

CAN THE MITOCHONDRIAL MALONDIALDEHYDE CONTENT BE AN USEFUL TOOL TO DISTINGUISH ECOLOGICAL QUALITY OF *PETROMYZON MARINUS* HABITAT?

M. Candeias(1), I. Alves-Pereira(1,2), M. J. Lança(1,3), A.F. Ferreira(5), B.R. Quintella(5,6),

P. R. Almeida(4,5), and R. Ferreira(1,2)

⁽¹⁾ICAAM-Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora, Portugal, Phone Number: +351 266 745 311, e-mail: raf@uevora.pt

⁽²⁾Departamento de Química, ECT, Universidade de Évora, Évora, Portugal.

⁽³⁾Departamento de Zootecnia, ECT, Universidade de Évora, Évora, Portugal.

⁽⁴⁾Departamento de Biologia, ECT, Universidade de Évora, Évora, Portugal.

⁽⁵⁾Centro de Oceanografia, Faculdade de Ciências, Universidade de Lisboa, Portugal.

⁽⁶⁾Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Portugal

1. Introduction – The sea lamprey, *Petromyzon marinus* L., 1758, is a cyclostome widely distributed along the North Atlantic which is present in several European rivers basins. Sea lamprey has been decreasing in Portugal, being classified as ‘Vulnerable’ in the Portuguese IUCN Red List of Threatened Vertebrate Species. Chemical pollution is one of the factors contributing to the decrease in *P. marinus* populations. Larval ammocoetes generally spend 4–7 years in the substrate of rivers and streams before undergoing a period of metamorphosis with marked morphological and physiological changes that ends with the start of the juvenile (i.e. macrophthalmia) trophic migration to the sea. Aerobic metabolism is influenced by the capacity of mitochondria to generate ATP to meet the energetic demands of tissues with continuous production of reactive oxygen species (ROS). Oxidative stress arise when balance between oxidant species, as O_2^- , OH^\cdot or H_2O_2 , and antioxidant species, as glutathione (GSH) is disturbed. Chronic oxidative stress may occur if chemical pollutants presents in the sea lamprey habitat contribute to increase lipid peroxidation and cause cell damages. This response may eventually compromise the success of *P. marinus* seawater acclimation. So, the aim of this study was to compare the glutathione and MDA levels of gills and liver mitochondria of sea lamprey juveniles captured in Minho, Lima and Vouga river basins.

2. Experimental - Sampling occurred at the beginning of the *P. marinus* downstream migration in the Minho, Lima and Vouga rivers basins of Portugal. The sampled *P. marinus* juveniles were transported alive to the laboratory in appropriate life support conditions and maintained in 200 L tanks. The salinity raised gradually from 0 psu to 35 psu, following a three step procedure with a time interval of 8 days for 30 days, or in freshwater. Mitochondria obtained by centrifugation at 15000 g, 30 min, 4 °C, of gills and livers homogenates, prepared in 50 mM Tris-HCl pH 7.5 buffer, were stored at -80°C for subsequent determination of protein, by spectrophotometry, ROS, MDA, GSH and GSSG, by fluorescence. All values were presented as mean of five pools of gill (#5) or liver (#8) \pm SEM. The statistical analysis were performed by ANOVA I and Duncan test to determine significant differences ($p < 0.05$) between treatments, using SPSS for Windows, version 22, licensed to University of Évora.

3. Results and Discussion - The results showed that sea lamprey juveniles from Lima basin exhibit the highest mitochondrial MDA levels of gills. However, no significant changes were detected in markers of oxidative stress and cell damages in the mitochondria of the gills of the animals captured in the three basins, during salt acclimation. The hepatic mitochondrial contents in proteins, GSH, ROS as well as GSH/GSSG ratio of macrophthalmia, captured in the Minho basin, decreased to the levels detected in the animals captured in the Lima and Vouga basins, during salt acclimation, although the MDA level in the liver mitochondria doubled in the animals from the Vouga basin.

4. Conclusions - The highest level of mitochondrial damages, detected in the gills from animals captured in the Lima basin, may suggest increased difficulties in seawater acclimation. However, this study did not detect an induction of oxidative stress, neither an increase of mitochondrial damages caused by salt acclimation of *P. marinus* juveniles captured in the three basins of Portugal. The steady pattern of stress markers detected in the hepatic mitochondria of macrophthalmia, suggest that the animals from the three basins are metabolically prepared to the parasitic phase. However, the increase in oxidative damages of hepatic mitochondria of macrophthalmia from Vouga basin suggests the occurrence of metabolic failures which may disturb the adaptation of these animals to the parasitic feeding phase. So, the mitochondrial MDA levels of gills and liver of *P. marinus*, may eventually prove a useful tool to discriminate sea lamprey habitat quality.