Methane-Carbon Flow into the Benthic Food Web at Cold Seeps – A Case Study from the Costa Rica Subduction Zone

Helge Niemann1,2*, Peter Linke3,4, Katrin Knittell2, Enrique MacPherson5, Antje Boetius2,6, Warner Brückmann3,4, Gaute Larvik2, Klaus Wallmann3,4, Ulrike Schacht3*, Enoma Omorogie2,7, David Hilton8, Kevin Brown9, Gregor Rehder3,9

1 Department of Environmental Sciences, University of Basel, Basel, Switzerland, 2 Max Planck Institute for Marine Microbiology, Bremen, Germany, 3 Sonderforschungsbereich 574, University of Kiel, Kiel, Germany, 4 Helmholtz Centre for Ocean Research Kiel, GEOMAR, Kiel, Germany, 5 Centro de Estudios Avanzados de Blanes (CEAB-CSIC), Blanes, Spain, 6 Alfred Wegener Institute for Marine and Polar Research, Bremerhaven, Germany, 7 Centro de Astrobiología (CSIC/INTA), Instituto Nacional de Técnica Aeroespacial Torrejón de Ardoz, Madrid, Spain, 8 Scripps Institution of Oceanography, University of California, San Diego, United States of America, 9 Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Rostock, Germany

Abstract

Cold seep ecosystems can support enormous biomasses of free-living and symbiotic chemoautotrophic organisms that get their energy from the oxidation of methane or sulfide. Most of this biomass derives from animals that are associated with bacterial symbionts, which are able to metabolize the chemical resources provided by the seeping fluids. Often these systems also harbor dense accumulations of non-symbiotic megafauna, which can be relevant in exporting chemosynthetically fixed carbon from seeps to the surrounding deep sea. Here we investigated the carbon sources of lithodid crabs (Paralomis sp.) feeding on thiotrophic bacterial mats at an active mud volcano at the Costa Rica subduction zone. To evaluate the dietary carbon source of the crabs, we compared the microbial community in stomach contents with surface sediments covered by microbial mats. The stomach content analyses revealed a dominance of epsilonproteobacterial 16S rRNA gene sequences related to the free-living and epibiotic sulfide oxidiser Sulfurovum sp. We also found Sulfurovum sp. as well as members of the genera Arcobacter and Sulfurimonas in mat-covered surface sediments where Epsilonproteobacteria were highly abundant constituting 10% of total cells. Furthermore, we detected substantial amounts of bacterial fatty acids such as i-C15:0 and C17:1v9c with stable carbon isotope compositions as low as −53% in the stomach and muscle tissue. These results indicate that the white microbial mats at Mound 12 are comprised of Epsilonproteobacteria and that microbial mat-derived carbon provides an important contribution to the crab’s nutrition. In addition, our lipid analyses also suggest that the crabs feed on other 13C-depleted organic matter sources, possibly symbiotic megafauna as well as on photosynthetic carbon sources such as sedimentary detritus.

Citation: Niemann H, Linke P, Knittell K, MacPherson E, Boetius A, et al. (2013) Methane-Carbon Flow into the Benthic Food Web at Cold Seeps – A Case Study from the Costa Rica Subduction Zone. PLoS ONE 8(10): e74894. doi:10.1371/journal.pone.0074894

Editor: Hauke Smidt, Wageningen University, The Netherlands

Received January 4, 2013; Accepted August 7, 2013; Published October 7, 2013

Copyright: © 2013 Niemann et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Financial support for the M66-Subflux cruise came through the Collaborative Research Center (SFB) 574 (“Volatiles and Fluids in Subduction Zones”) at Kiel University funded by the DFG. This work was further supported by NSF (OCE-0242034 and OCE-0242091), the Max Planck Society, the Helmholtz Association and the University of Basel. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: helge.niemann@unibas.ch

Current address: CO2CRC, Australian School of Petroleum, The University of Adelaide, Adelaide, Australia

Introduction

Most deep-sea ecosystems on Earth are considered to be energy limited, because they depend on a small fraction of photosynthetically produced organic carbon (C), which sinks from the productive ocean surface to the seafloor [1,2]. They are contrasted by chemosynthetic ecosystems such as hydrothermal vents and seeps, which are fueled by chemical energy transported with subsurface fluids. Especially cold seeps, which form around mud, gas and oil escape structures and which are characterized by high methane effluxes [3,4], support high biomasses of deep-sea life, comprising chemosynthetic microbial mats and megafauna as well as associated heterotrophic animals [5–9]. The key biogeochemical process at cold seeps is the anaerobic oxidation of methane with sulfate (AOM), which is a net conversion of methane and sulfate to carbon dioxide and sulfide [10–12]. The seeping sulfide fuels aerobic thiotrophic communities comprising free-living and symbiotic bacteria. The free-living thiotrophs often form dense microbial mats above gassy sediments [13–15]. Symbiotic megafauna such as bathybiomastixi bivalves and siboglinid tubeworms host thiotrophic bacteria in specialized cells and tissues [7]. Oxidised cold seep surface sediments may also support free-living aerobic methanotrophs [16–19]. These are not known to form dense mats at cold seeps, but they also occur as endosymbiotic associations with megafauna, such as bivalves and tube worms [20–23]. Furthermore, some highly adapted, hydrothermal vent or cold seep endemic annelids [24], gastropods [25] and crustaceans...
farm chemosynthetic, microbial epibionts on their skin and shells, which they graze upon. An important question in the ecology of vent and seep ecosystems remains as to how chemosynthetically fixed carbon is transferred to the deep-sea food web [5,9,26–30]. Current knowledge is mostly based on measurements of the stable C isotope ratio of faunal bulk tissue [5,9,31,32]. At cold seeps, both methane and its oxidation product CO2 are strongly depleted in 13C [33]. Consequently, methanotrophic and thiotrophic bacteria, which incorporate 13C-depleted methane and/or 13CO2 in their biomass are characterized by δ13C-values much lower than −15 to −30‰, which is the range typical for photosynthetically fixed C [33,34]. Consumer species feeding on free-living chemosynthetic microbes previously measured a total CH4 flux of 0.2% of the CH4 produced by the subduction zone. We combined bulk- and compound-specific isotopic analysis of lipid components to investigate the relevance of compound-specific δ13C-values, for example of fatty acids (FAs), which are contained in cellular membranes [36,37]. These lipids are incorporated from the food sometimes without significant alteration into the consumer biomass; e.g., essential fatty acids [30]. Furthermore, some lipids are diagnostic biomarkers because they are synthesized by specific source organisms. The analysis of their presence and specific C-isotope composition help to identify multiple dietary C-sources utilized by a consumer. However, the isotopic composition of biomass typically integrates over significant parts of an organism’s lifetime. In order to investigate food sources that a consumer ingested only recently, the analyses of stomach content, including DNA, are frequently used in food web studies [39–41].

