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Physiology/Physiologie

## First respiration estimates of cold-seep vesicomimid bivalves from *in situ* total oxygen uptake measurements

*Première estimation des taux de respiration des bivalves Vesicomiyidae des sources de fluides froids, par des mesures in situ de flux d'oxygène*

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## ARTICLE INFO

## Article history:

Received 3 November 2011

Accepted after revision 6 March 2012

Available online 24 April 2012

## Keywords:

Vesicomimid bivalves  
Total oxygen uptake  
Methane flux  
Respiratory rate  
Benthic chamber  
Cold-seep

## Mots clés :

Bivalves Vesicomiyidae  
Flux total d'oxygène  
Flux de méthane  
Taux de respiration  
Chambre benthique  
Sources de fluides froids

## ABSTRACT

Vesicomimid bivalves are one of the most abundant symbiont-bearing species inhabiting deep-sea reducing ecosystems. Nevertheless, except for the hydrothermal vent clam *Calyptogena magnifica*, their metabolic rates have not been documented, and only assessed with *ex situ* experiments. In this study, gathering benthic chamber measurements and biomass estimation, we give the first *in situ* assessment of the respiration rate of these bivalves. The giant pockmark Regab, located at 3160 m depth along the Congo-Angola margin, is a cold-seep site characterised by dense assemblages of two species of vesicomiyids: *Christineconcha regab* and *Laubiericoncha chuni* with high dominance of *C. regab*. Two sites with dense aggregates of vesicomiyids were selected to measure total oxygen uptake (TOU), and methane fluxes using IFREMER's benthic chamber CALMAR deployed by the ROV *Quest 4000* (MARUM). Photographs were taken and bivalves were sampled using blade corers to estimate density and biomass. Total oxygen uptake was higher at Site 2 compared to Site 1 (respectively 492 mmol.m<sup>-2</sup>.d<sup>-1</sup> and 332 mmol.m<sup>-2</sup>.d<sup>-1</sup>). However, given vesicomimid densities and biomass, mean oxygen consumption rates were similar at both sites (1.9 to 2.5 μmol.g total dry mass<sup>-1</sup>.h<sup>-1</sup> at the Site 1 and 1.8 to 2.3 μmol.g total dry mass<sup>-1</sup>.h<sup>-1</sup> at Site 2). These respiration rates are higher than published *ex situ* estimates for cold-seep or hydrothermal vent bivalves. Although methane fluxes at the base of sulphide production were clearly higher at Site 2 (14.6 mmol.m<sup>-2</sup>.d<sup>-1</sup>) than at Site 1 (0.3 mmol.m<sup>-2</sup>.d<sup>-1</sup>), they do not seem to influence the respiration rates of these bivalves associated to sulphide-oxidizing symbionts.

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## R É S U M É

Les bivalves Vesicomiyidae sont l'une des familles d'espèce symbiotrophiques dominantes dans les écosystèmes réducteurs profonds. Cependant, à l'exception de l'espèce hydrothermale *Calyptogena magnifica*, leur métabolisme est peu connu et uniquement estimé à partir d'expérimentations *ex situ*. Dans cette étude, le taux de respiration de ces bivalves est estimé *in situ* pour la première fois, à l'aide d'une cloche benthique manipulée par un ROV et de la quantification de leur biomasse. Le pockmark géant Regab, localisé à 3160 m de profondeur le long de la marge Congo-Angolaise, est un site d'émission de fluides froids caractérisé par des agrégats denses de bivalves Vesicomiyidae des espèces *Christineconcha regab* qui domine, et *Laubiericoncha chuni* plus rare. Deux sites ont été

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sélectionnés pour mesurer le flux total d'oxygène et le flux de méthane avec la cloche benthique CALMAR (IFREMER) manipulée par le ROV *Quest 4000* (MARUM). Des photographies des agrégats ainsi que des prélèvements quantitatifs ont été réalisés pour estimer la densité et la biomasse de bivalves. Le flux total d'oxygène est plus élevé sur le Site 2 que sur le Site 1 (respectivement  $492 \text{ mmol.m}^{-2}.\text{j}^{-1}$  et  $332 \text{ mmol.m}^{-2}.\text{j}^{-1}$ ). Cependant, en prenant en compte la densité de bivalves et leur biomasse, le taux moyen de consommation d'oxygène est similaire sur les deux sites ( $1,9$  à  $2,5 \mu\text{mol.g poids sec total}^{-1}.\text{h}^{-1}$  au Site 1 et  $1,8$  à  $2,3 \mu\text{mol.g poids sec total}^{-1}.\text{h}^{-1}$  au Site 2). Ces valeurs sont supérieures à celles publiées sur des estimations ex situ de taux de respiration de bivalves hydrothermaux. Bien que les flux de méthane, à la base de la production de sulfure, soient plus élevés sur le Site 2 ( $14,6 \text{ mmol.m}^{-2}.\text{j}^{-1}$ ) que sur le Site 1 ( $0,3 \text{ mmol.m}^{-2}.\text{j}^{-1}$ ), ils ne semblent pas influencer le métabolisme des bivalves associés à des bactéries symbiotiques sulfo-oxydantes.

