Supporting Information

Life-history trade-offs and limitations associated with phenotypic adaptation under future ocean warming and elevated salinity

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Enzyme assay methodology

Determination of citrate synthase and electron transport system activities

Citrate synthase and ETS activities were normalized to protein content. Measurements were taken using a UV/VIS microplate spectrophotometer (Perkin Elmer Envision, Foster City, CA, USA). Samples were homogenized in 120 μL of ice-cold 100 mM phosphate buffer, 20 mM EDTA (pH 8.00). CS, ETS and protein content were quantified using the same homogenate. CS activity was measured in 0.1 mM 5,50-dithiobis (2-nitrobenzoic acid) (DTNB), 0.1 mM acetyl-CoA and 0.15 mM oxaloacetate (pH 8.00). CS activity was measured in triplicate at 27°C and was calculated from the increase in absorbance at 412 nm over 3 min (e412 = 13.6 mL cm^{-1} μmol^{-1}), caused by the reduction in DTNB (1). ETS activity was measured in 0.85 mM b-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate, 2 mM Iodonitrotetrazolium chloride (INT) and 0.03% TritonTM X-100 (Sigma-Aldrich, Mississauga, ON, Canada) (pH 8). Activities were measured in triplicate at 27°C by following the increase in absorbance due to the reduction in INT at 490 nm for 4 min (e490 = 15.9 mL cm^{-1} μmol^{-1}) (2). Total protein content was determined on homogenates using the bicinchoninic acid method (3).

References

1. Thibeault M, Blier PU, Gurderley H. 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss)*. Fish Physiology and Biochemistry. (1997); 16:139–155.
2. Bergmeyer HU. Methods of enzymatic analysis, deerfield beach. Verlag Chemie, Florida (1993).
3. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Analytical Biochemistry. (1985) 1:76–85.