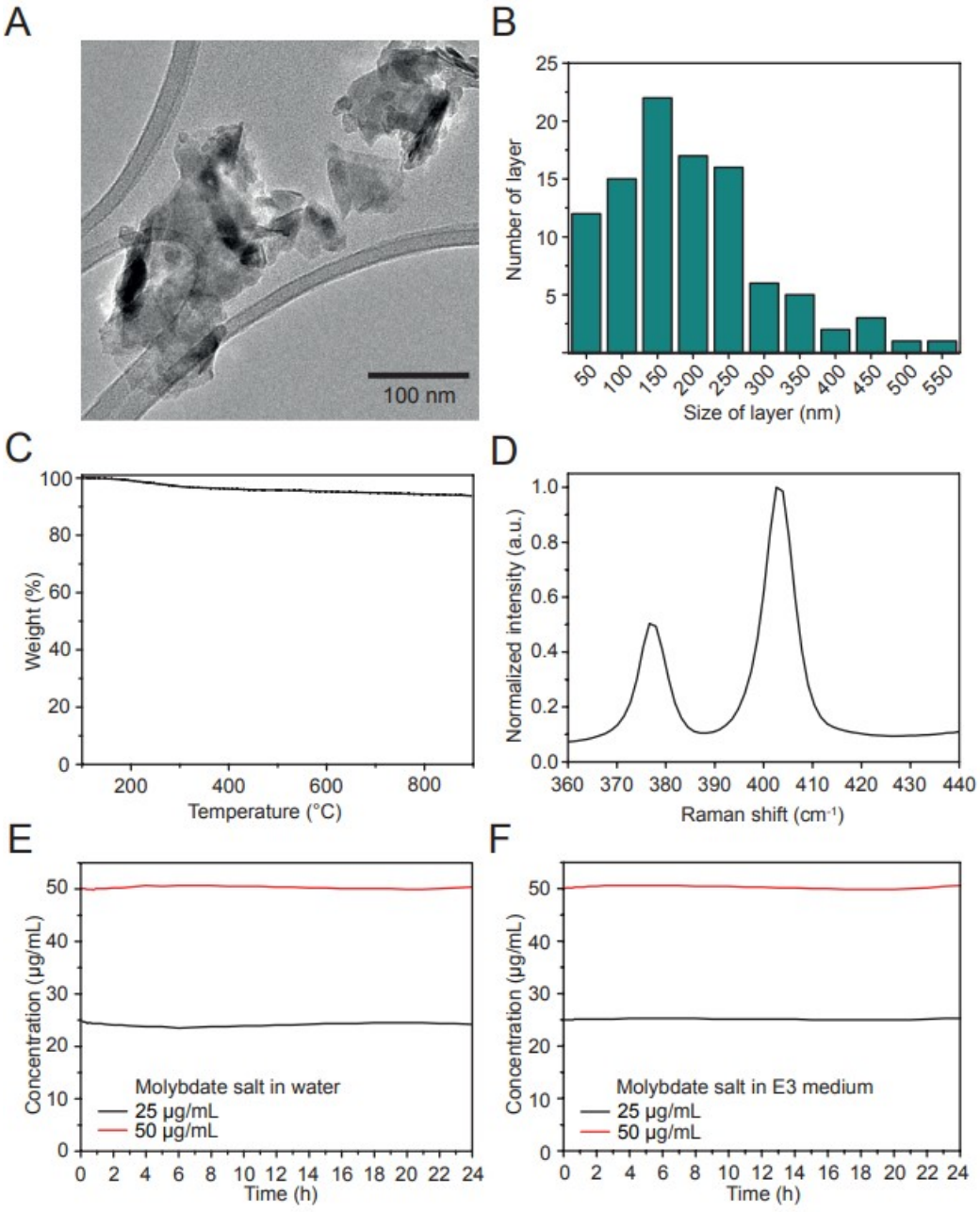
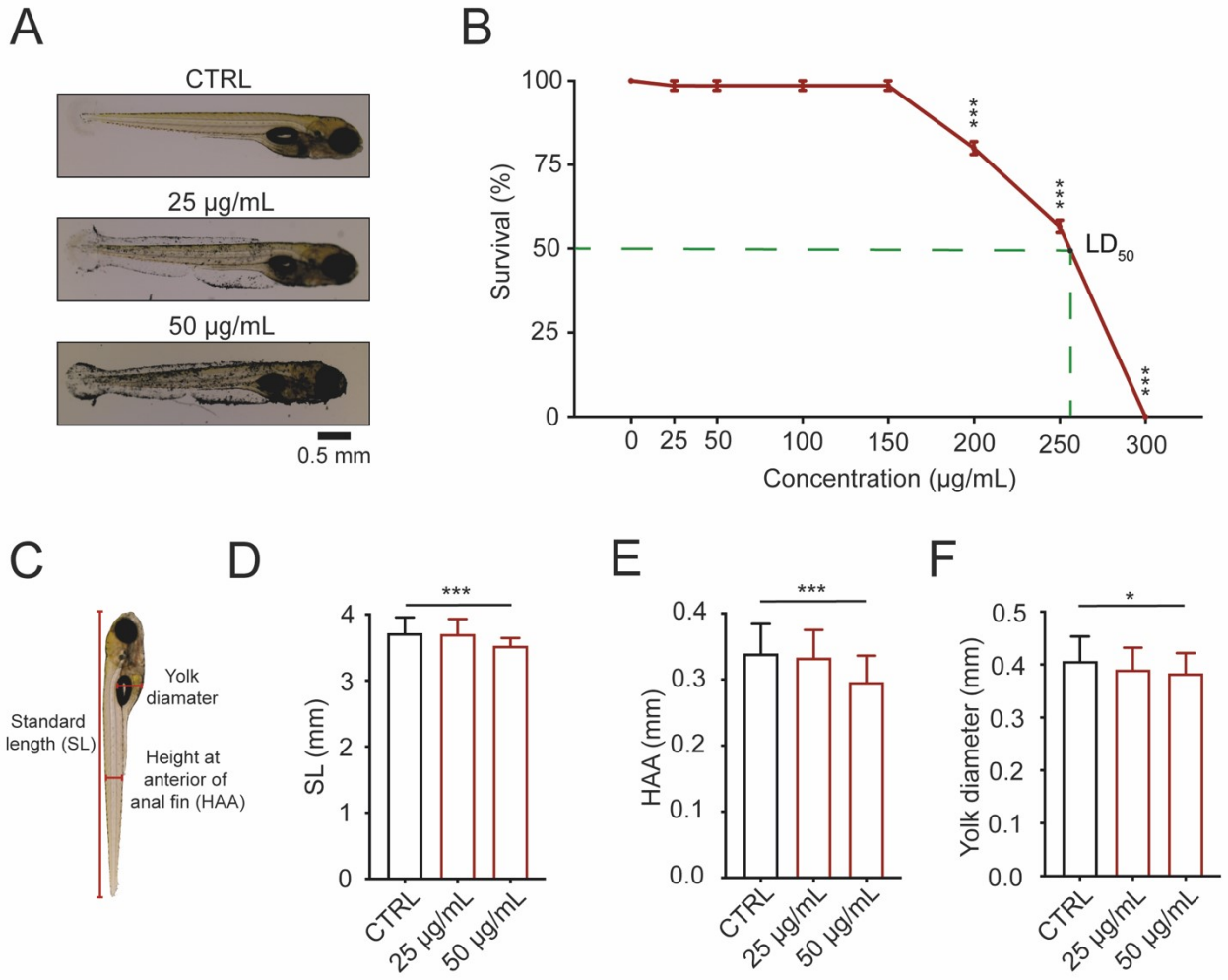
Electronic Supplementary Material (ESI) for Nanoscale Horizons.