Materials and Methods

Site description

Mound 12 (Md. 12) is an active mud volcano located at the Central America convergent margin off the coast of Costa Rica at 1020 m water depth (8° 55.85’ N, 84° 18.75’ W; [42]). It belongs to a series of cold seeps along the Costa Rican Pacific margin, which are related to the subduction of the Cocos plate and erosion of continental material, subsequent dehydration of subducted clay minerals as well as production of thermogenic CH4 [43–45]. At Md. 12, CH4, geofluids and mud ascend to the seafloor along faults, which cut deeply through the basement and upper plate sediments [46]. Diapirism and mudflows have formed a rounded (≈800 m diameter) cone-shaped relief (<30 m) with an irregular pinnacle in the NE and a lower profile ridge in the SW [42,47]. The mudflows are intercalated with slope sediments, indicating that Md. 12 is frequently active, alternated by low-activity phases. At present, the mound seems to be most active at its pinnacle and the SW flank, which is characterized by dense microbial mats and other chemosynthetic organisms (mullid mussels and Lamellibrachia tube worms) [47–49]. At a microbial mat site, we previously measured a total CH4 flux of ~ 10 mol m⁻² yr⁻¹ of which only half was oxidized with SO4²⁻ [47]. Indeed, bottom waters above Md. 12 were enriched in CH4 with 1–2 orders of magnitude higher concentrations compared to background values, indicating that a significant fraction of the seeping CH4 can escape into the water column [49].

Sea floor observations and sampling

We visited Md. 12 during two consecutive cruises with R/V Atlantis (AT11-28) and R/V Meteor (M66-2) in June and September 2005, respectively. Direct and/or video observations were carried out in June with DSV Alvin (Woods Hole Oceanographic Institute, USA) and in September with ROV Quest (Marum, Germany). In addition, we also photographed the sea floor during cruise M66-2 over a time period of 408 hours with a frequency of 2 pictures per hour. For this approach, a downward-facing digital still camera (Ocean Imaging Systems, North Falmouth, USA, 6.1 Mpix) was mounted on a lander frame (Deep-sea Observation System – DOS [48]) resulting in a field of view of 0.4 m². The lander was deployed on top of a microbial mat (8° 55.69’ N, 84° 18.78’ W), which covered ~60% of the cameras field of vision.

A specimen of the abundantly observed lithodid crab (see results and discussion section for a taxonomic assessment), which was apparently feeding on microbial mats, was sampled using DSV Alvin’s manipulators (8° 55.72’ N, 84° 18.83’ W). The crab was stored in a basket until surfacing of the submersible and directly thereafter photographed and dissected. A tissue sample from a leg muscle and the stomach were removed and frozen at −20°C until further analyses in the home laboratory. A ~6 m wide sediment strip (8° 55.69’ N, 84° 18.82’ W) covered by the whitish, thiotrophic microbial mats as well as bare sediments 1–2 m adjacent to the microbial mat were sampled by push coring with ROV Quest.

Taxonomic identification of lithodid crabs

The lithodid crabs were taxonomically identified from photographs that we recorded in situ (i.e., with the deep-sea camera of the DOS lander; e.g., Fig. 1b), and on board from the specimen recovered with Alvin (e.g., Fig. 1c, d). Identification was based on morphological features such as spines, spindles and granules according to our previous work [50].

Lipid analyses and determination of C and N contents

Extraction of lipids, separation and derivatization was carried out as described previously [51,52]. Briefly, total lipid extracts (TLEs) were obtained from subsamples of the muscle tissue (~500 mg wet weight – ww.) and stomach (including its contents; ~400 mg ww.) by ultrasonication with organic solvent mixtures (methanol and dichloromethane) of decreasing polarity. The TLEs were then saponified and subsequently separated into fractions containing (i) fatty acids (FAs), (ii) hydrocarbons, (iii) ketons and (iv) alcohols (including glycerol ethers). FAs and alcohols were methylated prior to extraction using BF3 in methanol and bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form fatty acid methyl esters (FAMES) and trimethylsilyl (TMS) ethers, respectively. Separation of single lipid compounds, their identification, quantification and the determination of their stable carbon isotope composition was achieved by gas chromatography (GC) coupled to flame ionization detection (GC-FID), quadrupole mass spectrometry (GC-MS) and isotope ratio mass spectrometry (GC-IRMS), respectively [53]. Bulk stable carbon isotope composition was measured from CO2, released after flash combustion of ~100 mg (ww.) of muscle tissue in an automated elemental analyzer (Thermo Flash EA, 1112 Series) coupled to an isotope ratio mass spectrometer (Finnigan DeltaPlus XP, Thermo Scientific).
Determination of bulk C and N contents was carried out according to standard methods (www.geomar.de/en/research/fb2/fb2-mg/benthic-biogeochemistry/mg-analytik/determination-of-cn/). Briefly, all inorganic and organic C and N compounds in sediment samples were flash combusted in a CNS analyzer (Carlo Erba Instruments, LTD) and the resulting combustion gases were analyzed with a thermal conductivity detector yielding total C and N contents. Organic C was determined in a similar fashion subsequently to the removal of carbonate-bound C with HCl. C:N-ratios are reported as the molar ratio of organic C versus total N.

DNA extraction and clone library construction

Total DNA of the microbial community in the crab’s stomach was extracted from ~350 mg (ww.) of stomach material using the FastDNA spin kit for soil (Q-Biogene, USA) as described elsewhere [53]. PCR amplification of 16S rRNA genes, cloning, and sequencing was conducted according to [16]. For the construction of the epsilonproteobacterial clone library, a subsample of 50 µl of formaldehyde-fixed sediment sample (the same sample as used for CARD-FISH, see next section) was centrifuged and the pellet was washed with 1× PBS and finally resuspended in 50 µl H2O. Subsequently, we sonicated the sample (2×30 sec, 35 kHz) in a water bath sonicator. 1 µl of a 100-fold dilution was used as template for specific amplification of epsilonproteobacterial 16S rRNA gene sequences using primers Epsi682F (5’-TGTGTAGGGGTAAAAATCCG 3’) and GM4. The PCR conditions were as follows: 32 cycles, annealing temperature 44°C. Ten parallel PCRs of each sample were pooled, purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany) and eluted in 30 µl H2O. Cloning reactions were performed with the TOPO TA Cloning Kit (Invitrogen, San Diego, CA, USA) and inserts sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an ABI PRISM 3130xl Genetic Analyzer. Sequences were checked for chimeras using the program UCHIME [54] and phylogenetically analyzed with the ARB software package using database SSURef-_SILVA_111 (July 2012, 739,633 sequences) downloaded from ARB SILVA resources [55]. The sequence data from the stomach sample will be published in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession numbers HE974888 to HE974904 as well as HF559372 and HF559373. Sequences from the epsilonproteobacterial clone library will be published under the accession numbers HG321335-HG321366.

