

**METABOLIC COMPARISON OF HATCHERY AND WILD  
SALMONIDS USING OTOLITH MICROSTRUCTURE AND ENZYME  
ACTIVITY**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

Hatchery culture of salmonids has become a standard in aquaculture and wild population restoration and enhancement. Because of frequent interactions between wild and hatchery-reared salmonids, domestication selection is potentially a serious problem and the physiological and selective mechanisms underlying domesticated behavior deserve close attention. We hypothesize that domesticated behavior in salmonids is caused by selection for elevated metabolic rates in the hatchery environment. Fish with an innate tendency for a higher metabolic rate (MR) may be favored in hatcheries because they develop faster, start exogenous feeding earlier, and are more aggressive competitors for food. A prerequisite for selection of high metabolic rate is that natural variation for relatively high or low metabolic rate exists and that this tendency is heritable. Although MR in fish varies greatly with temperature, body weight, life stage, and even time-of-day, there is inter-individual, probably persistent, variation in MR under identical environmental conditions (Metcalfé et al., 1995; Cutts et al., 1999). Egg development of individuals with a high MR has been shown to be accelerated. (Metcalfé et al., 1995; Cutts et al., 1999) and individuals with a higher MR are more aggressive and dominate over low MR individuals (Metcalfé et al., 1995; Yamamoto et al., 1998; Cutts et al., 1999). Growth rate of the otolith in larval fish is a function of basal MR rather than

somatic growth rate (Wright, 1991). In salmon from different populations raised under identical conditions, otolith size variation should reflect differences in MR at a given life stage. Similarly, although there is extensive variation in metabolic enzyme activity, often resulting from environmental effects or body-size scaling, the potential exists for heritable variation in enzyme function (Leonard and McCormick, 2001).

In this study, we examined commonly reared larvae from hatchery-maintained and wild lake trout (*Salvelinus namaycush*) from the same genetic origin (Marquette Harbor, MI) for differences in metabolic parameters. We also examined hybrid larvae produced by crossing wild females with hatchery males. For these hybrid crosses, wild females were selected that had also been spawned in wild pairs (i.e. clutches in both the hybrid and wild groups were produced using the same mothers). Spawnings were conducted from mid-October to early November 2001. Fertilized eggs (and larvae) from each adult crossing were maintained separately throughout the study under constant 12°C conditions. Three months after fertilization, larvae were sampled; larvae were pre-swim-up, yolk-sac stage.

At sampling, individuals for otolith microstructure analysis were weighed and the sagittal otoliths were removed. The left otolith was fixed to a glass slide, ground in the sagittal plane and polished using lapping film of 12, 6, and 3  $\mu$  grain size. Digital microphotographs were taken with a microscope-mounted camera and the files analyzed with imaging software (NIH image). Two measurements were taken in the posterior quadrant of each otolith: a) the distance between the nearest nucleus and the posterior edge on a line transecting all central nuclei and b) mean width of ten daily increments just inside of an otolith check that was thermally induced by subjecting the alevins to lower temperature water (6°C) on January 2-4 and 7-8. Otoliths are still being processed and analyzed at this writing, but data from this portion of the study will be presented during the conference.

Individuals for enzyme analysis were immediately frozen at -70°C. Citrate synthase (CS), lactate dehydrogenase (LDH), pyruvate kinase (PK) and malate dehydrogenase (MDH) activities were assessed on whole body homogenates of individual fish. All enzymes were analyzed at 25°C on the same homogenate using a microplate spectrophotometer (for further details on enzyme protocols and assays used, see Leonard and McCormick, 2001). Data were assessed for the effect of parental origin (wild, hatchery or wild-hatchery hybrid) and for maternal effects (i.e. inherited effects based on mother).

For the enzyme data, we found no significant effect of parental origin using either multivariate or univariate ANOVA techniques (Fig 1; ANOVAs  $p > 0.09$ ; MANOVA (Wilks)  $p = 0.29$ ). To analyze the potential effects of maternal contribution to MR, we only assessed the hybrid and wild offspring (there were no matching mothers in the hatchery crosses). Using these two groups and MANOVA, we found a significant maternal effect ( $p = 0.03$ ), but no effect ( $p = 0.52$ ) of overall parental group (hybrid or wild). Using single enzyme ANOVA, we demonstrated a maternal effect on CS ( $p = 0.02$ ), LDH ( $p = 0.01$ ). The maternal effect on PK was marginally significant (ANOVA  $p = 0.05$ ; MANOVA  $p = 0.18$ ). Although there is variation in the post hoc comparisons (using least significant difference testing), typically a mother produced offspring with either consistently high, low or moderate enzyme activities (Fig. 2). For example, offspring of females 6 and 7, regardless of paternity (hatchery or wild), typically demonstrated significantly elevated enzyme levels, while offspring of females 2 and 5 were usually significantly lower in relative enzyme activity.

