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Geo-Biological Coupling of Authigenic Carbonate Formation and Autotrophic Faunal Colonization at Deep-Sea Methane Seeps II. Geo-Biological Landscapes

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.78978>

Abstract

Deep-sea methane seeps are typically shaped with authigenic carbonates and unique biomes depending on methane-driven and methane-derived metabolisms. Authigenic carbonates vary in $\delta^{13}\text{C}$ values due probably to $\delta^{13}\text{C}$ variation in the carbon sources (directly carbon dioxide and bicarbonate, and ultimately methane) which is affected by the generation and degradation (oxidation) of methane at respective methane seeps. Anaerobic oxidation of methane (AOM) by specially developed microbial consortia has significant influences on the carbonate $\delta^{13}\text{C}$ variation as well as the production of carbon dioxide and hydrogen sulfide for chemoautotrophic biomass production. Authigenesis of carbonates and faunal colonization are thus connected. Authigenic carbonates also vary in Mg contents that seem correlated again to faunal colonization. Among the colonizers, mussels tend to colonize low $\delta^{13}\text{C}$ carbonates, while gutless tubeworms colonize high-Mg carbonates. The types and varieties of such geo-biological landscapes of methane seeps are overviewed in this chapter. A unique feature of a high-Mg content of the rock-tubeworm conglomerates is also discussed.

Keywords: lithotrophy, chemoautotrophy, thiotrophy, methanotrophy, stable carbon isotope, $\delta^{13}\text{C}$, isotope fractionation, $\Delta^{13}\text{C}$, calcite, dolomite, anaerobic oxidation of methane (AOM), sulfate-methane transition zone (SMTZ), *Lamellibrachia* tubeworm, *Bathymodiolus* mussel, *Calyptragenia* clam

1. Introduction

Aristotle separated the world into two realms, *nature* and *living things* (originally animals), the latter having structures, processes, and functions of spontaneous formation and voluntary

movements [1]. He argued the difference in the manner similar to “living organisms produce stones (e.g., calculi, shells, and other bio-mineralized materials [2]), but stones do not generate living things”. However, in the view of modern science, which has an origin in the Democritean atomism that Aristotle rejected, stones may sustain, not to say generate, life as exemplified by the biological term “lithotrophy.” Etymologically, the term was coined from the Greek words *lithos* and *tréphō* that correspond to English words of “stone” and “nourishment,” respectively. Therefore, lithotrophy is interpreted as “stone-eating,” and the corresponding organisms, that is, lithotrophs, are “stone-eaters.”

Lithotrophy or stone-eating is a figurative expression and is biologically interpreted as “living on inorganic sources.” For instance, human and animals feed on organic foods and thus are not lithotrophs (but organotrophs). Lithotrophs in this chapter are defined as the organisms that derive life-sustaining energy from redox processes of inorganic materials such as hydrogen sulfide and methane. It should be noted that methane in this argument is taken as semi-inorganic (and semi-organic) as discussed in the former chapter.

It should also be noted that the term “chemoautotrophy” takes the place of lithotrophy in this chapter, because chemoautotrophy (a.k.a., chemosynthesis) is more widely used in general and provides a clearer idea about energy sources in reference to light-driven photoautotrophy (a.k.a. photosynthesis). Autotrophy indicates the inorganic carbon source, that is, CO_2 , for organic (biomass) production in both photoautotrophy and chemoautotrophy (Note 1). Methane, CH_4 , serves as an energy source and occasionally as a carbon source via “methanotrophy,” part of which is regarded as a variety of autotrophy as explained later.

Authigenic formation of *lithos* (stones and rocks) at methane seeps is associated with the generation of inorganic sources that feed living organisms via chemoautotrophic and methanotrophic metabolisms. In addition, their metabolisms in turn facilitate the formation of authigenic carbonates. These rock-forming (geological) and biomass-producing (biological) processes are interwoven, and they literally, more than metaphorically, interweave to form “conglomerates” of rocks and organisms [3].

Authigenic carbonates and associated unique faunas thus represent the typical landscape of methane seeps. Methane seeps are formed by various settings as reviewed in the former chapter, and a variety of carbonates and faunas (and rock-fauna conglomerates) are formed, accordingly. This chapter provides an overview of the types of authigenic carbonates and faunas based on chemoautotrophy and methanotrophy. Profiles of a stable carbon isotope signature ($\delta^{13}\text{C}$) of authigenic carbonates and faunal tissues are summarized. In addition, high-magnesium (Mg) contents of the conglomerate carbonates, which are potentially associated with certain geo-biological processes, will be discussed.

2. Backgrounds for landscapes of deep-sea methane seeps

Landscapes of deep-sea methane seeps are characterized by unique and exotic biological communities based on microbial chemo(thio)autotrophic and methanotrophic biomass production, not on photosynthesis (photo-autotrophy), in the dark. Representatives of the methane seep biota are mussels, clams, and gutless tubeworms (**Figure 1**). The landscapes are also featured



Figure 1. Landscape of a methane seep at 1100 m depth, off Hatsushima Island, Sagami Bay, Central Japan. The *Bathymodiolus* Kenk and Wilson, 1985 mussels and *Lamellibrachia* Webb, 1969 tubeworms colonize the authigenic carbonate rocks. The *Calyptogena* Dall, 1891 clams are half-buried in the sediment to move around carbonate rocks to exploit available sulfide. Photo by JAMSTEC.

by occurrences of authigenic carbonate rocks that take forms of seafloor pavements, cementations, and slabs buried in sediments (**Figure 1**). The biological processes (biomass production, production and consumption of methane, etc.) and carbonate-forming processes are interrelated in rather a complex manner, and the interrelationships and the involved processes will be outlined from a geochemical point of view, with particular respect to stable carbon isotopes.

In addition to abiogenic carbonates, some organisms such as mollusks and foraminiferas produce carbonate as protective shells through the process of biomineralization. Biomineralized carbonates, particularly dolomite, are also produced by microorganisms as exemplified by *Desulfovibrio brasiliensis* Warthmann et al., 2005 [5], for dolomite formation or dolomitization [4–6] and *Bacillus subtilis* (Ehrenberg, 1835) John, 1872, possessing the *etfa* gene (involved in energetic electron transfer) for CaCO_3 deposition [7], although it is uncertain whether they are actually involved in carbonate formation in methane seeps. Carbonate formation in methane seeps is likely facilitated by the conversion of CH_4 to HCO_3^- , that is, oxidation of methane, which is mediated by microorganisms. Oxidation of methane is done both aerobically and anaerobically, and anaerobic oxidation of methane (AOM) coupled with sulfate reduction (sulfate respiration) produces sulfide, HS^- , that supports chemo(thio)autotrophic biomass production in the dark.

2.1. Stable carbon isotope deviation ($\delta^{13}\text{C}$) and fractionation ($\Delta^{13}\text{C}$)

Methane, CH_4 , is often geochemically characterized by the ratio of stable isotopes of $^{13}\text{C}/^{12}\text{C}$ and $^2\text{H}/^1\text{H}$ or D/H (D stands for deuterium). The ratios are generally expressed as deviations (depletion or enrichment; δ) from the reference standard ratios such as the Vienna Pee Dee Belemnite (VPDB) with the $^{13}\text{C}/^{12}\text{C}$ ratio of 0.0112372 for $\delta^{13}\text{C}$, and the Vienna Standard Mean Ocean Water (VSMOW) with the $^2\text{H}/^1\text{H}$ (D/H) ratio of 0.00015576 for $\delta^2\text{H}$ (δD) as well as the $^{18}\text{O}/^{16}\text{O}$ ratio of 0.00200520 for $\delta^{18}\text{O}$. The unit of ‰ (per mil) instead of % (percent) is generally

used for these deviations. For example, if a $^{13}\text{C}/^{12}\text{C}$ ratio of 0.0101135 is determined for biogenic methane, its $\delta^{13}\text{C}$ is -100‰ , as calculated as $(0.0101135/0.0112372 - 1) \times 1000$.

These deviation parameters ($\delta^{13}\text{C}$, $\delta^2\text{H}$ (δD) and $\delta^{18}\text{O}$) are regarded as the indicative of dynamics (origins, sources, processes, pathways, sinks, changes, etc.) of methane and carbonates as shown in **Figure 3** of the former chapter, with careful cautions for interpretations required. For example, methane that has high $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values (enriched in ^{13}C and ^2H) is generally regarded as abiotic origins (geothermal and thermogenic sources); however, the “enriched” methane may also be interpreted as “leftover” or residual of methane oxidation that removes lighter isotopes (^1H and ^{12}C) faster and leaves heavier isotopes (^2H and ^{13}C) behind, or isotope fractionation (discrimination), resulting in the isotopically “enriched” (heavy) residual methane, as discussed later.

The product of methane oxidation (carbon dioxide, CO_2) and the derived carbonate rock (CaCO_3) contain no hydrogen isotopes but stable oxygen isotopes, ^{16}O and ^{18}O , of geochemical interest. The deviation (depletion or enrichment) parameter $\delta^{18}\text{O}$ of carbonates is indicative of oxygen dynamics (origins, sources, processes, pathways, sinks, etc.) but is more readily influenced by temperature, fluid inclusion, diagenesis, and so on, than $\delta^{13}\text{C}$ [8]. Therefore, this chapter deals mainly with $\delta^{13}\text{C}$ that may serve as a “signature” of carbon dynamics as

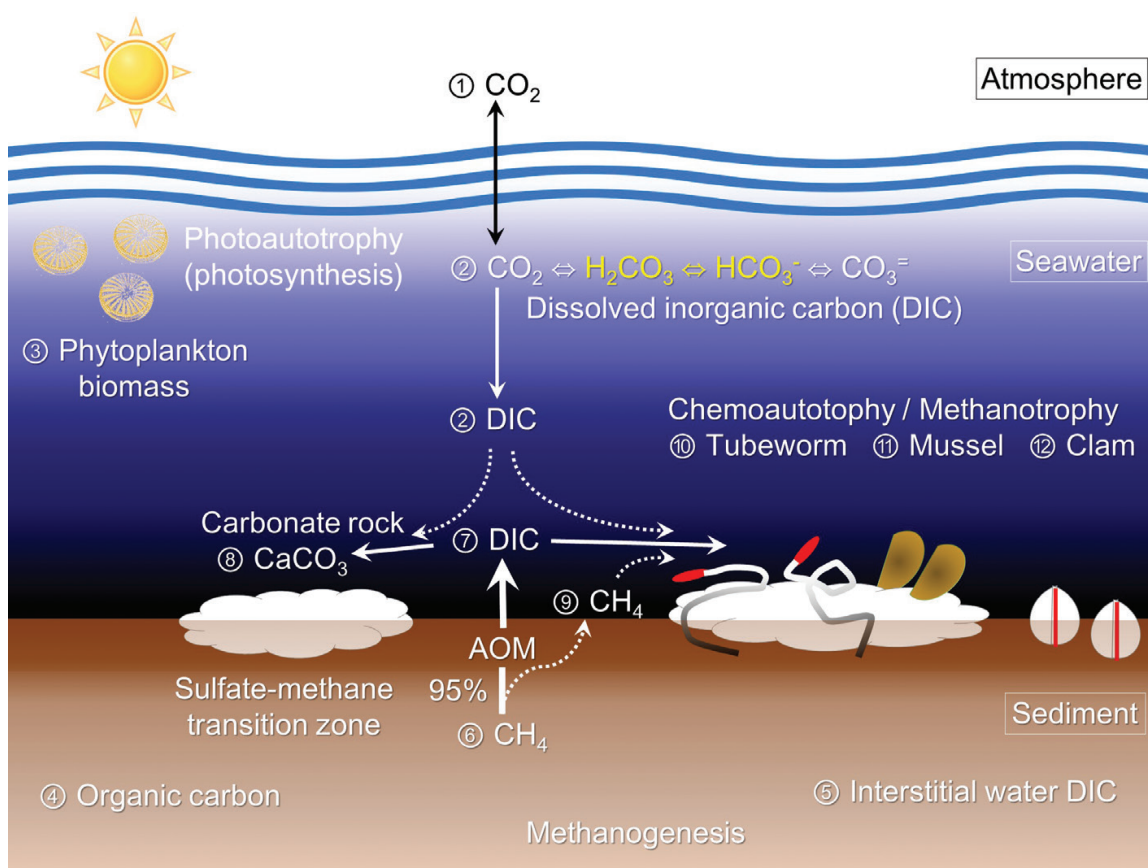


Figure 2. An illustrated general view of carbon dynamics with reference to the processes occurring at a methane seep. Numbers in circles from 1 to 12 correspond to the numbers in **Tables 2** and **3**.

	Carbon species	Form	$\delta^{13}\text{C}$ (‰)	Reference
1	CO_2	Gas (atmospheric)	-8.00	[9]
2	DIC	ca. 90% HCO_3^-	0.00	[9]; Bjerrum plot [13] Figure 1.1.2, p.5
3	Organic C	Phytoplankton biomass	-22.0	[9]
4	Organic C	Sedimentary	-27.0 to -22.0 -30.0 to -22.0 -80.0 to -47.0	In general [9] Methane seeps [23, 24, 26, 144] Brine seep at Florida Escarpment [27, 28]
5	CO_2	Interstitial water	-50.0 to -5.00 (DIC) -38.0 to +11.0 (DIC) -21.0 to +1.00 (CO_2) -27.5 (CO_2)	[25, 40, 145] [26] [26] [33]
6	CH_4	Sedimentary	-100.0 to -40 -101.3 to -27.2	In general [32] [23, 26, 31, 33]
7	post-AOM CO_2	CH_4 -derived (oxidized)	Not found	
8	CaCO_3	Authigenic carbonate	-60.0 to +26.0	[50, 56]
9	post-AOM CH_4	Residual (unoxidized)	Possibly -27.2	Eel River Basin [33]
10	Tubeworm (<i>Lamellibrachia</i>)	Soft tissue Tube (chitin + protein)	-55.0 to -18.0 -28.1 to -19.9	[23, 31, 41–48] [24, 41–44]
11	Mussel (<i>Bathymodiolus</i>)	Soft tissue Shell (CaCO_3)	-76.0 to -36.4 -6.80 to -2.60	[27, 31, 49, 146, 147] [27, 147]
12	Clam (<i>Calyptogena</i>)	Soft tissue Shell (CaCO_3)	-69.2 to -32.5 -2.00 to +0.35	[23, 43, 44, 146] [23, 43, 144, 148, 149]

Table 1. Stable carbon isotope ratio deviation, $\delta^{13}\text{C}$ (‰), of different carbon species and forms involved in the carbon dynamics associated with methane seepage.

depicted in **Figure 2** and summarized in **Table 1**, and fractionation parameters ($\Delta^{13}\text{C}$) in the processes and pathways of carbon dynamics (focusing on methane dynamics) is also discussed later and summarized in **Table 2**.

