



The first record of active methane (cold) seep ecosystem associated with shallow methane hydrate from the Indian EEZ

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Here we report the discovery of cold-seep ecosystem and shallow methane hydrates (2–3 mbsf) associated with methane gas flares in the water column from the Indian EEZ for the first time. The seep-sites are located in the Krishna–Godavari (K–G) basin at water depths of 900–1800 m and are characterized by gas flares in the water-column images. The occurrence of methane gas hydrates at very shallow depths (2–3 mbsf) at some of the seep-sites is attributed to high methane flux and conducive P–T conditions, necessary for the stability of methane hydrate. Chemosymbiont bearing Bivalves (Vesicomidae, Mytilidae, Thyasiridae and Solemyidae families); Polychaetes (Siboglinidae family) and Gastropods (Provannidae family) are also identified from seep-sites.

Keywords. Cold seep; gas flares; methane hydrate; methane and hydrogen sulfide gases; chemosymbiont.

1. Introduction

Emission of methane and hydrogen sulfide across the sediment–water interface at cold bottom water temperature conditions lead to the proliferation of cold seep ecosystem (Levin 2005; Levin *et al.* 2016). The cold seep biotic community represents a dominantly endemic ecosystem, characterized by chemosynthetic and heterotrophic fauna. The faunal diversity and spatial distribution of such ecosystem are primarily controlled by fluxes of H₂S and CH₄ at the sediment–water interface (Portail *et al.* 2015). On the other hand, growth and sustenance of such ecosystems depend on the

continuous supply of methane and hydrogen sulfide gases.

Marine cold seeps and associated ecosystems are reported from numerous sites across the globe (Sibuet and Olu-Le Roy 2002; Levin 2005; Vanreusel *et al.* 2009) including Hikurangi basin (off New Zealand); upper, middle and lower Louisiana slope/Florida escarpment (Gulf of Mexico); Nankai Trough (Japan); Congo-Angola and Nigeria margins (off West Africa), Barbados Trench, off Papua New-Guinea, Makran coast (off Pakistan), Nordic margin, Gulf of Cádiz and areas of Nile deep sea site (Eastern Mediterranean); Queen Charlotte Basin (off the Pacific north coast of British

Columbia) and below the Larsen Ice Shelf off Antarctic (Niemann *et al.* 2009).

Global interest in the genesis/sustenance of methane seeps and proliferation of the extreme ecosystems are attributed to the role of methane seepage in global warming, application in methane hydrate exploration, understanding evolutionary biology and potential in bioprospecting and ocean acidification (Le Bris *et al.* 2016). The present study is the first report on the discovery of active methane seepage sites with tell-tale chemosymbiont bearing and associated heterotrophic biotic assemblages from the Indian exclusive economic zone (EEZ). We also report here occurrences of shallow methane hydrate deposits (2–3 mbsf) from some of the cold-seep sites. The seeps were recorded off the Krishna–Godavari basin (K–G basin, Bay of Bengal) within water depths ranging from 900 (pressure: 9 MPa and temperature: 7.15°C) to 1800 m (pressure: 18 MPa and temperature: 3.55°C).

2. Geology

K–G basin is a petroliferous, pericratonic rift basin located in the eastern continental margin of India. The general stratigraphy of K–G basin comprises sediments from Early Cretaceous to Recent (Rao 1993). Owing to high sedimentation rate during

Neogene, abnormal formation pressure is observed in the deposited shale strata (Rao and Mani 1993). The presence of these deeply buried, over pressured mobile shale strata has resulted in gravity-driven shale tectonism in the K–G basin (Choudhuri *et al.* 2011). The surface imprints of the shale tectonism such as diapiric mounds and bathymetry ridges formed due to mobile shale and toe-thrust faults respectively (Dewangan *et al.* 2010). The mounds and ridges are often associated with fluid/gas migration features and deep-seated faults. In the compression zones, the advective flow of methane-rich fluid is reported along the fault fracture zones in the K–G basin (Dewangan *et al.* 2011; Mazumdar *et al.* 2012).

