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Discovery of asphalt seeps in the deep Southwest Atlantic off Brazil

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ABSTRACT

The discovery and description of cold seeps with deep-sea chemosynthetic communities in the Southwest Atlantic Ocean are still incomplete, despite the large proven oil and gas reserves off the coast of Brazil. In the southeastern Brazilian continental margin, where over 71% of the country's oil and gas production takes place, there are previous geological and qualitative biological evidence of seep biota associated with pockmarks on the upper slope of the Santos Basin. In order to further study seep ecosystems on the Brazilian margin, a deep-sea investigation named *Iatá-Piúna cruise* was conducted using the human-occupied vehicle *Shinkai 6500* off Brazil's southeast continental margin. Asphalt seeps were discovered on the seafloor of the North São Paulo Plateau from depths of 2652–2752 m, representing only the third discovery of this type of seep worldwide, following those in the Gulf of Mexico and off Angola. Video and isotopic analyses indicated a number of megabenthic animals in the asphalt seeps in the North São Paulo Plateau and revealed typical deep-sea heterotrophic and photosynthesis-based fauna occupying hard substrates provided by the asphalt seep. There was no evidence of chemosynthesis-based megabenthic fauna such as vesicomyid clams, *Bathymodiolus* mussels, and siboglinid tube worms, or any sediment bacterial mats, gas seepage, and carbonate rock in/around the seeps. The benthic fauna was composed mainly of sponges (ca. 15 species), such as the hexactinellids *Caulophacus* sp., *Poliopogon amadou*, *Saccocalyx pedunculatus*, *Farrea occa* and cf. *Chonelasma choanoides*; besides typical deep-sea isidid octocorals, brisingid starfishes and galatheid crabs. The $\delta^{13}C$ values of poriferan sponges suggested a heterotrophic and pelagic nutrition. Geochemical analyses of asphalt revealed a heavy biodegradation of hydrocarbon molecules, supported by the depletion of light *n*-alkanes and other labile compounds. This advanced asphalt biodegradation is the likely reason for the absence of chemosynthetic communities at these seep sites.

1. Introduction

Deep-sea chemosynthetic ecosystems have been widely discovered in the world ocean, with over 280 known hydrothermal vent fields and cold seeps globally (Baker and German 2004; Levin, 2005; Baker et al., 2010; German et al., 2011). Chemosynthetic organisms inhabiting such areas normally live under variable levels of sulfide and hydrocarbon (Van Dover, 2000; Levin, 2005) and their numeric densities and biomasses are high when compared with the background food-limited deep-sea communities based on photosynthesis (Bernardino et al., 2012). Typical megafaunal organisms have a unique ecology and physiology, relying on chemosynthesis for part or most of their nutrition, and may achieve high biomass levels in these ecosystems (Gage and Tyler, 1992; Van Dover, 2000; Fujikura et al., 2008; Kiel, 2010). Vesicomyid clams, *Bathymodiolus* mussels, and siboglinid tube worms are typical endemic benthic invertebrates present in seep and vent communities in various regions of world oceans. These endemic taxa show a concurrence in different areas in spite of great geographical

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Table 1
Dive log of the HOV Shinkai 6500 on the São Paulo Plateau, off Brazil, during the 2nd leg of the *Iatá-piúna*cruise.

Dive no.	Date	Area	Site	Start point			End point		
				Latitude (S)	Longitude (W)	Depth (m)	Latitude (S)	Longitude (W)	Depth (m)
1340	12 May, 2013	South São Paulo Plateau	Point 3	22°57.20′	38°35.96′	3148	22°55.60′	38°35.38′	3079
1341	13 May, 2013	South São Paulo Plateau	Point 4	22°57.28′	38°18.89′	3602	22°57.18′	38°21.64′	3434
1342	14 May, 2013	South São Paulo Plateau	Point 1	22°56.44′	39°08.14′	2997	22°54.38′	39°06.07′	2967
1343	15 May, 2013	North São Paulo Plateau	Point 6	20°41.63′	38°38.20′	2728	20°40.24′	38°38.10′	2456
1344	16 May, 2013	North São Paulo Plateau	Point 5	20°38.03′	38°47.13′	2499	20°39.12′	38°46.88′	2307
1345	17 May, 2013	North São Paulo Plateau	Point 6	20°43.20′	38°39.20′	2708	20°41.48′	38°38.16′	2706
1346	19 May, 2013	North São Paulo Plateau	Point 6	20°44.52′	38°40.14′	2730	20°43.24′	38°39.19′	2692
1347	20 May, 2013	North São Paulo Plateau	Point 6	20°45.48′	38°41.32′	2804	20°44.26′	38°40.19′	2642
1348	21 May, 2013	South São Paulo Plateau	Point 2	22°57.82′	38°56.13′	3127	22°56.39′	38°56.42′	3058

distance. To understand biogeographical distribution processes, deep-sea biologists are attempting to discover chemosynthetic communities in unexplored deep-sea areas.

Major deep-sea chemosynthetic communities include hydrothermal vents of active volcanoes and cold seeps of subduction zones, mainly distributed along oceanic plate boundaries (Sibuet and Olu, 1998; Ramírez Llodra et al., 2003; Fujikura et al., 2008; Van Dover, 2011). Chemosynthetic communities also associated with hydrocarbon seeps (also known as asphalt, gas, oil or petroleum seeps) were found along the Angolan margin off Africa and in the Gulf of Mexico, North Sea, and Sea of Okhotsk (Kennicutt et al., 1985; Zonenshayn et al., 1987; Hovland and Thomsen, 1989; MacDonald et al., 2004; Jones et al., 2014; Sahling et al., 2016). However, information on communities associated with these seeps remains limited, and only two fields, off Angola and in the Gulf of Mexico have been studied with respect to their biota (MacDonald et al., 2004; Jones et al., 2014; Sahling et al., 2016).

In the South Atlantic, cold seep communities with methane rich habitats were also discovered on the African and Brazilian margins (Olu et al., 2009; Ritt et al., 2011). South Atlantic African seeps typically support active ecosystems with gas seepages originating from pockmarks and mud volcanoes, and the seep sediments harbor distinct fauna (Ritt et al., 2011). Recognition and careful scientific description of seep ecosystems off Brazil are still lacking, despite consistent prospecting for industrial oil and gas. The lack of proper funding for scientific expeditions has hampered the description of seeps on the deep Brazilian margin for decades, and the first evidence of seep biota came from sampling pockmarks at 700 m in the Campos Basin in the early 2000s (Sumida et al., 2004), but there are further geological evidence for presence of seep sites on the upper slope of Campos Basin (e.g. Kowsmann and de Carvalho, 2002), and new seeps have recently been described on the southern Brazilian margin in areas that have attracted industrial interest in exploitation for methane hydrates (Miller et al., 2015; Giongo et al., 2015).