2.1.1. $\delta^{13}\text{C}$ values before and after methanogenesis

Taking atmospheric CO_2 as the starting material of methanogenesis, its $\delta^{13}\text{C}$ value is currently estimated at about -8‰ (**Table 1**) [9] with an estimate of a decrease rate of 0.05–0.06‰ year⁻¹ [10], possibly due to the input of lighter carbon (^{12}C) into atmosphere by artificial fossil fuel combustion, that is, the so-called “Suess effect” [11]. Air-to-seawater transport prefers lighter $^{12}\text{CO}_2$ to heavier $^{13}\text{CO}_2$ and thus decreases its $\delta^{13}\text{C}$ by 2‰ ($\Delta^{13}\text{C}$ of -2‰, **Table 2**) [12]. Part of

Process	Carbon isotope fractionation process	$\Delta^{13}\text{C}$ (‰)	Reference
1 → 2	Air-to-sea transfer of CO_2	-2.00	[12]
2 → 1	(Sea-to-air transfer of CO_2)	(-10.0)	[12]
2	Hydration of CO_2 (^{13}C -enrichment in HCO_3^-)	+8.00	[14]
2 → 3	HCO_3^- to CO_2 by carbonic anhydrase	+10.0	[15]
2 → 3	Photoautotrophy (photosynthesis in seawater)	-29.0 to -11.0	[16–19]
3 → 4	Diagenesis (shallow burial)	-4.00 to -5.00	[22, 150]
	Diagenesis (deep burial)	few	[150]
4 → 5	Anaerobic oxidation (organic C to CO_2)	Not found	
4 → 6	Methanogenesis (organic C to CH_4)	-61.5 to -31.1	Peatland [29]
		-83.0 to -72.0	From methanol [30]
6 → 7	AOM	-29.00	With nitrate (not sulfate) [36]
7 → 8	Carbonate authigenesis (from CO_2 or DIC)	Almost 0	In general [9]
		+0.08, +19.44	Certain seep cases [28]
7 → 10	Chemoautotrophy	-33.0 to -24.0	Calvin-Benson cycle [151]
		-11.0 to -10.0	Reductive TCA cycle [152, 153]
	Biominingalization (shell formation)	A few; +10.0	To DIC; to food [154]

Table 2. Stable carbon isotope fractionation, $\Delta^{13}\text{C}$ (‰), during geo-biological processes of carbon dynamics associated with methane seepage.

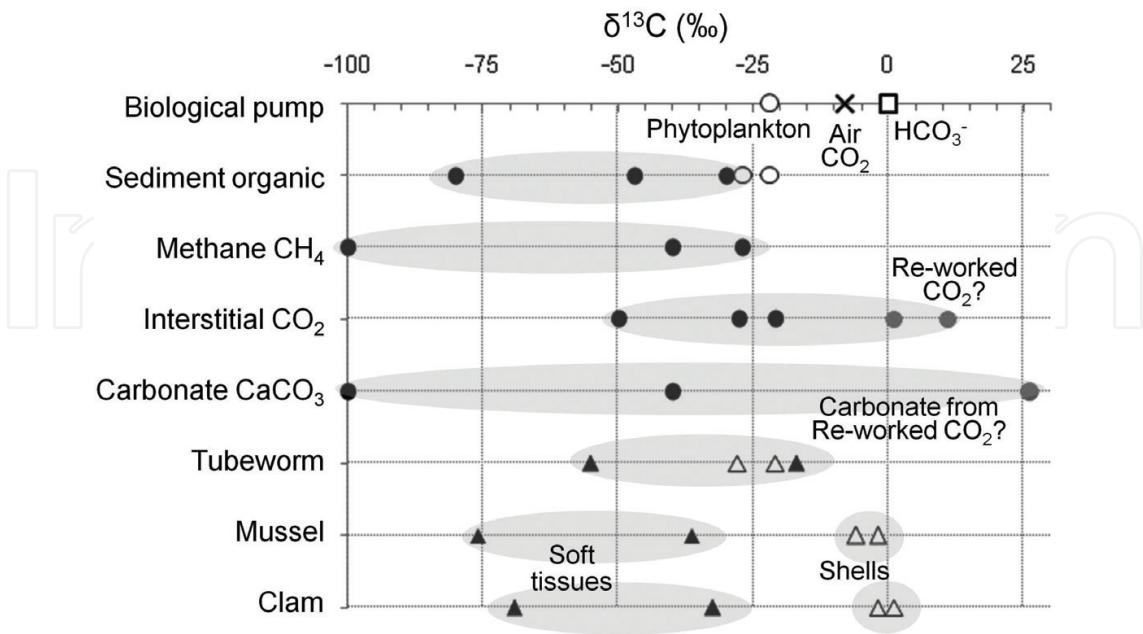


Figure 3. A schematized view of $\delta^{13}\text{C}$ variability from atmospheric CO_2 to authigenic carbonate and autotrophic fauna. The $\delta^{13}\text{C}$ values are taken from Table 2.

CO_2 in seawater will be hydrated toward a chemical equilibrium of $\text{CO}_2\text{-H}_2\text{CO}_3\text{-HCO}_3^-\text{-CO}_3^{2-}$ (virtually $\text{CO}_2\text{-HCO}_3^-\text{-CO}_3^{2-}$), and these are collectively termed “dissolved inorganic carbon” (ΣDIC or simply “DIC”). Among DIC, HCO_3^- is the predominant ion form at seawater pH of 8.00 [13]. Fractionation between ^{12}C and ^{13}C also occurs during hydration of CO_2 into HCO_3^- , back-conversion (by carbonic anhydrase) from HCO_3^- to photosynthetically available CO_2 , and photosynthesis mainly by phytoplankton that assimilate CO_2 via C3 pathway (Calvin-Benson cycle, **Table 2**) [14–19]. After all fractionations together, $\delta^{13}\text{C}$ of eventually (photosynthetically) produced biomass is generally taken as -22.0‰ [9].

About 0.50% of the photosynthetically produced organic matter is buried in the sediment [20], where the process is often termed as “biological pump” shown in **Figure 3** [21]. The $\delta^{13}\text{C}$ values of the “biologically pumped” sedimentary organic matter decreases from -22.0‰ in shallow (fresh) to -27.0‰ in deep (old) layers, due to diagenetic fractionation [22]. Almost the same $\delta^{13}\text{C}$ values, -22.0 to -30.0‰ , of sedimentary organic matter from methane seepstwere reported [23–26]. However, very depleted $\delta^{13}\text{C}$ values, as low as -47.0 to -80.0‰ , of sedimentary organic matter were also reported from the methane seep at the base of Florida Escarpment in the Gulf of Mexico [27, 28], which may be associated with “brine” seepage from very old organics or organics of non-photosynthetic origins.

Strong fractionation occurs during methanogenesis, for example, as strong as $\Delta^{13}\text{C}$ of $>-60.0\text{‰}$ in a Finnish peatland [29] and $>80\text{‰}$ from methanol in the laboratory [30]. Therefore, methane generated from sedimentary organic matter often shows depleted $\delta^{13}\text{C}$ values, as low as -40.0 to -100‰ [26, 31–33]. Care should be taken to such cases that even biogenic methane may be enriched (having high $\delta^{13}\text{C}$ values) due to microbial oxidation as described subsequently and thus mistakenly regarded as abiogenic (thermogenic) methane [34].

2.1.2. $\delta^{13}\text{C}$ values after anaerobic oxidation of methane

In methane oxidation, heavier methane ($^{13}\text{CH}_4$) is less preferably oxidized, and therefore $\delta^{13}\text{C}$ of the resultant CO_2 is depleted by 5.00 to 30.0‰, that is, $\Delta^{13}\text{C}$ of $\text{CH}_4 \rightarrow \text{CO}_2$ is generally -5.00 to -30.0‰ [34]. A decrease in $\delta^{13}\text{C}$, or $\Delta^{13}\text{C}$, during aerobic microbial methane oxidation is reported to be -15.0 to -30.0‰ [35]. AOM with nitrate (not sulfate) is reported to result in $\Delta^{13}\text{C}$ of about -30.0‰ [36]. Although $\Delta^{13}\text{C}$ of “AOM with sulfate” has not been determined, it would not be too far from these fractionation values.

Theoretically, $\delta^{13}\text{C}$ values of the resultant DIC forms (CO_2 , HCO_3^- , and CO_3^{2-}) after AOM are depleted and actually determined to be -41.0‰ for DIC (virtually HCO_3^-) in interstitial water, compared with $\delta^{13}\text{C}$ -24.0‰ of sedimentary organic matter [25]. It should be remembered that $\Delta^{13}\text{C}$ of $\text{CO}_2 \rightarrow \text{HCO}_3^-$ is about $+8.00\text{‰}$ [14] (**Table 2**), leading to a generally accepted idea and the fact that $\delta^{13}\text{C}$ of DIC in non-seep, non-vent seawater is -2.00‰ or roughly 0‰ . Returning to seep-methane-derived DIC, $\delta^{13}\text{C}$ -41.0‰ of DIC would possibly correspond to $\delta^{13}\text{C}$ -49.0‰ of CO_2 . However, such a theoretical view is often challenged by reality. An example of sequential $\delta^{13}\text{C}$ values from sedimentary organic matter (-22.0‰) and biogenic methane (-68.0‰) to CO_2 (-20.0‰ to $+1.00\text{‰}$) and DIC (-38.0 to $+11.0\text{‰}$) [26] is tough to be elucidated.

Common name	Functional name	Autotrophy	$\Delta^{13}\text{C}$ from DIC (‰)	Reference
Calvin-Benson cycle	C3; Reductive pentose phosphate cycle	Photo (O/A), Chemo, Methanol	-33.0 to -24.0	[151]
Hatch-Slack pathway	C4 carbon fixation	Photo (O)	-16.0 to -10.0	[151]
CAM pathway	Crassulacean acid metabolism pathway	Photo (O)	-20.0 to -10.0	[151]
Reverse Krebs cycle	Reverse tricarboxylic acid (rTCA) cycle	Photo (A), Chemo	-11.4, -10.0	[152, 153]
Wood-Ljungdahl pathway	Reductive acetyl CoA pathway	Chemo (Methanogenesis)	-36.0	[152]
3-Hydroxypropionate pathway		Photo (A), Chemo	-14.0 + 3	[155, 156]
3-Hydroxypropionate/4-hydroxybutyrate cycle		Chemo	+2.50	[157]
Dicarboxylate/4-hydroxybutyrate cycle		Chemo	Not found	

Photo, oxygenic (O), and anoxygenic (A) photosynthesis or photoautotrophy; Chemo, chemoautotrophy; and, Methano, methanotrophy.

Table 3. Pathways and fractionation factors, $\Delta^{13}\text{C}$ (‰), of autotrophic CO_2 assimilation.

2.1.3. $\delta^{13}\text{C}$ values of authigenic carbonates and autotrophic faunas

Generally, fractionation in $\delta^{13}\text{C}$, that is, $\Delta^{13}\text{C}$, from DIC to carbonates is regarded to be zero [9]. Therefore, authigenic carbonates in methane seeps should have depleted $\delta^{13}\text{C}$ values, as authigenesis starts with oxidation of depleted methane and proceeds with the resultant depleted CO_2 , as typically shown by the $\delta^{13}\text{C}$ values of around -50‰ in the matrices, nodules, and cements of authigenic seep carbonates [37–39]. However, again, theories are challenged by unexpectedly high $\delta^{13}\text{C}$ values (**Figure 3**), for example, as high as +16 and +24‰ in carbonates of the Eel River Basin seeps [37, 40]. The involvement of ordinary (non-seep, non-vent) DIC and “leftover” DIC that had not been incorporated into earlier carbonate authigenesis (therefore enriched), as well as “re-worked” DIC that had been generated from leftover (enriched) CH_4 , is imaginable.

The seep methane and derived CO_2 after AOM will also be incorporated into faunal biomass via thioautotrophic or methanotrophic biomass production by symbiotic bacteria, respectively. As the source methane and CO_2 are depleted in $\delta^{13}\text{C}$, the biomass $\delta^{13}\text{C}$ should be depleted accordingly. The observed $\delta^{13}\text{C}$ values in soft tissue of the gutless tubeworms are within the range from -55.0 to -18.0‰ [23, 31, 41–48]. The $\delta^{13}\text{C}$ values of tubeworm soft tissue are relatively higher (enriched) compared with those in the soft tissue of mussels and clams inhabiting the same seeps (**Table 1; Figure 3**) [46, 47, 49, 50], which may be related to the possible dual CO_2 -fixation pathways (C3 pathway and rTCA cycle, **Table 3**) in seep tubeworms as predicted for the endosymbiont of the vent tubeworm *Riftia pachyptila* Jones, 1981, by metaproteomics [51]. Hard tissues such as shells of mussels and clams show generally higher $\delta^{13}\text{C}$ values than those in soft tissues, some of which are close to that of ordinary DIC (**Table 1; Figure 3**).

