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Fatty acid compositions and trophic relationships of shelled molluscs from the Kuril–Kamchatka Trench and the adjacent abyssal plain

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ABSTRACT

Fatty acid (FA) compositions of 12 species of shelled molluscs (gastropods, bivalves, and scaphopods) from the Kuril–Kamchatka Trench and the adjacent abyssal plain were studied. According to the results of multivariate statistical analysis, molluscs were divided into three groups. Group I consisted of three scaphopod species, the bivalve *Nucula profundorum* and the gastropod *Solariella delicata*. FA compositions of this group were characterized by high levels of 20:4(n-6). We suggest that the FA pattern found in scaphopods with high values of 20:4(n-6) is most likely typical for that of benthic organisms feeding preferentially on foraminiferans. Group II included the bivalves *Neilonella politissima*, *Bentharca asperula*, and *Rhinoclama filatovae*. Bivalves from the second group had elevated concentrations of 22:6(n-3), and the ratio of 20:4(n-6) to 20:5(n-3) was lower than 1. Bivalves from the second group had elevated concentrations of 22:6(n-3). We propose that high concentrations of this FA can be used as a specific marker for a carnivorous feeding mode of deep-sea benthic invertebrates. The bivalve *Bathyspinula calcarella* as well as the scaphopod *Polyschides sakuraii* could not unambiguously be assigned to one group. Within the similarity analysis they rather clustered together with the foraminiferans feeders (group I), but forming an own subgroup. In the PCA on the other hand, *P. sakuraii* showed a position close to the other bivalves, while *B. calcarella* had an intermediate position between all three groups. Group III consisted of the gastropods *Tacita holoserica* and *Paracteocina* sp., which contained high concentrations of 20:5(n-3) and 22:5(n-3). Both are known to exhibit a carnivorous/scavenging feeding strategy. The very low content of DHA in both species is on first sight not consistent with the suggested carnivorous feeding behavior. A characteristic feature of *Paracteocina* sp. and *T. holoserica* was a high level of 22:5(n-3), and HUFA ratios indicate that DHA might be replaced by DPA and EPA in the structural lipids. The comparison of FA compositions of abyssal molluscs showed that different FA patterns are related more to the feeding type than to taxonomic classification.

1. Introduction

Molluscs (especially gastropods, bivalves, and scaphopods) form a significant proportion of the abyssal benthos. Abundance of bivalves represents approximately 10% of all abyssal animals and within these, the subclass Protobranchia is numerically dominant (Allen, 1979; Allen and Sanders, 1996). Gastropoda and Scaphopoda can also form important components of the abyssal benthos in terms of abundance and diversity (Rex, 1981; Reynolds, 2002; Sokolova, 1997). Despite their obvious importance, the dietary resources and trophic relationships of these abundant groups are insufficiently studied. Information about feeding preferences of bivalves and gastropods is often derived from shallow water congeners and has been primarily obtained by microscopic techniques that have a number of limitations (e.g., the bias toward harder body parts, which are digested more slowly). Accordingly, to study the trophic relationships and dietary resources of abyssal molluscs, the additional methods, such as analysis of fatty acid (FA) composition, can be highly informative. Many potential food sources have specific FA signatures that can be used to trace their utilization by consumers. For this reason, FA analyses have been used extensively to study the trophic relationships in marine food webs (Budge et al., 2006; Dalsgaard et al., 2003; Kelly and Scheibling, 2012).

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The northwest part of the Pacific Ocean, including the Kuril–Kamchatka Trench and the adjacent abyssal plain, is one of the most productive areas of this ocean (Filatova, 1969). It has been characterized as a near-continental eutrophic area in which deposit-feeders dominated in terms of biomass, whereby shelled clams average about 10% of the total biomass of the benthic fauna (Sokolova, 1997). In the hadal zone of the Kuril–Kamchatka Trench, bivalves can account for up to half the total benthic biomass (Filatova, 1969, 1971). Despite the fact that this area has been investigated in detail over several expeditions, little is known about the diet of these shelled molluscs. The aim of this study was to identify the main dietary resources and to analyze the trophic interactions of common species of shelled molluscs (gastropods, bivalves, and scaphopods) of the abyssal plain adjacent to the Kuril–Kamchatka Trench (4862–5430 m in depth) using FA markers. The most abundant species of bivalve molluscs in this area (Kamenev, 2015), and common species of gastropods and scaphopods were sampled for analysis.

2. Materials and methods

2.1. Study site and species sampling

Sampling was performed in July–August 2012 during the joint German–Russian expedition KuramBio (Kurile–Kamchatka Biodiversity Study) on board the R.V. *Sonne* (SO 223) to the Kuril–Kamchatka Trench and the adjacent abyssal plain. The molluscs were collected at 7 abyssal stations (Fig. 1) using an Agassiz trawl and an epibenthic sledge (Malyutina and Brandt, 2013). Animals were dissected on board after collection, and whole individuals (bivalves and scaphopods) or muscle tissue subsamples (larger gastropods) were processed. For smaller species, up to 27 individuals were pooled into one sample in order to obtain sufficient biomass for FA analysis. The particulate organic matter from water–sediment interface was collected with a boxcorer. After collection, samples of overlying water were pre-filtered through a sieve with a mesh size of 300 μm. All remaining visible animals were removed under a stereomicroscope, and samples were subsequently sieved through 250, 125 and 63 μm sieves and processed in the same way as the invertebrate samples.