The Campos and Santos Basins on the Southeast Brazilian margin are responsible for over 71% of Brazil's oil production, but no seep communities have been described in this area preventing an adequate basin-wide management of those vulnerable ecosystems (Cordes et al., 2016; Almada and Bernardino, 2017). This margin is relatively well studied with respect to its oceanographic patterns and biological communities (Bernardino et al., 2016), but there is yet no evidence of chemosynthetic ecosystems in this region where abundant oil and gas reservoirs exist. Although it is impossible to conduct scientific investigations in these basins due to commercial oil production, we instead conducted deep-sea biological and geochemical investigations during the joint Japanese-Brazilian *Iatá-Piúna* cruise in the São Paulo Plateau, on the lower slope depths of the Espírito Santo Basin (2500–3600 m), just north of Campos Basin. Legal obligations did not allow investigations on upper slope areas where most evidence for seep communities exists (e.g., Sumida et al., 2004; Kowsmann and de Carvalho, 2002). Using the human-occupied vehicle (HOV) *Shinkai 6500* of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), we found asphalt seeps at a depth of 2700 m on the São Paulo Plateau colonized by non-chemosynthetic megafaunal organisms.

Our results indicated that these asphalt seeps do not sustain chemosynthetic production and endemic symbiont-bearing fauna, but resemble other non-seep ecosystems with diverse benthic biota. Geochemical results indicated heavy biodegradation of hydrocarbon molecules on the asphalt seeps, suggesting that advanced biodegradation is the likely reason for the absence of chemosynthetic communities at these seeps.

2. Materials and methods

2.1. Survey area and dive surveys

The deep seafloor survey for seep sites was conducted during the 2nd leg of the *Iatá-Piúna* cruise in the São Paulo Plateau from May 10–24, 2013, using the HOV *Shinkai 6500* and her support vessel *Yokosuka* of JAMSTEC. Six survey sites were chosen (points 1 to 4 were on the South São Paulo Plateau, and points 5 and 6 were on the North São Paulo Plateau) after multichannel seismic surveys that indicated the existence of geologic faults and salt diapirism. Nine diving surveys were carried out at points 1–6 (Table 1, Fig. 1). Before the diving surveys, we conducted topographic mapping of the area using a ship-mounted Kongsberg Maritime AS EM122 multibeam echo sounder. After mapping, nine submersible dives were carried out along all sites (Table 1). *In-situ* observations were made with two high-resolution color TV cameras (NEC NC-H1000, Tokyo, Japan, and Sony FCB-H11, Tokyo, Japan) and a digital still camera (Olympus E-PL6, Olympus, Tokyo, Japan). Submersible positioning was performed using a super-short baseline transponder coupled to a GPS. Water temperature, dissolved oxygen, and salinity were measured with a CTD (Seabird SBE-19 + SBE-43, Sea-Bird Electronics, Bellevue, WA, USA).

2.2. Image surveys and sample collection

During each dive, video surveys were recorded along the planned HOV track to identify epibenthic organisms and demersal fauna along the selected target areas. Megabenthic specimens were collected by the manipulators and suction sampler attached to the HOV for taxonomic identification and isotopic analysis. Image analysis and taxonomic identification of fauna were conducted based on both video and photo images and on laboratory identification by experts. Biological specimens were fixed in 99.5% ethanol or frozen at –80 °C. Asphalt and sediment samples were also collected by the manipulator and preserved for geochemical and isotopic analysis.

Particulate organic matter (POM) was separated from the seawater samples obtained at sites #5 and #8 in Fig. 2 by filtration using preheated (450 °C, 3 h) glass-fiber filters (GC-50, Advantec, Tokyo, Japan) onboard the research vessel *Yokosuka*. Sediment core samples were collected at sites #5 and #7 in Fig. 2 by push corers operated by

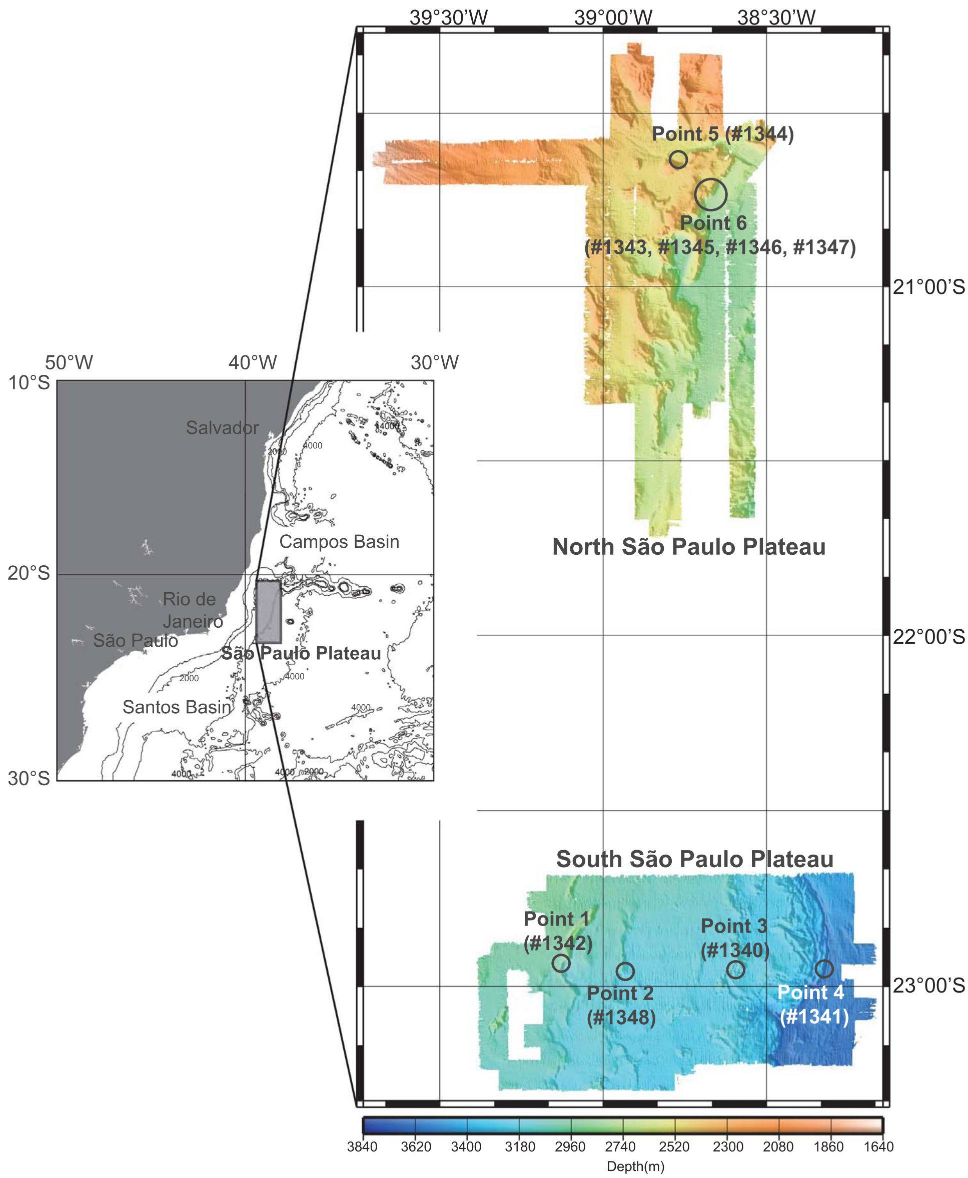

Fig. 1. Bathymetric map and location of the diving survey points on the São Paulo Plateau, off Brazil. A: Black circles indicate dive sites (points 1–6) by the HOV *Shinkai 6500*. Points 1 to 4 are on the South São Paulo Plateau and points 5 and 6 are on the North São Paulo Plateau.