3. Authigenic carbonate rocks

Authigenic carbonates have recently been recognized as an important “carbon sink” in the global carbon cycling next to marine carbonates and organic matters [52], and the authigenesis occurs mainly at methane seeps via anaerobic oxidation of methane (AOM). In other words, methane seeps are often accompanied by authigenic carbonate rocks, that is, carbonate rocks that are formed in situ (autochthonously) in the seabed of methane seeps. The process generates structurally and compositionally multi-staged carbonates that are explained by not simply geochemical but biogeochemical or even microbial involvements [53]. The process may be accelerated due possibly to microbial involvements, as shown by “fresh” ages of authigenic carbonates, as fresh as 195 years old to almost zero for the carbonates exposed on the shelf slope of the Gulf of Mexico [126]; otherwise, from 53,400 to 1700 years old (53.4–1.70 kiloyears ago, ka) in the Gulf of Mexico, 45.5–3.00 ka in the Cong Fan, and 1.60–1.10 ka in the Black Sea [54], as well as 6.40–0.80 ka in the Hydrate Ridge [55].

A simplified mechanism of carbonate rock formation, or authigenesis, is as follows: methane is oxidized (mainly anaerobically with sulfate in the sediment) to generate DIC ($\text{CO}_2\text{-HCO}_3^-\text{-CO}_3^{2-}$), which will react with Ca^{2+} and Mg^{2+} to precipitate Ca(Mg)CO_3 . The precipitates will grow into aggregates and conglomerates of visible sizes. The real processes are not that simple [56], and they often take the forms of slabs in the sediment and pavements on the sediment, as well as half-buried aggregates with wide-ranged $\delta^{13}\text{C}$ values. Modes of carbonate occurrence are regarded as affected not only by microorganisms but also by carbonate-dwelling macro-fauna such as tubeworms and mussels [57].

Gutless tubeworms are occasionally incorporated in aggregates (**Figure 1**), as they require physically hard substrates for settlement and chemically aerobic-anaerobic boundaries, at which carbonates are exactly deposited though anaerobic oxidation of methane (AOM) with sulfate. Sulfide, an AOM byproduct, is aerobically oxidized by symbiotic bacteria (of the host tubeworms) with O_2 to obtain metabolic energy for thioautotrophic chemosynthesis; the symbionts are localized inside of the host cells and are termed “endosymbionts.” The gutless tubeworms, therefore, colonize the hard substrates that lay in the zone where sulfide and O_2 coexist, that is, exactly the zone of carbonate deposition, almost overlapping the sulfate–methane transition zone (SMTZ).

Mussels that harbor thioautotrophic and/or methanotrophic symbiotic bacteria are epibenthic and colonize the carbonate rocks that extrude the sediment for settlement (**Figure 1**). By contrast, clams depending on bacterial thioautotrophy are semi-endobenthic and live half-buried in the sediment between and around carbonate rocks (**Figure 1**).

Types of authigenic carbonate rocks are generally grouped into calcites (as well as aragonites) and dolomites. Calcite is further divided into high- and low-Mg calcite according to their Mg contents. Interrelations between carbonate rock types and seep faunal types, that is, between calcites-dolomites and mussels-clams-tubeworms, are hypothesized as discussed later.

Extreme ^{13}C depletion is seen in the authigenic carbonates, when they are formed from depleted DIC ($\text{CO}_2\text{-HCO}_3^-\text{-CO}_3^{2-}$) via AOM against biogenic-depleted methane having $\delta^{13}\text{C}$ values as

low as -125‰ [58]. On the other hand, enriched DIC is derived from oxidation (probably aerobic oxidation after AOM) of residual ^{13}C -enriched methane and yields ^{13}C -enriched carbonates accordingly. Therefore, the $\delta^{13}\text{C}$ ranges of authigenic carbonates are relatively greater than those of seep mussels, clams, tubeworms, and so on.

3.1. Authigenic calcite and aragonite

Among the carbonates, calcite/aragonite and dolomite are often seen in the methane seep environs. Calcite and aragonite are the carbonate polymorphs used in many marine organisms (mollusks, brachiopods, foraminiferans, corals, etc.); they have the same chemical formula (CaCO_3) but are different in crystal structures [59]. Aragonite is about 1.50 times as soluble (unstable) as calcite in seawater. Calcite that has lower contents of impurity magnesium, Mg, is more stable than high-Mg calcite. Therefore, the stability order on the deep seafloor is generally low-Mg calcite, high-Mg calcite, and aragonite.

Despite the stability next to low-Mg calcite, high-Mg calcite is often seen in the methane seep environs, with occasional association with tubeworm settlement. By contrast, low-Mg calcite is associated with mussel colonization. The formation and occurrence of less stable high-Mg calcite has not yet been fully elucidated. It may be associated with the attachment and growth of colonies of tubeworms and will probably provide a platform to investigate mineral-animal (and bacteria) interaction from a geo-biological point of view.

3.2. Authigenic dolomites

Dolomite, $\text{CaMg}(\text{CO}_3)_2$ in an ideal chemical formula, is formed by replacing calcium ions of calcite with magnesium ions. Or, high-Mg calcite may be regarded as an intermediate form of dolomitization. Once there was a paradox about dolomitization at low temperatures, that is, at physiological temperatures, it was solved by the laboratory experiment using sulfur-reducing (sulfate-respiring) bacteria [4–6].

Authigenic dolomite in methane seeps has rarely been studied, and an example from the naturally exhumed fossil seep in Greece showed depleted $\delta^{13}\text{C}$ values as low (light, depleted) as -8.00 to -29.0‰ , maybe indicative of dolomite formation at the AOM zone in the sediment [60]. Authigenic dolomite in drill-cores from the oil fields in the Santa Barbara Basin, off California, showed $\delta^{13}\text{C}$ values of -16.0 to $+9.00\text{‰}$; lighter values are also indicative of dolomite formation in the relatively shallower zone of AOM with sulfate reduction, while heavier values may come from the relatively deeper zone of methanogenesis [61].

4. Autotrophic faunas: Tubeworms, mussels, and clams

Methane seeps and hydrothermal vents, as well as organic falls such as whale carcasses, are located not too far from each other, particularly along the Pan-Pacific “Ring of Fire.” Similarities in taxonomic structures and energetic metabolisms (including chemoautotrophy and methanotrophy) between seep and vent fauna have been studied. While the importance of the β -diversity, that is, site-specific diversity, among the seeps and vents worldwide has been pointed out [62],

faunal assemblages in the semi-enclosed Guaymas Basin in the Gulf of California (six seeps and four vents without topo-/geographic barriers) share species compositions [63]. In the same but a greater way, the ring-of-fire-type array of seeps and back-arc basin vents in the active margins (convergent margins) contribute to biogeographic connectivity of vent and seep faunas but does not support the “stepping stone” hypothesis by whale carcasses [64].

The gutless tubeworms that represent the exotic seep/vent faunas had already been known (but only sporadically sampled) since the beginning of the twentieth century, before the massive colonies were discovered in association with deep-sea volcanism at the Galápagos Rift in 1977 [65]. It took 4 years to propose that the gutless tubeworms (polychaetes) depend for their nutrition on sulfide-/sulfur-oxidizing chemoautotrophic (thiotrophic or thioautotrophic) biomass production by endosymbiotic bacteria in their specialized sac-like tissue, trophosome [66]. The trophosome is a natural “culture vessel” of, for example, not-yet-cultured thiotrophic gamma-proteobacterial *Candidatus Endoriftia persephone* in the case of the giant vent tubeworm *R. pachyptila* Jones, 1981 [67], and has still been enthusiastically investigated from not only biological but also biomedical and biotechnological points of view [68].

It also took 3–4 years after the first discovery of methane seeps in the Gulf of Mexico [69] for scientists to reveal that seep mussels depend not on thiotrophic but on methanotrophic endosymbionts in gills [70, 71]. In 1987, methanotrophic symbiosis was also found in a non-vent, non-seep gutless tubeworm (*Siboglinum poseidoni* Flügel and Langhof, 1983 from polychaetes) from the sediment of the central Skagerrak strait [72, 73] and some other vent/seep mussel species, but not for clams [74]. While many bivalve and gastropod mollusks have chemo-/methanotrophic symbionts [75], this chapter focuses on bivalve mussels (*Bathymodiolus* Kenk and Wilson, 1985) and clams (*Calypptogena* Dall, 1891) from seep (and vent) habitats.

Symbiosis with more than one symbiotic species in one host, which is dual or multiple symbiosis, is known for gutless tubeworms; an example is the vent-dwelling tubeworm that hosts multiple thiotrophic species as endosymbionts [76]. In addition, dual symbiosis with both thio- and methanotrophic endosymbionts has been known for the seep mussels (*Bathymodiolus* spp.) in, for example, the Gulf of Mexico [77] and off-Congo passive margins [78], as well as other invertebrates (mostly gutless oligochaetes) dwelling non-vent, non-seep habitats (Note 2).

In addition to thiotrophy and methanotrophy, in 2011, hydrogenotrophy (chemoautotrophy based on hydrogen oxidation) appeared as the third way of a vent mussel (and possibly for seep mussels) [79].

4.1. Thiotrophy and methanotrophy

Photosynthesis and chemoautotrophy differ in energetic processes but share the CO₂-fixing pathways, for example, the best-known Calvin-Benson cycle (reductive pentose phosphate cycle). Currently, eight autotrophic CO₂-fixing pathways, including Calvin-Benson cycle, are known for life even in the dark chemoautotrophy (**Table 3**) [80]. Dual pathways are widely known in chemoautotrophy-based macro-organisms, such as siboglinid (formerly known as vestimentiferan and pogonophoran) tubeworms, of hydrothermal vents and methane seeps [48].

In addition to a variety of CO₂-fixation pathways, a battery of energetic pathways with diverse reductants and oxidants (electron donors and acceptors) is also known for chemoautotrophy [81, 82]. The most representative one in the methane seeps is the oxidation of sulfide. If chemoautotrophy is based on sulfide oxidation, it is correspondingly termed “thiotrophy” or “thioautotrophy.” While aerobic oxidation of sulfide is most common, anaerobic oxidation of sulfide with nitrate, $\text{HS}^- + \text{NO}_3^- \rightarrow \text{HSO}_4^- + \text{N}_2$ (Note 3), (nitrate reduction, nitrate respiration, or denitrification), is possible, although its occurrence in methane seeps is not necessarily evident.

Both aerobic and anaerobic oxidation of H₂ can also provide an energetic basis for chemoautotrophy. Aerobic oxidation of H₂ with O₂ has recently been recognized as widespread among the hydrothermal vent chemoautotrophy [79, 83]. H₂ is also oxidized anaerobically with CO₂, which corresponds to the autotrophic CO₂ respiration, or autotrophic hydrogenotrophic methanogenesis, and represented by the thermophilic species of *Methanothermobacter thermoflexus* (Kotelnikova et al. 1994) Boone 2002 and *M. thermautotrophicus* (Zeikus and Wolfe 1972) Wasserfallen et al. 2000. However, in a non-thermophilic environment such as artificial anaerobic digesters, non-autotrophic CO₂ respirers dominate the microflora [84], which gives an implication for considering CO₂ respirers in the “cold” methane seeps.

Methanotrophy, feeding methane as “food” or “fuel,” may be placed between autotrophy and heterotrophy, because methane can be placed between inorganic and organic matter [85]. For most methanotrophs, methane serves as the dual sources for metabolic energy (catabolism, dissimilation) and biomass production (anabolism, assimilation). In this context, if methane is taken as half-organic and half-inorganic, methanotrophs are accordingly regarded as half-autotrophs and half-heterotrophs. However, the “type X” (or type Ib) methanotrophs and Verrucomicrobia-related methanotrophs are known to possess the CO₂-fixing enzyme, RuBisCO, and assimilate CO₂ via the Calvin-Benson cycle [86], and they are probably more widespread than previously presumed [87, 88].

Anaerobic oxidation of methane (AOM) is also a form of methanotrophy that is conducted by more than one microbial species, that is, archaeal-bacterial consortia, as described earlier. Both archaeal methanogens and bacterial sulfate-respirers (sulfate-reducing bacteria) are reported to assimilate CH₄-derived (CH₄-oxidized) CO₂ autotrophically via the Calvin-Benson cycle [89].

4.2. Tubeworms

The gutless tubeworms, or siboglinid veriforms, represent the most enigmatic and intriguing organisms of the seep fauna in terms of body plan, morphology, life cycle, metabolisms, endosymbioses, and so on [90]. The first specimen of the gutless tubeworms was dredge-sampled in 1900 during the Siboga Expedition (1899–1900) from 462 m deep, off Selayar Island, Flores Sea, Indonesia [91], where methane seepage is presumed to occur in the active margin [92], and was later described as the new species *S. weberi* Caullery, 1944 [93], with proposals of the new genus *Siboglinum* Caullery, 1914, and the new family Siboglinidae [94] (Note 4).

Thereafter, siboglinid worms, typically >10-mm long and <1-mm wide, were collected sporadically from various oceanographic sites. Due to their unique and enigmatic body plan with

Year	Species	Genus	Family	Order	Class	Phylum
Current	236 accepted [158]	32 accepted [158]	Siboglinidae [94]	(Sabellida) [159]	(Polychaeta) [160])	(Annelida) [158])
2015	<i>Lamellibrachia sagami</i> Kobayashi, Miura and Kojima, 2015 [96]					
1997			Siboglinidae [94, 161, 162]			
1985	<i>Escarpia spicata</i> Jones, 1985, <i>Escarpia laminata</i> Jones, 1985 [104]	<i>Escarpia</i> [104]				Vestimentifera
1981	<i>Riftia pachyptila</i> Jones, 1981 [163]	<i>Riftia</i> [163]				Pogonophora
1975	<i>Lamellibrachia luymesii</i> van der Land and Nørrevang, 1975 [103]					
1969					Vestimentifera	
1964	<i>Lamellibrachia barhami</i> Webb, 1969 [164]	<i>Lamellibrachia</i> [164]	Lamellibrachiidae			
1944	<i>Siboglinum weberi</i> Caullery, 1944 [93]					Pogonophora
1937					Pogonophora	
1933			Sabellidae			
1914		<i>Siboglinum</i> [94]	Siboglinidae [94]			
Other marine gutless worms (mostly belonging to the subclass Oligochaeta, class Clitellata) are not listed. Only accepted highest taxa, that is, accepted parents, as well as representative species and genera, are listed with accepted references. Current supra-taxa are also listed but in parentheses. Currently (as of May 2018), the family Siboglinidae is the highest taxon (parent) of all the vent/seep gutless tubeworms.						