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## 1. Introduction

Vesicomimid bivalves are one of the most abundant chemosynthetic faunal species inhabiting deep-sea reducing ecosystems, including hydrothermal vents, whale falls and cold-seep ecosystems [1–3]. About 100 vesicomimid species are described worldwide mainly at bathyal and abyssal depths [4,5], and colonise the great majority of cold-seep sites. Vesicomimid bivalves live in symbiosis with sulphide-oxidising bacteria located in their gills [6,7]. These symbionts produce organic compounds used by bivalves for their nutrition and, in turn, the bivalve hosts supply their symbionts with oxygen, carbon dioxide and sulphide. Oxygen and carbon dioxide are directly available in the ambient seawater and are transported through the inhalant siphon to gills and the bacteria therein. Sulphide comes from the sediment and goes through the foot into the haemolymph and is thus transported to the gill [8]. Their metabolism is indirectly based on methane anaerobic oxidation (AOM) [9] producing sulphide used as energy source by the vesicomimid symbionts.

Metabolic rates of vesicomimids are not well known. Three studies on oxygen consumption rates have been performed *ex situ* measurements on hydrothermal vent vesicomimids in stainless steel vessels to maintain *in situ* pressure conditions during the experiment [8,10,11]. Arp et al. [8] assessed oxygen consumption rates in *Calyptogena magnifica* Boss and Turner, 1980 [12], and observed values were as high as those from shallow-water bivalves. Two other smaller species, *Calyptogena elongata* Dall, 1916 [13] and *Calyptogena pacifica* Dall, 1891 [14], show much lower oxygen consumption rates (average  $0.2 \mu\text{mol.g wet weight [without shell]}^{-1}.\text{h}^{-1}$  [11]). Only one *in situ* measurement of oxygen consumption rate has been performed on *C. magnifica* on the Galapagos rift, giving an average rate of  $0.4 \mu\text{mol.g wet weight}^{-1}.\text{h}^{-1}$  [11].

The first *in situ* measurements of methane, oxygen and carbon fluxes were carried out in several deep-sea environments using benthic chambers [15–18]. However, these devices deployed from the surface cannot specifically target selected biogenic habitats in highly heterogeneous environments such as cold-seeps or hydrothermal vents. A relatively new approach is to deploy benthic chambers with submersibles. These devices have been used at seeps to measure both methane and oxygen fluxes, to assess the

role of microbial activity, especially anaerobic oxidation of methane [19,20], and the role of macrofauna, particularly through symbioses [20–22] in the regulation of seabed methane emissions (benthic biological filters). Differences in total oxygen uptake (TOU) have been observed between active seeps and “normal” sediments (from 2 to 20 times higher in active seeps) [20,21] and among seep habitats [20,22,23], revealing higher fluxes in macrofauna clusters than in microbial mats on Håkon Mosby mud volcano (HMMV) [22]. In these papers, TOUs have been measured *in situ* by autonomous or ROV-manipulated chambers in microbial mats, vesicomimid beds, siboglinid or ampharetid polychaete fields. Only one *in situ* oxygen uptake estimation on vesicomimid bivalves has been obtained with a benthic chamber deployed from the surface, remotely guided for habitat selection, but without estimation of the density and biomass of individuals [20]. TOUs also vary according to advective methane fluxes and show a negative relationship across the HMMV ecosystem [22].