the manipulators of *Shinkai 6500*, and after collection the core samples were divided into several layers at 4- to 5-cm intervals onboard the *Yokosuka* and stored in a freezer at –80 °C until analysis. Asphalt samples were also collected by the manipulators at sites #5 and #9 in Fig. 2 and stored in a freezer at –20 °C until analysis. Fissure filling-type asphalt samples were sampled for the analysis and one large hexactinellid sponge collected at site #7 was stored in a freezer at –80 °C until analysis.

2.3. Stable isotope and chemical analysis

The $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions of benthic specimens, sediment cores, POM and oil samples were analyzed. Preparation of the specimens for carbon and nitrogen isotopic measurements followed the procedures described in previous studies (Mizota et al., 1999; Yamanaka et al., 2008). Each specimen was separated randomly into four parts and those parts were treated with 1 N HCl to remove carbonate, then freeze-dried and pulverized. Only top 4 cm layer of the core samples was cut vertically into three columns, then each column was freeze-dried and pulverized using an agate mortar and pestle. Total organic carbon (TOC) and total nitrogen (TN) concentrations in the sediment samples and POM on the filters were measured using dry samples, after adding 1 N HCl followed by vacuum drying to remove carbonates and analyzed by the dry combustion method using an elemental analyzer (EuroVector EA3000, GV Instruments, UK). Isotope carbon and nitrogen signatures of the pulverized specimens, carbonate-free sediment and POM samples and oil samples were determined using a EuroVector EA3000 analyzer coupled to an isotope ratio mass spectrometer (IsoPrime, GV Instruments, Manchester, UK). All values are expressed as common $\delta^{13}C$ and $\delta^{15}N$ notations, per mil variation (‰) relative to V-PDB and air dinitrogen, respectively. The analytical precision of the $\delta^{13}C$ and $\delta^{15}N$ measurements was greater than ± 0.2‰ during the present study.

Elemental compositions of asphalt samples were measured by the dry combustion method using the elemental analyzer (EA3000, GV Instruments). The chemical compositions of oil samples were measured according the procedure described by Yamanaka et al. (2000). The oil samples were quantitatively separated by silica-gel column chromatography into three fractions: aliphatic (F1) and aromatic hydrocarbons (F2); and nitrogen, sulfur, and oxygen (NSO) plus asphaltic compounds

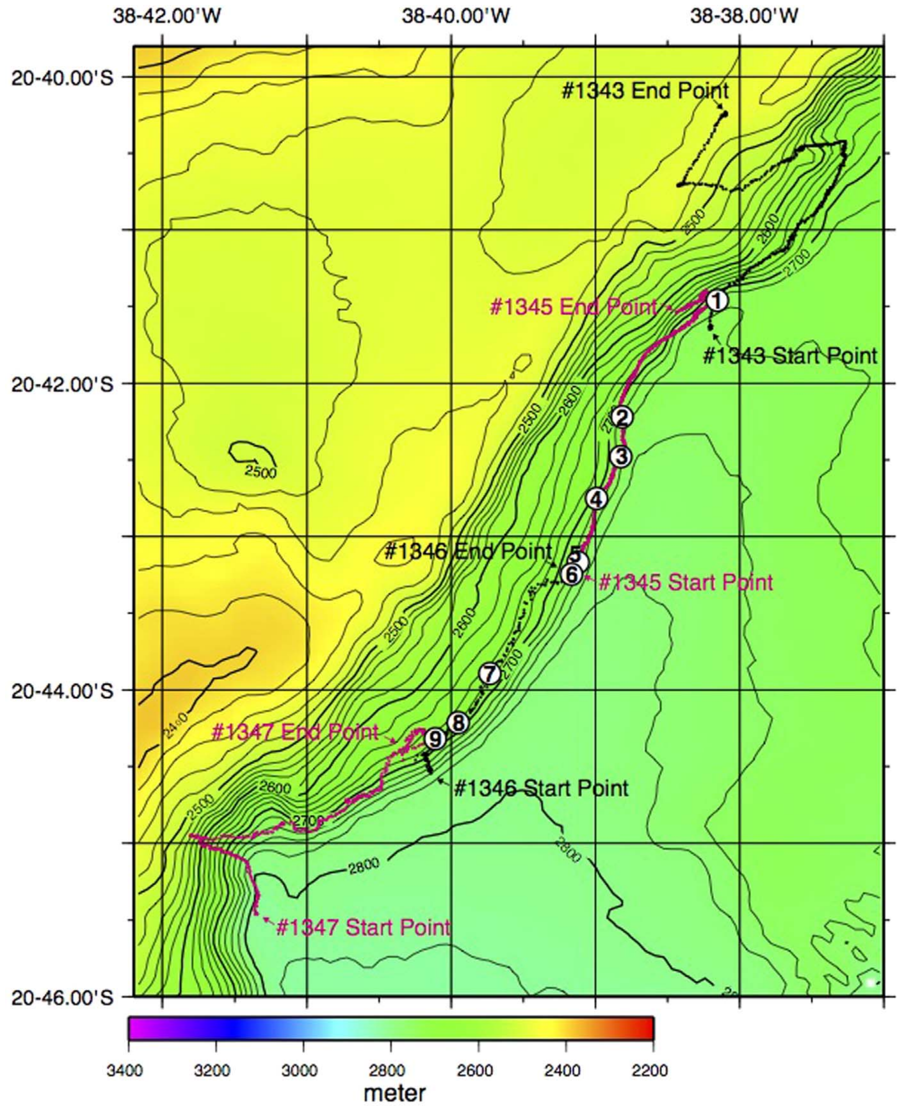

Fig. 2. Asphalt seep sites (white circles) along the dive tracks (#1343, #1345, #1346, and #1347) of the HOV *Shinkai 6500* at point 6, the North São Paulo Plateau. Asphalt samples were collected at sites #5 and #9, sediment core samples were collected at sites #5 and #7, seawater samples for POM analysis were collected at sites #5 and #8, and sponge sample provided for analyses was collected at site #7.