Table 4. Taxonomic changes in positions and statuses of “gutless tubeworms” (mostly belonging to the class Polychaeta) that inhabit hydrothermal vents and methane seeps.

no mouth, no anus, and no digestive tract, their way of living was interpreted to depend on dissolved organic matter absorbed from somewhere of body surface including the anterior “beard”; they were often called “beard worms.” The interpretation was based on the common belief that animals are heterotrophic. The heterotrophic animal view is still correct even now in a strict sense (that siboglinid worms depend eventually on organic matter produced by symbiotic bacteria), but the less-defined phrase “autotrophic animal” was advocated for siboglinid worms in a broad sense [66].

The gutless siboglinid worms often host a single thiotrophic endosymbiont species. A non-vent, non-seep gutless tubeworm (*S. poseidoni* Flügel and Langhof, 1983) harbors a methanotrophic symbiont [72, 73] probably of one species. However, gutless tubeworms may occasionally possess a single but probably methanotrophic endosymbiont, or thiotrophic but multiple endosymbionts. For example, the sediment-dwelling *Oligobranchia mashikoi* Imajima, 1973, hosts a symbiont that bears the genes of methanotroph-related 16S rRNA and CO₂-fixing enzyme RuBisCO, suggesting the possible involvement of a CO₂-fixing methanotroph, that is, “type X” or type Ib methanotroph [95]. Another sediment-dwelling worm (*S. poseidoni*) also has a methanotrophic endosymbiont [73]. Regarding multiple symbioses, some individuals of *Lamellibrachia* sp., currently described as *Lamellibrachia sagami* Kobayashi et al. [96], from the off-Hatsushima seep, Sagami Bay, central Japan, were reported to have four distinct thiotroph-like symbionts in their trophosome tissue [97–100], and *L. anaximandri* Southward, Andersen and Hourdez, 2011, despite vent-dwelling, also hosts multiple thiotrophic species as endosymbionts [76].

Due to their unique and enigmatic body plan, their taxonomic position and status have been confused and subject to not a few, not minor changes (**Table 4**), and still investigated by modern phylogenetic and phylogenomic approaches [101, 102]. As to their physiology, the “giant tubeworm” or *R. pachyptila* that inhabits hydrothermal vents is probably the most popular, well known, and well studied. The methane seep counterparts would be *L. luymesii* van der Land and Nørrevang [103] and *Escarpiia laminata* Jones, 1985, inhabiting the base of Florida escarpment in the Gulf of Mexico [104], which are known to have extreme longevities as long as possibly >300 years [105–107].

The giant tubeworm *R. pachyptila* grows quickly to >2 m high as far as the worm can uptake sulfide emitted from the vents (**Figure 4A**). By contrast, the seep tubeworm *L. luymesii* may also grow over 2 m long (not high) only slowly over >200-year longevity [108], as well as *E. laminata* may grow over 300 years [107]. Individual worms of the colonies of *Lamellibrachia* Webb 1969 often look “trimmed” within the limits of seeped methane/sulfide (**Figure 4B**). Not only trimmed, their bodies are often twisted to crawl on seafloor and even buried in the sediment. It has been pointed out that the posterior extension, or “root,” of *Lamellibrachia* worms functions to “sip” sulfide from the sulfate–methane transition zone (SMTZ) in the sediment [109] as well as to “dump” sulfate and protons (H⁺) to SMTZ [110]. Sulfate dumped into or below SMTZ will facilitate anaerobic oxidation of methane (AOM) with sulfate, and sulfide will be regenerated from the dumped sulfate and protons via AOM.

A whole process including symbiotic thiotrophy (aerobic oxidation of sulfide) and AOM in sediment may be viewed as an “extended symbiosis” that circulates the regeneration of

sulfide and sulfate as if it functions as a “coffee percolator” (Note 5). The $\text{H}_2\text{S}/\text{SO}_4^{2-}$ percolator is driven by methane supplies, eventually leading to the generation of CO_2 - HCO_3^- - CO_3^{2-} from methane and thus to authigenesis of carbonates. The *Lamellibrachia* worms are often associated with carbonates and even incorporated in carbonates occasionally to form conglomerates as discussed later (Figure 5).

The tubeworm soft tissue that contains an amount of endosymbiotic thiotrophic bacteria is relatively higher (more enriched) in $\delta^{13}\text{C}$ than soft tissues of mussels and clams of the same

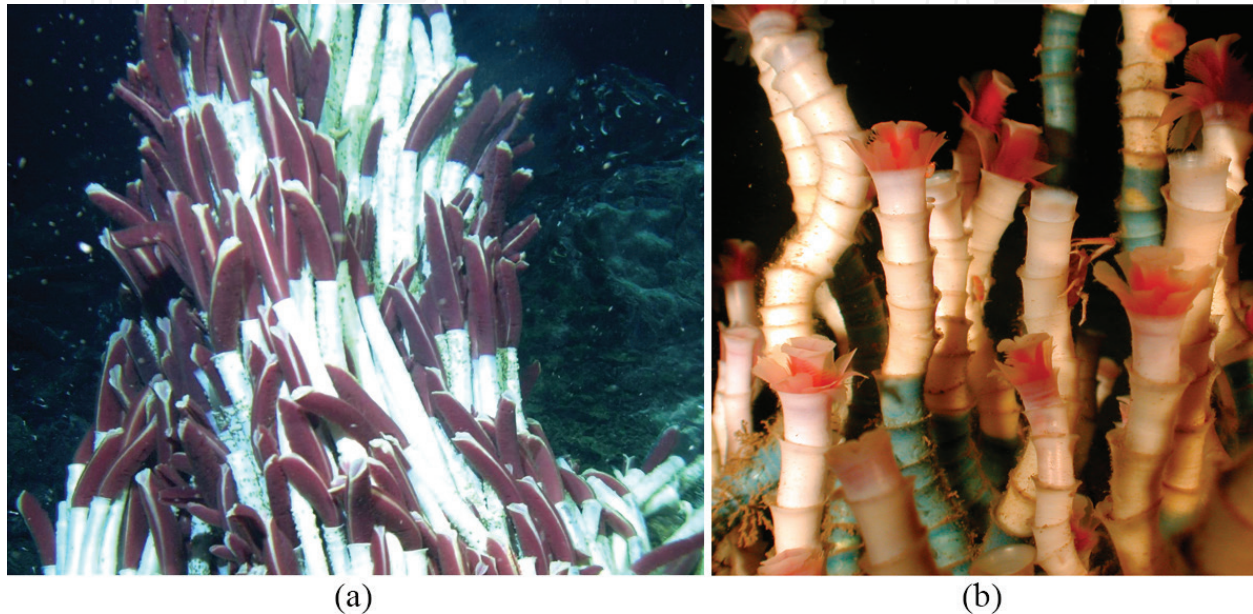


Figure 4. The gutless tubeworms of a hydrothermal vent and a methane seep. (A) *Riftia pachyptila* Jones, 1981, inhabiting a vent site of the East Pacific rise at 2500 m depth [167]. (B) *Lamellibrachia luymesii* van der Land and Nørrevang [103], from a methane seep at 550 m depth in the Gulf of Mexico. The blue-stained tube indicates approximately 14 months of growth [168]. Photograph by Charles R. Fisher.



Figure 5. The seep tubeworm *Lamellibrachia sagami* Kobayashi et al. [96], being embedded in authigenic carbonate formed in the off-Hatsushima methane seep at 1100 m depth, Sagami Bay, Central Japan. The width of the photograph corresponds to 7 cm. Photograph by the author.

seep (**Figure 3**) [46]. Tubes of the worms show intermediate $\delta^{13}\text{C}$ values compared with those of soft tissues, with a weak tendency of $\delta^{13}\text{C}$ becoming heavier toward the posterior end of the tubes [104]. This may be associated with the phenomenon that lower (more depleted) $\delta^{13}\text{C}$ values are seen in the tubeworm-associated high-Mg carbonates than those colonized by *Bathymodiolus* mussels [39, 50, 57, 111].

4.3. Mussels

The mussels belonging to the genus *Bathymodiolus* Kenk and Wilson, 1985, represent the vent and seep fauna as well and harbor endosymbiotic bacteria eventually in their gills. While juveniles of vent mussels host epi-symbionts almost everywhere on their surfaces, endosymbiotic colonization becomes restricted to gill tissue during the mussel growth [112], and even adults are subject to continued bacterial colonization attacks throughout their lifetimes [113]. Although the process was observed with vent *Bathymodiolus* mussels, it may similarly occur to the seep *Bathymodiolus* mussels, as it is known for the shallow-water tropical lucinid clam *Ctena orbiculata* (Montagu, 1808) cited as its synonym *Codakia orbiculata* (Montagu, 1808) [114].

Their endosymbionts are thiotrophs or methanotrophs or both. The vent mussel *B. thermophilus* Kenk and Wilson, 1985, in the East Pacific Rise has only thiotrophic symbionts [66, 115], while the seep mussel *B. childressii* Gustafson, Turner Lutz and Vrijenhoek, 1998, in the Gulf of Mexico hosts only methanotrophic symbionts [70]. A vent *Bathymodiolus* species in a western Pacific harbors a CO_2 -fixing methanotroph, that is, “type X” or type Ib methanotroph [116].

Dual symbiosis, in which a single host harbors both thiotrophic and methanotrophic bacteria, has been described for seep mussels from the Gulf of Mexico (*B. brooksii* Gustafson, Turner, Lutz and Vrijenhoek, 1998) [71], off Congo passive margins [78] and other seep mussels, as well as vent mussels. A vent *Bathymodiolus* mussel is known to harbor hydrogen-oxidizing autotrophic bacteria [79], which process is now regarded as probably more widely and ubiquitously distributed in deep-sea environments [83].

The small mytilid mussel, *Idas* sp., inhabiting carbonate crusts at the off-Nile fan seep harbors six endosymbionts probably of thiotrophs, methanotrophs, and previously unrecognized roles [117]. Similarly, *B. heckerae* Turner, Gustafson, Lutz and Vrijenhoek, 1998, inhabiting the “asphalt” seep in the Gulf of Mexico [118, 119] harbors multiple endosymbiotic bacterial species, one of which belongs probably to the genus *Cycloclasticus* Dyksterhouse, Gray, Herwig, Lara and Staley, 1995 [120]. The symbiotic *Cycloclasticus* appeared to degrade and derive carbon and energy from short-chain alkanes such as ethane and butane [121], despite lack of genes responsible for the degradation of polyaromatic hydrocarbons that are seen in other *Cycloclasticus* species [122].

Overall, it can be said that the seep mussels possess high plasticity and flexibility in harboring and depending on a variety of endosymbionts and their energetic metabolisms: from single via dual to multiple symbioses with thiotrophs and methanotrophs via hydrogenotrophs to asphalt-degrading heterotrophs. This symbiotic plasticity may facilitate evolution and ubiquity of these mussel species [123–125].

The *Bathymodiolus*, as well as *Idas*, mussels attach and settle on hard substrates such as basalts in hydrothermal vents and carbonates in methane seeps, like tubeworms do. However,

	Modern carbonate in the off-Hatsushima methane seep, Sagami Bay		Miocene carbonate (17.2–14.4 Ma), Hayama Group, Miura Peninsula	
	With tubeworms	Without tubeworms	With fossil tubes	Without fossil tubes
$\delta^{13}\text{C}$	–28.0 to –27.0‰	–34.0 to –33.0‰	Not determined	
CaCO_3 (%)	34.0–43.0	52.0–86.0	39.0–40.0	66.0–67.0
MgCO_3 (%)	12.0–15.0	<0.10	14.0–15.0	<0.10
FeO_2 (%)	4.00–6.00	5.00–15.0	4.00–5.00	28.0–29.0
SiO_2 (%)	28.0–31.0	<0.10–37.0	28.0–29.0	<0.10
AlO_2 (%)	9.00–10.0	<0.10–12.0	9.00–10.0	<0.10

Table 5. Comparison of $\delta^{13}\text{C}$ values (‰) and compositions of selected chemical species (weight-to-weight %) in modern and Miocene carbonates with reference to the presence/absence of living tubeworms or fossil worm tubes [3, 100, 165, 166].

Bathymodiolus mussels tend to colonize ^{13}C -enriched carbonates, compared with tubeworm association with ^{13}C -depleted high-Mg carbonates [39, 50, 57, 111].

4.4. Clams

The “giant white clams” or the vesicomid clams belonging to the genus *Calymene* Dall, 1891, also represent the fauna of both hydrothermal vents and methane seeps. They are mostly dependent on endosymbiotic bacterial thiotrophs for their nutrition, not on methanotrophs [74]. However, a thyasirid clam, *Axinulus hadalis* Okutani et al. [126], living within (but a few meters deeper from) the deepest methane seep community [127], was reported to have dual endosymbionts, which were speculated to be thio- and hydrogenotrophs, that is, sulfur- and hydrogen-oxidizing chemoautotrophs, respectively [128].

The *Calymene* clams are semi-endobenthic and live half-buried in the sediment between and around carbonate rocks. Therefore, the clams are not associated with carbonate rocks for living, although they occur in close vicinities. This may be confirmed by the difference in $\delta^{13}\text{C}$ values of the clam shells and carbonate rocks, which would reflect different carbon sources despite their habitat vicinities [129].