3. Methodology

The expedition (SSD-045) was conducted in the K–G basin during the 12th January to 6th February 2018 onboard *ORV Sindhu Sadhana*. The active seep sites (figure 1a) were detected in the K–G basin by analyzing the water-column (WC) images of the multi-beam echosounder (Atlas-Hydrosweep DS). CTD (figure 2) and sound velocity profiling (SVP) of our study area were generated using a Seabird CTD profiler. The depth corrections for multi-beam data were carried out using salinity,

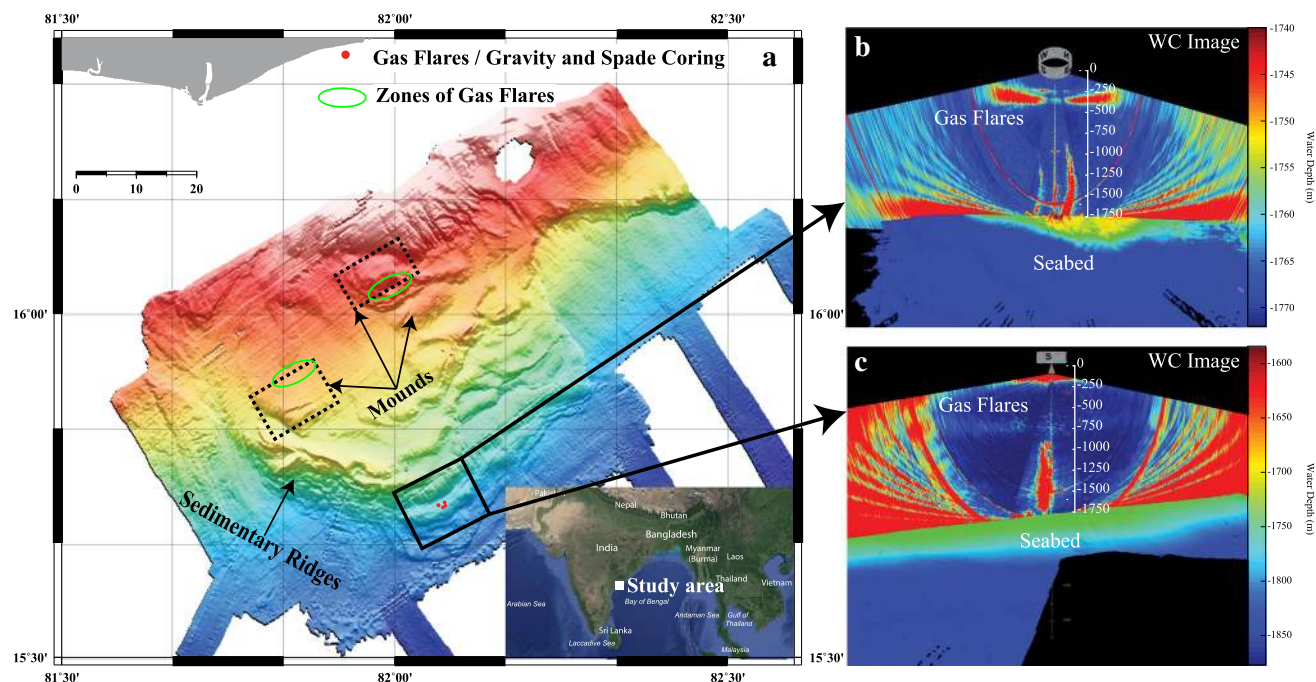


Figure 1. (a) The bathymetry map of the K–G offshore basin, showing the locations of active cold seep sites as red dots within the toe-thrust region (solid rectangle) and solid ellipse within the diapiric mounds (dashed rectangle). The region encompassing the ellipse indicate multiple overlapping gas seepages. The inset in (a) shows the study area. (b & c) show the representative WC images where gas flares are identified. The gas flares rise from the seabed up to a depth of 700 mbsl.

