SHORT COMMUNICATION

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Methane Ice Worms: *Hesiocaeca methanicola* Colonizing Fossil Fuel Reserves

Abstract During a research cruise in July 1997 in the Gulf of Mexico we discovered a gas hydrate approximately 1 m thick and over 2 m in diameter which had recently breached the sea floor at a depth of 540 m. The hydrate surface visible from the submarine was considerably greater than that of any other reported hydrate. Two distinct color bands of hydrate were present in the same mound, and the entire exposed surface of the hydrate was infested (2500 individuals/m^2) with 2 to 4 cm-long worms, since described as a new species, *Hesiocaeca methanicola*, in the polychaete family Hesionidae (Desbruyères and Toulmond 1998). *H. methanicola* tissue stable isotope values are consistent with a chemoautotrophic food source. No evidence of chemoautotrophic symbionts was detected, but geochemical data support the presence of abundant free living bacteria on the hydrate. The activities of the polychaetes, grazing on the hydrate bacteria and supplying oxygen to their habitats, appears to contribute to the dissolution of hydrates in surface sediments.

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Gas hydrates are icelike crystalline solids composed of gasses, predominantly methane, but also hydrogen sulfide (Kastner et al. 1998), encased within rigid cages of water molecules. Formed under pressure, hydrate stability depends on temperature, pressure, and chemical composition (MacDonald et al. 1994). Globally, methane associated with hydrates comprises on the order of 10^4 gigatons of carbon and may represent the most abundant store of fossil fuel on the earth (Kvenvolden 1993, 1995). No nonmicrobial life has previously been reported living in or on hydrate structures. Most hydrate deposits are deeply buried by marine sediments and have been stable during recent geological time. Hydrates also occur in surface sediments in areas of active hydrocarbon seepage (Brooks et al. 1991, 1984), where formation and dissociation of hydrates is dynamic owing to interactions of the hydrate with bottom water (MacDonald et al. 1994).

Analysis of small-volume water samples taken from the surface of the discovered hydrate and molecular analysis of pore water samples from the overlying sediments confirm that the hydrate is decomposing. Samples taken adjacent to polychaetes contained up to 900 μM methane and 100 μM hydrogen sulfide, which are not present in micromolar quantities in the ambient bottom water. Furthermore, the elevated concentrations and low δ^{13}C values of the carbon dioxide in the sediments reflect bacterial oxidation of hydrocarbon gasses over the hydrate (Table 1). The yellow color in the upper band of the gas hydrate is correlated with the presence of high molecular weight hydrocarbons (oil) in this band, whereas the lower white band lacked oil. The molecular characteristics of the hydrate gases from both bands are quite similar and consistent with structure II gas hydrates formed from an unaltered single source of thermogenic vent gas (Fig. 1, Table 1) (Sassen et al. 1998).

The faunal aggregation on the entire exposed surface of the hydrate, and surfaces revealed by removal of sediment by the submersible, was monospecific *H. methanicola* (Fig. 2). Most individuals were between 2

Table 1 $\delta^{13}C$ values (‰ relative to the Peedee belemnite standard) and percent abundance of small molecular weight hydrocarbons and carbon dioxide in the gas hydrate and associated samples (*IW* interstitial water in the mud)

	C1	C2	C3	i-C4	n-C4	i-C5	n-C5	CO$_2$
Vent gas								
$\delta^{13}C$	−48.7	−29.2	−26.5	−28.1	−25.4	−26.6	−25	2.9
%	92.9	2.4	2.3	0.5	0.6	0.2	0.1	0.8
White hydrate								
$\delta^{13}C$	−48.3	−30.0	−26.2	−27.5	−27.3	−26.6	−26.2	1.9
%	71.4	9.0	13.8	2.9	1.8	0.2	0.1	0.9
Yellow hydrate								
$\delta^{13}C$	−48.4	−29.4	−26.4	−28.4	−24.9	ND	ND	ND
%	73.5	12.5	9.9	1.6	1.8	0.2	0.1	<0.1
IW over hydrate								
$\delta^{13}C$	−51.7	−24.0	ND	−30.5	ND	−22.5	ND	−40.5
%	60.9	10.1	15.1	1.7	0.0	2.0	0.0	10.2

Fig. 1 Gas hydrate on the sea floor at 540 m depth. Note the two distinctly colored bands separated by a layer of sediment

and 4 cm in length and inhabited oval depressions that averaged roughly 4 cm long, 3 cm wide, and 2 cm deep. In over 95% of the exposed depressions only a single polychaete was visible. Full-sized polychaetes were rarely seen sharing the same depression, although several depressions housed multiple smaller animals. The depressions abutted one upon another over all visible surfaces of the hydrate. Based on measurements from video images, we calculated that an average density of 2500 individuals/m^2 was visible immediately following the discovery.