The giant Regab pockmark off Gabon, located at 3160 m depth along the Congo-Angola margin, is characterised by dense assemblages of vesicomimids, mytilids and escarpids distributed along a gradient of methane concentrations decreasing from the centre to the periphery [24]. Two species of vesicomimids – *Christineconcha regab* Cosel & Olu, 2009 [4,25] and *Laubiericoncha chuni* Cosel & Olu, 2008 – are found in the pockmark, where *C. regab* largely dominates. The giant pockmark was re-visited by the ROV *Quest 4000* (MARUM, University Bremen, Germany) in 2008. We selected several vesicomimid beds in the centre and at the south-west border of the active area to: (1) perform *in situ* benthic fluxes measurements to estimate vesicomimid respiratory rates; and (2) compare TOU on clam beds of various density with different methane levels along this cold-seep ecosystem.

## 2. Materials and methods

### 2.1. Study site

The giant pockmark Regab (800 m wide and 15 m deep) was discovered in 2001 at 3160 m depth along the Congo-Angola margin [24]. Dense assemblages of symbiont-bearing species including Bathymodioidae, Escarpiidae and Vesicomiyidae have been observed along a NE-SW axis

**Table 1**

Study sites at the REGAB pockmark, number of individuals sampled and number of individuals counted under CALMAR with photos for *C. regab* (Cr) and *L. chuni* (Lc).

Site (Marker GUINECO)	Latitude	Longitude	Depth (m)	Dive number (Pangaea labels)	No. of individuals sampled	No. of individuals under CALMAR
Centre (M7)	S 5° 47.8674	E 9° 42.6881	3171	M76/3b 355, D220	80 Cr	46 Cr
South-West (M10)	S 5° 47.9761	E 9° 42.4825	3170	M76/3b 379, D225	143 Cr + 8 Lc	121 Cr + 4 Lc

Further cruise data are archived at the World Data Center (WDC) Mare [www.pangaea.de](http://www.pangaea.de).

[26]. Their distribution is consistent with the methane concentration in water above the sediment which is higher in the centre of the pockmark, where mytilids and siboglinids dominate, than in the periphery dominated by clam beds. Previous studies had enabled the sampling of *C. regab* all along the pockmark while *L. chuni* only in the periphery [4].

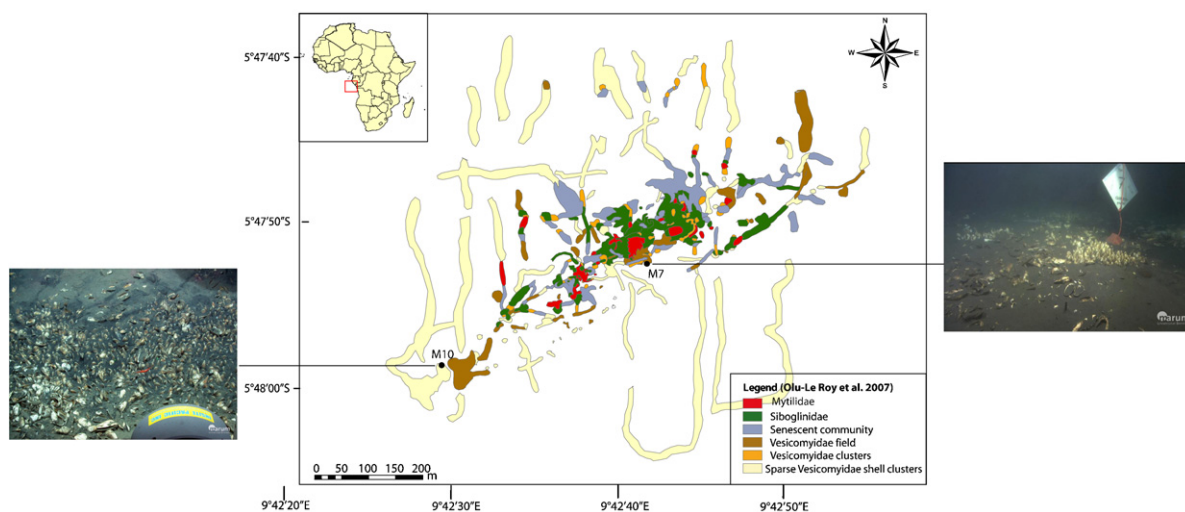
During the GUINECO expedition M76-3b aboard the RV *Meteor* (Chief scientist: A. Boetius), the Regab pockmark was re-explored with the ROV *Quest 4000* (MARUM, University Bremen, Germany). Following previous studies, two sites were studied along the NE-SW axis: (1) at the centre (Site 1, Marker GUINECO 7) where the highest methane levels in vesicomid beds were recorded in 2001 (3.9–4.4  $\mu\text{M}$ ); and (2) to the south-west (Site 2, Marker GUINECO 10) where methane concentrations were roughly four times lower (0.9–1.0  $\mu\text{M}$ ) (Table 1, Fig. 1). At each site, water temperature below the sea-floor recorded by the ROV *Quest 4000* was 2.6 °C. As previously observed [4], these sites were characterised by dense aggregates of vesicomid bivalves. During the GUINECO cruise, *C. regab* being sampled at both sites, whereas a few individuals of *L. chuni* was also sampled in the SW vesicomid bed.

