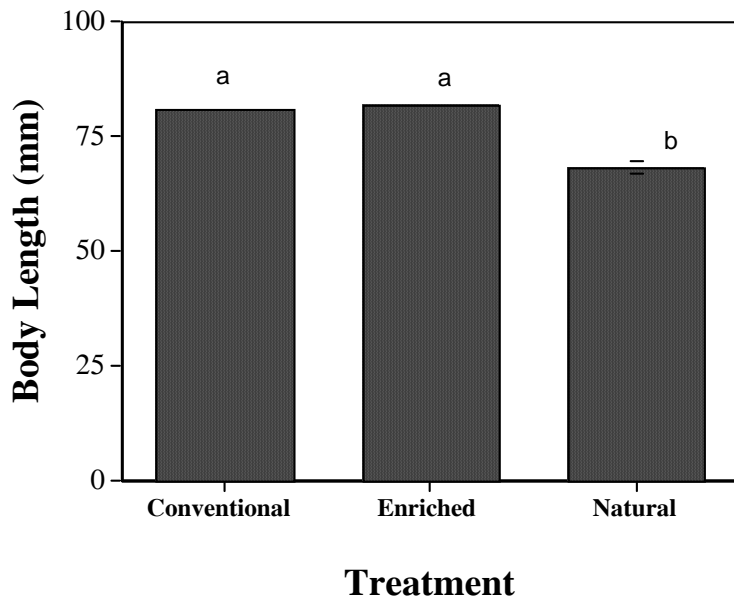


Figure 8. Body lengths of steelhead reared on three rearing environments. Data are mean +/- SEM. Significant differences are indicated ($p < 0.05$).

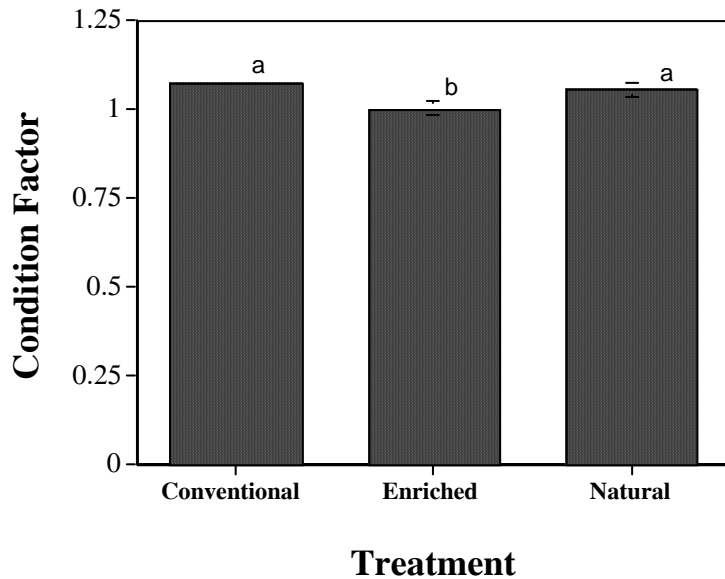


Figure 9. Condition factor of steelhead reared on three rearing environments. Data are mean \pm SEM. Significant differences are indicated ($p < 0.05$).

Brain volume measurements have not yet been made. We are in the process of establishing methods to process the samples histologically and testing various image analyzing software. We will begin testing the BrDU labeling techniques for quantifying cell proliferation from January 2001 –February 2002 using juvenile coho salmon reared at the NWFSC Montlake hatchery.

5. Status of Timeline: Provide a summary of your timeline to complete the activity.

Sectioning and staining brains from steelhead samples: December 2001- February 2002 .

Image analysis: January 2001-February 2002

Final Data Analysis and Draft Manuscript: March –April 2002

Test of BrDU labeling: Immunostaining of samples February-March 2002.

6. Major Problems Encountered or Anticipated: The major problem will be quantifying brain volume in fish that were too small to perfuse with fixative. Whole fish had to be fixed and processed for histology. We anticipate difficulty with processing the tissue fixed in this manner.

The second major problem may be with our inability to process large numbers of samples for this study. The sample number may be too small to detect differences in the treatment groups.