Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information Figures:

Ecotoxicological assessment of nanoparticle-containing acrylic copolymer dispersions in fairy shrimp and zebrafish embryos

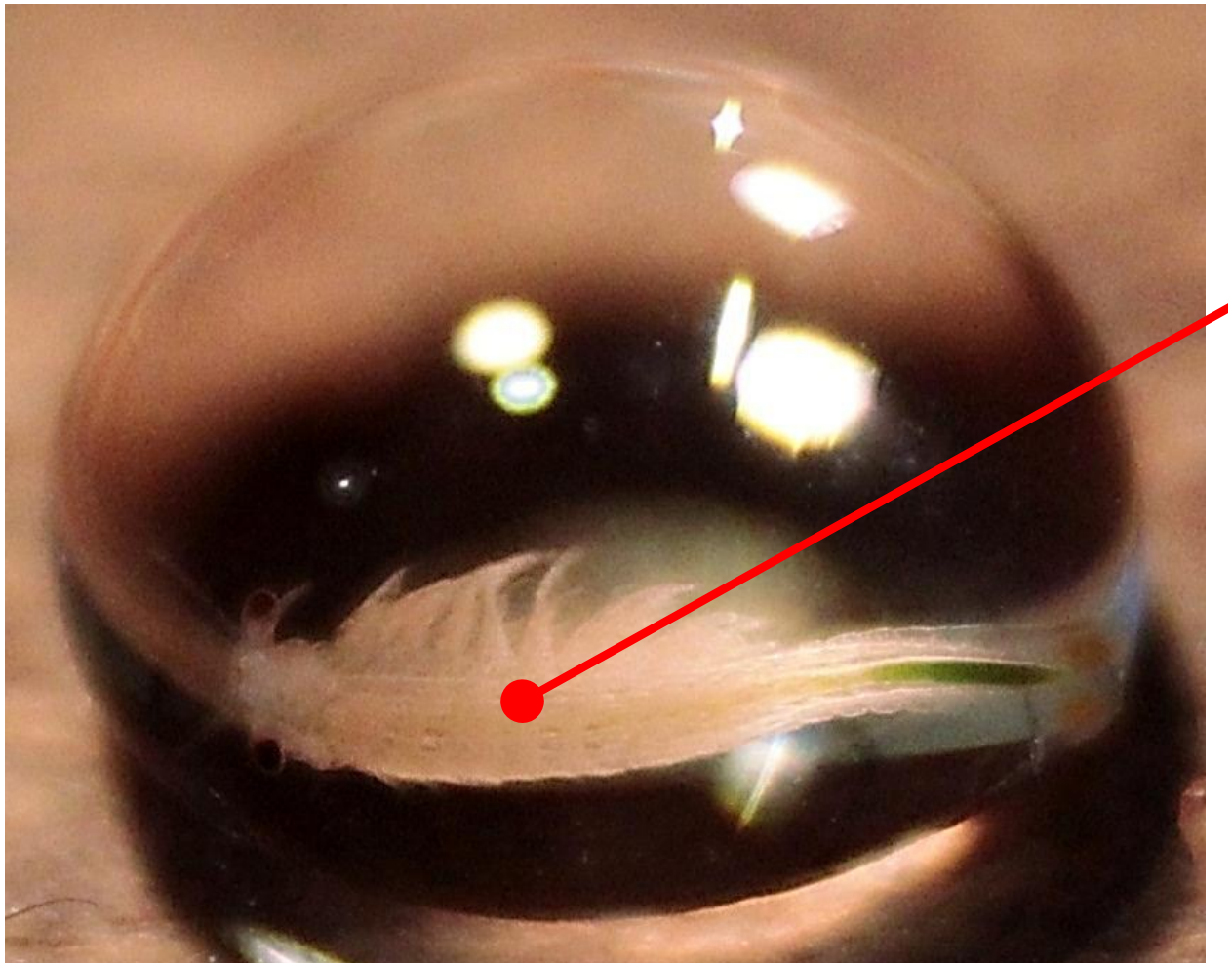

Figure SI-1: A photograph of the fairy shrimp (*T. platyurus*), whose spectral profile is presented in the article in Figure 1A. The marker denotes the position where the laser was focussed.

