

Bromine enrichment in Hadal amphipod *Hirondellea gigas*

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Many animal groups in the deep-sea have evolved to adopt to low temperature, high pressure, and nutrition-poor environment. especially, at hadal environments >6000 m depth. Except chemosynthetic environments, biomass decreases in the deep sea because of a lack of primary production. One example of large hadal animal is the hadal amphipod *Hirondellea gigas*, found in the deepest trenches of the Pacific and thought to have evolved two features in adaptation to the harsh environment. Firstly, the amphipod possesses special types of cellulase that work under high pressure, which is considered to digest polysaccharides such as cellulose under poor nutritional environment. Another is the use of "aluminum gel" to prevent calcium carbonate (calcite) to dissolve at depth deeper than the carbonate compensation depth (CCD), based on scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDS) coupled with dissolution experiment.

We found that the SEM/EDS-based previous research ambiguous, and we re-analyzed *H. gigas* obtained from the Boso Triple Junction (Figure a) using SEM/EDS and electron microprobe analysis (EPMA), with special care to eliminate possible artefact signals from aluminum contaminant sources. Our SEM/EDS and EPMA investigation of the semi-thin sections of resin-embedded amphipod showed the absence of aluminum in the exoskeleton, but the presence of magnesium and bromine, the latter having similar characteristic X-ray energy to that of aluminum, was detected. Further anatomical analysis of elemental distribution using the dissected dried animal showed the preferential accumulation of bromine on setae and most preferentially on the ossicle spines of the gastric mill (Figure b, c). The mis-assignment in the previous literature would be traced back to the X-ray signals generated by the SEM chamber, which was excited by backscattered electron from the charged amphipod during non-coated SEM/EDS analysis. In this report, we discuss the bromine accumulation in the setae and gastric mill, as well as the magnesium localization in the exoskeleton of *H. gigas*. We also discuss elemental artefacts during SEM/EDS investigation.

Keywords: Deep-sea organism, *Hirondellea gigas*, bromine, SEM/EDS

