Stress Experiments

Samples from *Eisenia andrei (*EAND) and *Hirudo medicinalis* (HMED) were subjected to several types of abiotic stress related to their terrestrial/freshwater ecological niches, respectively: exposure to visible and UV-B light, hyperoxia, hypoxia, and osmotic stress. Samples under visible light were kept under natural light (ie, close to a window in the laboratory) for 15 minutes in an empty tray with no space to hide. For exposure to UV-B light, specimens were exposed under a UV-B lamp (302 nm) for 2 minutes. Animals were allowed to recover in darkness 24 hours in a dark chamber at a constant temperature of 16ºC. Animals under no recovery conditions were collected as well. Hyperoxia experiments were carried out by adding pure oxygen into a controlled chamber until reaching 38-44% oxygen for 20 minutes. Oxygen concentration was continuously monitored by an oxygen sensor located at the interior of the hyperoxia chambers (Presens OXY-1 ST Fiber). Hypoxia experiments were done in a HypoxyLab™ (Oxford Optronix) at 8% oxygen concentration for aquatic species (HMED) and 15% oxygen concentration for terrestrial species (EAND) for 20 minutes, based on previous information on what constitutes hypoxic conditions in freshwater or terrestrial environments without causing mortality. For the osmoregulation experiment, HMED was immersed in sea water for 2 minutes to mimic osmotic stress, making sure that the exposure time was causing stress but not mortality and EAND after dried out with paper was left in a 9 cm petri dish for 15 minutes, considering it as a low-humidity condition for the animals. Control samples were directly processed without any additional treatment. All samples were left in starvation for at least 24h before any experiment to reduce gut content. Five biological replicates per species and experiment were included. After the experiments, all samples were dissected into head, mid body and tail, flash frozen in liquid nitrogen and kept <-70ºC until further processing.