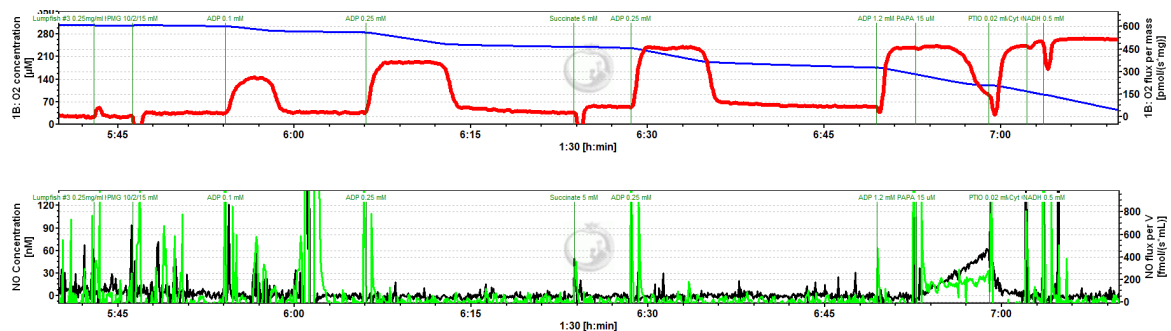


**Supplementary Figure S1**

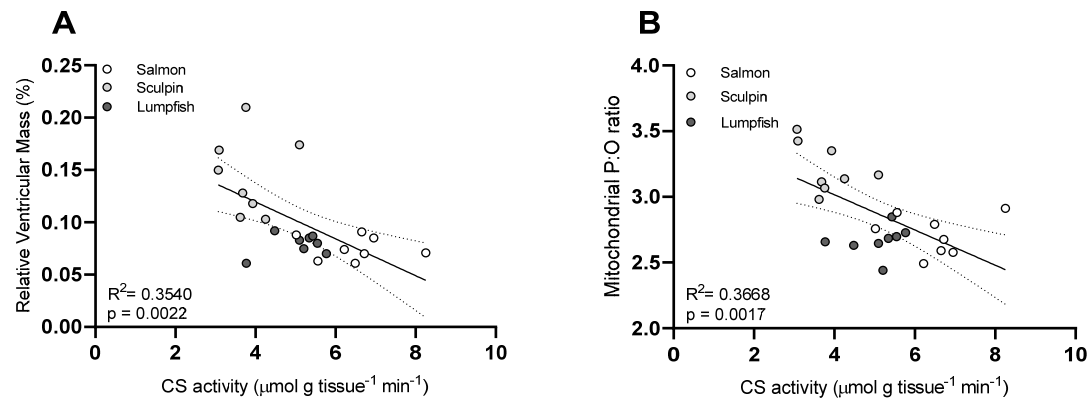
**Supplementary Figure S1.** Representative recording of Experiment 1. The figure shows the simultaneous measurement of O<sub>2</sub> consumption (flux) and ROS release rate (flux) during OXPHOS (State 3) and leak (State 4) respiration in the presence of substrates of complex-I alone (pyruvate, malate and glutamate) and complexes-I+II (+ succinate). O<sub>2</sub> concentration (in μM) is represented by the blue line, O<sub>2</sub> flux [i.e., mitochondrial respiration rate (in pmol O<sub>2</sub> (s\*mg)<sup>-1</sup>)] by the red line, H<sub>2</sub>O<sub>2</sub> concentration (in μM) by the black line and H<sub>2</sub>O<sub>2</sub> flux [i.e., mitochondrial ROS release rate (in pmol H<sub>2</sub>O<sub>2</sub> (s\*mg)<sup>-1</sup>)] by the green line. Events and concentrations of chemical used are labeled on the recording. Figure shows data from cardiac mitochondria (0.25 mg mL<sup>-1</sup> chamber) of lumpfish incubated at 12°C.

**Supplementary Figure S2**



**Supplementary Figure S2.** Representative recording of Experiment 2. The figure shows the measurement of mitochondrial respiration during OXPHOS (State 3) in the presence of substrates of complexes-I+II (pyruvate, malate, glutamate and succinate, PMGS) and excess ADP; following the initiation of NO production (using the NO donor PAPANONOate); after PTIO was added to the chamber to scavenge NO and re-establish OXPHOS respiration; and finally after cytochrome c and NADH were added to examine mitochondrial integrity. This protocol was used to determine the sensitivity of mitochondrial respiration to NO (i.e., its IC<sub>50</sub>). O<sub>2</sub> concentration (in μM) is represented by the blue line, O<sub>2</sub> flux [i.e., mitochondrial respiration rate (in pmol O<sub>2</sub> (s\*mg)<sup>-1</sup>)] by the red line, NO concentration (in μM) by the black line and NO flux (in fmol NO (s\*mg)<sup>-1</sup>) by the green line. Events and concentrations of chemical used are labeled on the recording. Figure shows data from cardiac mitochondria (0.25 mg mL<sup>-1</sup> chamber) of lumpfish incubated at 12°C.

### Supplementary Figure 3



**Supplementary Figure S3.** Relationships between ventricular citrate synthase (CS) activity, and relative ventricular mass (RVM) and mitochondrial efficiency (P:O ratio), of Atlantic salmon, short-horned sculpin and lumpfish. The solid line represents the overall linear pairwise regression and the dotted lines represent the 95% confidence limits of the fitted line. Symbols represent individual paired values. Values are means  $\pm$  s.e.m. (N=8 per group). A  $P < 0.05$  was considered significant.