temperature and SVP data. Seabed samples were collected using a spade-corer (48 cm (L) × 48 cm (B) × 44 cm (H)) and gravity corer (PVC liner; inner diameter: 10 cm). Organisms (1+ cm size) were handpicked from the sediment collected in the spade core and stored in either buffered formalin solution or −20°C refrigerator for the shore-based analysis. Authigenic carbonates and hard shells were cleaned, dried at room temperature and stored in polyethylene bags for chemical analysis and taxonomic identifications, respectively. Sediment pore- fluid/gas-extraction and preservation for onshore analyses of concentrations and isotope ratios (Mazumdar *et al.*, in preparation) were carried out on board. Gas hydrate samples recovered from the gravity cores were stored in liquid nitrogen and in gas-tight tubes for on-shore carbon isotope ratio measurements.

4. Results and discussion

4.1 Methane gas flares in K-G basin

The analysis of the WC images shows four distinct gas flares (marked on the figure 1) in the ridge area. In addition, regions with multiple overlapping

flares recorded on the mounds are highlighted in the map. The gas bubbles rise from the surrounding seafloor depth of 1750 m to a depth of 700 m. The strong impedance contrast between the water and the free gas generate intense backscatter data from the gas bubbles which helps in their detection. These gas flares are tell-tale signatures of methane ebullition from the seabed (Skarke *et al.* 2014; Hong *et al.* 2017; Mau *et al.* 2017). The mapping of water column coupled with precision sediment sampling led to the discovery of active cold seeps. Here, we report the occurrence of cold seep associated biological community and shallow gas hydrate from the sites located within the toe thrust fault system (study area marked by a thick rectangle in figure 1). Sampling sites in the study area are marked in red dots (figure 1) and listed in table 1.

4.2 Chemosymbiont bearing and heterotrophic faunal communities

Characteristic endosymbiont bearing and chemo-heterotrophic faunal communities (live and relict: figure 3) are identified from the cold seep sites. The endosymbiont bearing bivalves recorded in the present study belong to the genus *Bathymodiolus* (figure 3a: family: Mytilidae, Subfamily: Bathymodiolinae), *Calyptogena* (figure 3b–c, family: Vesicomidae), *Conchocele* (figure 3d, family: Thyasiridae) and *Acharax* (figure 3e, family: Solemyidae). Intact relict shells of *Calyptogena magnifica* are recorded from the gas flare sites. The chemosynthetic bivalves imbibe H₂S and CH₄ using specialised body parts (foot) and transfer the same to the symbionts (thiotrophic and/or methanotrophic bacteria) hosted by bacterocytes in gill tissues, where H₂S and/or CH₄ are oxidized by the symbionts using oxygen from the overlying seawater (Duperron *et al.* 2013).

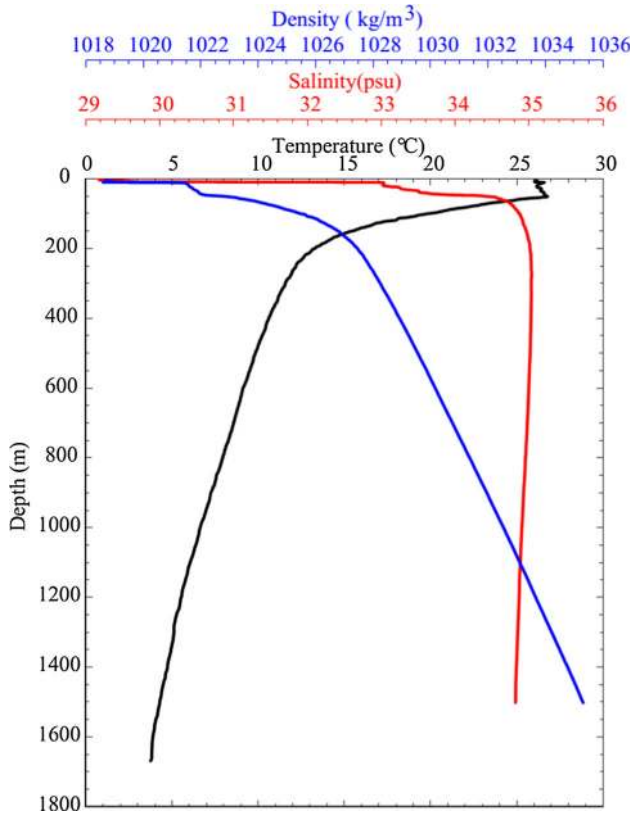


Figure 2. CTD based salinity, density and temperature profiles from the study site (solid rectangle in the figure 1).