(F3). Fractions F1 and F2 were measured using a gas chromatography/flame ionization detector (GC/FID; model GC-17A, Shimadzu, Kyoto, Japan).

3. Results

3.1. Asphalt seeps

The asphalt seeps were distributed on the lower slope of the Southeast Brazilian margin at depths of 2652 m to 2752 m at point 6 on the North São Paulo Plateau (Fig. 2). Visual observations indicated dark-colored hard bottoms suggestive of an oil origin, covered by living epifaunal organisms, intermingled or lightly covered by foraminiferan ooze. These asphalt extrusions were clearly distinguishable from rocks coated by manganese oxide as the angular edge and smooth surfaces of manganese oxide rocks were identified due to the very thin manganese oxide layer. The asphalt samples (dark-colored substrates) were easily collected by the manipulator of the HOV and rapidly changed to a sticky oil and taffy-like state upon atmospheric exposure and at the temperature onboard. Video images suggested various types of asphalt/oil extrusion forms in the area, including reticulate filling types in the fissures of mudstone outcrops (Fig. 3A, B), linearly extruding types (Fig. 3C), lump types (Fig. 3D), scattered-stick types (Fig. 3E), and mound types (Fig. 3F). We did not observe any indication of active seepages including gas gushing and bacterial mats around the asphalt seeps.

The São Paulo Plateau background seafloor was typically covered by a calcium carbonate ooze containing dead shells of planktonic gastropods and foraminiferans. Outcrops of mudstone also occurred. Most of those outcrops were thin and covered by black manganese oxide except in the asphalt seep areas. Manganese oxide crusts and nodules were also common on the São Paulo Plateau. By collapses of gas hydrate, topographic pockmarks occasionally occurred on deep-sea seafloor. However, no pockmarks were observed on the lower slope of the São Paulo Plateau in the topographic surveys. Near bottom water temperature, salinity, and dissolved oxygen values ranged from 2.51 to 2.63 °C, 34.90 to 34.92, and 5.06 to 5.21 ml/l, respectively. There was no evidence of active oil or gas seepages at the other points investigated (points 1 to 5).

3.2. Chemicals and stable isotopes of asphalt

Geochemical analysis of asphalt samples revealed a high variability in carbon content (*c.* 60–90%, Table 2), but with similar nitrogen

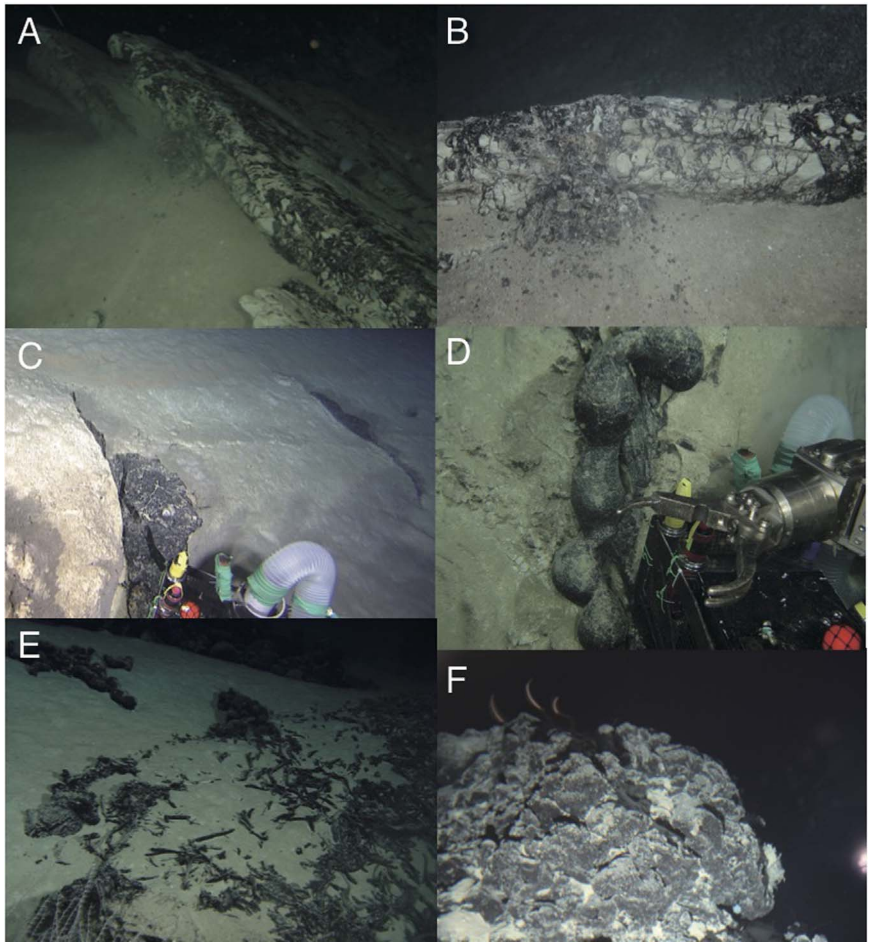

Fig. 3. Various types of seeped asphalt at point 6 on the North São Paulo Plateau. (A) Reticulate filling type at a depth of 2721 m, (B) reticulate filling type (observed from the opposite side) at a depth of 2721 m, (C) linearly extruding type at a depth of 2727 m, (D) lump type at a depth of 2720 m, (E) scattered-stick type at a depth of 2720 m, and (F) mound type at a depth of 2667 m.

content (about 3%). The carbon content of asphalt sample 6K#1345 (*c.* 60%) was significantly lower than that of sample 6K#1346 (*c.* 90%). This may be due to differences in their water contents, which were not measured precisely although sample 6K#1345 was richer in water relative to the other (Table 2). Despite the δ^{15}N values differed slightly between samples, bulk isotopic ratios were close to 0‰ (Table 2). The asphalt δ^{13}C values were almost uniform at –25‰. Hydrocarbon compositions (F1, F2, and F3) showed that the asphalt samples were abundant in F3 components but also contained aliphatic and aromatic hydrocarbons (F1 and F2) (Table 2). Despite the lack of distinguishable apparent peaks of fractions F1 and F2 in GC/FID chromatograms, significant humps of an unresolved complex mixture (UCM) were observed (Fig. 4).

3.3. Representative organisms

Megafaunal organisms associated with the asphalt seeps on the São Paulo Plateau included sponges, gorgonians (Isididae; Fig. 5A, D, G), sea stars (Brisingidae; Fig. 5B), anomuran crabs (*Munidopsis*; Fig. 5A, D), and tunicates (*Megalodicopia*; Fig. 5H). Those benthic animals except for anomuran crabs were sessile and obviously attached to the hard substrates formed by the seeps (Fig. 5). Sponges were visually dominant with at least 16 species, most of them hexactinellids. Despite their occurrence on the seep areas, many species were also present on mudstone outcrops at background areas (Fig. 5I). Sponges that could be identified included the hexactinellids *Caulofacus* sp. (Fig. 5A, D), cf. *Chonelasma choanoides*, *Farrea occa* (Fig. 5C, E), *Poliopogon amadou* (Fig. 5A, B, I) and *Saccocalyx pedunculatus* (Fig. 5F).