Cell enumeration and catalyzed reporter deposition fluorescence in situ hybridization (CARD–FISH)

Sediment samples for CARD-FISH were fixed in formaldehyde solution, washed in PBS and stored at –20°C as described previously [16]. CARD-FISH was carried out on two parallel surface sediment samples (0–2 cm) from the microbial mat habitat and on one sediment sample from the adjacent, non-covered sediment as described previously [56] with the following modifications: Samples were sonicated before filtration (20 s an amplitude of 42 µm <10 W; MS73 probe, Sonopuls HD70, Bandelin, Germany) and endogenous peroxidases were inactivated by incubation in 0.5% H2O2 in methanol for 30 min at room temperature. Cell walls were permeabilized with 10 mg ml⁻¹ lysozyme in 1×TE-buffer for 45 min at 37°C [57]. For the specific detection of Epsilonproteobacteria, we used the HRP-labeled probe Epsi682 (5’-CGGATTTTACCCCTACACM- 3’; biomers.net) [58] applied at 20% formamide. Cells were stained with DAPI, embedded in mounting medium and counted under an epifluorescence microscope in 20–100 independent microscopic fields.

Methane oxidation- and sulfate reduction rate measurements

Microbial turnover of CH4 and SO4²⁻ in sediments of Md. 12 was measured with radiotracer assays according to previously published works [51,59,60]. Briefly, CH4 oxidation and sulfate reduction (SR) rates were determined from 6 push cores distributed over the ~6 m wide sediment strip covered with bacterial mats and from 3 push cores recovered 1–2 m away from this mat.
Results and Discussion

Sea floor observations and biogeochemical environment

Sea floor habitat. We visited Md. 12 in 2005 and investigated the seafloor with DSV Alvin and ROV Quest. Visually, we could identify several habitats: reduced sediments covered by whitish microbial mats (e.g. Fig. 1a, b) and adjacent bare sediments without microbial mats (movie S1 in the supplement), colonies of bathymodiolin mussels (Bathymodiolus sp.) or siboglinid tubeworms (Lamellibrachia sp.) and CH$_4$-derived carbonate pavements. As reported previously [47–49], these habitats were distributed in a patchy fashion, interspersed by olive-green sediments. The size of the microbial mat patches varied from decimeters to several meters in diameter. The whitish color of the mats suggested that they consisted of thiotrophic bacteria, but the morphology of the mats differed in thickness and structure from those present at most known cold seep systems formed by large sulfur bacteria such as Beggiatoa, Thiomargarita or Thioploca [6,15,53,61]. They resembled more the Arcobacter-type mats known from mud volcanoes, such as of the Eastern Mediterranean [14,62]. The sediments below the mats strongly smelled of sulfide, and previous measurements found ~15 mM sulfide in porewaters from this habitat [47]. All cores recovered from the microbial mats were also rich in CH$_4$ as indicated by their degassing during recovery, and the oversaturated CH$_4$ concentrations of ≥1.4 mM in recovered sediments (data not shown). Ex situ rate measurements of AOM and SR showed peak values of up to 225 and 327 nmol cm$^{-2}$ d$^{-1}$, and integrated rates of 7.4 and 6.5 mol m$^{-2}$ yr$^{-1}$ for AOM and SR, respectively (Tab. 1). The high sulfide concentrations are thus explained by AOM-dependent SR. We found a high variability in rate measurements when comparing replicates (Tab. 1), possibly related to heterogeneous fluid flow regimes [47]. Just ~1 m outside the microbial mat habitat, sediments barely smelled of sulfide, and AOM and SR rates were <5 nmol cm$^{-2}$ d$^{-1}$, equivalent to areal rates of <0.3 mol m$^{-2}$ yr$^{-1}$ (Tab. 1).

Lithodid crabs grazing on microbial mats. We frequently observed one type of lithodid crab, which dwelled and apparently fed on the microbial mats of Md. 12 (Fig. 1a–d, movie S1). Based on the shape of the carapace, rostrum and abdomen documented by high-resolution photography, we identified this species as Paralomis sp. [50]. Its morphology is similar to *P. diomedeae*, known to populate continental margins from Costa Rica to Peru, but it differs by the granules on the dorsal carapace surface and the armature of the chelipeds and walking legs. This suggests that the Paralomis type of Md. 12 could be a new Paralomis species, closely related to *P. diomedeae*. A conclusive determination of the crab’s taxonomic status requires collection of new material and in-depth morphological and genetical investigations. We did not conduct off-site surveys during our sampling campaigns so that we can only speculate about the biogeography of the *P. diomedeae* related crabs and potential adaptations for the consumption of chemosynthetic biomass. Little is known about the ecology of *P. diomedeae* but the mouth parts (mandibles, maxillae, maxillulae and maxillipeds) of the previously examined specimens from the eastern Pacific Ocean off Costa Rica [50] and the ones of Md. 12 indicate that both are adapted to an omnivorous diet including detritus. Indeed, during submersible and ROV dives, we observed that the *Paralomis* sp. of Md. 12 grazed on the microbial mats (or on surface sediments including the mats) leaving clearly distinguishable feeding tracks of bare sediments behind (Fig. 1a, b). Other members of the genus *Paralomis*, possibly opportunistic scavengers or predators, have also been observed at other cold seeps and hydrothermal vents constituting a potential link for the export of seep carbon to the surrounding deep sea [28,63,64]. However, to our knowledge, only one other publication has reported similar, direct observations from a cold seep setting, i.e. hermit crabs feeding on *Beggiatoa* mats at the Gullfaks seeps, North Sea [65]. The longer-term recordings of the lander-mounted still camera provided further evidence that the microbial mats apparently attracted *Paralomis* sp. (movie S1). During the 408 hours of observation with the lander-mounted camera, we counted 184 sightings of this crab species on a microbial mat patch while only 6 sightings were recorded from surrounding sediments (Tab. 1). Our observations, furthermore, indicate a pattern where intensive grazing was followed by a time period between 8 and 33.5 h of little or no grazing during which the mat regrew until it was grazed of again. We could also record the occurrence of a larger food fall, i.e. a Pyrosome (tunicate colony), which was also consumed by the *Paralomis* sp. (movie S1), confirming that they are opportunistic predators/scavengers. In addition to the *P. diomedeae* relative, we also noticed a second but rather rarely occurring *Paralomis* species (Fig. 2), which we tentatively identified as *P. patitilla* or a relative of this species [50]. However, the few available photo documents did not allow for a more reliable identification. One specimen of the so-called Yeti Crab (*Kiwa paraeide*) could be seen once on the photo material of the lander mounted camera. We could not observe the *P.*