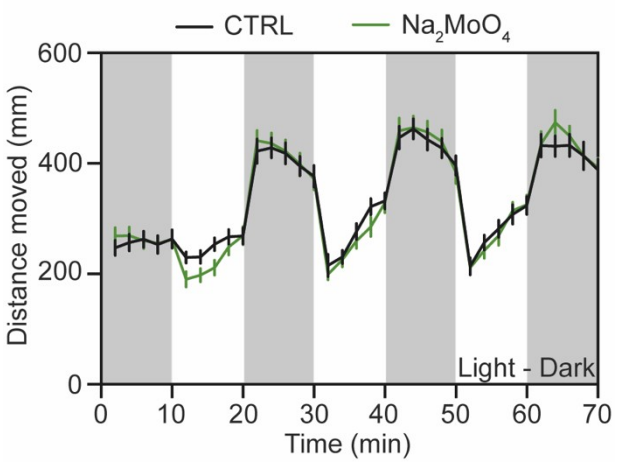
Supplementary Information



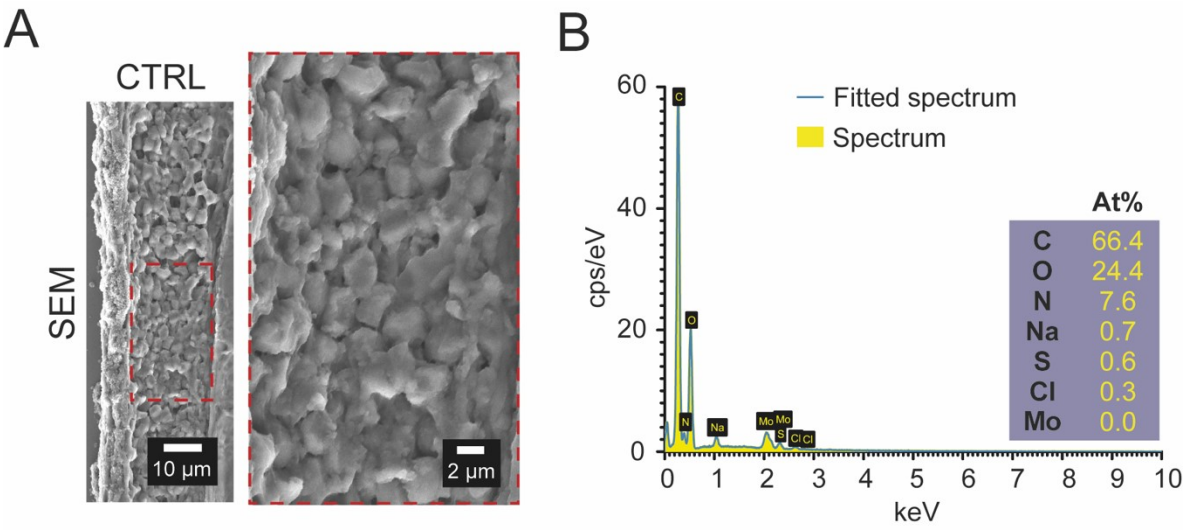
Supplementary figure S1. Additional characterization of MoS_2 nanomaterials. (A) HRTEM image with corresponding (B) layer size distribution. (C) TGA result in an inert atmosphere. (D) Raman spectra of the nanomaterial and (E) colloidal stability in zebrafish E3 medium at 25 and 50 µg/mL during 24 h. Colloidal stability of molybdate salt in water (E) and E3 medium (F) at 25 µg/ml and 50 µg/ml.



