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Respiration of bivalves from three different deep-sea areas: Cold seeps, hydrothermal vents and organic carbon-rich sediments

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ABSTRACT

We studied bivalves (vesicomyids and mytilids) inhabiting four different areas of high sulfide and methane production: (1) in the Gulf of Guinea, two pockmarks (650 m and 3150 m depth) and one site rich in organic sediments in the deepest zone (4950 m average depth), (2) at the Azores Triple Junction on the Mid-Atlantic Ridge, one hydrothermal site (Lucky Strike vent field, 1700 m depth). Two types of Calmar benthic chambers were deployed, either directly set into the sediment (standard Calmar chamber) or fitted with a tank to isolate organisms from the sediment (modified Calmar chamber), to assess gas and solute exchanges in relation to bivalve bed metabolism. Fluxes of oxygen, total carbon dioxide, ammonium and methane were measured. At the site with organic-rich sediments, oxygen consumption by clams measured *in situ* with the standard benthic chamber was variable (1.3–6.7 mmol m^{-2} h^{-1}) as was total carbon dioxide production (1–9.6 mmol m^{-2} h^{-1}). The observed gas and solute fluxes were attributed primarily to bivalve respiration (vesicomyids or mytilids), but microbial and geochemical processes in the sediment may be also responsible for some of variations in the deepest stations. The respiration rate of isolated vesicomyids (16.1–0.25.7 μmol g^{-1} dry weight h^{-1}) was always lower than that of mytilids (33 μmol g^{-1} dry weight h^{-1}). This difference was attributed to the presence of a commensal scaleworm in the mytilids. The respiratory coefficient (QR) ≥ 1 indicated high levels of anaerobic metabolism. The O:N index ranged from 5 to 25, confirming that vesicomyids and mytilids, living in symbiosis with bacteria, have a protein-based food diet.

1. Introduction

In hydrothermal sites, cold seeps or areas rich in organic carbon, high fluxes of methane and sulfide support chemoautotrophic free-living and symbiotic bacteria. These fluxes provide the basis for complex microbial and metazoan communities. Many biological studies in these particular habitats have focused on the distribution, structure, nutrition, and food web architecture of faunal communities as well as on their interaction with the geochemistry of their environment. However, the metabolism of these organisms is poorly documented. Bivalves are one of the most abundant chemosynthetic organisms inhabiting deep-sea reducing ecosystems with production of methane and sulfur (Lutz and Kennish, 1993; Sibuet and Olu, 1998; Fiala Medioni et al., 2002; Duperron et al., 2005; Cosel Von and Olu, 2009). Vesicomyids and mytilids at these sites feed via symbiotic sulfide-oxidizing bacteria that inhabit bacteriocytes in their gills (Fiala-Medioni and Le Pennec, 1988). The vesicomyids provides its symbionts with sulfides taken up by its foot buried in the sulfide-rich sediments and mytilids directly uptaken via gill-associated symbionts. Oxygen and carbon dioxide are absorbed from the seawater that flows through the bivalve siphons above the sediment (Arp and Childress, 1983; Childress et al., 1984; Roeselers and Newton, 2012).

To study the necessary physiological requirements for deep-sea bivalve life, oxygen consumption and carbon and nitrogen excretion rates represent the gains and losses of energy associated with metabolism. The physiological responses of vesicomyids or mytilids to changes in the environment are extremely variable (Widdows et al., 1984; Tedengren et al., 1990; Navarro and Gonzalez, 1998). Bivalve-bacterial associations can thrive in sulfide-rich environments, surviving on the oxidation of sulfide. Bivalves uptake both sulfide and oxygen which do not normally exist together in water column due to rapid oxidation of sulfide. They are assumed to be involved in numerous interactions with the surrounding environment and organisms due to their size and abundance, (Olu-Le Roy et al., 2007; Marcon et al., 2014).

Research directed at studying the unusual physiological adaptations of these animals is necessary to understand how these bivalves can live in such hostile environments, largely unhospitable to other animals. Studies on oxygen consumption rates have

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been performed using *in situ* measurements on hydrothermal vent mussels (Smith Jr., 1985) and other deep-sea habitats maintained under *in situ* pressure conditions (Arp et al., 1984; Childress and Mickel, 1982, 1985; Henry et al., 2008). In these experiments, observed oxygen consumption rates are generally as high as those measured in shallow-water mussels.

The objective of our study was to estimate *in situ* the respiratory rate of vesicomyids and mytilids at four different deep-sea sites in the Gulf of Guinea and on the Mid-Atlantic Ridge. Measurements of oxygen, total dissolved inorganic carbon, methane and ammonium were obtained *in situ* using two different benthic chambers and were assessed along with vesicomyids or mytilids density and biomass. The results were examined according to the type of benthic chamber used, explored sites and bivalve species.

2. Materials and methods

2.1. Study area

The study was carried out in three sites in the Gulf of Guinea explored during the WACS cruise (February 2011), the CongoLobe cruise (December 2011–January 2012) and in one site on the Mid-Atlantic Ridge in the Lucky Strike vent field during the Momarsat 2015 cruise (April 2015) on the R/V *Pourquoi Pas?*. All the deployments of Calmar were realized using the ROV *Victor*.

