

**THE SEASONAL PRODUCTION OF GLYCEROL IN RAINBOW  
SMELT (*OSMERUS MORDAX*) IS ASSOCIATED WITH INCREASED  
ACTIVITY OF LIVER GLUCONEOGENIC AND GLYCEROL  
PRODUCING ENZYMES.**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

Rainbow smelt (*Osmerus mordax*) are an anadromous species from the family Osmeridae that inhabit inshore waters along the North American Atlantic coastline. During the winter, waters in this area are frequently ice covered and can reach temperatures as low as  $-1.9^{\circ}\text{C}$ . Rainbow smelt remain in these waters during the winter; therefore they must employ a biochemical means of freeze resistance. Previous studies have shown smelt to produce low levels of type II antifreeze protein (AFP). However, their main method of freeze resistance appears to come from the production and accumulation of high levels of glycerol (200-400 mM), which lowers the freezing point of the blood via normal colligative means (Ewart & Fletcher, 1990; Raymond, 1992). The production of glycerol in the smelt occurs in the liver through the breakdown of amino acids via gluconeogenesis (Raymond & Driedzic, 1997).

The main objective of this study was to perform a long term acclimation study in order to determine the seasonal pattern of the antifreeze response in smelt and to examine key liver enzymes responsible for amino acid breakdown, gluconeogenesis and glycerol production as potential regulating loci for increased glycerol production. Wild caught smelt were maintained in a laboratory setting from October 2000 to June 2001, at two different temperature regimes, one which tracked ambient seawater temperature ( $11^{\circ}\text{C}$  to  $-1.5^{\circ}\text{C}$ ) and

the other which was maintained at 8-10°C throughout the study. A subgroup of fish from the ambient treatment were moved to chilled seawater in March, in order to determine the response of smelt to extreme temperatures.

### Seasonal Glycerol Production

The initial upregulation of the antifreeze response occurred well before the onset of subzero temperatures. Glycerol levels increased to 80 mM in December when water temperatures were between 5-6°C. The accumulation of glycerol in the blood was found to be inversely related to temperature, as the highest concentrations (200-300 mM) were found at the lowest water temperatures (-1.0 to -1.5°C).

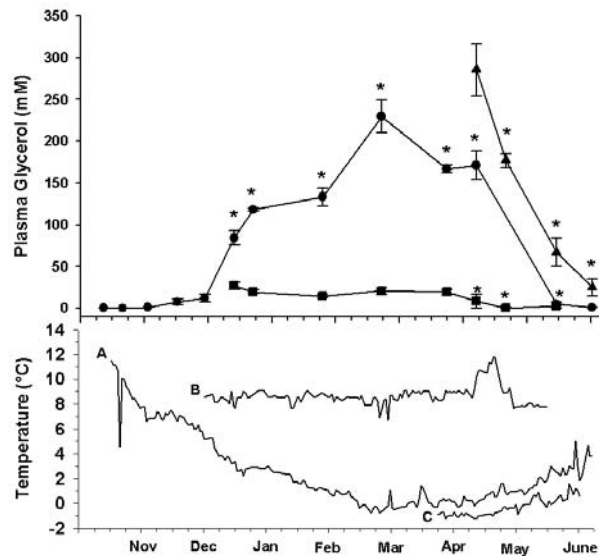


Figure 1. Seasonal pattern of plasma glycerol levels (mM) (□ ambient, □ heated, □ chilled) and temperature profile (A=ambient, B=heated, C=chilled) for smelt held in a long-term acclimation study from October 2000 to June 2001. Symbols represent mean  $\pm$  SEM (n=5), \* indicates significance from initial sampling point within a treatment.

Fish in the heated seawater treatment maintained slightly elevated levels of glycerol (10-20 mM) throughout the winter despite the warm water temperatures. Fish in all three treatments lost glycerol from the blood at the same point of time, regardless of the temperature they were held at, indicating that the loss of glycerol from the blood is controlled by a factor other than temperature. One potential trigger for the loss of glycerol in the spring could be a photoperiod cue of increasing day length.