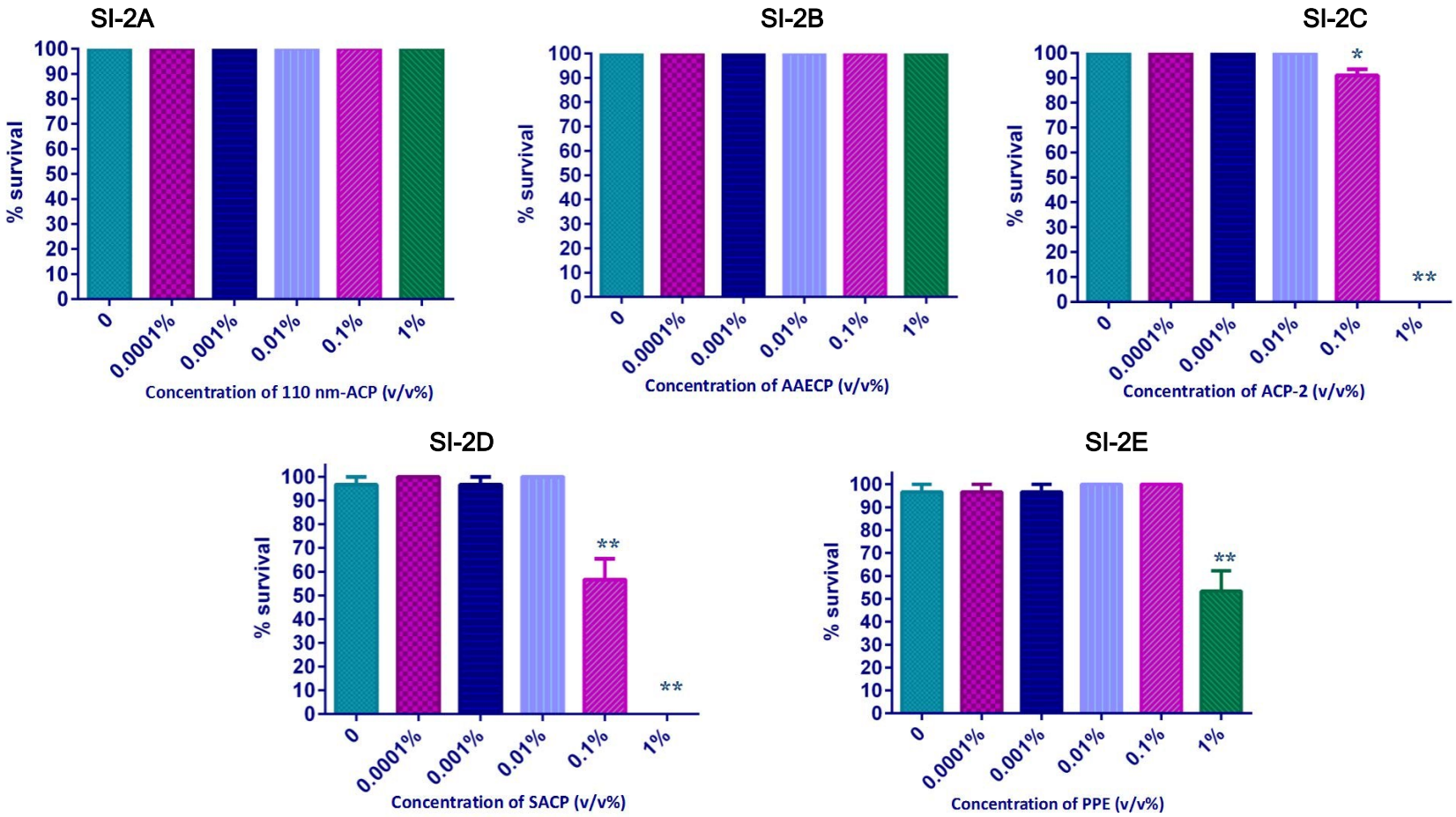

Figure SI-2: Range finding study assessing 1, 10, 100, 1000 and 10000 mg/L (corresponding to 0.0001-1% (v/v)) of the respective test material in fairy shrimp (*T. platyurus*) (24-hour exposure; counting 30 animals / concentration). Statistical significance was calculated by ANOVA with SPSS Statistics for windows version 21.0 (SPSS Inc., Chicago, USA), ** $p < 0.01$ and * $p < 0.05$.