Table 1. Latitude, longitudes and water depths of active seep sites in the ridge area marked in figure 1.

Sl. no.	Latitude (°)	Longitude (°)	Water depth (m)
1	015.70°N	082.05°E	~1752
2	015.71°N	082.18°E	~1752
3	015.73°N	082.06°E	~1754
4	015.73°N	082.08°E	~1756

Gravity and spade core sampling were carried at these sites.



Figure 3. (a) Cluster of *Bathymodiolus* sp. (marked as Bm) in black sulfidic sediments recovered in spade cores from the cold seep sites; (b & c) *Calyptogena* sp. showing growth layers; (d) *Conchocele* sp. shell with soft body; (e) *Acharax* sp. shell with soft body; (f) Bivalve shell belonging to family the Pectinidae; (g) Limpet shell with soft body; (h) Limpet shell attached to *Bathymodiolus* sp.; (i) Gastropoda shell belonging to the family Provannidae showing soft body (foot); (j) Gastropoda shell belonging to family Neritidae with soft body; (k) Tube of *Sclerolinum* sp.; (l) Polychaete worm belonging to the family Glyceridae; (m) Mantle cavity of *Bathymodiolus* sp. showing presence of polychaete worm *Branchipolynoe seepensis*; (n & o) Dorsal views of squat lobsters belonging to the family Galatheidae and Munidopsidae; (p) Brittle star belonging to the family Amphiodia; (q) Goose Barnacle belonging the Genus *Neolepas* attached to the shell of *Bathymodiolus* sp.; (r) Expanded image of *Neolepas* Sp. showing the capitulum.

The erythrocytic hemoglobin bearing blood in the circulatory system binds the oxygen/hydrogen sulfide and carry it to the gill tissues (Zal *et al.* 2000; Decker *et al.* 2017). Energy produced (ΔG^0) via microbially mediated oxidation of methane and/or H_2S is used in the faunal biomass production. Dual

symbiosis (thiotrophic and methanotrophic) is reported only amongst the members of *Bathymodiolus* sp. (Ponnudurai *et al.* 2017). In contrast, members of Thyasiridae, Solemyidae, and Vesicomidae families have mostly thiotrophic bacteria in the gill tissues. Compared to the *Bathymodiolus*

sp., which are epibenthic in nature, *Calyptogena* sp., *Acharax* sp. and *Conchocele* sp. burrow into the soft substratum and extend their foot deeper into the sediment for a steady supply of reduced sulfur species (primarily H_2S). The burrowing activities lead to deeper penetration of oxygen into the sediments as well as formation of bioturbation (trace fossils) structures often preserved as fecal pellets and calcareous tubes (Mazumdar *et al.* 2011). In this study, we have recorded extremely high HS^- concentrations in the sediment porewaters ranging up to 35 mM (Mazumdar *et al.*, in preparation) which can support the chemotrophic processes essential for the sustenance of the cold seep ecosystem. Organisms thriving the methane seep ecosystem have variable tolerance limit to H_2S and specialized survival mechanism (Levin 2005). The non-symbiont bearing bivalves belonging to family Pectinidae (figure 3f) were also recorded at this site.

At our study sites, dominant Gastropods are represented by Limpet (figure 3g and h), Provannidae (figure 3i), Neritidae (figure 3j) families. Amongst them, some species belonging to the Provannidae family are reported to have chemosymbionts in their gills (Childress and Girguis 2011), whereas, some species belonging to the family Neritidae are associated with *Bathymodiolus* (at cold seep sites) and feed on bacteria and decomposing periostracum of *Bathymodiolus* shells (Zande and Carney 2001). At the studied cold seep sites, Limpets are also observed to be attached to the *Bathymodiolus* shells (figure 3g).