5. Conglomerates of carbonates and tubeworms

Authigenic carbonates “grow” in the zone anaerobic oxidation of methane (AOM) and sulfate–methane transition zone (SMTZ) in the sediment of methane seeps, as shown by low $\delta^{13}\text{C}$ values. Similarly, the gutless tubeworms such as *Lamellibrachia* species grow toward posterior rather than anterior to “percolate” (exploit and regenerate) sulfide in the horizon of AOM zone overlapped with SMTZ, also as indicated by the tendency of lower $\delta^{13}\text{C}$ toward the posterior end of the tube [24]. Sipping of sulfide and dumping of sulfate at the root (posterior end) of the tubeworms may change the local pH and thus affect the conditions for favored

formation calcite to aragonite [57, 130]. These growths of authigenic carbonates and autotrophic tubeworms occur concomitantly near the posterior of worm tubes [130] and often intercalate each other to form mineral-animal “conglomerates” (**Figure 5**). Although detailed observation suggested that a nodular high-Mg calcite is formed before tubeworm settlement [130], the coupling of high-Mg calcite and tubeworm colonization has not been elucidated.

The conglomerates of calcite and *L. sagami* in the off-Hatsushima methane seep, Sagami Bay, central Japan, were studied with reference to $\delta^{13}\text{C}$ and Mg contents and were compared with those from the past authigenic carbonate formed in the Miocene, 17.2–14.4 million years ago (Ma), from the Hayama Group, Miura Peninsula, central Japan. The two sites, that is, modern and past methane seeps, are only <50.0 km distant, and the data from the modern and past carbonates revealed that the high content (~15.0%) presence of Mg in relatively high $\delta^{13}\text{C}$ (<30.0‰ against >30.0‰) calcite is closely associated with conglomeration with tubeworms in both modern and fossil specimens (**Table 5**) [3, 100, 131].

6. Conclusive remarks

Authigenic carbonates and autotrophic faunas provide unique and typical landscapes of methane seeps (**Figures 1 and 2**). The carbonates and faunas are formed and maintained not independently but interactively via microbial activities of methanogenesis, anaerobic (and aerobic) oxidation of methane, anaerobic respirations such as sulfate reduction, and carbonate (particularly dolomite) formation. During these microbial processes, the stable carbon isotope ratios ($\delta^{13}\text{C}$ values) do shift with respective ranges of isotopic fractionation ($\Delta^{13}\text{C}$, **Tables 2 and 3**). Not only microorganisms but also macro-fauna may contribute to form and shape authigenic carbonate rocks. In the case of rock-tubeworm conglomerates, tubeworms actively recycle sulfide and sulfate in sediment, resulting in the acceleration of “growth” of the conglomerates.

Acknowledgements

The author is obliged to the crew and operation teams of manned and unmanned deep-sea vehicles and support ships of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) for deep-sea expeditions. The photograph of the methane seep landscape (**Figure 1**) was courteously provided by JAMSTEC.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Notes

(Note 1) In the strict sense, chemolithoautotrophy should be used instead of lithotrophy or chemoautotrophy; however, the term is only used by experts in rather a narrow area of microbiology and sounds too technical for non-expert audience.

(Note 2) Endosymbiosis is widespread in non-vent, non-seep organisms [123]. Chemoautotrophic bacterial endosymbiosis was characterized with the gutless oligochaetes *Inanidrillus leukoderma-tus* (Giere, 1979) from 5-m-deep sediment in a Bermuda inlet [135] and *Olavius crassitunicatus* Finogenova, 1986, from 270- to 359-m-deep sediment in the Peruvian passive margin [136], as well as a marine nematode *Astomonema* sp. from coral reef sediments in the Bahamas [137]. Multiple endosymbiosis of the gutless oligochaete *Olavius algarvensis* Giere, Erséus and Stuhlmacher, 1998, was subject to genetically and biochemically dissected by metaproteomics [138] that revealed unusual pathways, for example, use CO and H₂ for energetic metabolism [139]. Animal-bacterial chemo-/methanotrophic symbioses have recently been recognized as more widespread and ubiquitous in anaerobic sediments, besides vents, seeps, and organic falls, than previously presumed [140]. A microbiome of an individual of the gutless oligochaete *I. exumae* Erséus, 2003, differs markedly from those of other 22 individuals [141], which also demonstrates the symbiotic plasticity that may facilitate the adaption and evolution of this group of the gutless tubeworms.

(Note 3) Anaerobic oxidation of sulfide with nitrate, which is anaerobic thiotrophy (thioautotrophy) based on nitrate-respiration, is seen in bacterial species belonging to the genus *Beggiatoa* Trevisan, 1842 [132]. This energetic metabolism was once expected for the endosymbiont of the hydrothermal vent tubeworm (*R. pachyptila* Jones, 1981) [133], but the possibility was denied later [134].

(Note 4) Maurice Jules Gaston Corneille Caullery (1868–1958), a French zoologist, studied the first specimen of the “beard worm” and proposed the new family Siboglinidae and new genus *Siboglinum* Caullery, 1914, presumably named after the Dutch “Siboga” Expedition (1899–1900), during which the specimen was dredge-sampled. “Siboga” was the name of the vessel, which originally was a 50-m-long gunboat owned by the government of the Dutch East Indies [142]. Caullery described the new family Siboglinidae in 1914 based on the simultaneously proposed new genus *Siboglinum*. The type species of the genus, *S. weberi* Caullery 1944, was described subsequently 30 years later, in 1944. It is reasonably considered that the specific epithet “*weberi*” was named after the leader of the “Siboga” expedition, Max Carl Wilhelm Weber, a German-Dutch zoologist.

(Note 5) The sulfide/sulfate “percolator” in the seep system can be viewed as “extended symbiosis” between gutless host tubeworms, thiotrophic endosymbionts, and sulfate-consuming AOM microbial consortia. In these relationships, biologically true symbiosis is postulated only between host worms and internal thiotrophs, and the involvement of external AOM consortia is regarded as “extended”. By contrast, true endosymbiosis of a host worm and two bacterial symbionts, that is, dual symbiosis of sulfur-oxidizing and sulfate-reducing bacteria is seen in the gutless oligochaete worm *Olavius algarvensis* Giere, Erséus and Stuhlmacher, 1998, and *Olavius crassitunicatus* Finogenova, 1986, that host multiple (more than dual) endosymbionts [136, 143].

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References

- [1] Plochmann GK. Nature and the living thing in Aristotle's biology. *Journal of the History of Ideas*. 1953;**14**:167-190. DOI: 10.2307/2707469
- [2] Lowenstam HA. Minerals formed by organisms. *Science*. 1981;**211**:1126-1131. DOI: 10.1126/science.7008198
- [3] Naganuma T, Okayama Y, Hattori M, Kanie Y. Fossil worm tubes from the presumed cold-seep carbonates of the Miocene Hayama group, Central Miura peninsula, Japan. *Island Arc*. 1995;**4**:199-208. DOI: 10.1111/j.1440-1738.1995.tb00143.x
- [4] Vasconcelos C, McKenzie JA, Bernasconi S, Grujic D, Tiens AJ. Microbial mediation as a possible mechanism for natural dolomite formation at low temperatures. *Nature*. 1995;**377**:220-222. DOI: 10.1038/377220a0
- [5] Warthmann R, Vasconcelos C, Sass H, McKenzie JA. *Desulfovibrio brasiliensis* sp. nov., a moderate halophilic sulfate-reducing bacterium from Lagoa Vermelha (Brazil) mediating dolomite formation. *Extremophiles*. 2005;**9**:255-261. DOI: 10.1007/s00792-005-0441-8
- [6] Bontognali TRR, Vasconcelos C, Warthmann RJ, Lundberg R, McKenzie JA. Dolomite-mediating bacterium isolated from the sabkha of Abu Dhabi (UAE). *Terra Nova*. 2012;**24**:248-254. DOI: 10.1111/j.1365-3121.2012.01065.x
- [7] Marvasi M, Casillas-Santiago LM, Henríquez T, Casillas-Martinez L. Involvement of *etfA* gene during CaCO₃ precipitation in *Bacillus subtilis* biofilm. *Geomicrobiology Journal*. 2017;**34**:722-728. DOI: 10.1080/01490451.2016.1248254
- [8] Swart PK. The geochemistry of carbonate diagenesis: The past, present and future. *Sedimentology*. 2015;**2**:1233-1304. DOI: 10.1111/sed.12205
- [9] Boutton TW. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: Coleman DC, editor. *Carbon Isotope Techniques*. San Diego: Academic Press; 1991. pp. 173-185. DOI: 10.1016/B978-0-12-179730-0.50016-3
- [10] Longinelli A, Lenaz R, Ori C, Selmo E. Concentrations and $\delta^{13}\text{C}$ values of atmospheric CO₂ from oceanic atmosphere through time: Polluted and non-polluted areas. *Tellus Series B: Chemical and Physical Meteorology*. 2005;**57**:385-390. DOI: 10.3402/tellusb.v57i5.16567
- [11] Keeling CD. The Suess effect: ¹³Carbon-¹⁴Carbon interrelations. *Environment International*. 1979;**2**:229-300. DOI: 10.1016/0160-4120(79)90005-9

- [12] Siegenthaler U, Munnich KO. $^{13}\text{C}/^{12}\text{C}$ fractionation during CO_2 transfer from air to sea. In: Bolin B, editor. SCOPE 16 Carbon Cycle Modelling. Chichester: Wiley; 1981. pp. 249-257. https://dgc.carnegiescience.edu/SCOPE/SCOPE_16/SCOPE_16_1.5.05_Siegenthaler_249-257.pdf
- [13] Zeebe RE, Wolf-Gladrow DA. CO_2 in Seawater: Equilibrium, Kinetics, Isotopes. Amsterdam: Elsevier; 2001 (First edition; 2005 Third impression with corrections). p. 360. ISBN-13: 978-0444509468
- [14] Fogel ML, Cifuentes LA. Isotope fractionation during primary production. In: Engel MH, Macko SA, editors. Organic Geochemistry. Topics in Geobiology. Vol. 11. Boston: Springer; 1993. pp. 73-98. DOI: 10.1007/978-1-4615-2890-6_3
- [15] Paneth P, O'Leary MH. Carbon isotope effect on dehydration of bicarbonate ion catalyzed by carbonic anhydrase. Biochemistry. 1985;**24**:5143-5147. DOI: 10.1021/bi00340a028
- [16] Roeske CA, O'Leary MH. Carbon isotope effects on enzyme-catalyzed carboxylation of ribulose biphosphate. Biochemistry. 1984;**23**:6275-6284. DOI: 10.1021/bi00320a058
- [17] Raven JA, Johnston AM. Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnology and Oceanography. 1991;**36**:1701-1714. DOI: 10.4319/lo.1991.36.8.1701
- [18] Boller AJ, Thomas PJ, Cavanaugh CM, Scott KM. Low stable carbon isotope fractionation by coccolithophore *RubisCO*. Geochimica et Cosmochimica Acta. 2011;**75**:7200-7207. DOI: 10.1016/j.gca.2011.08.031
- [19] Hoins M, Eberlein T, Van de Waal DB, Sluijs A, Reichart GJ, Rost B. CO_2 -dependent carbon isotope fractionation in dinoflagellates relates to their inorganic carbon fluxes. Journal of Experimental Marine Biology and Ecology. 2016;**481**:9-14. DOI: 10.1016/j.jembe.2016.04.001
- [20] Dunne JP, Sarmiento JL, Gnanadesikan A. A synthesis of global particle export from the surface ocean and cycling through the ocean interior and on the seafloor. Global Biogeochemical Cycles. 2007;**21**:GB4006. DOI: 10.1029/2006GB002907
- [21] Thomsen L, Aguzzi J, Costa C, De Leo F, Ogston A, Purser A. The oceanic biological pump: Rapid carbon transfer to depth at continental margins during winter. Scientific Reports. 2017;**7**:10763. DOI: 10.1038/s41598-017-11075-6
- [22] Spiker EC, Hatcher PG. Carbon isotope fractionation of sapropelic organic matter during early diagenesis. Organic Geochemistry. 1984;**5**:283-290. DOI: 10.1016/0146-6380(84)90016-0
- [23] Sakai H, Gamo T, Endow K, Ishibashi J, Ishizuka T, Yanagisawa F, Kusakabe M, Akagi T, Igarashi G, Ohta S. Geochemical study of the bathyal seep communities at the Hatsushima site, Sagami Bay, Central Japan. Geochemical Journal. 1987;**21**:227-236. DOI: 10.2343/geochemj.21.227
- [24] MacDonald IR, Boland GS, Baker JS, Brooks JM, Kennicutt MC II, Bidigare RR. Gulf of Mexico hydrocarbon seep communities II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill. Marine Biology. 1989;**101**:235-247. DOI: 10.1007/BF00391463

- [25] Masuzawa T, Handa N, Kitagawa H, Kusakabe M. Sulfate reduction using methane in sediments beneath a bathyal "cold seep" giant clam community off Hatsushima Island, Sagami Bay, Japan. *Earth and Planetary Science Letters*. 1992;**110**:39-50. DOI: 10.1016/0012-821X(92)90037-V
- [26] Paull CK, Lorenson TD, Borowski WS, Ussler W III, Olsen K, Rodriguez NM. Isotopic composition of CH₄, CO₂ species, and sedimentary organic matter within samples from the Blake ridge: Gas source implications. *Proceedings. Ocean Drilling Program. Scientific Results*. 2000;**164**:67-78. DOI: 10.2973/odp.proc.sr.164.207.2000
- [27] Paull CK, Jull AJT, Toolin LJ, Linick T. Stable isotope evidence for chemosynthesis in an abyssal seep community. *Nature*. 1985;**317**:709-711. DOI: 10.1038/317709a0
- [28] Paull CK, Chanton JP, Neumann AC, Coston JA, Martens CS, Showers W. Indicators of methane-derived carbonates and chemosynthetic organic carbon deposits: Examples from the Florida escarpment. *PALAIOS*. 1992;**7**:361-375. DOI: 10.2307/3514822
- [29] Galand PE, Yrjälä K, Conrad R. Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems. *Biogeosciences*. 2010;**7**:3893-3900. DOI: 10.5194/bg-7-3893-2010
- [30] Penger J, Conrad R, Blaser M. Stable carbon isotope fractionation by methylotrophic methanogenic archaea. *Applied and Environmental Microbiology*. 2012;**7**:7596-7602. DOI: 10.1128/AEM.01773-12
- [31] Paull CK, Martens CS, Chanton JP, Neumann AC, Coston J, Jull AJT, Toolin LJ. Old carbon in living organisms and young CaCO₃ cements from abyssal brine seeps. *Nature*. 1989;**342**:166-168. DOI: 10.1038/342166a0
- [32] Roberts HH, Feng D, Joye SB. Cold-seep carbonates of the middle and lower continental slope, northern Gulf of Mexico. *Deep Sea Research, Part II*. 2010;**57**:2040-2054. DOI: 10.1016/j.dsr2.2010.09.003
- [33] Orphan VJ, Ussler IIIW, Naehr TH, House CH, Hinrichs K-U, Paull CK. Geological, geochemical, and microbiological heterogeneity of the seafloor around methane vents in the Eel River basin, offshore California. *Chemical Geology*. 2004;**205**:265-289. DOI: 10.1016/j.chemgeo.2003.12.035
- [34] Barker JF, Fritz P. (1981) carbon isotope fractionation during microbial methane oxidation. *Nature*. 1981;**293**:289-291. DOI: 10.1038/293289a0
- [35] Feisthauer S, Vogt C, Modrzynski J, Szlenkier M, Krüger M, Siegert M, Richnow H-H. Different types of methane monooxygenases produce similar carbon and hydrogen isotope fractionation patterns during methane oxidation. *Geochimica et Cosmochimica Acta*. 2011;**75**:1173-1184. DOI: 10.1016/j.gca.2010.12.006
- [36] Rasigraf O, Vogt C, Richnow H-H, Jetten MSM, Ettwig KF. Carbon and hydrogen isotope fractionation during nitrite-dependent anaerobic methane oxidation by *Methylomirabilis oxyfera*. *Geochimica et Cosmochimica Acta*. 2012;**89**:256-264. DOI: 10.1016/j.gca.2012.04.054