Overall, the species biodiversity of megabenthic and benthopelagic animals did not differ between the North and South São Paulo plateaus. Hexactinellid sponges, isidid and primnoid gorgonians, *Psychropotes* spp. sea cucumbers (Elasipodida), brittle stars (Ophiurida), *Munidopsis* squat lobsters, shrimps (Aristeidae and Pandalidae), cirrate octopuses (*Cirrithauma* and *Opisthoteuthis*), and fish (Bathysauridae and Ipnopidae) were rarely seen as representative animals.

Table 2
Isotopic and elemental compositions of asphalt samples and their chemical compositions. HC: hydrocarbon, NSO: nitrogen, sulfur, oxygen, TOC: total organic carbon, TN: total nitrogen.

Sample ID no.	Number of sample	Isotopic ratios		Contents		Aliphatic HC	Aromatic HC	Asphalt + NSO
		δ^{13}C (‰)	δ^{15}N (‰)	TOC (wt%)	TN (wt%)	F1 (wt%)	F2 (wt%)	F3 (wt%)
6K#1345R01	3	–25.2 ± 0.2	+ 0.4 ± 0.3	60 ± 4	2.8 ± 0.6	8.2	10.3	81.5
6K#1346R01	3	–25.0 ± 0.2	+ 1.2 ± 0.3	90 ± 3	3.0 ± 0.6	13.4	17.5	69.1

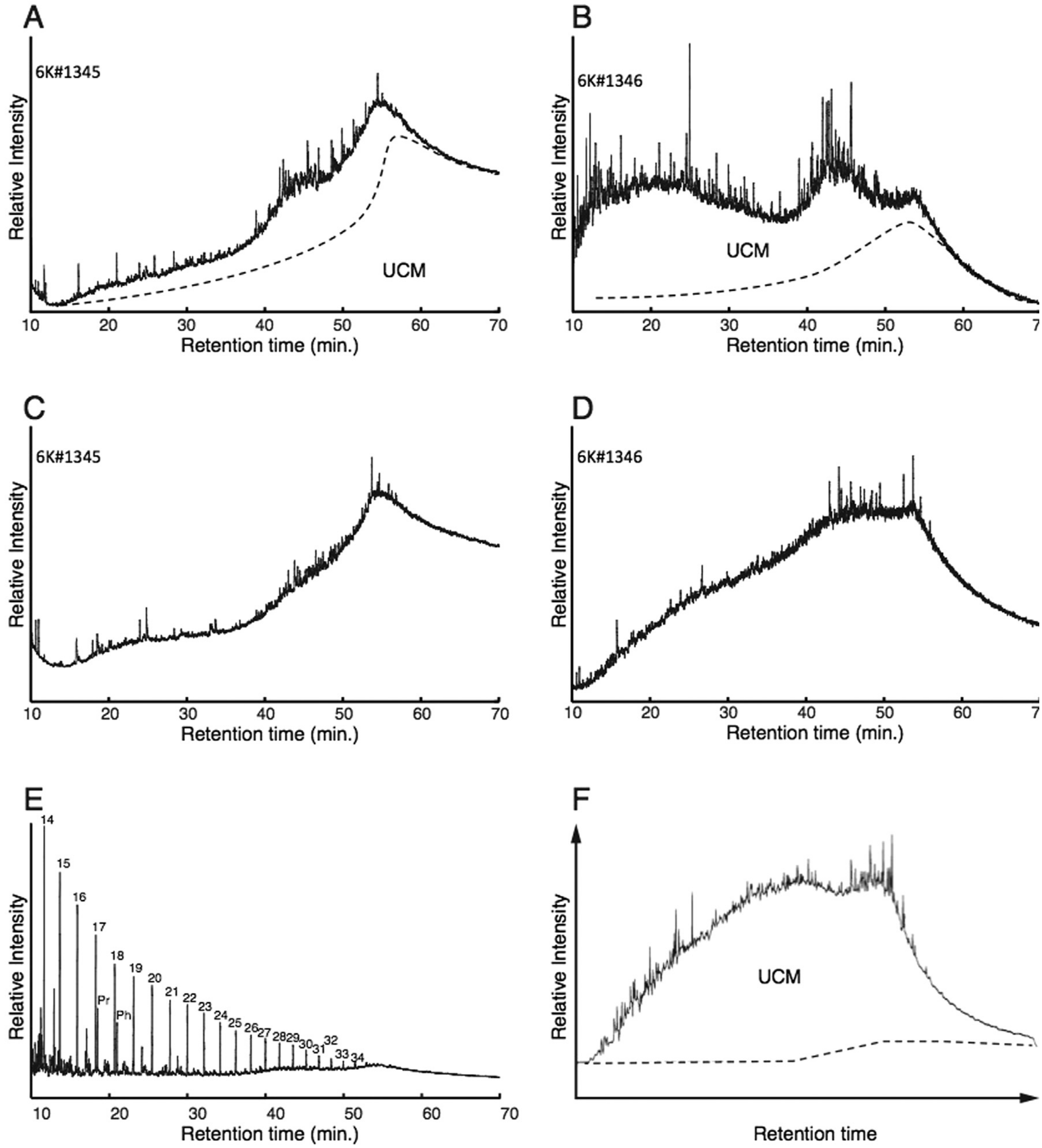

Fig. 4. Gas chromatograms of the asphalt from point 6. (A, B) Aliphatic fractions (F1) of the asphalt samples collected in dives #1345 and #1346, respectively. The broken lines show the approximate zero signal. Significant humps of the unresolved complex mixture (UCM) are observed. (C, D) Aromatic fractions (F2) of the asphalt samples collected in dives #1345 and #1346, respectively. Similar humps of UCM observed in A and B also appeared in this fraction. (E) Example of a whole-oil gas chromatogram from crude oil recovered from an oil well located in the eastern Atlantic Ocean (unpublished data by T. Yamanaka). The numbers indicate n-alkanes by carbon number; Pr and Ph indicate pristine and phytane, respectively. (F) The total hydrocarbon fraction from a heavily biodegraded oil sample appearing in Killops and Killops (2005; p. 304, Fig. 7.8). The broken line shows the approximate zero signal.

3.4. Stable isotopes, organic carbon and nitrogen

Chemical and isotopic compositions of sponges, surface sediments, and POM are shown in Table 3. The δ^{13}C values of those samples had relatively narrow ranges of from –24 to –20‰, while their δ^{15}N values were slightly variable, ranging from +2 to +11‰. Total organic carbon (TOC) and total nitrogen (TN) contents of the surface sediment samples were *c.* 0.1 and *c.* 0.03 wt%, respectively, and TOC and TN of the POM samples were 50–100 μg/L and 2–6 μg/L, respectively. The δ^{13}C value of sponge samples showed intermediate values between the surface sediment and POM samples, while the δ^{15}N values of sponges were the highest among the samples. The carbon and nitrogen contents of the sponges were *c.* 2% and 0.1%, respectively. The sponges are considered to belong to the Hexactinellida, and therefore their major constituent is inorganic silica mineral.