Panels: SI-2A: Acrylic copolymer 110 nm-ACP; SI-2B: Anionic acrylic ester copolymer (AAECP); SI-2C: Acrylic copolymer ACP-2; SI-2D: Straight acrylic copolymer (SACP); SI-2E: Polyester-polyurethane elastomer (PPE).

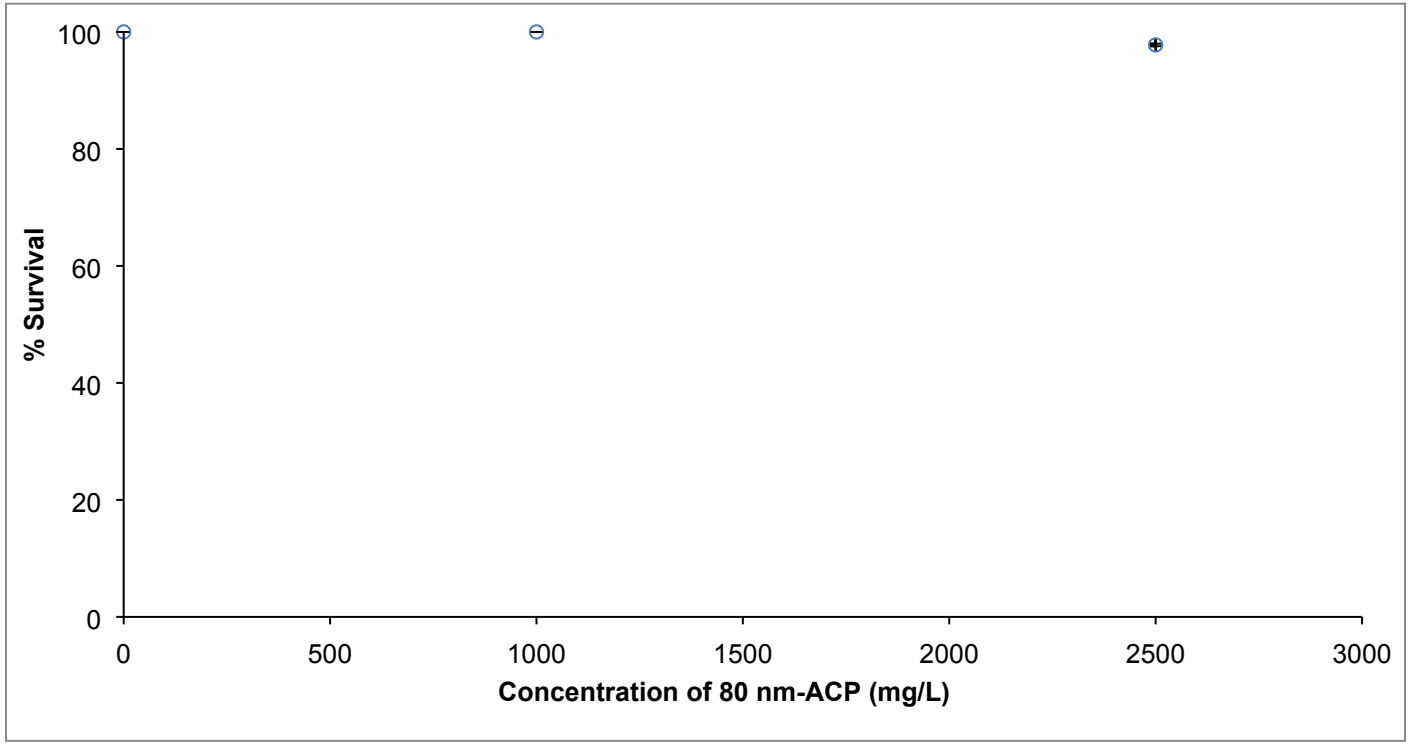

Figure SI-3: Survival of fairy shrimp (*T. platyurus*) upon 24 h-exposure to 1000 and 2500 mg/L 80 nm-ACP (relative to negative control).

