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Are Rhodophyceae a dietary component for deep-sea holothurians?

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Eicosatetraenoic acid (20:4(n-6)), a polyunsaturated fatty acid (PUFA), was found in deep-sea holothurians at a depth of nearly 5000 m in the temperate north-east Atlantic. It has been suggested that deep-sea animals may be able to synthesize this compound *de novo*. Since this fatty acid is typical of Rhodophyceae, which has previously been found incorporated in gelatinous material captured in the deep sea, an alternative origin of this fatty acid in the deep-sea holothurians is proposed.

The sedimentation of particulate organic matter (POM) to the sea-floor is subject to seasonal variations coupled to the productivity cycle of the phytoplankton. Billett et al. (1983) discovered a rapid seasonal deposition of phytodetritus to the deep-sea floor in the temperate North Atlantic Ocean. Large and fast sinking aggregates are the dominant mechanisms transporting organic matter into the deep sea, and they are important for the nutrition of the deep-sea community. Occasionally, large faecal mats from salps and remains of dead and decaying organisms, together with phytoplankton spring bloom material, form large aggregates of gelatinous material that can be sampled by closing nets at bathyal and abyssal depths (see Koppelman, 1994).

Holothurians, the dominant epibenthic megafauna in many deep-sea areas, often feed on the superficial sediment and are therefore important in the degradation of organic material. In the present paper two investigations demonstrate that there is an indication that deep-sea holothurians may occasionally use Rhodophyceae as a food source.

The holothurians were sampled in August 1998 during cruise M42/2 of RV 'Meteor' to the Porcupine Abyssal Plain (PAP, 48°50'N 16°30'W) in the north-east Atlantic. The water depth at this site is ~4850 m. The animals were caught with an epibenthic sledge. After recovery of the sledge, the gonads of *Oneirophanta mutabilis* and *Psychropotes longicauda* were dissected and stored at -80°C for seven months. In order to avoid oxidation processes in the vials, the air was displaced by nitrogen. The lipids were extracted with a mixture of dichloromethane and methanol (2:1, v:v). For the gas chromatographic analysis of the fatty acid composition, an aliquot of the extracted samples was converted into methyl esters by transesterification with 3% concentrated sulphuric acid in methanol for 4 h at 80°C. After extraction with hexane, 1 µl was injected into a Chrompack CP 9000 gas-liquid chromatograph (column 30 m × 0.25 mm; film thickness: 0.25 µm; liquid phase: DB-FFAP (Durabond®-Free Fatty Acid Phase)). The methyl esters were identified with standard mixtures and quantified with nonadecanacidmethyl ester as an internal standard. δ¹³C values of the fatty acids were measured using a GC-C-IRMS (Finnigan MAT) relative to the PDB standard.

The most abundant fatty acids in the gonads of *O. mutabilis* and *P. longicauda* were 20:4(n-6) together with 20:5(n-3) (Figure 1). These polyunsaturated fatty acids (PUFAs) are usually components of the phospholipids which increase the fluidity of the cell membranes (Sargent & Falk-Petersen, 1981). This is especially important for deep-sea organisms due to the high pressure and

low temperature conditions of their environment. The same fatty acid composition was found in shallow water holothurians, but deep-sea species possess greater proportions of unsaturated fatty acids than shallow-living species (Cossins & Macdonald, 1986). The PUFAs are essential dietary components of marine animals, and their production has been conventionally considered to be supported by algae only (Sargent et al., 1993). The major algae groups differ significantly in their fatty acid composition: the planktonic diatoms and dinoflagellates contain large concentrations of the (n-3) PUFAs, while the (n-6) PUFAs are typical for littoral benthic algae. The shallow water members of the holothurians can feed directly on benthic littoral algae, but in sediments at abyssal depths the concentrations of polyunsaturated fatty acids are generally low (Santos et al., 1994).