4. Discussion and conclusions

4.1. Asphalt seeps without benthic chemosynthetic communities

This is the first investigation using an HOV for the discovery of deep-sea chemosynthetic biological communities off Brazil. We discovered *in-situ* asphalt seeps at point 6 on the North São Paulo Plateau, but no gas seeps were observed in the survey area. Authigenic carbonates are usually produced as a result of the oxidation of methane from the source in active hydrocarbon seeps (e.g., Cordes et al., 2007; Jones et al., 2014), but they were also not observed on the São Paulo Plateau. These image evidences indicate that the asphalt seep in the Sao

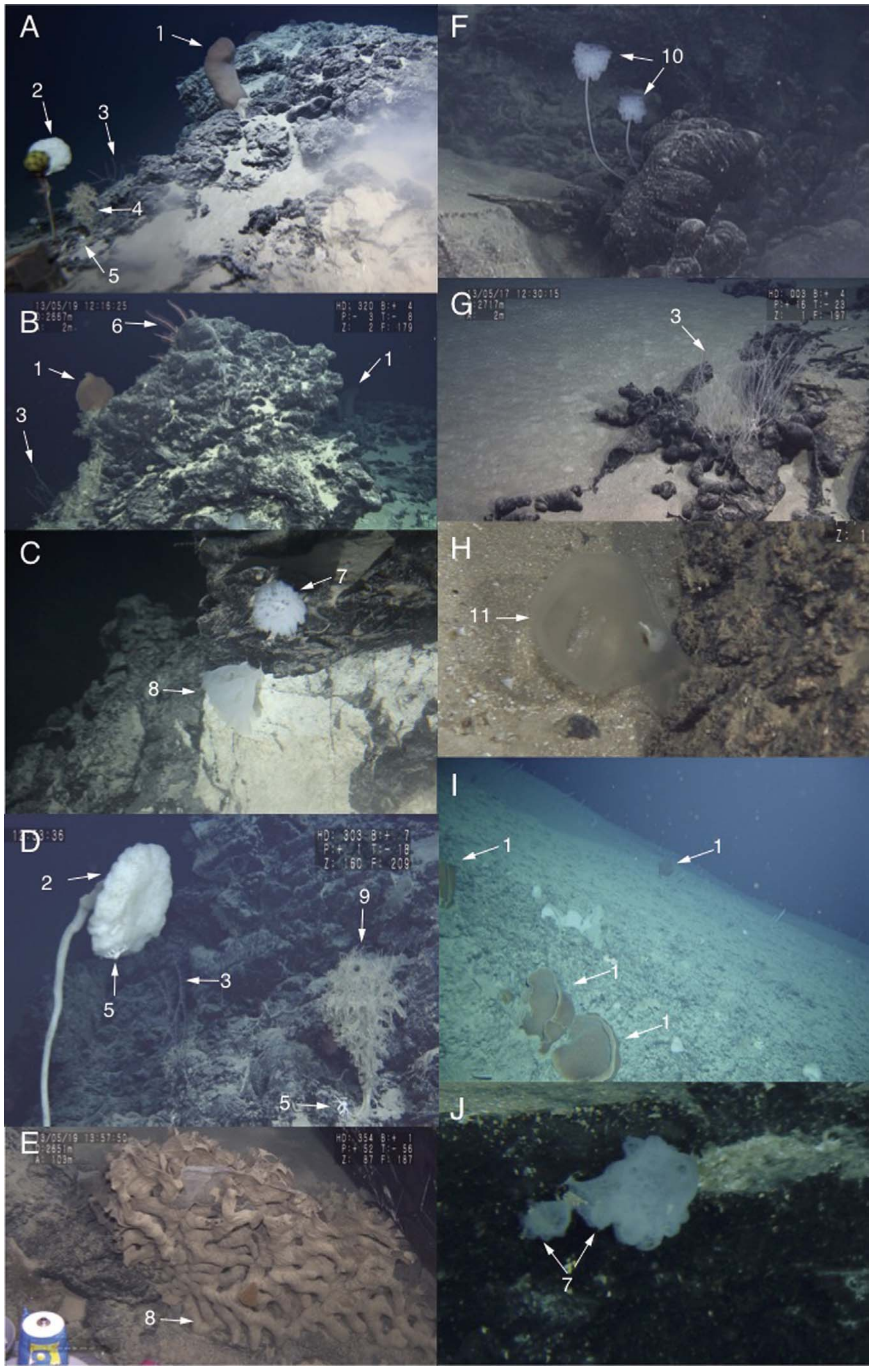

Fig. 5. *In-situ* images of representative benthic animals in the asphalt seeps (A to H) and outside of the asphalt seeps (I and J) at point 6, the North São Paulo Plateau. (A) Two species of hexactinellid sponges, the brown *Poliopogon amadou* (1) and *Caulophacus* sp. (2); one species of gorgonian of the family Isididae (3) and one not identified organism (4); and an anomuran crab, *Munidopsis* sp. (5). (B) *Poliopogon amadou* (1); an isidid gorgonian (3); and a sea star of the order Brisingida (6). (C) An unidentified hexactinellid sponge (7) and the hexactinellid *Farrea occa* (8). (D) *Caulophacus* sp. (2); an isidid gorgonian (3); unidentified organism (9); and two anomuran crabs, *Munidopsis* sp. (5). (E) *Farrea occa* (8). Note that most of the sponge tissue is dead (brown coloration). (F) The hexctinellid *Saccocalyx pedunculatus* (10). (G) An isidid gorgonian (3). (H) The ascidian *Megalodicopia* sp. (11). (I) Several sponges including brown *Poliopogon amadou* (1) living outside the seep. (J) Two unidentified hexactinellid sponges (7) living outside the seep. The black surface is caused by manganese oxide, not by asphalt seepage. Because the angular edge and smooth surfaces of manganese oxide rocks were seen due to very thin manganese oxide layer.

Paulo Plateau is not active and does not support typical chemosynthetic communities at this site, contrary to other asphalt seeps in the southern Gulf of Mexico and off Angola (Kennicutt et al., 1985; MacDonald et al., 2004; Jones et al., 2014). But, the hard substrata provide habitat for a number of deep-sea epifaunal organisms including sponges and echinoderms.