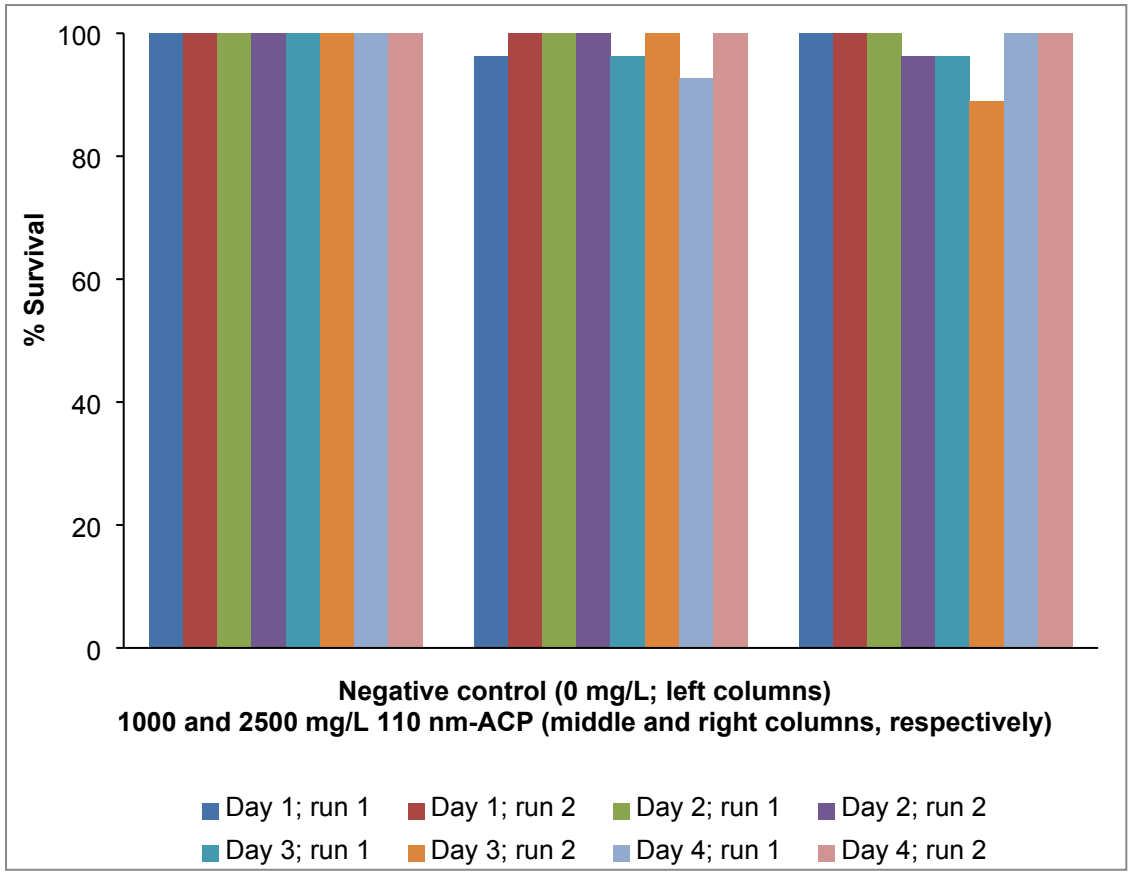

Figure SI-4: Survival of fairy shrimp (*T. platyurus*) upon 24 h-exposure to 1000 and 2500 mg/L 110 nm-ACP (relative to negative control). Measurements from 8 individual assays.