| Habitats of Md. 12 sediments covered- and devoid of microbial mats. |
|---|
| **Table 1.** |
| **microbial mat** | **adjacent sediments** |
| tot. No. of crabs observed | 184 | 6 |
| oxidation state | strongly reduced | oxic/anoxic/slightly reduced |
| organic C (wt%) | 2.6 (±0.1) | 2.5 (±0.1) |
| C:N-ratio | 9.9 (±0.5) | 10.0 (±0.4) |
| sediment depth of AOM max. (cm) | 3 cm | - |
| AOM max. (nmol cm$^{-2}$ d$^{-1}$) | 225 (±60) | 5 (±1) |
| areal AOM (mol m$^{-2}$ yr$^{-1}$) | 7.5 (±1.8) | 0.2 (±0.04) |
| SR max. (nmol cm$^{-2}$ d$^{-1}$) | 328 (±107) | 8 (±5) |
| areal SR (mol m$^{-2}$ yr$^{-1}$) | 6.5 (±1.8) | 0.3 (±0.18) |

Total number of crabs was counted from still camera images (2 pictures h$^{-1}$) during an observation period of 408 h. Note that we did not account for feeding tracks without a photo record of the originator and that single specimens could have been counted repeatedly. Org. C contents and C:N-ratios were averaged over the first 10 cm- and AOM and SR rates were integrated over the first 16 cm of surface sediment. Errors are presented as standard error. doi:10.1371/journal.pone.0074894.t001
pepilata relative or the Yeti Crab feeding on the mats but we noticed snails, which seemed to feed on the mats (movie S1).

Dietary carbon sources for the Paralomis diomedeae relative

Sediment C and N contents. To investigate whether the P. diomedeae relative preferentially feeds on microbial mats compared to regular sediments as suggested by our observations, we compared the bulk chemical composition of surface sediments. Both habitat types were characterized by high contents of organic C (≈2.5 weight%) and low C:N-ratios (≈10, Tab. 1) throughout the upper 10 cm of surface sediments. These values are comparable to seafloor sediments from the highly productive upwelling regions of Peru [66] or Chile [67] at ~1000 m water depth and are indicative for a high fraction of fresh organic matter. This may be explained by the high pelagic primary production in the region of the Costa Rica Dome [68]. The organic deposits in sediments surrounding the seeps of Md. 12 could thus also serve as a relevant carbon source for the Paralomis sp. Nevertheless, the nutritional value of the microbial mat is probably higher than that of sediment detritus because of the low C:N-ratio of bacteria (typically 4–5), caused by a relatively high cellular protein content (~50%) [33]. As the bacterial mat was very thin, the rather coarse sediment sampling of 2 cm sections may thus have masked this signal. Besides the microbial mats and the sedimentary detritus, also the symbiotic megafauna at Md. 12 (i.e., Bathymodiolus sp. and Lanellibrachia sp.) could be an attractive food source for the crabs. Bivalves and annelids typically contain very high protein contents, which may comprise >70% of their organic matter [34]. However, we did not observe the crabs to feed on these potential food sources.

To further investigate the dietary importance of chemosynthetic vs. photosynthetic carbon for the Paralomis sp. we analyzed the molecular signatures of stomach contents, muscle tissue and surface sediments covered by microbial mats (see next 2 sections).

16S rRNA gene libraries and FISH. CARD-FISH analyses of two parallel samples of the microbial mat and underlying sediments with the Epsilonproteobacteria-specific probe EPSI682 indicated that Epsilonproteobacteria constituted 9.5 and 11.1% of single cells. In contrast, in the surface layer of the adjacent, bare sea floor, we could only detect <2% Epsilonproteobacteria. With respect to the morphological appearance of the mat, this confirmed dominance by Epsilonproteobacteria rather than by large gammaproteobacterial thiotrophs (Beggioa, Thiomargarita or Thioploca). We used probe EPSI682 as a specific forward primer together with the general bacterial primer GM4 in a PCR to resolve the diversity of CARD-FISH-detected Epsilonproteobacteria in the microbial mat habitat. Of the epsilonproteobacterial 16S rRNA genes (53 epsilonproteobacterial sequences from 72 clones analyzed in total, Tab. 2), six sequences grouped within the genus Sulfurovum. Other epsilonproteobacterial sequences belonged to the genera Arcobacter (25 sequences), Sulfurovumus (17 sequences), and Campylobacter (3 sequences).

From the stomach contents, we could amplify bacterial 16S rRNA gene sequences successfully but repeated attempts to amplify archaeal rRNA genes failed. This likely indicates a very low abundance of archaea in the stomach contents, which is in accordance with our biomarker analyses (see next section). From the amplified bacterial 16S rRNA genes, we analyzed a total of 79 clones. We identified Epsilonproteobacteria of the genus Sulfurovum as the dominant bacterial group in the stomach of the Paralomis sp. (Tab. 3). Two groups (8 and 17 sequences, respectively) with a high intragroup sequence similarity of 98–99% and 94–95% between the two groups were detected. Sequences of cluster 1 were 99.8% similar to sequences from Eel River Basin methane seeps ([69] e.g. acc.no.FJ264599) and those of the second cluster were 97.8% similar to a sequence obtained from particulate detritus from grabs of the vestimentiferan tubeworm Rudiella piaosea (Forget & Jupiter, database release, acc.no. JN662293). Furthermore, sediment Sulfurovum sp. was highly similar to the Sulfurovum sp. cluster 1 found in the crab’s stomach (96.8–99.8% sequence similarity). Also gut and sediment Campylobacter spp. showed a high degree of similarity (up to 98.7%). Although Arcobacter- and Sulfurovumus-related sequences were not retrieved from the crab’s stomach, these results provide evidence that Epsilonproteobacteria in the stomach originate from the thiotrophic mats, which the crab was observed to feed upon. Together with our observations of crabs feeding specifically on microbial mats, this strongly suggests that these mats are an important nutrition source for the P. diomedeae relative recovered from Md. 12.

