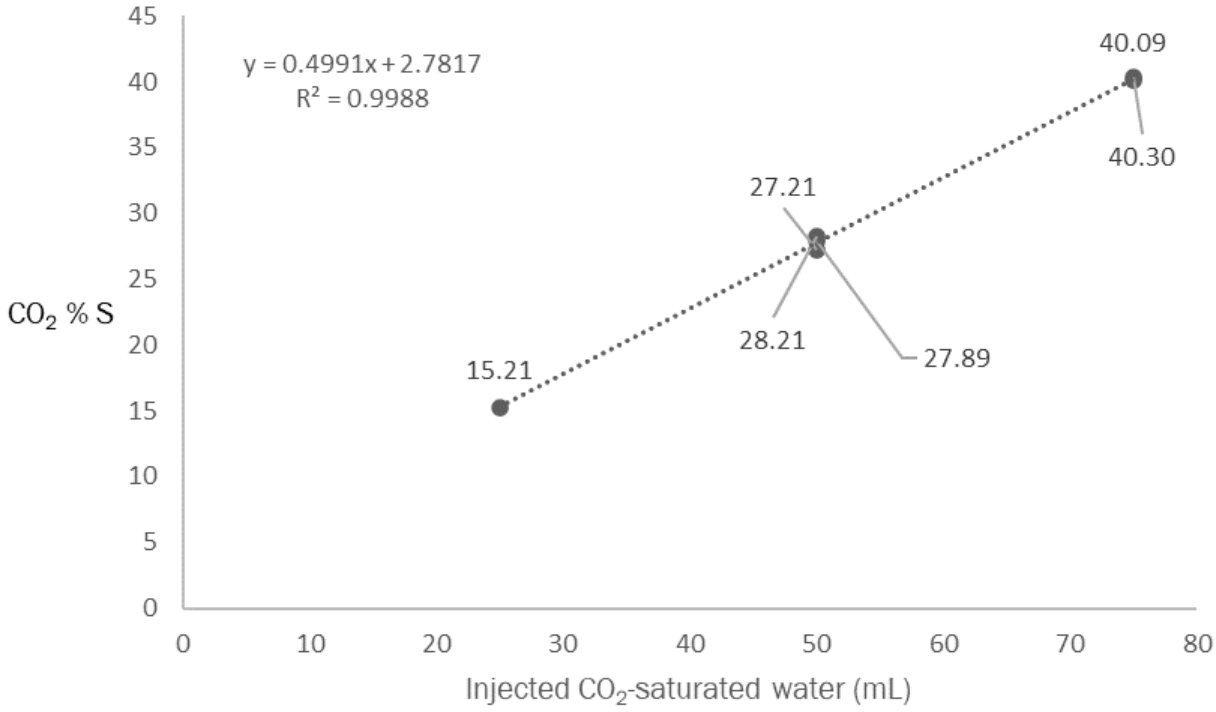
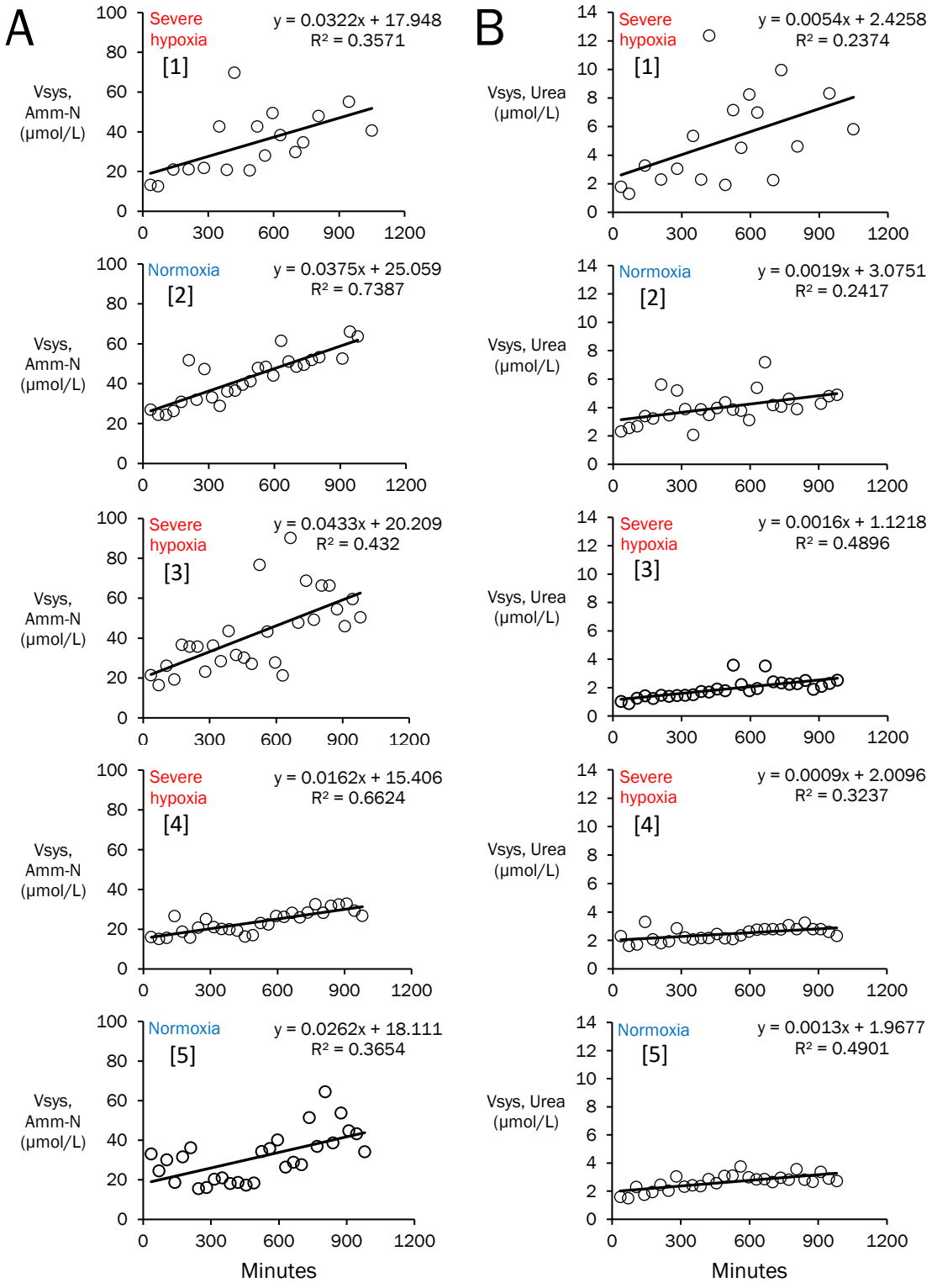
Fig. S1. Accuracy and repeatability of the artificial lung