## 2.2. Benthic chamber deployment and data acquisitions

To assess *in situ* TOU and methane fluxes, the CALMAR benthic chamber [21] was deployed by the ROV on one clam bed at each site. CALMAR is a cylinder of 41 cm

diameter opened at one end. It is equipped with six sampling cells controlled by a motor and a stirred to homogenize the water samples. The sampling sequence is programmed onboard, before the deployment. At each site, CALMAR was deployed during at least 6 hours (Fig. 2). Deployment time depended on the number of bivalves under the benthic chamber, previously estimated. In a 6-hour time lapse, oxygen concentrations in the CALMAR chamber could decrease and stabilise, making it possible to calculate oxygen fluxes. Penetration depth was estimated from video-monitoring of the ROV and the corresponding volume of the chamber is used for flux estimates. The maximum volume of water enclosed under the chamber is 40 L. When the benthic chamber was placed, the ROV removed the magnet starter to start the sampling sequence.

Variations of oxygen and methane concentrations in the chamber were obtained by analysis of sequential water samples (6 sampling cells) and oxygen optode probe (Aadi®) measurements. The analytical methods are described in detail in Caprais et al. [21]. Briefly, dissolved oxygen concentration was analyzed in water samples using the modified Winkler titration method [27] with a precision of 2  $\mu\text{mol.L}^{-1}$ . Methane was analyzed by gas chromatography coupled with a headspace system (GC-HSS) [28]. Using the oxygen probe, *in situ* data can be obtained with better temporal resolution (data acquisition every 5 s) and a precision of 5%. The fluxes were calculated from the slope of the linear regression before the plateau,



**Fig. 1.** Location of the two study sites in the Regab pockmark: Site 1 at the centre of the pockmark (Marker GUINECO 7, M3 [26]) and Site 2 in the south-western part of the pockmark (Marker GUINECO 10, VC [26]). Images ROV QUEST, MARUM University of Bremen.

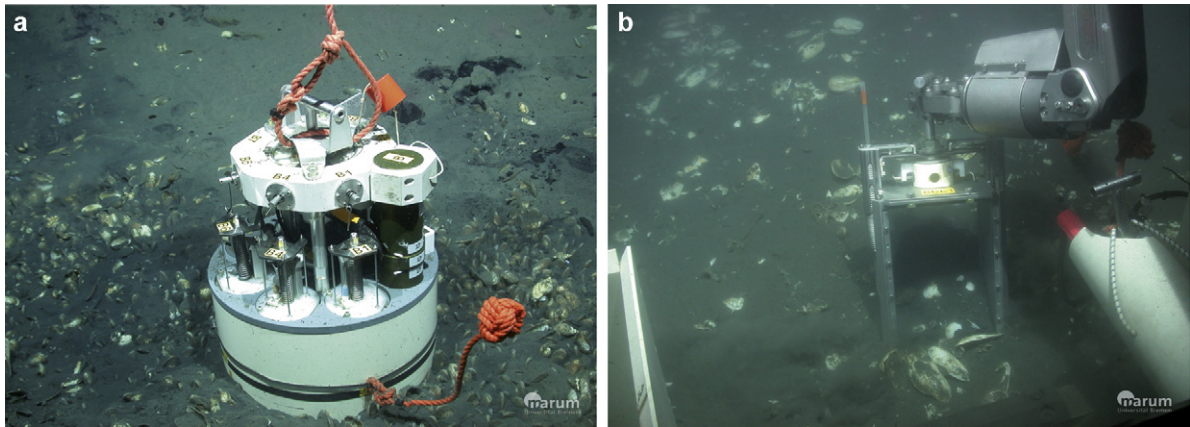


Fig. 2. Deployment of the benthic chambers: a: the CALMAR benthic chamber positioned over a vesicomid bed; b: sampling vesicomids using a blade corer (images ROV QUEST, MARUM, University of Bremen).

obtained with oxygen concentration versus time (Fig. 4). The plateau indicates a stop of oxygen consumption by the vesicomid bivalves. The oxygen concentration of  $35 \mu\text{M}$  (Site South-West) and  $50 \mu\text{M}$  (site Centre) could be considered as the threshold of hypoxia [29]. Below these concentrations, vesicomid bivalves could shift to anaerobic metabolism as demonstrate for costal bivalves or die [29].