Siboglinid polychaetes (figure 3k) which are essentially mouthless and gutless are predominant in the field represented by *Sclerolinum* sp. known to harbor sulfur-oxidizing bacteria in their bodies (Lösekann *et al.* 2008). The worms are hosted in gently contorted chitinous tubes having a diameter <0.5 mm and ranging in length from 15 to 30 cm. The tubes have reddish tinge likely due to the presence of hemoglobin pigments in body fluid essential for driving the chemosymbiosis (Childress and Girguis 2011; Georgieva *et al.* 2015). Other morphologically identified polychaete families at the study sites include Glyceridae (figure 3l). The deep red color of polychaete worms belonging to the family Glyceridae is possibly due to the presence of red blood pigment hemoglobin responsible for oxygen transportation in coelomic and vascular fluids under oxygen stressed conditions. Polychaete worms belonging to family Serpulidae are mostly observed living inside calcareous tubes cemented

on the hard shell surfaces. Within the pallial cavities of *Bathymodiolus*, blood-red, commensal polychaete belonging to the species *Branchipolynoe seepensis* (figure 3m) are observed. *B. seepensis* plays an important role in controlling the metal concentration in the host's body tissues/fluids (Bebianno *et al.* 2018).

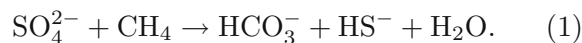
Decapod crustaceans (squat lobsters) belonging to the families Munidopsidae (figure 3n) and Galatheididae (figure 3o) are abundant at the study sites. They mostly graze on the mussel beds and microbial mats (Niemann *et al.* 2013). Additionally, the methane and sulfide-oxidizing bacteria farmed on the hair/comb-like setae grown on cheliped are important food sources (Thurber *et al.* 2011). Thus, the non-symbiotic heterotrophic fauna can accumulate microbial symbionts as well as chemosynthetically fixed carbon in their digestive tract and can transport the same beyond the seep site into the surrounding deep sea (Niemann *et al.* 2013).

The Ophiuroid, *Amphiudia* sp. (figure 3p, family: Amphiuridae) (Gondim *et al.* 2013) are ubiquitous at the K-G basin cold seep sites. They possibly graze on the bacteria and detritus in the sediment or in suspension.

Goose Barnacles belonging to the genus *Neolepas* (figure 3q and r; family: Eolepadidae) were found attached to the *Bathymodiolus* shells. Individuals with small scutum (juveniles) are also attached to the neck of the larger barnacles. They show distinct cirri and scutum (movable plates). These barnacles probably consume the bacteria (sulfur) present in the cirri as their food (Southward and Newman 1998).

4.3 Occurrence of shallow gas hydrate

The high flux of hydrogen sulfide (Mazumdar *et al.*, in preparation) across the sediment–water interface which supports the proliferation of cold seep community, is predominantly sustained by advective methane flux, and subsequent syntrophic microbial processes leading to sulfate reduction and anaerobic methane oxidation (Knittel and Boetius 2009, equation 1).



The advective methane flux at the sediment–water interface has resulted in the formation of shallow (2–3 mbsf at a water depth of ~1750 m) methane hydrate reported first time from K-G