Six CO_2-saturated water injections were made and measured in the order 50, 50, 25, 75, 75 and 50 ml.

Fig. S1 shows a pilot calibration test where eight injections where measured based on three different volumes of CO_2 injected (25 ml, 50 ml, and 75 ml (755, 1510 and 2265 µmol CO_2)), based on the method described in the CO_2 water measurement section (**2.3.3**). This validation of the apparatus shows the accurate repeatability and linearity of the system. A linear slope indicates that the calibration factor does not differ when different CO_2 concentrations are measured, which means that a single injection after each replicate is enough to accurately calibrate the system even when the fish varies in amount of CO_2 excreted during the measurement (e.g., high stress values vs low relaxed values). The accurate repeatability of the system is indicated by the overlapping measurements. The chamber had been flushed twice before injections to equilibrate the system.

Fig. S2. Ammonia and Urea baseline increase



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These plots show the concentration increase of total Ammonia-N (A) and Urea-N (B) in the whole system water volume (~240 L) for Arapaima gigas (n=5). Water treatment is shown by 'Normoxia' and 'Severe hypoxia'. Individual experiments are displayed with unique bracketed [ ] numbers.

We could not reliably measure the elevations of the concentrations of the N-wastes inside the chamber during each closed period. We instead considered the water samples made at the beginning of each closed period (note: just after flushing the chamber with header-tank water) as being a sample from the entire system's water volume (chamber + header-tank). Thus, we calculated an average excretion rate for the entire measurement period (~17.5h) by performing a linear regression on the system's concentration increase of both ammonia-N and urea-N (Fig. S2).