- [37] Loyd SJ, Sample J, Tripathi RE, Defliese WF, Brooks K, Hovland M, Torres M, Marlow J, Hancock LG, Martin R, Lyons T, Tripathi AE. Methane seep carbonates yield clumped isotope signatures out of equilibrium with formation temperatures. *Nature Communications*. 2016;**7**:12274. DOI: 10.1038/ncomms12274
- [38] Feng D, Chen D. Authigenic carbonates from an active cold seep of the northern South China Sea: New insights into fluid sources and past seepage activity. *Deep Sea Research, Part II*. 2015;**122**:74-83. DOI: 10.1016/j.dsr2.2015.02.003
- [39] Feng D, Roberts HH. Initial results of comparing cold-seep carbonates from mussel- and tubeworm-associated environments at Atwater Valley lease block 340, northern Gulf of Mexico. *Deep Sea Research, Part II*. 2010;**57**:2030-2039. DOI: 10.1016/j.dsr2.2010.05.004
- [40] Gieskes J, Mahn C, Day S, Martin JB, Greinert J, Rathburn T, McAdoo B. A study of the chemistry of pore fluids and authigenic carbonates in methane seep environments: Kodiak trench, hydrate ridge, Monterey Bay, and Eel River basin. *Chemical Geology*. 2005;**220**:329-345. DOI: 10.1016/j.chemgeo.2005.04.002
- [41] Kennicutt MC II, Brooks JM, Bidigare RR, Fay RR, Wade TL, McDonald TJ. Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. *Nature*. 1985;**317**:351-353. DOI: 10.1038/317351a0
- [42] Brooks M, Benjamin H, William C, Bryant R, Kennicutt MC II, Mann RG, McDonald TJ. Association of gas hydrates and oil seepage in the Gulf of Mexico. *Organic Geochemistry*. 1986;**10**:221-234. DOI: 10.1016/0146-6380(86)90025-2
- [43] Kulm LD, Suess E, Moore JC, Carson B, Lewis BT, Ritger SD, Kadko DC, Thornburg TM, Embley RW, Rugh WD, Massoth GJ, Langseth MG, Cochrane GR, Scamman RL. Oregon subduction zone: Venting, fauna, and carbonates. *Science*. 1986;**231**:561-566. DOI: 10.1126/science.231.4738.561
- [44] Saino T, Ohta S. $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios of vesicomyid clams and a vestimentiferan tube worm in the subduction zone east of Japan. *Palaeogeography Palaeoclimatology Palaeoecology*. 1989;**71**:169-178. DOI: 10.1016/0031-0182(89)90036-9
- [45] Kennicutt II, MC B, Jr RA, MacDonald IR, Brooks JM, Denoux GJ, Macko SA. Stable isotope partitioning in seep and vent organisms: Chemical and ecological significance. *Chemical Geology (Isotope Geoscience Section)*. 1992;**101**:293-310. DOI: 10.1016/0009-2541(92)90009-T
- [46] Olu-Le Roy K, Sibuet M, Fiala-Médioni A, Gofas S, Salas C, Mariotti A, Foucher J-P, Woodside J. Cold seep communities in the deep eastern Mediterranean Sea: Composition, symbiosis and spatial distribution on mud volcanoes. *Deep Sea Research, Part I*. 2004;**51**:1915-1936. DOI: 10.1016/j.dsr.2004.07.004
- [47] Becker EL, Macko SA, Lee RW, Fisher CR. Stable isotopes provide new insights into vestimentiferan physiological ecology at Gulf of Mexico cold seeps. *Die Naturwissenschaften*. 2011;**98**:169-174. DOI: 10.1007/s00114-010-0754-z
- [48] Thiel V, Hügler M, Blümel M, Thiel V, Hügler M, Blümel M, Baumann HI, Gärtner A, Schmaljohann R, Strauss H, Garbe-Schönberg D, Petersen S, Cowart DA, Fisher CR,

- Imhoff JF. Widespread occurrence of two carbon fixation pathways in tubeworm endosymbionts: Lessons from hydrothermal vent associated tubeworms from the Mediterranean Sea. *Frontiers in Microbiology*. 2012;**3**:423. DOI: 10.3389/fmicb.2012.00423
- [49] Becker EL, Raymond WL, Stephen AM, Fisher CR. Stable carbon and nitrogen isotope composition of hydrocarbon seep bivalves on the lower continental slope of the Gulf of Mexico. *Deep Sea Research, Part II*. 2010;**57**:1957-1964. DOI: 10.1016/j.dsr2.2010.05.002
- [50] Roberts HH, Feng D. Carbonate precipitation at Gulf of Mexico hydrocarbon seeps: An overview. In: Aminzadeh F, Berge TB, Connolly DL, editors. *Hydrocarbon Seepage: From Source to Surface*. Tulsa, OK: Society of Exploration – Geophysicists (SEG); 2013. pp. 43-61. DOI: 10.1190/1.9781560803119.ch3
- [51] Markert S, Arndt C, Felbeck H, Becher D, Sievert SM, Hügler M, Albrecht D, Robidart J, Bench S, Feldman RA, Hecker M, Schweder T. Physiological proteomics of the uncultured endosymbiont of *Riftia pachyptila*. *Science*. 2007;**315**:247-250. DOI: 10.1126/science.1132913
- [52] Zhao M-Y, Zheng Y-F, Zhao Y-Y. Seeking a geochemical identifier for authigenic carbonate. *Nature Communications*. 2016;**7**:10885. DOI: 10.1038/ncomms10885
- [53] Lein AY. Authigenic carbonate formation in the ocean. *Lithology and Mineral Resources*. 2004;**39**:1-30. DOI: 10.1023/B:LIMI.0000010767.52720.8f
- [54] Feng D, Roberts HH, Cheng H, Peckmann J, Bohrmann G, Edwards RL, Chen D. U/Th dating of cold-seep carbonates: An initial comparison. *Deep Sea Research, Part II*. 2010;**57**:2055-2060. DOI: 10.1016/j.dsr2.2010.09.004
- [55] Teichert BMA, Eisenhauer A, Bohrmann G, Haase-Schramm A, Bock B, Linke P. U/Th systematics and ages of authigenic carbonates from hydrate ridge, Cascadia margin: Records of fluid flow variations. *Geochimica et Cosmochimica Acta*. 2003;**67**:3845-3857. DOI: 10.1016/S0016-7037(03)00128-5
- [56] Naehr TH, Eichhubl P, Orphan VJ, Hovland M, Paull CK, Ussler W III, Lorenson TD, Greene HG. Authigenic carbonate formation at hydrocarbon seeps in continental margin sediments: A comparative study. *Deep Sea Research, Part II*. 2007;**54**:1268-1291. DOI: 10.1016/j.dsr2.2007.04.010
- [57] Feng D, Erik E, Cordes EE, Harry H, Roberts HH, Fisher CR. A comparative study of authigenic carbonates from mussel and tubeworm environments: Implications for discriminating the effects of tubeworms. *Deep Sea Research, Part I*. 2013;**75**:110-118. DOI: 10.1016/j.dsr.2013.02.002
- [58] Drake H, Åström ME, Heim C, Broman C, Åström J, Whitehouse M, Ivarsson M, Siljeström S, Sjövall P. Extreme ^{13}C depletion of carbonates formed during oxidation of biogenic methane in fractured granite. *Nature Communications*. 2015;**6**:7020. DOI: 10.1038/ncomms8020
- [59] Ries JB. Review: Geological and experimental evidence for secular variation in seawater mg/Ca (calcite-aragonite seas) and its effects on marine biological calcification. *Biogeosciences*. 2010;**7**:2795-2849. DOI: 10.5194/bg-7-2795-2010

- [60] Andrews J, Stamatakis M, Marca-Bell A, Stewart C, Millar IL. Exhumed hydrocarbon-seep authigenic carbonates from Zakynthos island (Greece): Concretions not archaeological remains. *Marine and Petroleum Geology*. 2016;**76**:16-25. DOI: 10.1016/j.marpetgeo.2016.05.022
- [61] Blättler CL, Miller NR, Higgins JA. Mg and Ca isotope signatures of authigenic dolomite in siliceous deep-sea sediments. *Earth and Planetary Science Letters*. 2015;**419**:32-42. DOI: 10.1016/j.epsl.2015.03.006
- [62] Bernardino AF, Levin LA, Thurber AR, Smith CR (2012) comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. *PLoS One*. 2012;**7**:e33515. DOI: 10.1371/journal.pone.0033515
- [63] Portail M, Olu K, Escobar-Briones E, Caprais JC, Menot L, Waeles M, Cruaud P, Sarradin PM, Godfroy A, Sarrazin J. Comparative study of vent and seep macrofaunal communities in the Guaymas Basin. *Biogeosciences*. 2015;**12**:5455-5479. DOI: 10.5194/bg-12-5455-2015
- [64] Kiel S. A biogeographic network reveals evolutionary links between deep-sea hydrothermal vent and methane seep faunas. *Proceedings of the Royal Society B: Biological Sciences*. 2016;**283**:20162337. DOI: 10.1098/rspb.2016.2337
- [65] Corliss JB, Dymond J, Gordon LI, Edmond JM, von Herzen RP, Ballard RD, Green K, Williams D, Bainbridge A, Crane K, van Andel TH. Submarine thermal springs on the Galápagos rift. *Science*. 1979;**203**:1073-1083. DOI: 10.1126/science.203.4385.1073
- [66] Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts. *Science*. 1981;**213**:340-342. DOI: 10.1126/science.213.4505.340
- [67] Robidart JC, Bench SR, Feldman RA, Novoradovsky A, Podell SB, Gaasterland T, Allen EE, Felbeck H. Metabolic versatility of the *Riftia pachyptila* endosymbiont revealed through metagenomics. *Environmental Microbiology*. 2008;**10**:727-737. DOI: 10.1111/j.1462-2920.2007.01496.x
- [68] Klose J, Aistleitner K, Horn M, Krenn L, Dirsch V, Zehl M, Bright M. Trophosome of the deep-sea tubeworm *Riftia pachyptila* inhibits bacterial growth. *PLoS One*. 2016;**11**:e0146446. DOI: 10.1371/journal.pone.0146446
- [69] Paull CK, Hecker B, Commeau R, Freeman-Lynde RP, Neumann C, Corso WP, Golubic S, Hook JE, Sikes E, Curray J. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. *Science*. 1984;**226**:965-967. DOI: 10.1126/science.226.4677.965
- [70] Childress JJ, Fisher CR, Brooks JM, Kennicutt MC 2nd, Bidigare R, Anderson AE. A methanotrophic marine molluscan (bivalvia, mytilidae) symbiosis: Mussels fueled by gas. *Science*. 1986;**233**:1306-1308. DOI: 10.1126/science.233.4770.1306
- [71] Cavanaugh CM, Levering PR, Maki JS, Mitchell R, Lidstrom ME. Symbiosis of methylo-trophic bacteria and deep-sea mussels. *Nature*. 1987;**325**:346-347. DOI: 10.1038/325346a0
- [72] Schmaljohann R, Flügel HJ. Methane-oxidizing bacteria in Pogonophora. *Sarsia*. 1987;**72**: 91-98. DOI: 10.1080/00364827.1987.10419707