Epsilonproteobacteria are known from a variety of hydrothermal vents [70] but have also been found at cold seeps [19,62,69,71] including brines [72,73]. Members of the genus Sulfurovum have been found as free-living bacteria [74,75], epibionts associated with a hydrothermal vent shrimp [70,76] and with the cold seep associated Yeti Crab (Kiwa puravida), the latter of which was also found at Md. 12 [26]. Members of the Sulfurovum clade were also found in the gut system of the Yeti Crab and a hydrothermal vent shrimp [26,77]. However, these Sulfurovum types shared only ~95% similarity with our sequences. The biogeochemical functioning of the Sulfurovum relatives constituting the microbial mats at Md. 12 is not clear. Known members of the genus Sulfurovum use elemental sulfur or thiosulfate as an electron donor, and nitrate or oxygen as electron acceptors [74,78,79]. Whole genome sequencing of a Sulfurovum strain (NBC37-1) revealed the presence of sox genes (coding for enzymes involved in sulfide oxidation) and the strain also had cytoplasmic and periplasmic sulfide-quinone oxidoreductases that oxidize sulfide to elemental sulfur [80].

The stomach contents also contained sequences of other, seep-related chemosynthetic microbes including aerobic organisms thriving in the upmost, oxic surface sediment layer as well as anaerobic strains from deeper sediment layers. We detected one sequence of a relative of Hypomicrobium and Acinetobacter, which were previously found to grow aerobically on chloro- or dichloromethane [81] and long-chain alkanes [82], respectively. Among the anaerobic strains, we detected two deltaproteobacterial sequences belonging to relatives of the Desulfobulbus/Seep-SRB3
cluster, one sequence of the SEEP-SRB2 cluster, which comprise SRB associated to ANME archaea [10,83], and three sequences related to Desulfocapsa, which is a typical SRB in marine sediments, including cold seeps [16]. Furthermore, we also found other bacteria of unknown biogeochemical function that have regularly been found in anoxic cold seep sediments, i.e. relatives of the Candidate Division OD1 and Propionibacterium (of which we found six and two sequences, respectively) [11,84,85]. However, the relatively low abundance of sequences of anaerobic cold seep microbes indicates that the crab specimen analyzed here mostly fed on oxic surface- and ingested rather little amounts of reduced sediments containing AOM biomass, at least during its last feeding activities. The relatively deep position of the AOM horizon (~3 cm, Tab. 1) could make archaeal biomass rather inaccessible for the P. diomedeae relative or the expectedly high sulfide contents of the AOM horizon [47] could be too toxic.

25 out of 78 sequences were affiliated with Candidatus Lumbricincola and Candidatus Bacilloplasma, relatives that most likely belong to the gut flora of the Paralomis sp. Candidatus Lumbricincola has yet only been found in the gut systems of annelids [86]. Candidatus Bacilloplasma relatives, on the other hand, were found in the guts of decapod crustaceans (Scylla sp.; Sun & Li, database release acc.no. AY360554 and Nephrops norvegicus [87]) isopods [88] and chordates (Wu & Wang, database release ac.no. GU293173). Members of the class Mollicutes are often pathogenic or parasitic, but also commensal and beneficial associations with their hosts have been found [41,89].

Stable carbon isotope and lipid analyses. The bulk stable carbon isotope composition of the muscle tissue was −46‰ (Fig. 3)

Table 2. Epsilonproteobacterial 16S rRNA gene library obtained from surface sediments (0–2 cm) covered with whitish microbial mats.

| Order                              | Family                  | Genus                     | No. of clones | Clone representative | Acc. No. |
|------------------------------------|-------------------------|---------------------------|---------------|----------------------|----------|
| Campylobacterales                   | Helicobacteraceae       | Sulfurovum, cluster 1     | 6             | CRsed_Md12_64_17A3   | HG321355 |
|                                    |                         | Sulfurimonas              | 17            | CRsed_Md12_64_82B11  | HG321360 |
| Campylobacterales                   | Campylobacter           | 5                         | CRsed_Md12_64_45E6 | HG321356    |
|                                    | Arcobacter              | 25                        | CRsed_Md12_64_66B9 | HG321357    |
and thus extremely $^{13}$C-depleted in comparison to organic matter in regular, recent marine sediments ($-10$ to $-35\%$), which are usually of photosynthetic origin (Calvin Benson Cycle) [33]. In eukaryotes, such negative carbon isotope signatures are typically attributed to a methanotrophic food chain [30,36,90–92]. However, also sulfate reducing bacteria and thiotrophs may show similar signatures by incorporating isotopically depleted CO$_2$ derived from methane oxidation and by further fractionation in autotrophic assimilation pathways [93,94]. Together with our observations of the Paralomis sp. feeding habits and the presence of Sulfurovum sequences in the crab’s stomach, the low $\delta^{13}$C-value of the muscle sample thus strongly indicates that the Paralomis sp. derives a substantial fraction of organic carbon from the thiotrophic microbial mats, apparently over significant parts of the crab’s lifetime. However, the bulk stable isotope composition may also comprise contribution from other chemosynthetic- and/or phototropic sources.

To investigate the potential dietary carbon sources in more detail, we analyzed lipids from stomach contents (including the stomach epithelium) and from muscle tissue of a walking leg. Only trace amounts of the isoprenoidal glycerol ethers archaeol and sn$_2$-hydroxyarchaeol, which are typical for AOM-mediating ANME archaea [95] were found in the stomach sample (data not shown). This directly implies that the stomach of the Paralomis sp. contained comparably little archaeal biomass, which is consistent with our 16s rRNA analyses (see above).