Deep-sea chemosynthetic communities at active seeping habitats are characterized by dense patches of living fauna known to have chemoautotrophic symbionts (i.e., chemosynthesis-based animals), including vesicomyid clams, *Bathymodiolus* mussels, and siboglinid tube worms. Asphalt cold seeps at the African margin and on the Gulf of Mexico also harbour pogonophoran tube worms and symbiont-harboring bivalves (Vesicomyidae, *Bathymodiolus,* and *Solemya*) at active seeping sites (e.g., MacDonald et al., 2004; Jones et al., 2014), but they were not observed on the São Paulo Plateau.

Instead, a rich non-chemosynthetic fauna including sponges (Hexactinellida, Porifera), gorgonians (Isididae, Cnidaria), sea stars (Brisingida, Echinodermata), anomuran crabs (*Munidopsis*,

Table 3
The $\delta^{13}C$ and $\delta^{15}N$ stable isotopic compositions and, carbon and nitrogen contents of sponges, POM and surface sediments at point 6 on the North São Paulo Plateau. n.d.: not determined, POM: particulate organic matter, TOC: Total organic carbon, TN: Total nitrogen.

Sample	ID no.	Number of samples	Isotopic ratio		Contents	
			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TOC	TN
Hexactinellida, Porifera	6K#1346B01	4[a]	–22.4 ± 0.3	+10.6 ± 0.4	2.0 ± 0.5 wt%	0.10 ± 0.04 wt%
POM	6K#1345NR	1	–23.4	n.d.	*c.* 50 µg C/L	*c.* 2 µg/L
	6K#1346NG	1	–23.6	+2.4	*c.* 100 µg C/L	*c.* 6 µg N/L
Surface sediments	6K#1345C1	3[b]	–20.7 ± 0.2	+7.6 ± 0.3	0.15 ± 0.02 wt%	0.02 ± 0.01 wt%
	6K#1346C1	3[b]	–21.1 ± 0.2	+7.2 ± 0.3	0.11 ± 0.02 wt%	0.03 ± 0.01 wt%

[a] Separately measured four parts from one large specimen.
[b] Top 4 cm-thick layer of the core samples was cut vertically into three columns and each column was homogenized using an agate mortar and pestle.

Arthropoda), and tunicates (*Megalodicopia*, Chordata) occurred above hard substrata in the asphalt seeps on the North São Paulo Plateau as representative taxa (Fig. 5), although none formed dense patches. Hexactinellid sponges, isidid gorgonians, and brisingid sea stars are filter feeders, and anomuran crabs and *Megalodicopia* tunicates are carnivores. In calculations of chemosynthetic community endemism, no sponges and 7 cnidarian species were recognized (Wolff, 2005). Recently, two species of sponge, *Characella* sp. at a hydrothermal vent site in the Ogasawara (Bonin) Islands area and *Pachastrella* sp. at a seep site in the Gulf of Mexico, both of the Class Demospongiae, have been found to harbor thioautotrophic symbiotic bacteria (Nishijima et al., 2010). However, all hexactinellid species from the present oil seeps differ in their taxonomic position at the class level from previously identified species. Several endemic cnidarian species belonging to the Actiniaria were described from hydrothermal vents and whale falls (e.g., Reimer et al., 2007; Zelnio et al., 2009) but they also differ in their taxonomic position at the class level from the present isidid species. Three galatheid species, *Kiwa hirsute*, *Kiwa puravida*, and *Shinkaia crosnieri* rely on visible epibiotic bacteria on their body setae as a food source (Goffredi et al., 2008; Thurber et al., 2011; Watsuji et al., 2014), but those epibiotic bacteria were not noted on the setae of *Munidopsis* sp. from the present asphalt seeps. Therefore, it seems that megabenthic animals in the asphalt seeps on the North São Paulo Plateau are photosynthesis-based, not chemosynthesis-based, animals, as further supported by isotopic signatures discussed below.

4.2. Chemical characteristics of asphalt seep and its origin

The samples from the asphalt seep were abundant in NSO plus asphaltic components, and their compositions in a ternary diagram were outside the typical crude oil range (Fig. 6). The elemental composition of typical crude oil was reported to be approximately 82–87% carbon and 0.1–1.5% nitrogen (e.g., Killops and Killops, 2005). The carbon contents of the samples were almost within the range of typical crude oil or less, but the samples were richer in nitrogen (Table 2). Those characteristics are considered to result from the lack of aliphatic and aromatic fractions and abundance of NSO plus asphaltic components in the samples relative to typical crude oil. In addition to those results, the lack of simple hydrocarbons such as *n*-alkanes observed in the GC analysis of fractions F1 and F2 (Fig. 4) suggests that the asphalt samples were subjected to heavy biodegradation (e.g., Killops and Al-Juboori, 1990) and the loss of easily mobile and labile hydrocarbon components during migration and in-reservoir alteration such as geochromatography, phase separation, gas de-asphalting, etc. (e.g., Mackenzie, 1984; Wenger et al., 2002). According to the geological and geochemical study the oil was filling fissures and faults observed on rock outcrops (Freire et al., unpublished data). The outcrops were considered to form along an active fault system driven by salt diapirism. Although when the outcrops were exposed on seafloor is not known, due to the excellent preservation condition of microfossils the host rocks may not have undergone deep burial or severe compaction (Freire et al., unpublished data). According to the appearance of asphalt (solid oil) filling fissures of the host rock as shown in Fig. 3, the oil was already solid when the outcrop was exposed. It means that the fluid oil had been already escaped before exposing. Some of the asphalt observed on the seafloor was covering the unconsolidated sediment, suggesting that they were seeping recently. But the morphological feature of seeped asphalt on the seafloor, such as lump and mound types, seems to seep slowly and intermittently. Those features imply that the observed asphalt was exposed on the seafloor by erosion and/or collapse of cliff in addition to intermittent slow seeping. Therefore, it is considered that those asphalt are subjected to biodegradation near seafloor before the seep and during the seeping. Those asphalt seeps are possibly still active as mentioned by Freire et al. (unpublished data) from the perspective of geological time scale. However, the flux of chemical species such as methane from the asphalt seep is insufficient for sustain of chemosynthesis-based fauna. Therefore, from the perspective of biological metabolism those seeps are plausibly inactive.

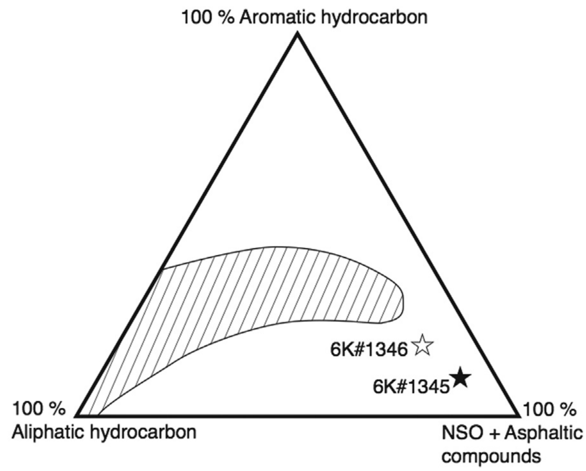

Fig. 6. Ternary diagram of aliphatic hydrocarbons (F1), aromatic hydrocarbons (F2), and NSO plus asphaltic compounds (F3). Typical crude oil falls within the hatched area (Tissot and Welte, 1984). Star symbols indicate the asphalt compositions in this study. Data are shown in Table 2.