- [73] Schmaljohann R. Oxidation of various potential energy sources by the methanotrophic endosymbionts of *Siboglinum poseidoni* (Pogonophora). Marine Ecology Progress Series. 1991;**76**:143-148
- [74] DeChaine EG, Cavanaugh CM. Symbioses of methanotrophs and deep-sea mussels (Mytilidae: Bathymodiolinae). Progress in Molecular and Subcellular Biology. 2006;**41**:227-249. PMID: 16623396
- [75] Cavanaugh CM, McKiness ZP, Newton ILG, Stewart FJ. Marine chemosynthetic symbioses. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes. Berlin: Springer; 2013. pp. 597-607. DOI: 10.1007/978-3-642-30194-0_21
- [76] Zimmermann J, Lott C, Weber M, Ramette A, Bright M, Dubilier N, Petersen JM. Dual symbiosis with co-occurring sulfur-oxidizing symbionts in vestimentiferan tubeworms from a Mediterranean hydrothermal vent. Environmental Microbiology. 2014;**16**:3638-3656. DOI: 10.1111/1462-2920.12427
- [77] Cavanaugh CM. Microbial symbiosis: Patterns of diversity in the marine environment. American Zoologist (currently Integr. Comp. Biol.). 1994;**34**:79-89. DOI: 10.1093/icb/34.1.79
- [78] Duperron S, Nadalig T, Caprais J-C, Sibuet M, Fiala-Médioni A, Amann R, Dubilier N. Dual symbiosis in a *Bathymodiolus* sp. mussel from a methane seep on the Gabon continental margin (southeast Atlantic): 16S rRNA phylogeny and distribution of the symbionts in gills. Applied and Environmental Microbiology. 2005;**71**:1694-1700. DOI: 10.1128/AEM.71.4.1694-1700.2005
- [79] Petersen JM, Zielinski FU, Pape T, Seifert R, Moraru C, Amann R, Hourdez S, Girguis PR, Wankel SD, Barbe V, Pelletier E, Fink D, Borowski C, Bach W, Dubilier N. Hydrogen is an energy source for hydrothermal vent symbioses. Nature. 2011;**476**:176-180. DOI: 10.1038/nature10325
- [80] Swan BK, Martinez-Garcia M, Preston CM, Sczyrba A, Woyke T, Lamy D, Reinthaler T, Poulton NJ, Masland EDP, Gomez ML, Sieracki ME, DeLong EF, Herndl GJ, Stepanauskas R. Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. Science. 2011;**333**:1296-1300. DOI: 10.1126/science.1203690
- [81] Nakagawa S, Takai K. Deep-sea vent chemoautotrophs: Diversity, biochemistry and ecological significance. FEMS Microbiology Ecology. 2008;**65**:1-14. DOI: 10.1111/j.1574-6941.2008.00502.x
- [82] Middelburg JJ. Chemoautotrophy in the ocean. Geophysical Research Letters. 2011;**38**:L24604. DOI: 10.1029/2011GL049725
- [83] Anantharaman K, Breier JA, Sheik CS, Dick GJ. Evidence for hydrogen oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:330-335. DOI: 10.1073/pnas.1215340110
- [84] Kouzuma A, Tsutsumi M, Ishii S, Ueno Y, Abe T, Watanabe K. Non-autotrophic methanogens dominate in anaerobic digesters. Scientific Reports. 2017;**7**:1510. DOI: 10.1038/s41598-017-01752-x

- [85] Petersen JM, Dubilier N. Methanotrophic symbioses in marine invertebrates. *Environmental Microbiology Reports*. 2009;**1**:319-335. DOI: 10.1111/j.1758-2229.2009.00081.x
- [86] Khadem AF, Pol A, Wieczorek A, Mohammadi SS, Francois K-J, Stunnenberg HG, Jetten MSM, Op den Camp HJM. Autotrophic methanotrophy in Verrucomicrobia: *Methylophilum fumariolicum* SolV uses the Calvin-Benson-Bassham cycle for carbon dioxide fixation. *Journal of Bacteriology*. 2011;**193**:4438-4446. DOI: 10.1128/JB.00407-11
- [87] van Teeseling MCF, Pol A, Harhangi HR, van der Zwart, Jetten MSM, Op den Camp HJM, van Niftrik L. Expanding the verrucomicrobial methanotrophic world: Description of three novel species of *Methylophilum* gen. nov. *Applied and Environmental Microbiology*. 2014;**80**:6782-6791. DOI: 10.1128/AEM.01838-14
- [88] Danilova OV, Suzina NE, Van De Kamp J, Svenning MM, Bodrossy L, Dedys SN. A new cell morphotype among methane oxidizers: A spiral-shaped obligately micro-aerophilic methanotroph from northern low-oxygen environments. *The ISME Journal*. 2016;**10**:2734-2743. DOI: 10.1038/ismej.2016.48
- [89] Kellermann MY, Wegener G, Elvert M, Yoshinaga MY, Lin Y-S, Holler T, Mollar XP, Knittel K, Hinrichs K-U. Autotrophy as a predominant mode of carbon fixation in anaerobic methane-oxidizing microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**2012**(109):19321-19326. DOI: 10.1073/pnas.1208795109
- [90] Bright M, Lallier FH. The biology of vestimentiferan tubeworms. In: Gibson RN, Atkinson RJA, Gordon JDM, editors. *Oceanography and Marine Biology: An Annual Review*. Vol. 48. Boca Raton: CRC Press; 2010. pp. 213-266. DOI: 10.1201/EBK1439821169-c4
- [91] Southward EC. Siboga-Expeditie Uitkomsten op Zoologisch, Botanisch, Oceanographisch en Geologisch gebied verzameld in Nederlandsch Oost-Indië 1899-1900; Pogonophora. *Siboga Expedition Monograph*. 1961;**25**(3):22
- [92] Wiedicke M, Weiss W. Stable carbon isotope records of carbonates tracing fossil seep activity off Indonesia. *Geochemistry, Geophysics, Geosystems*. 2006;**7**:Q11009. DOI: 10.1029/2006GC001292
- [93] Caullery M. 1944. *Siboglinum* Caullery, 1914. Type nouveau d'invertébrés, d'affinités à préciser. *Siboga Expedition Monograph* 25. Siboga-Expeditie Uitkomsten op Zoologisch, Botanisch, Oceanographisch en Geologisch gebied verzameld in Nederlandsch Oost-Indië 1899-1900; 1944. 26p
- [94] Caullery M. Sur les Siboglinidae, type nouveau d'invertébrés recueillis par l'expédition du Siboga. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*. 1914;**158**: 2014-2017. <https://www.biodiversitylibrary.org/page/7159983#page/2024/mode/1up>
- [95] Kimura H, Sato M, Sasayama Y, Naganuma T. Molecular characterization and in situ localization of endosymbiotic 16S ribosomal RNA and RuBisCO genes in the pogonophoran tissue. *Marine Biotechnology*. 2003;**5**:261-269. DOI: 10.1007/s10126-002-0073-2

- [96] Kobayashi G, Miura T, Kojima S. *Lamellibrachia sagami* sp. nov., a new vestimentiferan tubeworm (Annelida: Siboglinidae) from Sagami Bay and several sites in the north-western Pacific Ocean. *Zootaxa*. 2015;**4018**:97-108. DOI: 10.11646/zootaxa.4018.1.5
- [97] Naganuma T, Naka J, Okayama Y, Minami A, Horikoshi K. Morphological diversity of the microbial population in a vestimentiferan tubeworm. *Journal of Marine Biotechnology*. 1997;**5**:119-123. NII (National Institute of Informatics of Japan) Article ID: 10002211726
- [98] Kimura H, Higashide Y, Naganuma T. Endosymbiotic microflora of the vestimentiferan tubeworm (*Lamellibrachia* sp.) from a bathyal cold seep. *Marine Biotechnology*. 2003;**5**:593-603. DOI: 10.1007/s10126-002-0117-7
- [99] Naganuma T, Elsaied HE, Hoshii D, Kimura H. Bacterial endosymbioses of gutless tube-dwelling worms in non-hydrothermal vent habitats. *Marine Biotechnology*. 2005;**7**:416-428. DOI: 10.1007/s10126-004-5089-3
- [100] Naganuma T. Animal-bacterial endosymbioses of gutless tube-dwelling worms in marine sediments. In: Seckbach J, Grube M, editors. *Symbioses and Stress. Cellular Origin, Life in Extreme Habitats and Astrobiology*. Vol. 17. Dordrecht: Springer; 2010. pp. 99-120. DOI: 10.1007/978-90-481-9449-0_6
- [101] Hilário A, Capa M, Dahlgren TG, Halanych KM, Little CTS, Thornhill DJ, Verna C, Glover AG. New perspectives on the ecology and evolution of *Siboglinid* tubeworms. *PLoS One*. 2011;**6**:e16309. DOI: 10.1371/journal.pone.0016309
- [102] Yuanning L, Kocot KM, Whelan NV, Santos SR, Waits DS, Thornhill DJ, Halanych KM. Phylogenomics of tubeworms (Siboglinidae, Annelida) and comparative performance of different reconstruction methods. *Zoologica Scripta*. 2017;**46**:200-213. DOI: 10.1111/zsc.12201
- [103] van der Land J, Nørrevang A. The systematic position of *Lamellibrachia* (Annelida, Vestimentifera). *Zeitschrift für Zoologische Systematik und Evolutionsforschung*. 1975: 86-101
- [104] Jones ML. On the Vestimentifera, new phylum: Six new species, and other taxa, from hydrothermal vents and elsewhere. *Bulletin of the Biological Society of Washington*. 1985;**6**:117-158
- [105] Fisher CR, Urcuyo IA, Simpkins MA, Nix ER. Life in the slow lane: Growth and longevity of cold-seep vestimentiferans. *Marine Ecology*. 1997;**18**:83-94. DOI: 10.1111/j.1439-0485.1997.tb00428.x
- [106] Bergquist DC, Ward T, Cordes EE, McNelis T, Howlett S, Kosoff R, Hourdez S, Carney R, Fisher CR. Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. *Journal of Experimental Marine Biology and Ecology*. 2003, 2003;**289**:197-222. DOI: 10.1016/S0022-0981(03)00046-7
- [107] Durkin A, Fisher CR, Cordes EE. Extreme longevity in a deep-sea vestimentiferan tubeworm and its implications for the evolution of life history strategies. *The Science of Nature (Naturwissenschaften)*. 2017;**104**:63. DOI: 10.1007/s00114-017-1479-z

- [108] Bergquist DC, Williams FM, Charles R, Fisher CR. Longevity record for deep-sea invertebrate. *Nature*. 2000;**403**:499-500. DOI: 10.1038/35000647
- [109] Freytag JK, Girguis PR, Bergquist DC, Andras JP, Childress JJ, Fisher CR. A paradox resolved: Sulfide acquisition by roots of seep tubeworms sustains net chemoautotrophy. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:13408-13413. DOI: 10.1073/pnas.231589498
- [110] Dattagupta S, Miles LL, Barnabei MS, Fisher CR. The hydrocarbon seep tubeworm *Lamellibrachia luymesii* primarily eliminates sulfate and hydrogen ions across its roots to conserve energy and ensure sulfide supply. *The Journal of Experimental Biology*. 2006;**209**:3795-3805. DOI: 10.1242/jeb.02413
- [111] Campbell KA, Nelson CS, Alfaro AC, Boyd S, Greinert J, Nyman S, Grosjean E, Logan GA, Gregory MR, Cooke S, Linke P, Milloy S, Wallis I. Geological imprint of methane seepage on the seabed and biota of the convergent Hikurangi margin, New Zealand: Box core and grab carbonate results. *Marine Geology*. 2010;**272**:285-306. DOI: 10.1016/j.margeo.2010.01.002
- [112] Wentrup C, Wendeborg A, Huang JY, Borowski C, Dubilier N. Shift from widespread symbiont infection of host tissues to specific colonization of gills in juvenile deep-sea mussels. *The ISME Journal*. 2013;**7**:1244-1247. DOI: 10.1038/ismej.2013.5
- [113] Wentrup C, Wendeborg A, Schimak M, Borowski C, Dubilier N. Forever competent: Deep-sea bivalves are colonized by their chemosynthetic symbionts throughout their lifetime. *Environmental Microbiology*. 2014;**16**:3699-3713. DOI: 10.1111/1462-2920.12597
- [114] Gros O, Elisabeth NH, Gustave SD, Caro A, Dubilier N. Plasticity of symbiont acquisition throughout the life cycle of the shallow-water tropical lucinid *Codakia orbiculata* (Mollusca: Bivalvia). *Environmental Microbiology*. 2012;**14**:1584-1595. DOI: 10.1111/j.1462-2920.2012.02748.x
- [115] Raulfs E, Macko S, Van Dover C. Tissue and symbiont condition of mussels (*Bathymodiolus thermophilus*) exposed to varying levels of hydrothermal activity. *Journal of the Marine Biological Association of the UK*. 2004, 2004;**84**:229-234. DOI: 10.1017/S0025315404009087h
- [116] Elsaied HE, Kaneko R, Naganuma T. Molecular characterization of a deep-sea methanotrophic mussel symbiont that carries a RuBisCO gene. *Marine Biotechnology*. 2006;**8**:511-520. DOI: 10.1007/s10126-005-6135-5
- [117] Duperron S, Halary S, Lorion J, Sibuet M, Gaill F. Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). *Environmental Microbiology*. 2008;**10**:433-445. DOI: 10.1111/j.1462-2920.2007.01465.x
- [118] MacDonald IR, Bohrmann G, Escobar E, Abegg F, Blanchon P, Blinova V, Brückmann W, Drews M, Eisenhauer A, Han X, Heeschen K, Meier F, Mortera C, Naehr T, Orcutt B, Bernard B, Brooks J, de Faragó M. Asphalt volcanism and chemosynthetic life in the Campeche knolls, Gulf of Mexico. *Science*. 2004;**304**:999-1002. DOI: 10.1126/science.1097154