Contrary to the archaeal compounds, we detected substantial amounts of FAs in both, the stomach and the muscle sample (Fig. 3, Tab. 4). These lipids are of bacterial and/or eukaryotic origin. In the muscle sample, the FAs may originate from de novo synthesis, direct incorporation of food-derived compounds or a mix of both and can thus be used to trace chemosynthetic biomass in heterotrophs [37]. In the stomach sample, these lipids probably originate to a substantial degree from the crabs food source (however, note that the stomach sample contained not only stomach contents but also the stomach epithelium so that it comprises a mixed lipid signature of food and crab). The essential FAs C20:5$\omega$3, C20:4$\omega$6 constituted a major fraction of the analyzed FAs, in both samples (Fig. 3). These lipids cannot be synthesized by the crab de novo [30] and are thus derived from the crab’s food source. With respect to the depleted isotopic signatures of about $-40\%$ (C20:5$\omega$3) and $-37\%$ (C20:4$\omega$6), it is very likely that these compounds substantially originate from chemosynthetic bacterial biomass corroborating the molecular, and bulk stable isotope data. Moreover, the higher fractional abundance of C20:5$\omega$3 and C20:4$\omega$6 in the stomach- compared to the muscle sample indicates that these FAs were enriched in the stomach contents and thus originate from a recently ingested food source, possibly the microbial mats. Further evidence for the dietary importance of chemosynthetic biomass for the crab is provided by the presence of unusual, $^{13}$C-depleted FAs in the stomach and the muscle sample (Fig. 3), which contained substantial amounts of the iso- and anteiso-branched C15–C17 FAs, the moneonic FAs C16:1$\omega$5 and C17:1$\omega$6 as well as the cyclopropyl FA cyC17:0$\omega$5,6. Generally, these lipids are not found in crustaceans, but are representative of AOM-associated SRB and/or thiotrophic communities [17,52,95,96]. Just as for the essential FAs C20:5$\omega$3, C20:4$\omega$6, the depleted stable carbon isotope signature of these compounds with values as low as $-50.5$ and $-52.6\%$ (C17:1$\omega$6) in the stomach and muscle sample, respectively, point to CH$_4$-derived carbon as a dominant carbon source.

In addition to microbial mat biomass, our lipid data provide evidence that the crabs utilize detrital material as well. A second essential FA, C22:6$\omega$3, had a much higher fractional abundance in

![Figure 3. Fractional abundance and stable carbon isotope composition of fatty acids in a muscle- and a stomach sample of the Paralomis diomedeae relative.](https://www.plosone.org/doi/10.1371/journal.pone.0074894.g003)
the muscle tissue compared to the stomach sample (Fig. 3), which suggests that this compound originates from food sources not present in the stomach at the time of sampling. The high δ13C-value of C22:6Δ3 (about −28‰) indicates a photosynthetic origin of this FA. Most likely, the crab had consumed non-seep carbon during past feeding activities, for instance sedimented detrital organic matter, or food falls such as the Pyrosome colony (see Movie S1).

In comparison to the bulk stable carbon isotope composition of the muscle tissue (−46‰), the abundance-weighted, average FA δ13C-value was considerably less depleted (−36‰, Tab. 4). Therefore, the crab specimen must have consumed additional 13C-depleted compounds other than FAs. One such compound class are steroids, of which we found 13C-depleted cholesterol (cholest-5-ene-3β-ol) and its probable precursor desmosterol (cholest-5,24-diene-3β-ol) (Tab. 4). Just as the essential FAs, decapod crustaceans appear to lack the ability to synthesize steroids de novo [97,98] indicating a dietary origin of these compounds. Similar to the essential FA C22:6Δ3, we found a much higher fractional abundance of steroids in the muscle tissue compared to the stomach sample. One source of steroids could be infauna organisms such as polychaetes and nematodes, which, at other cold seeps, were found feeding on organic carbon from deeper sediment layers including the AOM horizon [5]. A second source of steroids could be symbiotic megafauna such as Bathymodiolus spp. and Lamellibrachia sp, which are also a potential food source for heterotrophic megafauna [36,99]. We did not measure δ13C-values of these organisms at Md. 12, but it is reasonable to assume that the bathymodiolin biomass is strongly 13C-depleted just as has been found at other cold seeps [22,90,92], so that Bathymodiolus sp. could be a source of the crab’s 13C-depleted sterol pool. Lamellibrachia sp., on the other hand, is often not 13C-depleted [92,100–102]. Nevertheless, a dietary mixture comprising symbiotic microbial mats, pelagic detritus and megafauna and/or infauna, probably accounts for the difference between bulk- and (abundance weighted) FA stable carbon isotope composition.

Conclusions

Our sea floor observations together with the analyses of ribosomal RNA genes, lipid biomarkers and stable carbon isotope composition provides evidence that at Md. 12, the lithodid crabs closely related to Paralolis diomedeae feed on chemosynthetic biomass. This includes the Epsilonproteobacteria (Sulfurimonas related spp., Anearchaeota spp. and Sulfurovum spp.), which form the thiotrophic microbial mats at Md. 12. Additionally, our analyses showed that other hydrocarbon degrading- and sulfate-reducing microbes as well as seep macro- and/or megafauna contribute to the nutrition of the crab. The stable carbon isotope- and lipid composition of the crab tissue confirmed that it is an opportunistic scavenger, using both, chemosynthetically as well as photosynthetically derived carbon in its diet. This agrees well with the shape of the crab’s feeding appendages, which are functionally similar to other lithodid deep-sea crabs with an omnivorous diet (including detritus) and an opportunistic and vagrant life style. The results of this study suggest that cold seeps may have an important ecological role not only for seep-endemic but also for opportunistic, mobile megafauna.

Supporting Information

Movie S1 Time-lapse movie of sea floor observation recorded from a stationary, downward facing camera (2 pictures per hour, field of vision = 0.4 m²). Lithodid crabs (Paralolis diomedeae relative), which were apparently grazing on a thiotrophic, microbial mat were the most common observable fauna type (184 sightings during 408 hours total observation time). (MP4)

Acknowledgments

We thank captain and crew of R/V Atlantis (cruise AT11-28) and R/V Meteor (cruise M66-2) for their excellent support with work at sea. We are particularly grateful to the teams of DSV Alvin and ROV Quest for their excellent help with sampling and observation. We thank Bernhard Bannert and Wolfgang Queisser for technical support during the lander deployments on R/V Meteor. We also thank Karen Stange for on-board methane and shore-based stable carbon isotope analysis, Viola Beier and Nicole Rodigier for excellent technical assistance with laboratory analyses, Maite Nicolai for video editing, Marcus Elvert for lipid identification and Lea Steinle and Moritz Lehmann for helpful comments on this manuscript.

Author Contributions

Conceived and designed the experiments: HN PL. Performed the experiments: HN PL GL KW EO. Analyzed the data: HN PL KK EM. Contributed reagents/materials/analysis tools: Viola Beier and Maike Nicolai for video editing, Marcus Elvert for lipid identification and Wolfgang Queisser for technical support during the lander deployments on R/V Meteor. We also thank Karen Stange for on-board methane and shore-based stable carbon isotope analysis, Viola Beier and Nicole Rodigier for excellent technical assistance with laboratory analyses, Maite Nicolai for video editing, Marcus Elvert for lipid identification and Lea Steinle and Moritz Lehmann for helpful comments on this manuscript.

