

Figure S1. Precision of 3D marker tracking during *in vivo* measurement conditions. (A) Histogram of the standard deviation of 306 intermarker distances from 51 startle response sequences. The mean standard deviation is 0.22 mm. (B) Histogram of the standard deviation of 24 intermarker distances from four stationary sequences. The mean standard deviation is 0.047 mm. Note that the mean standard deviation is approximately five times greater during the startle response. This decreased precision is likely due to motion artifacts. Unfortunately, the difficulty in visualizing markers through the large amount of water limited us to an exposure time of 500 μ s, which was not sufficient to remove all motion blur.

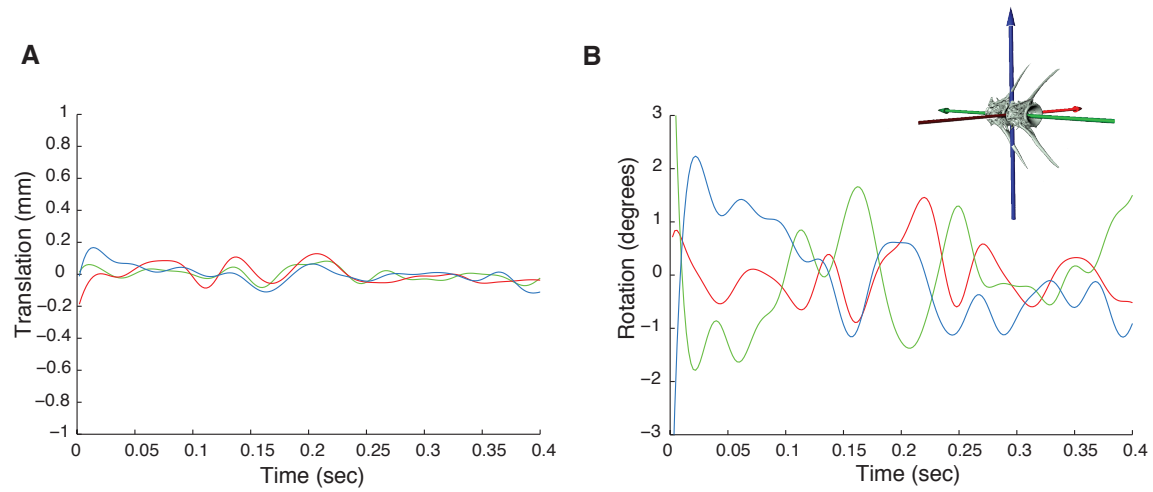


Figure S2. Root Mean Square Deviation (RMSD) of one representative stationary sequence portraying (A) translations and (B) rotations. Since the fish are stationary we expect these RMSD values to be zero. Mean RMSD translations for the four stationary sequences were indeed quite small: 0.05 mm mediolateral translation (red); 0.03 mm axial translation (green); and 0.04 mm dorsoventral translation resulting in peak-to-peak translations of 0.2-0.3 mm translational resolution. Mean RMSD rotations for the four stationary sequences: 0.45° for dorsoventral rotation (red); 0.52° for axial rotation (green); and 0.48° for lateral bending (blue) resulting in peak-to-peak rotations in a stationary fish of up to 2-3° rotational resolution.



Movie S1. Dorso-ventral camera view of *M. saxatilis* performing a startle response. The subject begins positioned with cranial towards the left of the screen and caudal towards the right of the screen. Video was captured at 500 frames s⁻¹, 500 μs exposure time, 70 kV X-ray voltage and 320 mA of X-ray current. It is important to note that as the water depth is disturbed, first by the stimulus and later by the movements of the fish, the exposure of those regions changes, causing more shallow areas to become temporarily brighter and deeper areas to become temporarily darker.