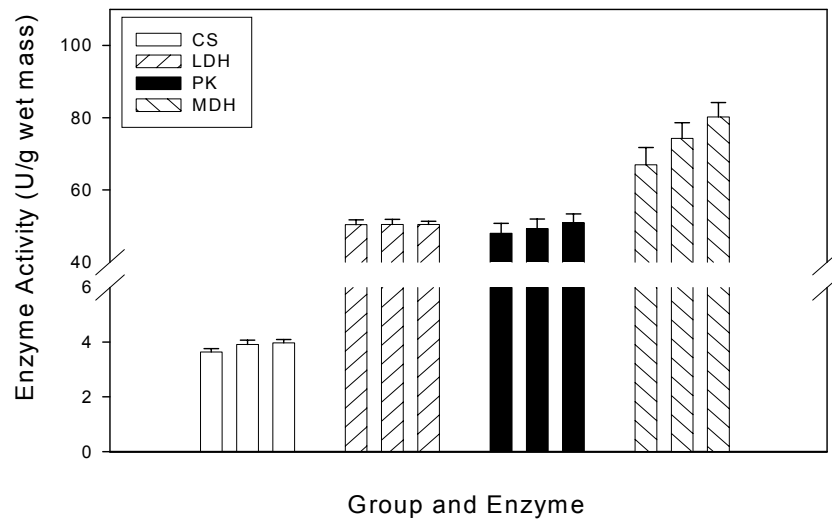


Figure 1. Comparison of the activity of four enzymes from whole body preparations of larval lake trout from groups of wild (W), hatchery-wild hybrid (H) and hatchery (cultured, C) parental origin. Bars are mean  $\pm$  standard error. None of the within enzyme comparisons are significant.

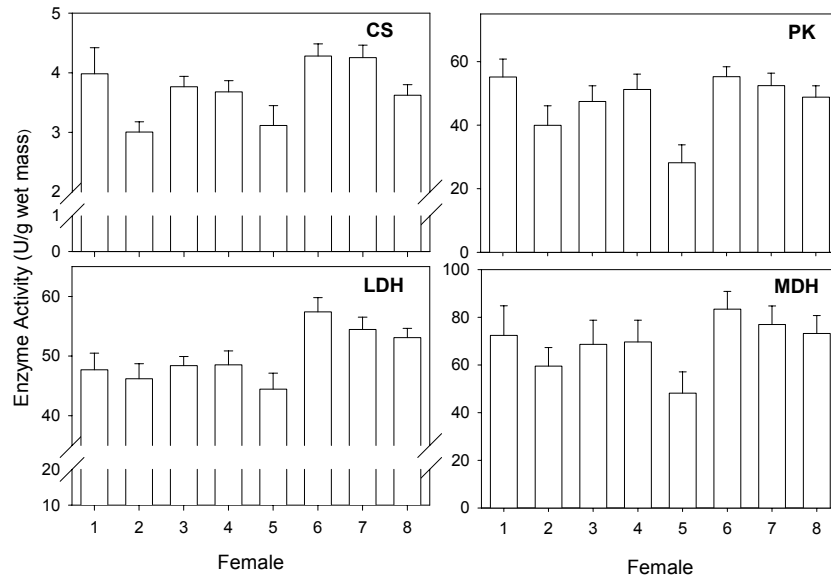


Figure 2. Comparison of the effects of mother on enzyme activity. Females were arbitrarily assigned a number, but numbers are constant between panels. Bars are mean  $\pm$  standard error. CS and LDH showed a significant difference in activities of offspring of different mothers and PK was marginally significant. For simplicity, results of post-hoc testing are not shown; however, in CS, LDH and PK, offspring of female 6 always had higher activities than offspring of females 2 and 5.

Although our results are still preliminary and we will offer an expanded data set in our presentation, our initial results are intriguing. We were not able to show a significant effect of parental origin on metabolic enzyme activity in lake trout; however, we were able to demonstrate the heritable nature of these parameters based on offspring of particular (female) individuals. This suggests that, although we did not see an effect of hatchery selection in this study, the initial prerequisite of genetic variation of metabolically important characters within strains does exist and warrants further investigation.

### References

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