Supplementary figure S2. Impact of MoS_2 nanosheets on zebrafish survival and anatomical treats. (A) Bright field images of larvae in control or exposed to MoS_2 for 24 hours. Note the deposition of nanomaterial aggregates on the body surface of zebrafish. (B) Survival analysis of zebrafish larvae after the dispersion of MoS_2 in the zebrafish E3 medium for 24 hours. Survival percentages were 100 ± 0 % in control, 98.6 ± 1.4 % in 25 µg/mL, 98.6 ± 1.4 % in 50 µg/mL, 98.6 ± 1.4 % in 100 µg/mL, 98.6 ± 1.4 % in 150 µg/mL, 80 ± 1.9 % in 200 µg/mL, 56.66 ± 1.9 % in 250 µg/mL and 0 ± 0 % in 300 µg/mL (N=30 for each condition, *** P values <0.001 are reported respect to control). The graph shows that LD_{50} is 256 µg/mL (green dotted lines). (C) Sketch of the anatomical traits measured to assess zebrafish development. Bar plots of SL (D), HAA (E) and yolk diameter (F). At the concentration of 25 µg/mL, no changes were found in SL (3.7 ± 0.03 mm in control and 3.7 ± 0.03 mm in MoS_2), HAA (0.33 ± 0.006 mm in control and 0.33 ± 0.006 mm in MoS_2) and yolk diameter (0.40 ± 0.006 mm in control and 0.39 ± 0.006 mm in MoS_2; N=45 animals for each condition, P>0.05). Differently MoS_2 at the concentration of 50 µg/mL induced reductions in larvae length (3.5 ± 0.01 mm; P<0.001), height (0.29 ± 0.005 mm; P<0.001) and yolk diameter (0.38 ± 0.005 mm; P<0.05; N=45 animals) respect to controls. *P<0.05, ***P<0.001.

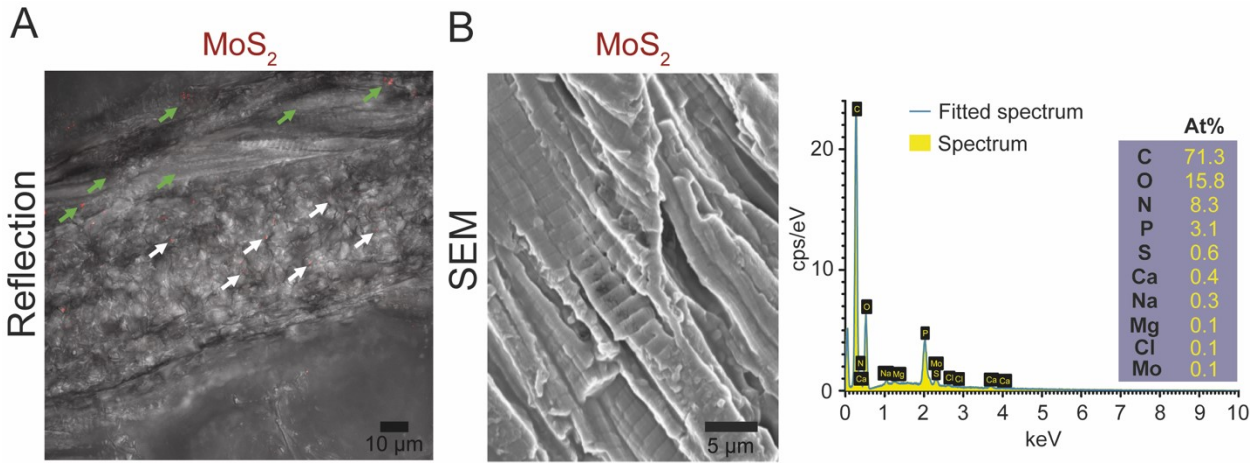


Supplementary figure S3. Behavioral locomotor screening of zebrafish larvae treated with Na_2MoS_4. Treatments with Na_2MoS_4 for 24 hours at 75 μg/mL (equimolar to 50 μg/mL of MoS_2) do not induce statistically significant differences in the swimming performance during light-dark transitions respect to controls. For the second 10 minutes long lasting dark period, distance moved was 2040 ± 89 mm for controls and 2062 ± 93 mm for Na_2MoS_4 treated larvae ($P>0.05$).