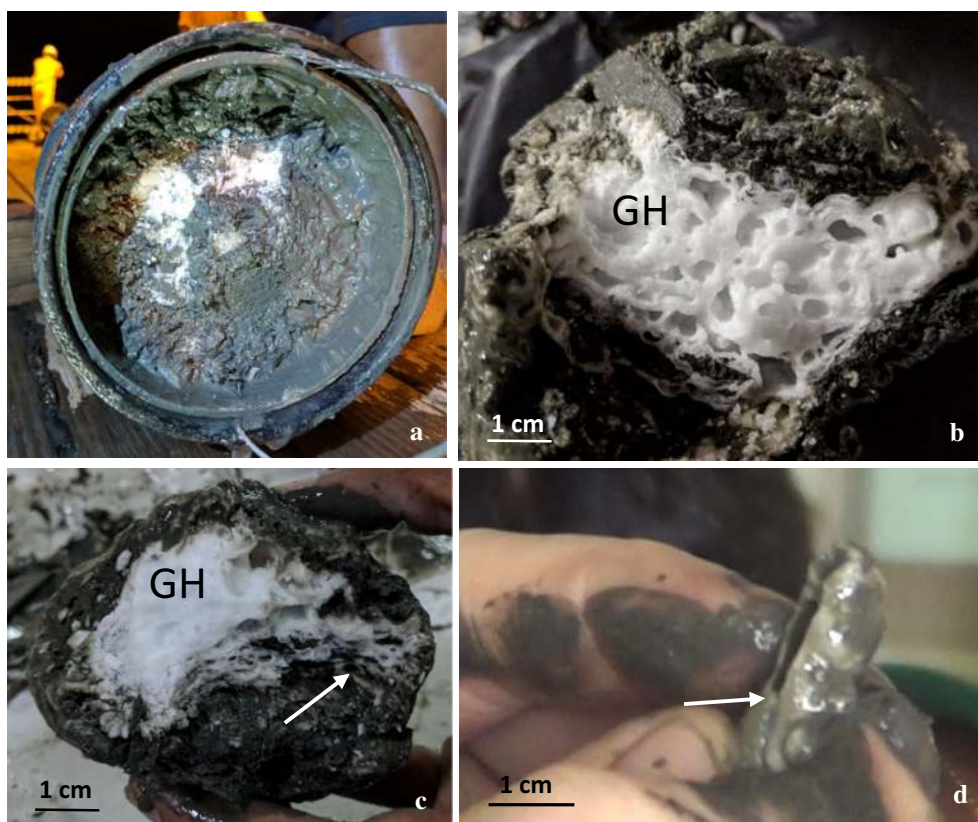


Figure 4. (a) Methane gas hydrate in the sediment core recovered from the cold seep sites; (b) Methane hydrate with gas cavities; (c) Hydrate filling up the fractures (arrow mark); and (d) Tubular-shaped methane hydrate (arrow mark).

basin (figure 4a–d). The hydrates are typically fracture filling types (figure 4a–c). Hydrate has also been observed as tubes (figure 4d) within the sediment. The average $\delta^{13}\text{C}_{\text{CH}_4}$ ($-73.7 \pm 0.7\text{‰}$ VPDB) of the methane hydrates from the studied cold seep sites (Mazumdar *et al.*, in preparation) indicate biogenic methane source (Mazumdar *et al.* 2012). The P–T conditions (figure 2) in the study area are conducive to the stability of the methane hydrates (Sloan 1990; Collett *et al.* 2008). The fracture generated gas plumbing system in the K–G basin is responsible for the vertical migration of deeper methane gas (Dewangan *et al.* 2011; Sriram *et al.* 2013).

5. Conclusion

We have presented here the first report on the discovery of methane gas flares in the water column and associated benthic biotic community at the cold seep sites off Krishna–Godavari Basin, Bay of Bengal. The occurrence of the shallow gas hydrates (2–3 mbsf) is also been reported in this study. High biogenic methane flux close to

the sediment–water interface has resulted in the crystallization of methane hydrate at shallow depths below the seafloor. The seep sites are characterized by chemosynthesis dependent organisms predominantly Bivalvia, Gastropods, and Polychaete. In addition, several non-chemosymbiont bearing heterotrophic fauna including Decapods, Ophiuroids, and Cirripeds are also reported here. The proliferation of chemosymbiont bearing organism at the cold seep sites is attributed to the emission of methane and hydrogen sulfide gases. The present discovery has brought India on to the global cold seep map and opened up the opportunity for future research on the possible role of methane emission on global warming, ocean acidification, extreme ecosystem, and bioprospecting.

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