The first site in the Gulf of Guinea (Fig. 1) is an organic carbon-rich area on the distal lobe complex of the Congo deep-sea fan. This area is characterized by brown sediment with several “black pools” composed of a surface of reduced black sediment. This site likely receives large terrestrial organic inputs from the African continent, transported via the Congo submarine canyon system (Khripounoff et al., 2003; Vangriesheim et al., 2009). Four stations (A, B, F and C) were sampled in this area from 4750 to 5070 m depth (Fig. 1). A distinctive ecosystem is associated with black pools and is characterized by a biological community that resembles those observed on pockmarks with microbial mats and vesicomyid bivalves (dominant species: *Christineconcha regab* and *Abyssogena southwardae*, Krylova et al. 2010). The three organic-rich stations (Fig. 1) were located in the main deposition zone of the Congo canyon along the track of the micro-channel that funnels the turbidity material to the terminal lobes except Station E, which was out of the turbidity input and was chosen as a deep-sea reference station.

The second site (Fig. 1), called Regab, is a giant pockmark 800 m in diameter at 3150 m depth, along the Congo margin (Olu-Le Roy et al., 2007). It is characterized by high habitat heterogeneity, with assemblages of the three major symbiont-bearing taxa encountered at cold-seeps: Vesicomyid bivalves (dominant species: *C. regab*), Mytilid bivalves (*Bathymodiolus* aff. *boomerang*) and Siboglinid polychaetes (*Escarpia southwardae*), as well as microbial mats.

The third site, called Guiness (Fig. 1), includes several small, less active pockmarks, from 580 to 690 m depth. The major benthic taxon observed was vesicomyids (*Calyptogena valdiviae,* and *Elenaconcha guiness*, Cosel Von and Olu, 2009). This pockmark is characterized by patchy vesicomyid beds associated with microbial mats.

The fourth site was the hydrothermal Lucky Strike vent field (1700 m average depth) at the Azores Triple Junction (ATJ) on the Mid-Atlantic Ridge. This area consists of a large central lava lake surrounded by three volcanic cones with several translucent smokers. Mytilid bivalves *Bathymodiolus azoricus* are the dominant megafaunal species and are distributed in patches of thousands of individuals (Desbruyères et al., 2001).

2.2. Calmar benthic chambers

To assess the *in situ* metabolism community of bivalves, the Calmar benthic chamber (Caprais et al., 2010) was deployed by the ROV *Victor 6000*. Basically, Calmar is a 41 cm diameter cylinder that is open at one end. The Calmar unit weighs 14 kg in water and it is equipped with six 100 ml sampling cells, an oxygen probe (Aadi, Norway) and a stirrer to homogenize the water in the chamber. The position of the sampling cells under the Calmar chamber and their closure mechanism preclude any suction and infiltration of uncontrolled water movement in the sealed Calmar chamber (Caprais et al., 2010). During the standard Calmar experiments (Fig. 2a), the incubation of bivalves directly on the sediment lasted for about 3 h. At each station, a ring of 50 cm in diameter was deployed to guide the Calmar positioning. The standard Calmar measured exchanges between water and bivalves lodged in the sediment was deployed two times during the WACS cruise on mytilid beds and three times during the CongoLobe cruise on vesicomyid beds. Two standard Calmars were also used to measure flux exchanges on sediment without bivalves and represent background references in this study. Measurements on undisturbed *B. azoricus* beds with standard Calmar were not possible because the chimney walls do not allow such measurements.

The respiration rate of isolated bivalves was measured *in situ* using a modified Calmar chamber fitted with a specific tank (Fig. 2b) (Khripounoff et al.; 2014). It was deployed by the ROV at all four sites: Lobe, Regab, Guiness and Lucky Strike (Table 1). At the beginning of the experiment, bivalve shells were sampled on the bottom by the ROV with a net or with the ROV arm and dropped into the cylindrical tank. Then, after about 1 h of stabilization, the Calmar unit was placed over this tank, immersing the bivalves in exactly 31 l of bottom water (Fig. 2b). The incubation then started and lasted for 3 h *in situ* on the bottom, close to the sampling area. The modified Calmar chamber fitted with a tank was used to incubate 7 shells of *C. regab* at the Lobe station, 17 shells of *C. regab* at the Regab station, 21 shells of *E. guiness* at the Guiness station and 24, 19 and 170 shells of *B. azoricus* at the Lucky Strike vent field.

2.3. Size, weight, density, biomass and elemental composition of vesicomyids and mytilids

After each experiment with the benthic chamber (standard or modified Calmar), the sampled bivalves were identified, washed and frozen (samples incubated with the modified Calmar) or fixed in 4% buffered formalin (samples incubated with the standard Calmar). In the laboratory, shells were measured and dry weight (dw) of bivalves was determined after the body, excised from the shell, had been dried for 24 h at 60 C. *B. azoricus* mussels generally have a commensal polychaete, *Branchipolynoe seepensis*. The biomass of each species was measured separately in each shell. Total carbon and sulfur concentrations of dried total body were analyzed with a Leco CS 125 (USA) elemental analyzer.

The *in situ* bivalve density under the standard Calmar was estimated with two methods using photos and quantitative core samples (Decker et al., 2012). First, a ring of 50 cm diameter was deployed on a bivalve bed and photos of this circle were taken by the ROV to estimate bivalve density before benthic chamber deployment (Khripounoff et al., 2015). Then, after the recovery of Calmar at the end of incubation, a blade core (0.036 m^2) was taken from within the ringed off area. Mean density was estimated from the average of these two measurements taken at each Calmar deployment. For each standard Calmar deployment, mean of biomass (g dw m^{-2}) were calculated using the mean density, estimated from photos and blade cores, and mean individual tissue dry weight calculated with the bivalves sampled with core. Only