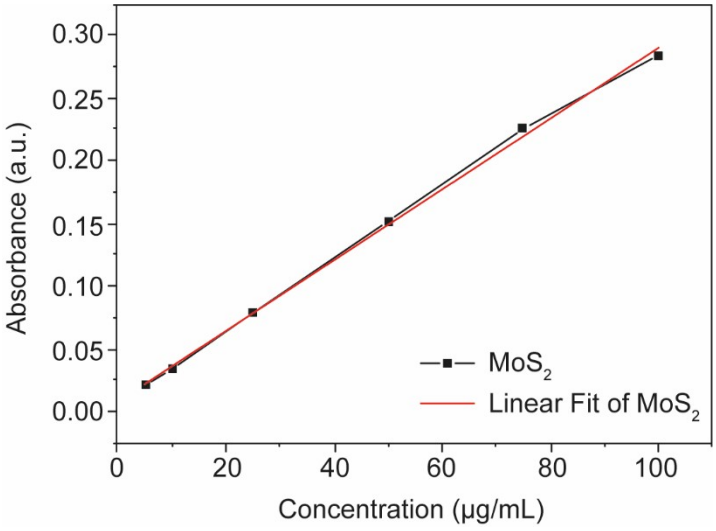


Supplementary figure S4. Cryo-SEM and EDS analysis of spinal tissue in untreated control zebrafish

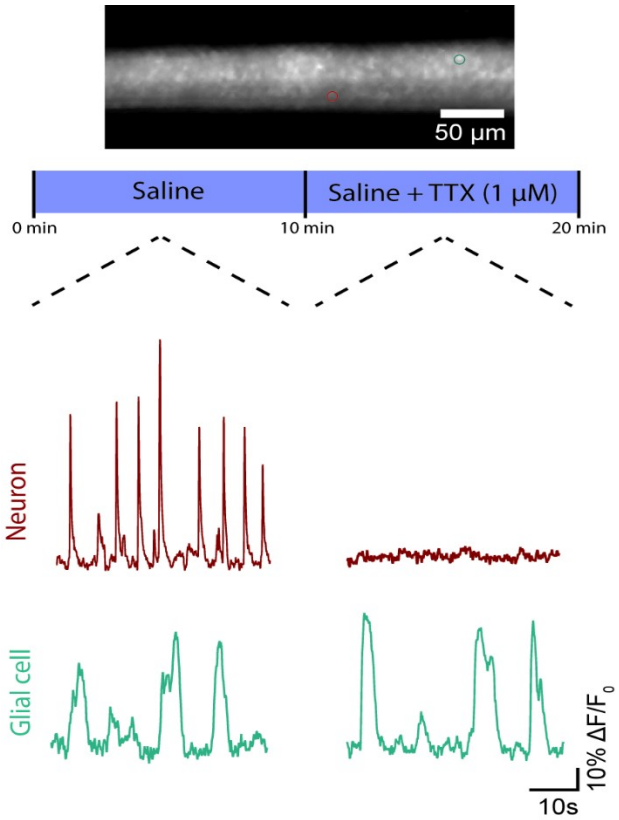
(A) SEM micrograph of the spinal cord of untreated animals. The area in the dashed red rectangular in the left image is magnified in the right one. (B) Compositional analysis performed by EDS.