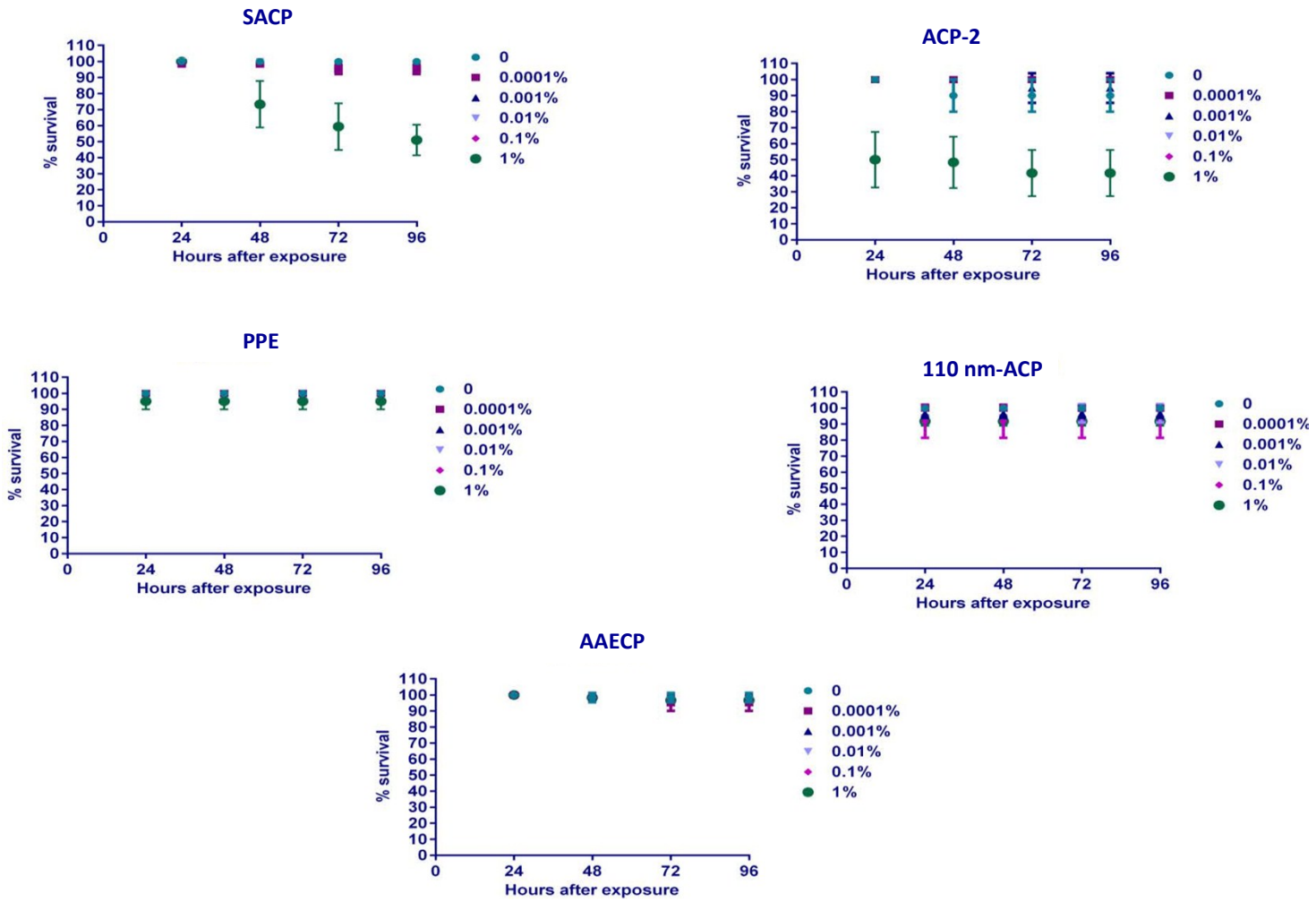

Figure SI-5: Range finding study assessing the five preliminary test materials at 1, 10, 100, 1000 and 10000 mg/L (corresponding to 0.0001-1% (v/v)) in zebrafish embryos (24-, 48, 72-, 96-hour exposure; counting 30 animals / concentration).