H. methanicola is a member of the polychaete family Hesionidae (Desbruyères and Toulmond 1998). Species of this small and poorly known family are most common in shallow water associated with hard substrates, but have also been found in the deep sea (Blake 1991). *H. methanicola* is pink in color with a large red dorsal vessel, reduced eyes, an eversible proboscis without robust jaws, and a functional digestive tract. The sexes are separate, and size-frequency analysis of histological sections from the gonads of ten females revealed oo-


Fig. 2 *H. methanicola* worms colonizing the exposed surface of a gas hydrate. Note the depressions occupied, and apparently created by the worms

cytes of all sizes and at all stages of development, a pattern typical of asynchronous gamete development. Some collected individuals spontaneously spawned, producing trochophore larvae within 74 h, which began to elongate 4 days later. Development ceased after 14 days, and all larvae died without settlement after 20 days. We infer from these observations a pattern of continuous reproduction in the adults, producing planktotrophic larvae with a planktonic dispersal phase of at least a few weeks duration.

Respiration rates and anaerobic tolerance were determined on *H. methanicola* collected from another hy-

drate in June 1998. Single worms were placed into sealed containers at a temperature 8–9 °C with an Orion 835 Dissolved Oxygen Meter inserted to measure oxygen levels. Oxygen concentration was checked every 5 min for 16 h. Worms from the experiment were then frozen and weighed in the laboratory. Oxygen consumption rates ranged from 2.9 to 67 μg O_2 min^{-1} g^{-1} wet weight in adults to juveniles, respectively (Fig. 3), which is within the expected range for polychaetes of these sizes at this temperature (Weber 1978). Anaerobic tolerance of 20 individuals was compared to that of a control group (Fig. 4). Eighteen of the animals survived 48 h in the absence of oxygen, and ten (50%) for 96 h. This level of tolerance to anoxia is not exceptional (Dales 1969) but is of adaptive significance to this polychaete, which is likely to experience extended periods of anoxia on the surface of shallowly buried hydrates.

During the dives and the 36 h deployment of a time-lapse video camera the worms on the exposed surface of the hydrate rarely left that surface unless physically disturbed. Both their fidelity to the hydrate and the presence of such a high density monospecific aggregation suggest a strong nutritional tie between the worms and the hydrate. Because only prokaryotes are known to utilize reduced gases as energy sources or to metabolize crude oil, we have focused our efforts on identifying this most probable intermediate in the worm-hydrate food chain. No external symbionts were identified during a complete survey of the external surface of two *H. methanicola* by scanning electron microscopy. The presence of a functional gut (freshly collected worms produced feces for 24 h) and absence of hypertrophied tissues argue against an internal symbiosis (Fisher 1990). A light and transmission electron microscope survey of selected internal tissues failed to reveal the presence of symbionts other than scattered bacteria in the gut. However, additional tests will be necessary before we can rule out the presence of a nutritional symbiosis in this organism.
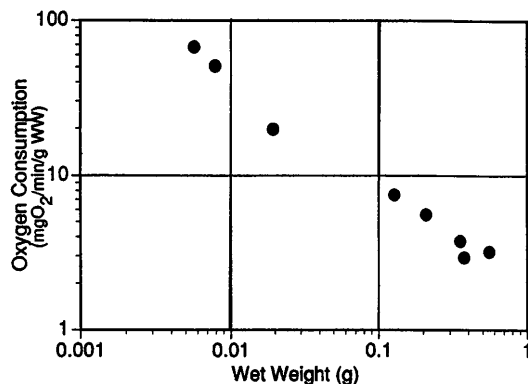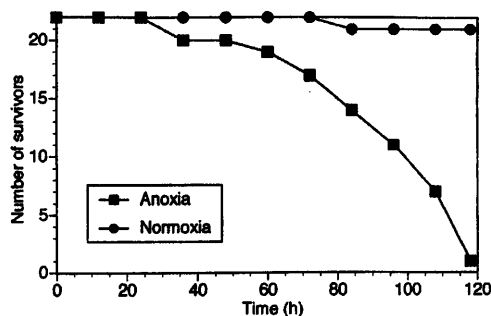
Fig. 4 Survival times of freshly collected *H. methanicola* kept under anoxic conditions at 8 °C. Twenty-two animals were kept in stoppered individual containers of sea water previously stripped of oxygen by bubbling for 1 h with nitrogen gas (*squares*) and another 22 animals in containers open to the atmosphere (*circles*). Survival was monitored every 12 h