Methane fluxes were also calculated from the slope of the linear regression obtained with methane concentration versus time (Fig. 5).

### 2.3. Sampling and sample processing

Vesicomid beds were visually selected during dives to ensure roughly equivalent bivalve densities. Bivalves were counted on images, based on their visible siphons, even though siphon length varies among species [25] (Fig. 3). Two or three blade cores (quantitative samples from  $0.036 \text{ m}^2$ , 20 cm in length) were taken at each site (Fig. 2), one associated with the benthic chamber, and one or two others in beds with approximately the same density. We



Fig. 3. An aggregate of vesicomid bivalve at site 2 (SW) with *C. regab* and *L. chuni*. Arrows show siphon of *L. chuni* (images ROV QUEST, MARUM, University of Bremen).

assessed the proportion of vesicomid biomass relative to total macrofauna biomass by comparing densities. Including the additional net samples from nearby beds, a total of 231 vesicomids were sampled (Table 1). After blade cores and nets were recovered, vesicomid species were identified, washed with seawater and fixed in 4% buffered formalin. Two species were collected: *C. regab* – the dominant species with 223 sampled individuals – and *L. chuni* with only 8 individuals sampled in the SW site (Site 2). In the blade cores associated to CALMAR deployments, only 2 individuals of *L. chuni* were sampled at Site 2. Some bivalves (50% from Site 1 and 100% from Site 2) were transferred to 70% ethanol after 2 days for further study.

### 2.4. Density, biomass and condition index estimations

At each site, vesicomid density ( $\text{ind.m}^{-2}$ ) was estimated using photos taken by the ROV before benthic chamber deployment and *via* quantitative sampling using blade cores ( $n = 2$  or 3) (Table 2). Due to large standard deviations, stemming from differences obtained between these two methods (lower density based on image data

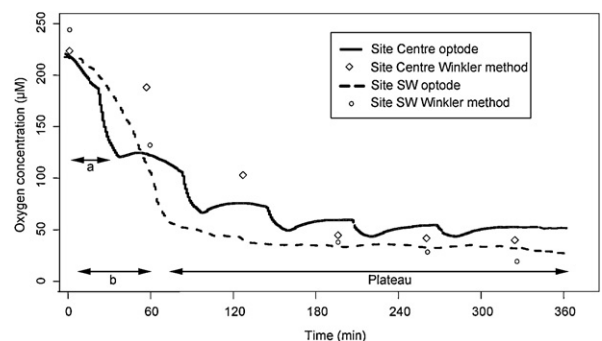


Fig. 4. Time evolution of oxygen concentrations under the CALMAR benthic chamber on both beds, obtained using chemical (Winkler) analysis of sequential samples and an oxygen probe. a: linear regression used for flux calculation for Centre site; b: linear regression used for flux calculation for SW site.

**Table 2**

Number of individuals weighed, minimum and maximum density and biomass estimations, and condition index at each site.

Site	Site 1-Centre	Site 2-South-West
Number of individuals weighed	39	29
Mean individual tissue wet mass (SD) (g)	10.9 (3.3)	n.d.
Mean individual tissue dry mass (SD) (g)	2.2 (0.7)	1.6 (0.4)
Mean individual total dry mass (SD) (g)	9.8 (2.3)	8.6 (2.1)
Mean individual AFDM (SD) (g)	1.9 (0.6)	1.5 (0.4)
Condition index (SD)	0.10 (0.3)	0.08 (0.01)
Mean density (SD) (ind.m <sup>-2</sup> )	681 (296)	1056 (218)
Min (images)	444	806
Max (blade cores)	917	1277
Mean biomass (SD) (g tissue wet mass.m <sup>-2</sup> )	7389.5 (3218.87)	n.d.
Min	3776.1	n.d.
Max	9950.4	n.d.
Mean biomass (SD) (g tissue dry mass.m <sup>-2</sup> )	1508.4 (657.1)	1655.8 (341.5)
Min	770.8	1263.8
Max	2031.2	2002.3
Mean biomass (SD) (g total dry mass.m <sup>-2</sup> )	6699.0 (2918.1)	9105.9 (1777.8)
Min	3423.3	6950.1
Max	9020.5	11011.6
Mean biomass (SD) (g AFDM.m <sup>-2</sup> )	1313.6 (572.2)	1558.7 (321.4)
Min	671.3	1189.6
Max	1768.9	1884.9