The carbon and nitrogen isotopic ratios of organic matter are good indications of the source organisms (e.g., Peterson and Fry, 1987), while those ratios, especially carbon isotopic ratios of primary producers, are known to have fluctuated significantly during the late Mesozoic era (Scholle and Arthur, 1980; Arthur et al., 1988). According to a study using fossil wood fragments by Gröcke et al. (1999), the range of $\delta^{13}C$ values for terrigenous primary producers (wood $\delta^{13}C$ = –25 to –22‰) that flourished during the early Cretaceous is slightly higher than those of the present terrigenous higher plants (i.e., $\delta^{13}C$ = –28 to –25‰; Denies, 1980). However, the $\delta^{13}C$ values of marine primary producers were more enriched in ^{13}C (mean $\delta^{13}C$ value is *c.* −22‰; Denies, 1980). The $\delta^{13}C$ values of the present oil samples (*c.* –25‰, Table 2) are lower than those of organic matters obtained around the seep, suggesting that the organic matter available to benthic organisms in the site were derived from marine primary production (Table 3). The low $\delta^{15}N$ values of the asphalt samples were also distinguishably lower than those of sampled epifauna and surface sediments. Such low $\delta^{15}N$ value is often observed to terrigenous plants (Denies, 1980). Those isotopic signatures suggest that the asphalt is mainly terrigenous organic matter in addition to marine primary production (some biomarkers indicating significant contribution of marine organisms is demonstrated by Freire et al. (unpublished data)). In fact, potential oil source rocks have been proposed to deposit under lacustrine and river condition formed during the sin-rift and sag phases of the Espírito Santo Basin from the Valanginian (ca. 140 Ma) to the Late Aptian (ca. 110 Ma) and shallow to deep marine condition formed since the Early Albian (ca. 110 Ma) (França et al., 2007). Detailed geological and geochemical studies of the asphalt/oil will be reported elsewhere.

4.3. Isotope signatures of benthic organisms

Sessile sponges were major community constituents around and on the asphalt seeps. The isotopic signatures of recovered sponges were clearly different from the associated asphalt substrate (i.e. oil) (Tables 2 and 3), meaning that the asphalt was not assimilated directly by the sponges. On the other hand, POM and surface sediments, which are other possible food sources for benthic animals, were closely related to the sponges. The $\delta^{13}C$ value of sponges was similar to that of the surface sediment and POM, while the $\delta^{15}N$ value of sponges was enriched in ^{15}N relative to the other samples. The $\delta^{15}N$ values in sponges were about 3‰ greater than those in the surface sediments. Although the $\delta^{15}N$ value in POM was only measured in one sample, it was about 8‰ lower than that in the sponges. This means that the surface sediments are a plausible food source for the sponges. Sponges are generally filter feeders, and therefore POM is their most likely food source. However, in the current study environment, sponges rely on the organic matter in the surface sediment, which is possibly resuspended due to bottom current flow, and POM may be in short supply. In addition, the ultimate source of organic matter in the surface sediment is POM in the bottom water. The isotope signature of organic matter in the surface sediment enriched in ^{13}C and ^{15}N suggests that the sedimentary organic matter is subjected to microbial alteration in the sediment surface since microbial alteration of organic matter involves small isotope fractionation (Fenton and Ritz, 1988). In this study, we measured the 4-cm thick sediment sample from seafloor, suggesting that the altered organic matter is predominated in the analyzed samples.

Another potential nutrient source for hydrocarbon-associated communities is chemosynthesis. Chemosynthesis-based animals have been found around hydrocarbon seeps on the deep-sea floor in the Gulf of Mexico and Sea of Okhotsk, where dense siboglinid tube worms and *Bathymodiolus* mussels or *Conchocele* clams have been observed (Kennicutt et al., 1985; Zonenshayn et al., 1987; MacDonald et al., 2004). The $\delta^{15}N$ values of marine animals depending on photosynthesis-based primary producers generally range from −22 to 17‰ for most and > −3‰ (Sackett et al., 1965; Minagawa and Wada, 1984; Fujikura et al., 2009). The isotopic signatures of chemosynthesis-based animals are usually distinguishable from those of photosyntheis-based animals relying on photosynthetic primary production (e.g., Yamanaka et al., 2015). For example, the $\delta^{13}C$ values of thioautotrophic bacteria are less than −30‰ for strains using the Calvin cycle during carbon fixation or greater than −15‰ for strains using the reverse tricarbocylic acid cycle (e.g., Sievert et al., 2008). When the isotopic signatures of sponges obtained from deep-sea hydrothermal fields in the Okinawa Trough and Izu-Ogasawara Arc were analyzed, some had significantly low $\delta^{13}C$ values approaching −30‰ (Yamanaka et al., 2015). Those ^{13}C-depleted specimens are considered to assimilate chemosynthetic production mainly by thioautotrophic bacteria using the Calvin cycle during carbon fixation. The isotopic signatures of sponge samples recovered from the North São Paulo Plateau were in the range of those of photosynthesis-based animals, and therefore the contribution of chemosynthetic production is insignificant. Light *n*-alkanes and other labile compounds that can support chemosynthetic primary producers and heterotrophic bacteria were almost completely absent in the asphalt samples from the North São Paulo Plateau, meaning that it would be difficult for that asphalt seep environment to support chemosynthesis-based and heterotrophic animals.

In conclusion, we discovered *in-situ* asphalt seeps on the North São Paulo Plateau off Brazil. This was the third such seep discovery after those in the Gulf of Mexico and on the Angolan margin. Deep-sea chemosynthetic communities formed in/around asphalt seeps in the latter two oil fields, but no chemosynthetic community was found in the North São Paulo Plateau asphalt seeps. It appears that the North São Paulo Plateau asphalt seeps cannot support chemosynthesis-based organisms due to the lack of hydrocarbon components with a result of heavy oil biodegradation along its upward migration from pre-salt diapirs. However, the hard substrata from Asphalt mounds create habitat for a number of benthic organisms, likely supporting a local distinct community from typical deep-sea soft sediments. Currently, large deep-sea areas off Brazil's margin such as the Campos and Santos Basins near the São Paulo Plateau are active oil production fields, with many topographic pockmarks that may host endangered seep communities that need conservation (Almada and Bernardino, 2017). Therefore, it is highly likely that chemosynthetic communities will be discovered there. To understand biogeography and the evolution of deep-sea chemosynthetic communities on a global scale, extensive deep-sea investigations in those basins should be undertaken in the near future.

Conflict of interest

The authors declare that they have no conflict of interest, financial or otherwise, to report in relation to the publication of this manuscript.

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