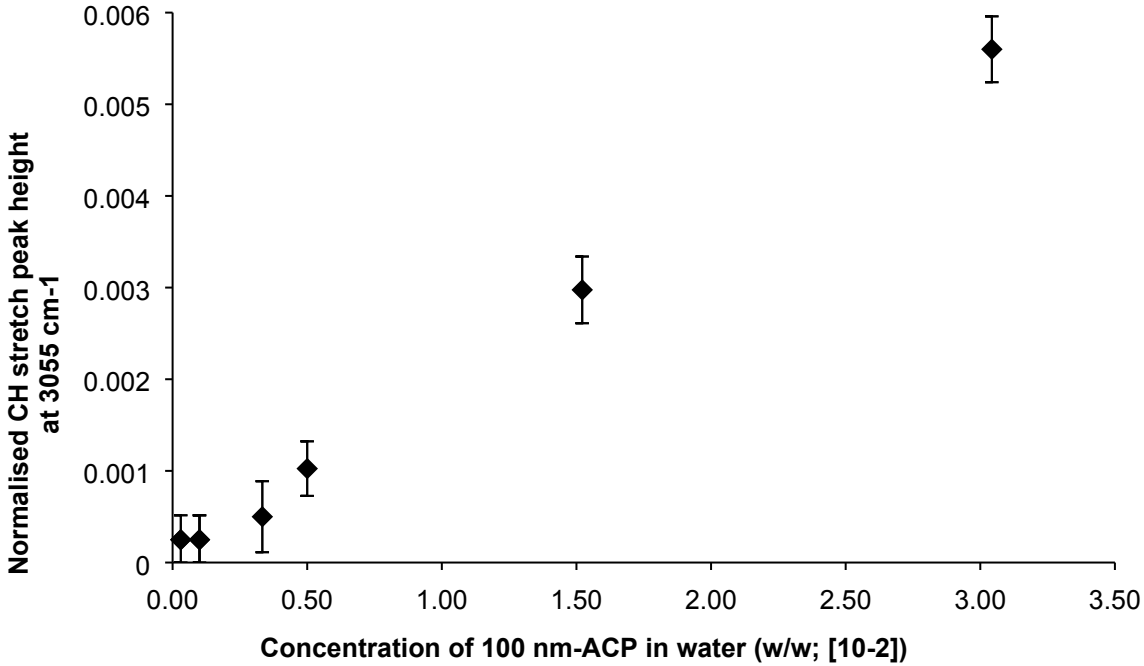

Figure SI-6: Limit of detection for 110 nm-ACP: SRS hyperspectral profiles were obtained from successive dilutions of 110 nm-ACP in standardised dilution water. The peak heights were measured relative to standardised dilution water and were normalised to the peak value for pure 110 nm-ACP, which was assigned a value of 1.

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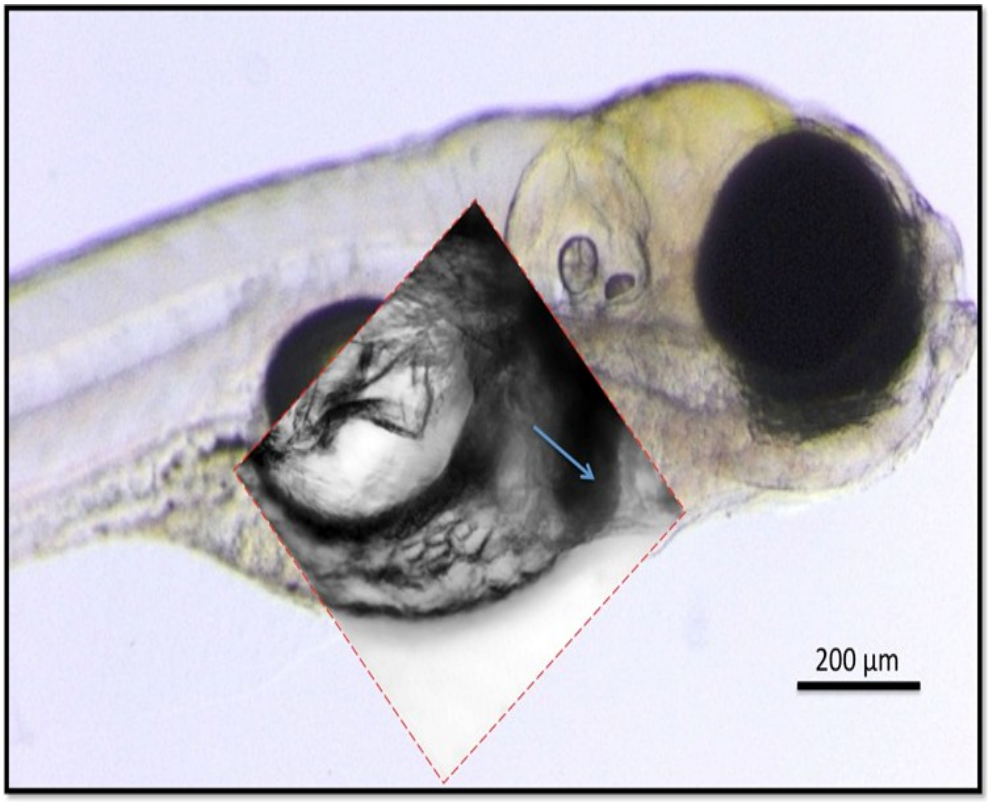