- [119] Sahling H, Borowski C, Escobar-Briones E, Gaytán-Caballero A, Hsu C-W, Loher M, MacDonald I, Marcon Y, Pape T, Römer M, Rubin-Blum M, Schubotz F, Smrzka D, Wegener G, Bohrmann G. Massive asphalt deposits, oil seepage, and gas venting support abundant chemosynthetic communities at the Campeche knolls, southern Gulf of Mexico. *Biogeosciences*. 2016;**13**:4491-4512. DOI: 10.5194/bg-13-4491-2016
- [120] Raggi L, Schubotz F, Hinrichs KU, Dubilier N, Petersen JM. Bacterial symbionts of *Bathymodiolus* mussels and Escarpia tubeworms from Chapopote, an asphalt seep in the southern Gulf of Mexico. *Environmental Microbiology*. 2013;**15**:1969-1987. DOI: 10.1111/1462-2920.12051
- [121] Rubin-Blum M, Antony CP, Borowski C, Sayavedra L, Pape T, Sahling H, Bohrmann G, Kleiner M, Redmond MC, Valentine DL, Dubilier N. Short-chain alkanes fuel mussel and sponge *Cycloclasticus* symbionts from deep-sea gas and oil seeps. *Nature Microbiology*. 2017;**2**:17093. DOI: 10.1038/nmicrobiol.2017.93
- [122] Chung WK, King GM. Isolation, characterization, and polyaromatic hydrocarbon degradation potential of aerobic bacteria from marine macrofaunal burrow sediments and description of *Lutibacterium anuloderans* gen. nov., sp. nov., and *Cycloclasticus spirillensus* sp. nov. *Applied and Environmental Microbiology*. 2001;**67**:5585-5592. DOI: 10.1128/AEM.67.12.5585-5592.2001
- [123] Dubilier N, Bergin C, Lott C. Symbiotic diversity in marine animals: The art of harnessing chemosynthesis. *Nature Reviews. Microbiology*. 2008 Oct;**6**(10):725-740. DOI: 10.1038/nrmicro1992
- [124] Petersen JM, Wentrup C, Verna C, Knittel K, Dubilier N. Origins and evolutionary flexibility of chemosynthetic symbionts from deep-sea animals. *The Biological Bulletin*. 2012;**223**:123-137. DOI: 10.1086/BBLv223n1p123 (Erratum in *Biol. Bull.* 2012;**223**(3):x)
- [125] McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**:3229-3236. DOI: 10.1073/pnas.1218525110
- [126] Okutani T, Fujikura K, Kojima S. Two new hadal bivalves of the family Thyasiridae from the plate convergent area of the Japan trench. *Venus*. 1999;**58**:49-54
- [127] Fujikura K, Kojima S, Tamaki K, Maki Y, Hunt J, Okutani T. The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan trench. *Marine Ecology Progress Series*. 1999;**190**:17-26. DOI: 10.3354/meps190017
- [128] Fujiwara Y, Kato C, Masui N, Fujikura K, Kojima S. Dual symbiosis in the cold-seep thyasirid clam *Maorithyas hadalis* from the hadal zone in the Japan trench, western Pacific. *Marine Ecology Progress Series*. 2001;**214**:151-159. DOI: 10.3354/meps214151

- [129] Fiala-Médioni A, Boulègue J, Ohta S, Felbeck H, Mariotti A. Source of energy sustaining the *Calymene* populations from deep trenches in subduction zones off Japan. Deep Sea Research, Part I. 1993;**40**:1241-1258. DOI: 10.1016/0967-0637(93)90136-Q
- [130] Haas A. Carbonate authigenesis and worm tube mineralization – biogeochemical and geobiological processes at methane seeps on the Congo deep-sea fan [thesis]. Bremen: Universität Bremen; 2008
- [131] Naganuma T, Hattori M, Hashimoto J, Kanie Y. Elemental distributions in the tubes of modern vestimentiferan worms, and carbonate formation in their habitats. Kaseki (Fossils, Palaeontological Society of Japan). 1996;**60**:26-31 (in Japanese with English abstract and captions). DOI: 10.14825/kaseki.60.0_26
- [132] Kamp A, Stief P, Schulz-Vogt HN. Anaerobic sulfide oxidation with nitrate by a freshwater *Beggiatoa* enrichment culture. Applied and Environmental Microbiology. 2006;**72**:4755-4760. DOI: 10.1128/AEM.00163-06
- [133] Hentschel U, Felbeck H. Nitrate respiration in the hydrothermal vent tubeworm *Riftia pachyptila*. Nature. 1993;**366**:338-340. DOI: 10.1038/366338a0
- [134] Girguis PR, Lee RW, Desaulniers N, Childress JJ, Pospesel M, Felbeck H, Zal F. Fate of nitrate acquired by the tubeworm *Riftia pachyptila*. Applied and Environmental Microbiology. 2000;**66**:2783-2790. PMCID: PMC92073
- [135] Dubilier N, Giere O, Distel DL, Cavanaugh CM. Characterization of chemoautotrophic bacterial symbionts in a gutless marine worm (Oligochaeta, Annelida) by phylogenetic 16S rRNA sequence analysis and in situ hybridization. Applied and Environmental Microbiology. 1995;**61**:2346-2350 PMCID: PMC167506
- [136] Blazejak A, Erséus C, Amann R, Dubilier N. Coexistence of bacterial sulfide oxidizers, sulfate reducers, and spirochetes in a gutless worm (Oligochaeta) from the Peru margin. Applied and Environmental Microbiology. 2005;**71**:1553-1561. DOI: 10.1128/AEM.71.3.1553-1561.2005
- [137] Musat N, Giere O, Gieseke A, Thiermann F, Amann R, Dubilier N. Molecular and morphological characterization of the association between bacterial endosymbionts and the marine nematode *Astomonema* sp. from the Bahamas. Environmental Microbiology. 2007;**9**:1345-1353. DOI: 10.1111/j.1462-2920.2006.01232.x
- [138] Kleiner M, Wentrup C, Lott C, Teeling H, Wetzel S, Young J, Chang YJ, Shah M, VerBerkmoes NC, Zarzycki J, Fuchs G, Markert S, Hempel K, Voigt B, Becher D, Liebeke M, Lalk M, Albrecht D, Hecker M, Schweder T, Dubilier N. Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:E1173-E1182. DOI: 10.1073/pnas.1121198109

- [139] Kleiner M, Wentrup C, Holler T, Lavik G, Harder J, Lott C, Littmann S, Kuypers MM, Dubilier N. Use of carbon monoxide and hydrogen by a bacteria-animal symbiosis from seagrass sediments. *Environmental Microbiology*. 2015;**17**:5023-5035. DOI: 10.1111/1462-2920.12912
- [140] Dubilier N, Blazejak A, Rühland C. Symbioses between bacteria and gutless marine oligochaetes. *Progress in Molecular and Subcellular Biology*. 2006;**41**:251-275 16623397
- [141] Bergin C, Wentrup C, Brewig N, Blazejak A, Erséus C, Giere O, Schmid M, De Wit P, Dubilier N. Acquisition of a novel sulfur-oxidizing symbiont in the gutless marine worm *Inanidrillus exumae*. *Applied and Environmental Microbiology*. 2018. DOI: 10.1128/AEM.02267-17 [online ahead of print]
- [142] Theberge AE. The Siboga Expedition. Hydro International [Internet]. 2011. Available from <https://www.hydro-international.com/content/article/the-siboga-expedition> [Accessed: 2017-04-04]
- [143] Dubilier N, Mülders C, Ferdelman T, de Beer D, Pernthaler A, Klein M, Wagner M, Erséus C, Thiermann F, Krieger J, Giere O, Amann R. Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. *Nature*. 2001;**411**:298-302. DOI: 10.1038/35077067
- [144] Yoshida N, Tsukahara H. $^{14}\text{C}/^{12}\text{C}$ and $^{13}\text{C}/^{12}\text{C}$ in the shell of *Calyptogena soyoe* from the subduction zone Sagami Bay and fluid flow from the deep part of the sea floor. *JAMSTEC Journal Deep Sea Research* 1991;**7**:25-31 (in Japanese with English abstract). http://www.godac.jamstec.go.jp/catalog/data/doc_catalog/media/shinkai07_04.pdf
- [145] Torres ME, Mix AC, Kinports K, Haley B, Klinkhammer GP, McManus J, de Angelis MA. Is methane venting at the seafloor recorded by $\delta^{13}\text{C}$ of benthic foraminifera shells? *Paleoceanography*. 2003;**18**:1062. DOI: 10.1029/2002PA000824
- [146] Yamanaka T, Shimamura S, Nagashio H, Yamagami S, Onishi Y, Hyodo A, Mampuku M, Mizota C. A compilation of the stable isotopic compositions of carbon, nitrogen, and sulfur in soft body parts of animals collected from deep-sea hydrothermal vent and methane seep fields: Variations in energy source and importance of subsurface microbial processes in the sediment-hosted systems. In: Ishibashi J-i, Okino K, Sunamura M, editors. *Subseafloor Biosphere Linked to Hydrothermal Systems: TAIGA Concept*. Tokyo: Springer; 2015. pp. 105-129. DOI: 10.1007/978-4-431-54865-2_10
- [147] Prouty NG, Sahy D, Ruppel CD, Roark EB, Condon D, Brooke S, Ross SW, Demopoulos WJ. Insights into methane dynamics from analysis of authigenic carbonates and chemosynthetic mussels at newly-discovered Atlantic margin seeps. *Earth and Planetary Science Letters*. 2016;**449**:332-344. DOI: 10.1016/j.epsl.2016.05.023
- [148] Sakai H, Gamo T, Ogawa Y, Boulegue J. Stable isotopic ratios and origins of the carbonates associated with cold seepage at the eastern Nankai trough. *Earth and Planetary Science Letters*. 1992;**109**:391-404. DOI: 10.1016/0012-821X(92)90101-Z

- [149] Lewis KB, Marshall BA. Seep faunas and other indicators of methane-rich dewatering on New Zealand convergent margins. *New Zealand Journal of Geology and Geophysics*. 1996;**39**:181-200. DOI: 10.1080/00288306.1996.9514704
- [150] Oehlert AM, Swart PK. Interpreting carbonate and organic carbon isotope covariance in the sedimentary record. *Nature Communications*. 2014;**5**:4672. DOI: 10.1038/ncomms5672
- [151] O'Leary MH. Carbon isotopes in photosynthesis: Fractionation techniques may reveal new aspects of carbon dynamics in plants. *Bioscience*. 1988;**38**:328-336. DOI: 10.2307/1310735
- [152] Preuß A, Schauder R, Fuchs G, Stichler W. Carbon isotope fractionation by autotrophic bacteria with three different CO₂ fixation pathways. *Zeitschrift für Naturforschung C*. 1989;**44**:397-402. DOI: 10.1515/znc-1989-5-610
- [153] Williams TJ, Zhang CL, Scott JH, Bazylinski DA. Evidence for autotrophy via the reverse tricarboxylic acid cycle in the marine magnetotactic coccus strain MC-1. *Applied and Environmental Microbiology*. 2006;**72**:1322-1329. DOI: 10.1128/AEM.72.2.1322-1329.2006
- [154] McConnaughe TA, Gillikin DP. Carbon isotopes in mollusk shell carbonates. *Geo-Marine Letters*. 2008;**28**:287-299. DOI: 10.1007/s00367-008-0116-4
- [155] van der Meer MTJ, Schouten S, van Dongen BE, Rijpstra WIC, Fuchs G, Damsté JSS, de Leeuw JW, Ward DM. Biosynthetic controls on the ¹³C contents of organic components in the photoautotrophic bacterium *Chloroflexus aurantiacus*. *The Journal of Biological Chemistry*. 2001;**276**:10971-10976. DOI: 10.1074/jbc.M009701200
- [156] van der Meer MTJ, Schouten S, Rijpstra WIC, Fuchs G, Damsté JSS. Stable carbon isotope fractionations of the hyperthermophilic crenarchaeon *Metallosphaera sedula*. *FEMS Microbiology Letters*. 2001;**196**:67-70. DOI: 10.1111/j.1574-6968.2001.tb10542.x
- [157] Jennings RM, Whitmore LM, Moran JJ, Kreuzer HW, Inskeep WP. Carbon dioxide fixation by *Metallosphaera yellowstonensis* and acidothermophilic iron-oxidizing microbial communities from Yellowstone National Park. *Applied and Environmental Microbiology*. 2014;**80**:2665-2671. DOI: 10.1128/AEM.03416-13
- [158] WoRMS. Annelida [Internet]. 2004. Available from <http://www.marinespecies.org/aphia.php?p=taxdetails&id=882> [Accessed: 2017-04-04]
- [159] Fauchald K. The polychaete worms, definitions and keys to the orders, families and genera. In: *Natural History Museum of Los Angeles County, Science Series*. Los Angeles, CA (USA); 1977. p. 188
- [160] Grube AE. Die Familien der Anneliden. *Archiv für Naturgeschichte*. 1850;**16**:249-364 <http://www.biodiversitylibrary.org/item/31180#page/257/mode/1up>

- [161] McHugh D. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**:8006-8009. PMID: PMC21546
- [162] Rouse GW, Fauchald K. Cladistics and polychaetes. *Zoologica Scripta*. 1997;**26**:139-204. DOI: 10.1111/j.1463-6409.1997.tb00412.x
- [163] Jones ML. *Riftia pachyptila*, new genus, new species, the vestimentiferan worm from the Galápagos rift geothermal vents (Pogonophora). *Proceedings of Biological Society of Washington*. 1981;**93**:1295-1313. <http://biodiversitylibrary.org/page/34600182>
- [164] Webb M. *Lamellibrachia barhami*, gen. nov. sp. nov. (Pogonophora), from the Northeast Pacific. *Bulletin of Marine Science*. 1969;**19**:18-47. <http://www.ingentaconnect.com/content/umrsmas/bullmar/1969/00000019/00000001/art00002>
- [165] Hattori M, Oba T, Kanie Y, Akimoto K. Authigenic carbonates collected from cold seepage area off Hatsushima Island, Sagami Bay, Central Japan. *JAMSTEC Journal of Deep Sea Research*. 1994;**10**:405-416 (in Japanese with English abstract and captions). http://www.godac.jamstec.go.jp/catalog/data/doc_catalog/media/shinkai10_33.pdf
- [166] Hattori M, Kanie Y, Oba T, Akimoto K. Environmental conditions of carbonates and chemosynthetic animal communities associated with cold seepage zones along the subduction zone in Sagami Bay, Central Japan. *Kaseki (Fossils, Palaeontological Society of Japan)*. 1996;**60**:13-22 (in Japanese with English abstract and captions). DOI: 10.14825/kaseki.60.0_13
- [167] Gollner S, Riemer B, P Arbizu PM, Le Bris N, Bright M. Diversity of meiofauna from the 9°50'N East Pacific rise across a gradient of hydrothermal fluid emissions. *PLoS One*. 2011;**5**:e12321. DOI: 10.1371/journal.pone.0012321
- [168] Boetius A. Microfauna–macrofauna interaction in the seafloor: Lessons from the tube-worm. *PLoS Biology*. 2005;**3**:e102. DOI: 10.1371/journal.pbio.0030102