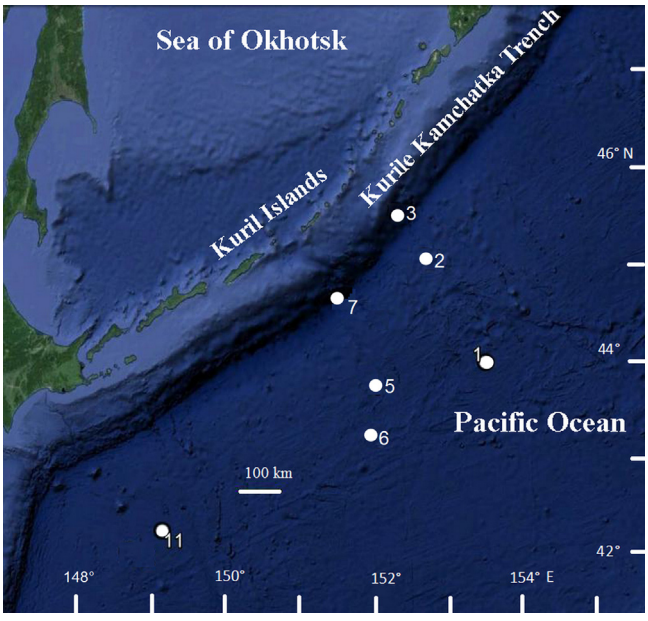

Fig. 1. Location of stations at the Kuril–Kamchatka Trench and the adjacent abyssal plain where molluscs were sampled.

2.2. Fatty acid analysis

The majority of samples was analyzed at the Institute of Hydrobiology and Fisheries Science (IHF), University of Hamburg. In order to create a more comprehensive data set, samples which were analyzed at the Laboratory of Comparative Biochemistry of the Institute of Marine Biology, Vladivostok using GC–MS were added to this study. A comparison of samples of the same species, which were analyzed in both laboratories respectively, indicated that both methods yielded in comparable results. Furthermore, samples from the IHF (GC–FID system) were measured at the Laboratory of Comparative Biochemistry with GC–MS to intercalibrate retention times and thus compound identification.

Samples for the IHF were frozen on board and stored at −80 °C prior to analysis. Prior to the lipid analysis, the tissues were freeze dried, weighed and allowed to extract in dichloromethane-methanol (v:v/2:1) for at least 48 h. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol and a washing procedure with aqueous KCl solution. The analysis of FA composition was performed with modifications as described in Kattner and Fricke (1986). FAs were converted to their methyl ester derivatives (FAME) in sulfuric methanol and analyzed using a gas chromatograph (HP 6890A) equipped with a programmable temperature vaporizer injector (Gerstel® CIS4plus) and a DB-FFAP column. The use of a large volume injector allows improving the sensitivity and measure very low lipid amounts. FAMEs and fatty alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards and samples of known composition. Samples for the Laboratory of Comparative Biochemistry were placed in vials containing a chloroform–methanol mixture immediately after capture and stored at −20 °C. Lipids were extracted from all samples using the extraction method of Bligh and Dyer (1959). FAMEs were prepared from the total lipids according to the procedure of Carreau and Dubacq (1978) and purified with preparative thin layer chromatography in benzene. Additionally, structures of FAs were identified based on GC–MS data from the FAMEs and from the 4,4-dimethyloxazoline derivatives of the FAs according to the method of Svetashev (2011). Mass spectrometry was performed on a Shimadzu GCMS QP5050A spectrometer using MDN 5S columns (temperature gradient from 170° to 290 °C at 2 °C min^{-1}, and then held for 25 min). All spectra were obtained using the electron impact method at 70 eV. Spectra were compared with the NIST library and FA mass spectra archive (Christie, 2013). Proportions of FAs are presented as percentage of total FAs throughout the text.

2.3. Multivariate statistical analyses

Only 12 FAs with concentration more than 10% in at least one sample were included in the multivariate statistical analysis. These FAs are presented in Tables 2 and 3. CLUSTER and SIMPER analysis was carried out using the PRIMER 6 software. No pre-treatment was applied to the FA data prior to computing a resemblance matrix based on Bray–Curtis similarity. The contributions of individual FAs to similarities and dissimilarities within and between sample groups were tested using similarity percentage analysis (SIMPER). A principle component analysis (PCA) was performed using SPSS 15.0 based on the correlation matrix, using untransformed FA data, extracting non-rotated components with eigenvalues >1. Only the first two principle components (PC1 and PC2) are presented.