Figure SI-7: Transmitted light image (red dashed box) indicating the region of the *Casper* zebrafish (fed with 10 g/kg 80 nm-ACP) that was selected for hyperspectral SRS imaging (presented in Figures 15 and 16)

The arrow indicates the position of the liver that was found to contain 80 nm-ACP signal.

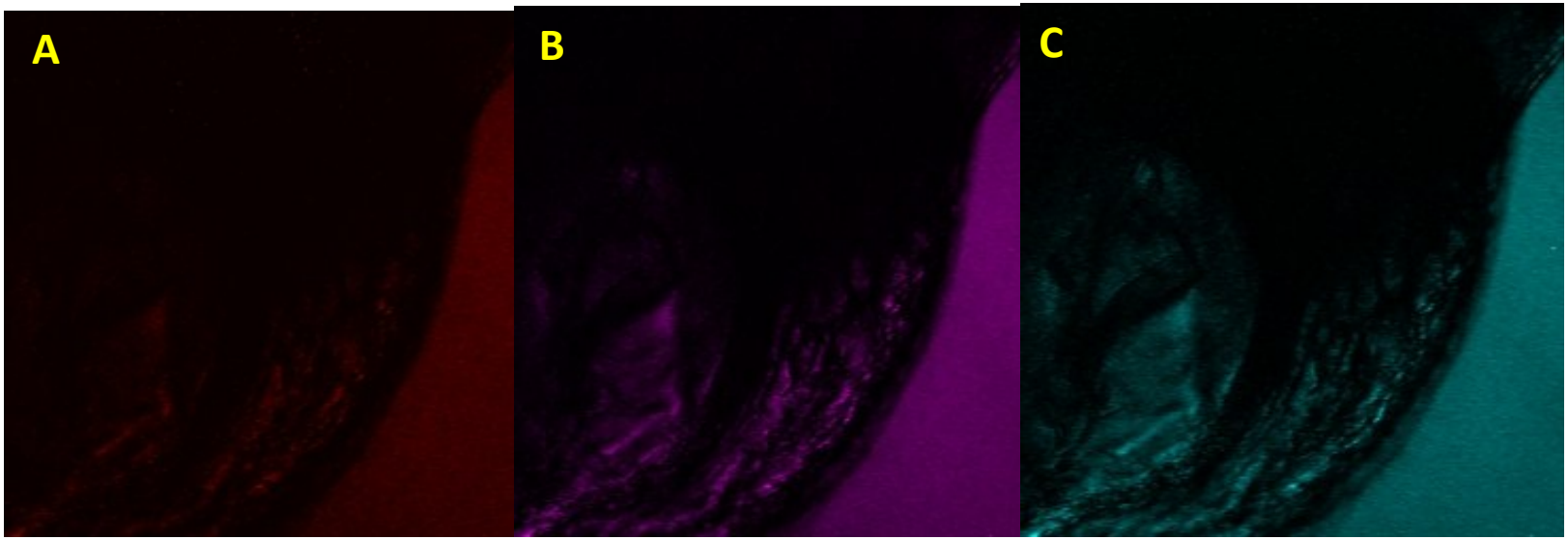

Figure SI-8: Identification of regions of the *Casper* zebrafish (presented in Figure 14) in which 80-nm ACP is present.

First, the relative signal intensities for the test material were exploited over three different regions of the hyperspectral data stack, i.e.

