

The ecological quality of the Sea lamprey habitat may negatively impact the recruitment of juveniles for trophic migration

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- 1. Introduction The Sea lamprey (*Petromyzon marinus* L., 1758) is a cyclostome that occurs in the river basins of Portugal. It has been classified as 'Vulnerable', according to the Portuguese IUCN Red List of Threatened Vertebrate Species, due to the continued decline of individuals. Its life cycle begins in fresh water with the hatching of eggs in rivers, producing ammocoetes that are buried in river beds in places with clean, oxygenated water, and they are fed by means of filtration. The larval phase lasts for 4-5 years and ends after metamorphosis, and they become transformers. These then embark on a process of trophic migration to the sea, where they remain for 2-3 years. Metamorphosis prepares the ammocoetes for the gradual increase in salinity that occurs on the journey from the fresh water environment to the estuarine environment. However, salinity may be toxic and is sometimes associated with delayed recruitment of individuals for trophic migration. The loss of quality in the fresh water environment due to the presence of industrial or agricultural pollutants may also affect the gills' capacity for xenobiotic biotransformation, inducing oxidative stress. The aim of this work was to characterise the gills' xenobiotic conjugation and the oxidative stress response systems of sea lamprey transformers, by observing individuals captured in two river basins: the Vouga and the Lima (which is less polluted) and exposing them to a salt gradient of 0-35 in tanks, at salinity 0, 10, 25 and 35.
- **2. Experimental** Sampling occurred at the beginning of the Sea lamprey downstream migration in the Lima and Vouga river basins in Portugal. The sampled transformers were then transported alive to the laboratory under suitable life support conditions and maintained in 200 L tanks. Salinity levels were raised gradually from 0 to 10, 25 and 35, following a three-step procedure with a time interval of 8 days for 30 days, or in fresh water. At each step, body length, body weight and wet weight of the gills of each individual captured in the Lima and Vouga basins were recorded. Post-mitochondrial supernatants obtained by means of centrifugation at 15,000 g, 30 min, at 4 °C of gill homogenates, prepared in 50 mM Tris-HCl pH 7.5 buffer, were centrifuged at 105,000 g, for 1 h at 4 °C. Aliquots of the resulting microsomes and cytosol were stored at -80 °C for subsequent determination of protein, mGST, GR, GPx, G6PD, CTT1 and cGST enzyme activities by means of UV/Vis spectrophotometry as well as GSH, GSSG and ROS contents by means of fluorescence. All values were presented as the mean of five pools of gills (#5) ± SEM. Statistical analysis was performed by using ANOVA I and the Duncan test.
- **3. Results and Discussion** The gradual acclimation of transformers captured in the Lima and Vouga basins to salinity did not affect gill enzyme activities, mGST, cGST, GR and CTT1, ROS content or GSH/GSSG ratio. A marked difference was also found between transformers captured in the two basins in terms of mGST activity and cGST activity, glutathione content and GSH/GSSG ratio at each instant of analysis (t₀-t₃₀), higher levels of conjugation enzymes being detected in juveniles of the Vouga basin and the higher levels of glutathione and GSH/GSSG ratio detected in individuals in the Lima basin.
- **4. Conclusions** The xenobiotic biotransformation capacity and oxidative stress markers of the gills of transformers captured in Vouga river basin were higher than those detected along the salinity gradient in transformers of the Lima basin. Environmental factors associated with the two basins and molecular mechanisms of the memory of Sea lamprey transformers may account for this response. The Lima river basin may thus be regarded as being cleaner than the Vouga basin. Failures in mechanisms of the xenobiotic biotransformation metabolism and stress response of the gills of Sea lampreys from the Vouga basin may negatively impact the recruitment of juveniles of this ancestral species, especially during prolonged late-summer heat conditions, which are tending to become more common in Portugal.