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MicroCT protocols for scanning of embryos and juvenile Hexaplex trunculus

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ABSTRACT

Micro-computed tomography (micro-CT) is a high-resolution 3D-imaging technique which is now increasingly applied in biological studies focusing on taxonomy and functional morphology. The creation of virtual representations of specimens can increase availability of otherwise underexploited and inaccessible samples. This protocol aims to standardise micro-CT scanning procedures for embryos and juveniles of the marine gastropod species *Hexaplex trunculus*.

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KEYWORDS

microCT, gastropods, embryos, juveniles, scanning, Hexaplex trunculus

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Sample	preparation 42m	
1	Removal of embryos of <i>Hexaplex trunculus</i> from their egg capsules.	10m
2	Anesthetization of embryos and juvenile gastropods with 7% MgCl _{2.}	30m
3	Sample placement inside a plastic white pipette tip without any scanning medium (in the air).	2m
microC	T scanning 2h 23m	h 23m

SkyScan 1172 micro-computed tomographer (microCT) 10L01170 Bruker Detail detectability: <1 µm Low contrast resolution (10% MTF): 5 μm Pixel size at maximum magnification: <0.8 μm X-ray source: Sealed microfocus X-ray tube, air cooled, >10,000h lifetime; Spot size $<5\mu m$ @ 4W, 20-100kV, 0-250 μA (10W max) X-ray detector (camera): 11 Megapixel (4000X2300) 12-bit digital CCD-camera with fibre optic coupling to scintillator Maximum object size: 50 mm in diameter using offset scan Radiation safety: $< 1 \mu Sv/h$ at any point on the

4.1 Scanning parameters for embryos and juvenile Hexaplex trunculus

2h 23m

Voltage: 59 kV Current :167 μ A Filter: none Pixel size: 2 μ m Camera binning: 1 × 1

Exposure time: 325 ms for embryos and 316 ms for juveniles

Rotation: 360° for embryos and 180° for juveniles Rotation step: 0.20° for embryos and 0.25° for juveniles Frame averaging: 3 for embryos and 5 for juveniles

Images reconstruction

instrument surface

5 Projection images were reconstructed into cross sections using the SkyScan's NRecon software (Bruker, Kontich, Belgium)

5.1 Upload projection images

5.2 Perform X-Y alignment

5.3 Reconstruction parameters 16m

 Smoothing: 2

Ring artifact correction: 20 Beam hardening correction: 59%

Attenuation coefficients: 0 - 1.127 for embryos and 0 - 1 for juveniles

Save as: 16-bit TIFF images