Both sulfide and methane are excellent bacterial substrates and our working hypothesis is that the polychaetes obtain the bulk of their nutrition by consuming free living bacteria that colonize the surface of the hydrate. Although these putative bacteria have not been cultured or characterized, microscopic examination of glutaraldehyde fixed samples revealed an abundant and varied population of bacteria, including *Beggiatoa*-like forms and bacteria with internal refractive granules. We suggest that the worms contribute to the growth of bacteria, as well as to the hydrate's decomposition, by creating water currents on the hydrate surface with their parapodia. This activity would increase the supply of the oxygen required by the worms and aerobic chemoautotrophic or methanotrophic bacteria, and may form the depressions the worms occupy. The depressions themselves would also increase the rate of hydrate decomposition by increasing its exposed surface area.

The tissue stable isotope values of the polychaetes are consistent with a microbial food source for the worms. Tissue $\delta^{13}C$ and $\delta^{15}N$ values were determined for six individuals, three from each of the two colored bands of hydrate, and a single individual from each band was analyzed for tissue $\delta^{35}S$ (Sackett et al. 1970; Schoell et al. 1983; compound specific isotope analyses were performed at the geochemistry laboratory of the Houston Area Research Council, Sassen et. al. 1998). The worms from the two colored hydrate bands were not significantly different in either C or N stable isotope content. The $\delta^{15}N$ values ranged from 5.32‰ to 6.32‰, which is quite light for deep-sea fauna in general but similar to values reported for other animals utilizing chemosynthetic or methanotrophic food sources (Fisher 1995). The $\delta^{13}C$ values, ranging from −23.8 to −24.7, are significantly heavier than methane in the hydrate (Table 1), suggesting that the worms do not obtain the bulk of their nutrition from methanotrophs (Fisher 1990). However, these values are lighter than ambient deep sea fauna in the Gulf of Mexico but within the range found in vestimentiferan tubeworms

Fig. 3 Oxygen consumption rates of freshly collected *H. methanicola* as a function of size of the animals. The rates were determined on individual animals in 130 ml respirometry vessels at 8 °C at oxygen tensions between 5 and 0.5 mg O_2/l, using Clark-type polarographic oxygen electrodes

(which harbor chemoautotrophic sulfide oxidizing symbionts) and local seep bacterial mats (Fisher 1996; Sassen et al. 1993). The $\delta^{35}S$ values of 1.88‰ and 3.62‰ found in the two individuals analyzed indicate that their sulfur source is not sea water sulfate and, as with the $\delta^{13}C$ values, are consistent with a chemoautotrophic food source for the polychaetes. The interface between the sulfide-rich hydrate and overlying oxic sea water would provide an optimum habitat for chemoautotrophic sulfur-oxidizing bacteria, and we hypothesize that such bacteria are the primary food source for the polychaetes on this nodule.

A variety of potential predators were observed swimming or walking along the surface of the hydrate in submersible and time-lapse video records. These included large crustaceans such as *Bathynomous gigantus* and several fishes. Careful examination of video-film failed to provide a single clear case of ingestion of a polychaete by any of these potential predators. When the hydrate mound was revisited 1 month after its initial discovery, no empty depressions were visible. In fact the apparent density of polychaetes had increased to 3000 individuals/m^2, with a higher frequency of multiple polychaetes in single depressions. The most probable explanation for this is that their population suffers little from predation, perhaps because they are unpalatable.

The presence of the polychaetes on the exposed hydrate surface leads us to question the degree to which these polychaetes colonize hydrates buried by sediments. In addition to the implications for the decomposition rate and perhaps even mining of these fossil carbon reserves, this question has serious implications for the physiology, reproductive biology, and ecology of the polychaetes. Clearing mud with a suction sampler from the surface of previously unexposed portions of hydrate deposits at several sites revealed both polychaetes and their burrows within the mud. Thus it is clear that the worms are colonizing hydrates buried by up to at least 10 cm of sediment. The possibility that they may be capable of reaching and colonizing hydrates buried substantially deeper in the sediments is an important and open question.

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