References

1. Suess E (1980) Particulate organic carbon flux in the oceans-surface productivity and oxygen utilization. Nature 288: 260–263.
2. Jahnke RA (1996) The global ocean flux of particulate organic carbon: Areal distribution and magnitude. Global Biogeochem Cy 10: 71–88.
3. Niemann H, Boetius A (2010) Mud Volcanoes. In: Timmis KN, editors. Handbook of Hydrocarbon and Lipid Microbiology. Berlin: Springer. 205–214.
4. Suess H (2010) Marine Cold Seeps. In: Timmis KN, editors. Handbook of Hydrocarbon and Lipid Microbiology. Berlin: Springer. 167–204.
5. Levin LA (2005) Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes. Oceanogr Mar Biol 43: 1–46.
6. Jørgensen BB, Boetius A (2007) Feast and famine — microbial life in the deep-sea bed. Nat Rev Microbiol 5: 770–781.
7. Dubillier N, Bergin C, Lott C (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat Rev Microbiol 6: 725–740.
8. vanreusel/A, Andersen AC, Boetius A, Connelly D, Cunha MR, et al (2009) Biodiversity of Cold Seep Ecosystems Along the European Margins. Oceanography 22: 110–127.
9. Bernardino AF, Levin LA, Thuerer AR, Smith C (2012) Comparative Composition, Diversity and Trophic Ecology of Sediment Macrofauna at Vents, Seeps and Organic Falls. PLoS ONE 7: e35515.
10. Knittel K, Boetius A (2009) Anoxic Oxidation of Methane: Progress with an Unknown Process. Annu Rev Microbiol 63: 311–334.
11. Holler T, Wegener G, Niemann H, Deuser C, Freidelman TG, et al (2011) Carbon and sulfur flux back during anaerobic microbial oxidation of methane and coupled sulfate reduction. P Natl Acad Sci USA 108: E1484–E1490.
12. Milucka J, Ferdelman TG, Polerecký L, Franzke D, Wegener G, et al. (2012) Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Hikurangi mud volcano (New Zealand: Pacific margin). Geomicrobiol J 29: 1–14.

13. Sahling H, Galkin SV, Salyuk A, Greinert J, Foerstel H, et al. (2003) Depth-dependent distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia margin, Oregon). Geomicrobiol J 20: 269–294.

14. Niemann H, Fischer D, Graefe D, Knittel K, Montiel A, et al. (2009) Methane methanogenesis and sulfate reduction at cold seeps of the deep Eastern Mediterranean Sea. Mar Geol 261: 114–127.

15. Niemann H, Fischer D, Graefe D, Knittel K, Montiel A, et al. (2009) Biogeochemistry of a low-acitivity cold seep in the Larsen B area, western Ross Sea, Antarctica. Biogeosciences 6: 2381–2393.

16. Childress JJ, Fisher CR, Brooks JM, Kennicutt II MC, Biligare R, et al. (1986) A methanotrophic marine mudholus (Bivalvia, Mytilidae): ssumises; mussels fueled by gas. Science 233: 1306–1308.

17. Fisher CR, Gates DJ (1989) Chemosynthetic and Methanotrophic Symbioses in Marine-Invertebrates. Rev Aquat Sci 2: 399–436.

18. Duperron S, Sibuet M, MacGregor BJ, Kypers MMM, Fisher CR, et al. (2007) Diversity, relative abundance and metabolic potential of bacterial endosymbioses in the bathymodiolus mussel species from cold seeps in the Gulf of Mexico. Environ Microbiol 9: 1425–1435.

19. Petersen JM, Dubilier N (2009) Methanotrophic symbioses in invertebrates. Environ Microbiol Rep 1: 319–335.

20. Cary SC, Costrell MT, Stein JL, Camahan R, Desbruyeres D (1997) Molecular identification and localization of filamentous symbiotic bacteria associated with the hydrothermal vent anemid Alvinaea pompeja. Appl Environ Microbiol 63: 1124–1130.

21. Geißfördter SK, Warren A, Orphan VJ, Van Dover CL (2010) Frontal metacommunity structure on a bathyal hydrocarbon seep: Implications for community assembly. Deep Sea Res Pt II 57: 253–254.

22. Decker C, Olu K (2012) Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin – Part 2: contribution of chemosynthetic and nutritional patterns. Mar Ecol 33: 231–245.

23. MacGavin MT, Mannino JM, Stahl DA, Clark DP (2012) Brock Biology of Marine Microorganisms. Upper Saddle River: Pearson Prentice Hall. 1152 p.

24. Cary SC, Costrell MT, Stein JL, Camahan R, Desbruyeres D (1997) Molecular identification and localization of filamentous symbiotic bacteria associated with the hydrothermal vent anemid Alvinaea pompeja. Appl Environ Microbiol 63: 1124–1130.

25. Goffredi SK, Waren A, Orphan VJ, Van Dover CL, Vrijenhoek RC (2004) Novel forms of structural integration between microbes and a hydrothermal vent gastropod from the Indian Ocean. Appl Environ Microbiol 70: 3082–3090.

26. Thurber AR, Jones WJ, Schnabel K (2011) Dancing for Food in the Deep Sea: Nutritional strategies for macrofauna in the deep sea. Annu Rev Mar Sci 3: 1–23.

27. Niemann H, Elvert M, Hovland M, Orcutt B, Judd AG, et al. (2005) Methane microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. Nature 434: 854–858.

28. Chevaldonna H, Olu K (1996) Occurrence of anomuran crabs (Decapoda: Anomura) at the Hikurangi Margin (New Zealand). Mar Geol 272: 223–232.

29. Sahling H, Galkin SV, Salyuk A, Greinert J, Foerstel H, et al. (2003) Methane methanogenesis and sulfate reduction at cold seeps of the deep Eastern Mediterranean Sea. Mar Geol 261: 114–127.

30. Niemann H, Fischer D, Graefe D, Knittel K, Montiel A, et al. (2009) Biogeochemistry of a low-acitivity cold seep in the Larsen B area, western Ross Sea, Antarctica. Biogeosciences 6: 2381–2393.

31. Childress J, Fisher CR, Brooks JM, Kennicutt II MC, Biligare R, et al. (1986) A methanotrophic marine mudholus (Bivalvia, Mytilidae): ssumises; mussels fueled by gas. Science 233: 1306–1308.

32. Fisher CR, Gates DJ (1989) Chemosynthetic and Methanotrophic Symbioses in Marine-Invertebrates. Rev Aquat Sci 2: 399–436.