### **In Vitro Liver Enzyme Activity**

Previous studies comparing smelt to other teleosts from similar environments and to capelin, another osmerid species, have indicated the liver of smelt to be specifically poised for increased glycerol production via gluconeogenesis, during the winter months. Enzymes responsible for amino acid breakdown, gluconeogenesis and glycerol production were all significantly higher in smelt than in these other teleost species (Driedzic et al., 1998; Treberg et al., 2002).

Results from this study show a seasonal increase in activity levels of alanine aminotransferase (AlaAT), phosphoenolpyruvate carboxykinase (PEPCK) and glycerol-3-phosphate dehydrogenase (G3PDH) which, respectively, are key enzymes of amino acid breakdown, gluconeogenesis and glycerol production. AlaAT and G3PDH show significant increases in activity at the same time as the initial upregulation of glycerol production in the fall of the year. As well, the increasing activity of these three enzymes during the study was positively correlated with increasing plasma glycerol levels in fish held at ambient seawater temperatures. These results indicate that AlaAT, PEPCK and G3PDH are associated with, but not necessarily the regulating loci for increased glycerol production in rainbow smelt. The seasonal activity of aspartate aminotransferase (AspAT) does not correlate with increasing glycerol production indicating that it is not as closely linked with glycerol production for use as an antifreeze as the other enzymes studied.

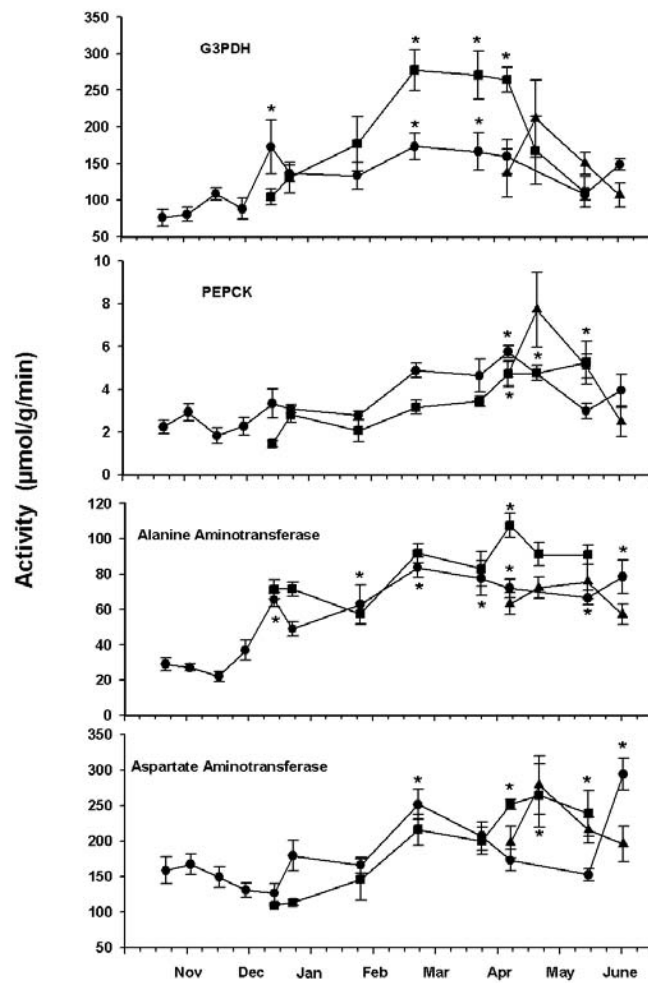


Figure 2. Seasonal pattern of in vitro liver enzyme activity ( $\mu\text{mol g}^{-1} \text{min}^{-1}$ ) for G3PDH, PEPCK, AlaAT and AspAT in rainbow smelt in  $\square$  ambient,  $\square$  heated,  $\square$  chilled sea water treatments. Symbols represent mean  $\pm$  SEM, \* indicates significance from initial sampling point within a treatment.

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