A. Off-resonance for the ACP peak (3025 cm^{-1});
B. On-resonance for the ACP peak (3055 cm^{-1});
C. The 'tail-end' of the OH stretch peak (3100 cm^{-1}).

For regions containing mostly water, the intensity of C will be greater than that of B and A.

For water-rich regions containing 80 nm-ACP, B will be greater than A and less than C.

For water-poor regions containing 80 nm-ACP, B will be greater than A and C.

For regions containing tissue alone, A will be greater than B with A relative to C varying depending on the water content of these tissues.

Once this mask is completed, the candidate pixel locations are transposed onto the hyperspectral stack, and each of their spectral profiles is compared against the 'standard' 110 nm-ACP spectrum.

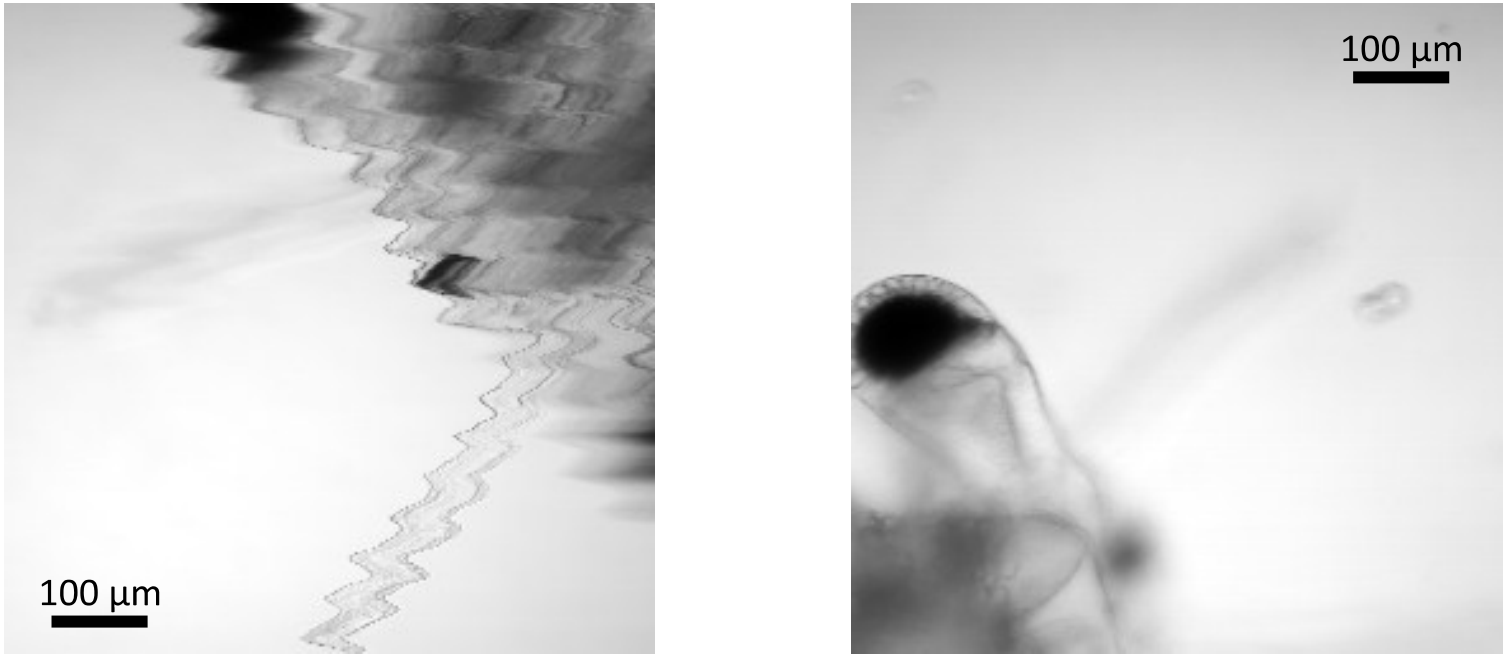

Figure SI-9: Clear transmitted light imaging of a fairy shrimp (*T. platyurus*) placed in a droplet of water directly onto a glass coverslip (20-fold magnification). Left: Scan time 20 seconds; Right: Scan time: 2 seconds