33.(attributes added)
84. Lanoil BD, Sassen R, La Duc MT, Sweet ST, Nealson KN (2001) Bacteria.
83. Kleindienst S, Ramette A, Amann R, Knittel K (2012) Distribution and in situ
81. Fetzner S (2010) Aerobic Degradation of Halogenated Aliphatics. In: Timmis
80. Nakagawa S, Takaki Y, Shimamura S, Reysenbach AL, Takai K, et al (2007)
79. Yamamoto M, Nakagawa S, Shimamura S, Takai K, Horikoshi K (2010) Colonization of Sulfitrovum sp. on the gill surfaces of Alvinocaris longirostris, a deep-sea hydrothermal vent shrimp. Mar Ecol 29: 106–114.
77. Durand L, Zbinden M, Cueff-Gauchard V, Duperron S, Roussel EG, et al (2010) Microbial diversity associated with the hydrothermal shrimp Rimicaris exoculata gut and occurrence of a resident microbial community. FEMS Microbiol Ecol 71: 291–303.
76. Borin S, Brescini L, Mapelli F, D’Auria G, Brusa T, et al (2009) Sulfur cycling and methanogenesis primarily drive microbial colonization of the highly sulfidic Uraiia deep hypersaline basin. P Natl Acad Sci USA 106: 9151–9156.
75. Schauer R, Roy H, Augustin KN, editors. Handbook of Hydrocarbon and Lipid Microbiology. Springer. 782–866–885.
74. Joye SB, Samarkin VA, Orcutt BN, MacDonald IR, Hinrichs KU, et al (2009) Molecular characterization of inorganic sulfur-compound metabolism in the hydrothermal chemolithoautotrophic isolates of Epsilonproteobacteria. Appl Microbiol Ecol 71: 291–303.
73. Borin S, Brescini L, Mapelli F, D’Auria G, Brusa T, et al (2009) Sulfur cycling and methanogenesis primarily drive microbial colonization of the highly sulfidic Uraiia deep hypersaline basin. P Natl Acad Sci USA 106: 9151–9156.
72. Joye SB, Samarkin VA, Orcutt BN, MacDonald IR, Hinrichs KU, et al (2009) The annual cycle and biological effects of the Costa Rica Dome. Deep-Sea Res Pt I 49: 321–338.
71. Beal EJ, House CH, Orphan VJ (2009) Manganese- and Iron-Dependent Marine Methane Oxidation. Science 325: 184–187.
70. Campbell BJ, Engel AS, Porter ML, Takai K (2006) The versatile epsilon-proteobacterial genomes provide insights into emergence and evolution of pathogens. P. Natl Aacad Sci USA 104: 12146–12150.
69. Fiedler PC (2002) The annual cycle and biological effects of the Costa Rica Dome. Deep-Sea Res Pt I 49: 321–338.
68. Fiedler PC (2002) The annual cycle and biological effects of the Costa Rica Dome. Deep-Sea Res Pt I 49: 321–338.
67. Thamdrup B, Canfield DE (1996) Pathways of carbon oxidation in continental margin sediments off central Chile. Limnol Oceanogr 41: 1629–1650.
66. Firefier PC (2002) The annual cycle and biological effects of the Costa Rica Dome. Deep-Sea Res Pt I 49: 321–338.
65. Bowles MW, Samarkin VA, Bowles KM, Joye SB (2013) Weak coupling between sulfate reduction and the anaerobic oxidation of methane in methane-rich seafloor sediments during ex situ incubation. Geochem Cosmochim Acta 75: 500–519.
64. Necitaylo TV, Timmis KN, Golyshin PN (2009) ‘‘ Candidatus Lumbricincola’’, a novel lineage of uncultured Mollicutes from earthworms of family Lumbricidae. Environ Microbiol 11: 1016–1026.
63. Meziti A, Ramette A, Mente E, Kormas KA (2010) Temporal shifts of the Norway lobster (Nephrops norvegicus) gut bacterial communities. FEMS Microbiol Ecol 74: 472–484.
62. Kostanjek R, Srus J, Augustin G (2007) ‘‘ Candidatus Bacillobaculum,’’ a novel lineage of Mollicutes associated with the hindgut wall of the terrestrial isoped Porcellio scaber (Crustacea : Isopoda). Appl Environ Microb 73: 5566–5573.
61. Whitcomb RF (1981) The Biology of Spiroplasmas. Annu Rev Entomol 26: 397–425.
60. Paull CK, Jul AFT, Toolin LJ, Linick T (1963) Stable isotope evidence for chemosynthesis in an abyssal seep community. Nature 317: 709–711.
59. Van Dover CL (2007) Stable Isotope Studies in Marine Chemoautotrophically Based Ecosystems: An Update. In: Michener R, Lajtha K, editors. Stable Isotopes in Ecology and Environmental Science. Blackwell Publishing Ltd. 202–237.
58. Thurber AR, Kroger K, Neira G, Wålhund H, Levin LA (2010) Stable isotope signatures and methane use by New Zealand cold seep benthos. Mar Geol 272: 260–269.
57. Boetius A, Ravenschlag K, Schubert C, Rickert D, Widdel F (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 462–463.
56. Losekann T, Rebador A, Niemann H, Knittel K, Boetius A, et al (2008) Endosymbioses between bacteria and deep-sea shogolid tube worms from an Arctic Cold Seep (Haison Mosby Mud Volcano, Barents Sea). Environ Microbiol 10: 3257–3254.
55. Niemann H, Elvert M (2008) Diagnostic lipid biomarker and stable carbon isotope signatures of microbial communities mediating the anaerobic oxidation of methane with sulphate. Org Geochem 39: 1668–1677.
54. Bergé J-P, Barnathan G (2000) Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Ecological Aspects. In: Le Gal Y, Uller R, editors. Marine Biotechnology I. Springer Berlin/Heidelberg. 49–123.
53. Blumenberg M, Seifert R, Pape T, Michaelis W (2004) Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. P Natl Acad Sci USA 101: 11111–11116.
52. Van den Oord A (1964) The absence of cholesterol synthesis in the crab, Porcellio scaber (Crustacea : Isopoda). Appl Environ Microbiol 73: 5566–5573.
51. MacDonald IR, Boland GS, Baker JS, Brooks JM, Kennicutt MC, et al (1989) Marine Methane Oxidation. Science 325: 184–187.
50. Nechitaylo TY, Timmis KN, Golyshin PN (2009) ‘‘ Candidatus Lumbricincola’’, a novel lineage of uncultured Mollicutes from earthworms of family Lumbricidae. Environ Microbiol 11: 1016–1026.
49. Meziti A, Ramette A, Mente E, Kormas KA (2010) Temporal shifts of the Norway lobster (Nephrops norvegicus) gut bacterial communities. FEMS Microbiol Ecol 74: 472–484.