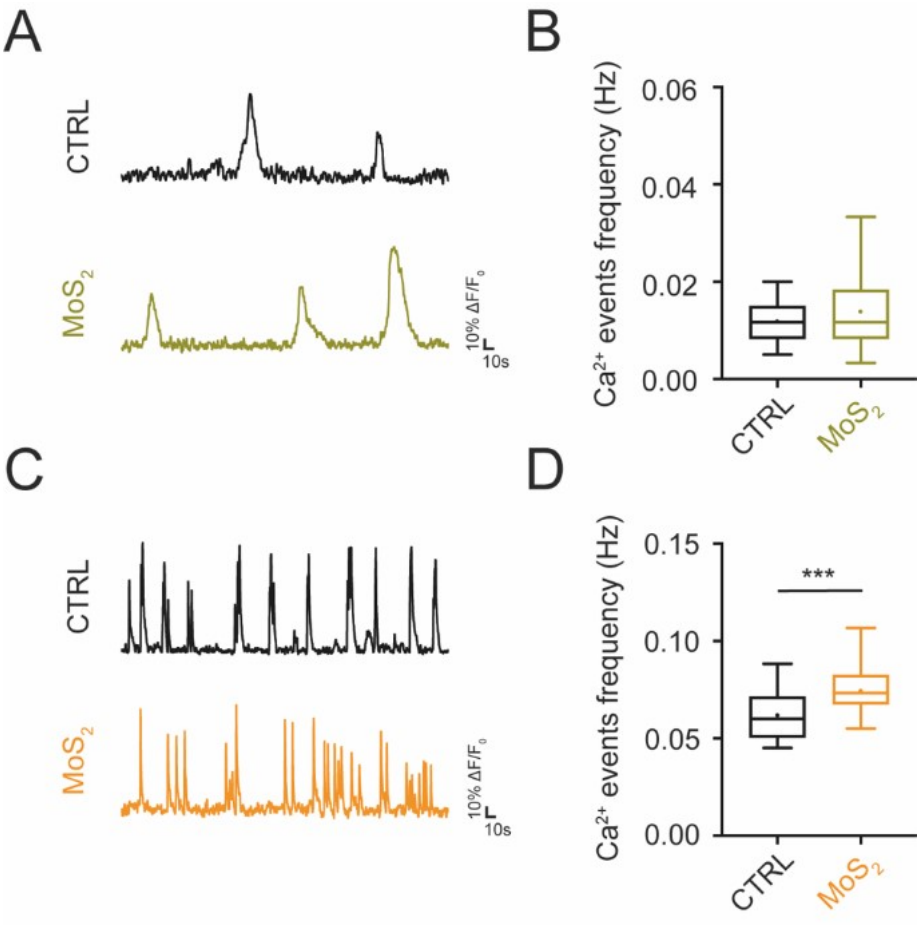
Supplementary figure S5: After ambient exposure, MoS2 nanosheets localized in the skeletal muscles. (A) Z-stack reconstructions of the dorsal portion of MoS_2 exposed zebrafish (50 µg/mL, 2 hours). Image shows an overlap of the bright field and reflection mode acquisitions. Reflection signals in the spinal cord are indicated by white arrows, while those in the skeletal muscles are indicated by green ones. (B) On the left, SEM micrograph of the spinal cord obtained from animals exposed to MoS_2 (50 µg/mL, 2 hours). On the right, compositional analysis performed by EDS.



Supplementary figure S6. Calibration curve of exfoliated MoS_2 in the zebrafish E3 medium.



Supplementary Figure S7: Neuronal and glial calcium oscillations are distinguishable for their different sensitivity to TTX. On the top, representative snapshot of a spinal cord portion during calcium imaging recording with exemplificative ROIs used for the analysis of glial (green) and neuronal (red) cells. Representative fluorescent traces of calcium oscillation activity from neuronal (in the middle) and glial (on the bottom) cells. Note that, by inhibiting action potential generation and related synaptic activity, the application of TTX (1 µM) silences neuronal calcium oscillations, while the glial ones are unaffected.



Supplementary figure S8: Spinal injections of 5 ug/mL MoS_2 nanosheets alter neuronal calcium signaling but not the glial one. (A) Representative fluorescent traces of calcium oscillation activity from glial cells. (B) Box plot of calcium events frequency for glial cells in control (0.0110 ± 0.0006 Hz, N=4 larvae, 53 cells) and MoS_2 (0.0130 ± 0.0007 Hz, N=6 larvae, 83 cells). (C) Representative fluorescent traces of calcium oscillation activity from ventral neurons. (D) Box plot of calcium events frequency for neurons in control (0.0610 ± 0.0015 Hz, N=4 larvae, 60 cells) and MoS_2 (0.074 ± 0.001 Hz, N=5 larvae, 65 cells). Dots in the box plot correspond to the mean values. *** P<0.001.

Samples	MoS_2 exfoliated
Intercept	0.00758
Slope	0.00283
R^2	0.99815
Linear range (µg/mL)	5-100

Supplementary table S1. Results from the statistical analysis of exfoliated MoS_2 in the zebrafish E3 medium.