Our identification of 20:4(n-6) in holothurians from nearly 5000 m depth at a remote ocean site is in accordance with analyses of Ginger et al. (2000) who found the same dominance of the polyunsaturated fatty acids 20:4(n-6) and 20:5(n-3) in *O. mutabilis* and *P. longicauda* and other deep-sea holothurians from the same region. These authors suggested that the animals may be able to biosynthesize these compounds *de novo* and thus belong to the very rare number of species which are able to synthesize these PUFAs. Our preliminary results of the δ¹³C composition gives evidence that the 20:4(n-6) is of marine plant origin, because of the low δ¹³C value of -22.8 psu and the difference of more than 3 psu to fatty acids that are normally synthesized *de novo* by marine animals, e.g. -19.4 psu of 18:1(n-9).

It is well-known that benthic algae can be torn off by wave activity and transported over long distances by surface currents (Hoek, 1987). Another possible source is the Sargasso Sea, where Woelkerling (1975) found 33 species of Rhodophyceae beside *Sargassum*. We have indirect evidence that the PUFAs may, indeed, result from a Rhodophycean food.

Filamentous Rhodophyceae (Figure 2) were found incorporated as an intrinsic element of gelatinous detrital material in the deep sea. The material was caught in a fresh condition in April/May 1988 at PAP and at the nearby BIOTRANS site (Koppelman, 1994) using a MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System) with 333-µm mesh aperture. Although we cannot exclude that the algae were incorporated in the flakes by shearing activity in the nets, they were certainly captured in the deep sea.

The appearance of filamentous algae in the deep sea may happen only occasionally, but it is possible that the holothurians do not depend on a frequent sedimentation of Rhodophyceae.

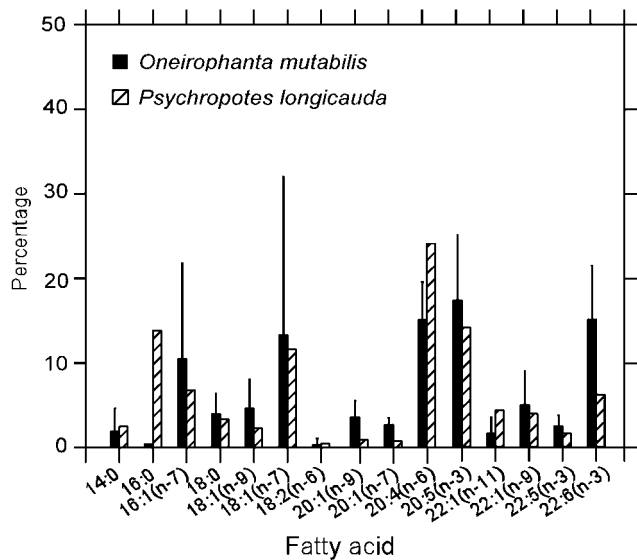


Figure 1. Fatty acid composition of *Oneirophanta mutabilis* and *Psychropotes longicauda*.

Holothurians might be able to use sporadic events and store 20:4(n-6) over a long period since phospholipids normally are not used to gain metabolic energy. We do not reject the hypothesis that holothurians are able to synthesize the polyunsaturated fatty acids *de novo*; however, our results give some indication that Rhodophyceae are a possible additional source of fatty acids for deep-sea holothurians.

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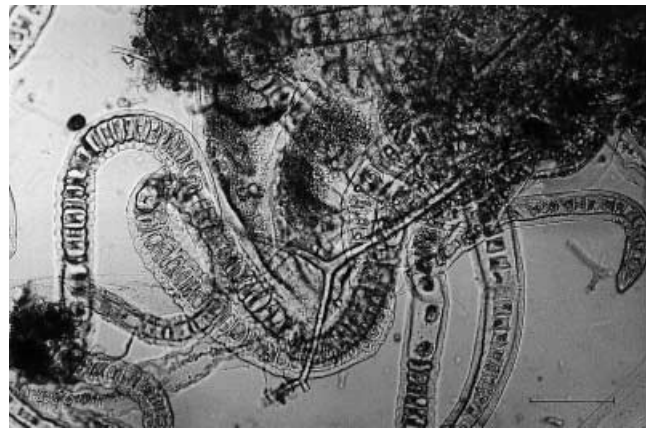


Figure 2. Filamentous Rhodophyceae found in detrital material from the deep sea. The bar in the lower right hand corner indicates a distance of 100 μm . Coordinates 47° 15'N 19° 30'W; date of sampling, 10 May 1988; sampling depth, 2750–3000 m; total water depth, 4550 m.

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