Standard deviations (SD) are given in parentheses.

compared to that estimated from blade cores) and from biases inherent to each method (abundance can be underestimated in images, surface sampled by blade core is smaller than that covered by the benthic chamber, and bed size can be smaller than chamber size), we chose to use both minimum and maximum densities to report a range of density and metabolism estimates.

At the laboratory, total dry mass (shell + tissue), tissue dry mass, and ash-free dry mass (AFDM) were determined to the nearest 0.1 g for *C. regab* stored in 4% buffered formalin from Site 1 (39 individuals) and for a subsample of individuals stored in 70% ethanol from Site 2 (29 individuals). Dry mass was obtained after tissues and shells were dried for 24 h at 60 °C. Ash weight was measured after combustion for 12 h at 550 °C. AFDM was determined after subtracting ash weight from dry weight. Individual total mass and AFDM were then compared among sites using the Student's *t*-test. For each site, mean, standard deviation and minimum and maximum biomass (g total mass.m<sup>-2</sup> and g AFDM.m<sup>-2</sup>) were calculated using minimum and maximum density, and mean individual total dry mass, tissue dry mass and AFDM. As *L. chuni* was not dominant [4 individuals counted under the benthic chamber (Table 1)], biomass estimates were based only on *C. regab*. Biomasses were compared among sites using non-parametric test (Wilcoxon-Mann-Whitney). Condition indexes (tissue dry weight/internal shell volume) [30] were estimated for individuals at each site (39 individuals from Site 1 and 29 individuals from Site 2). All analyses were performed using the free open-source R environment [31]. The Vegan library was used for the statistical tests [32].

### 2.5. Estimates of oxygen consumption rate

At each site, an attempt to estimate bivalve oxygen consumption rates (μmol.g total mass<sup>-1</sup>.h<sup>-1</sup> and μmol.g

AFDM<sup>-1</sup>.h<sup>-1</sup>) was made using TOU and biomass. The mean and standard deviation were also calculated. This value is considered as the upper estimate as experiment did not allowed one to assess the part of the TOU attributed to macrofauna and microorganism activities during the experiment with the CALMAR.

## 3. Results

### 3.1. Total oxygen uptake and methane fluxes

At both sites, oxygen measurements from both methods, sequential samples and oxygen probes, were correlated ( $R^2=0.92$ ) (Fig. 4). At Site 1, the oxygen concentration under CALMAR was 222.2 μmol.L<sup>-1</sup> at the beginning of incubation. After 6 h incubation, the oxygen concentration was 51.0 μmol.L<sup>-1</sup>. Despite a stepwise decrease in concentration, most of the oxygen was consumed after about 2 h (Fig. 4). The “zigzag” observed was probably related to a lack of homogenization of water under the chamber. Indeed, the stopping of the stirrer motor during sequential water sampling could have been too long in this case, compared to the other deployment [21]. At Site 2, oxygen concentrations under the CALMAR benthic chamber decreased from 215.1 μmol.L<sup>-1</sup> to 31.9 μmol.L<sup>-1</sup> after 6 h incubation (Fig. 4). As in Site 1, Site 2's profile shows a sharp decrease in concentration after 2 h. TOUs, calculated from the slope of the optode profiles, were 332 mmol.m<sup>-2</sup>.d<sup>-1</sup> at Site 1 and 492 mmol.m<sup>-2</sup>.d<sup>-1</sup> at Site 2 (Table 3).

At the beginning of incubation, the methane concentration was 1.1 μmol.L<sup>-1</sup> at Site 1 and 13.1 μmol.L<sup>-1</sup> at Site 2. Six hours later, concentrations were much higher at Site 1 (383.0 μmol.L<sup>-1</sup>) than at Site 2 (26.8 μmol.L<sup>-1</sup>) (Fig. 5). In contrast to TOU, methane flux was higher at Site 1 than at Site 2 (respectively: 14.6 mmol.m<sup>2</sup>.d<sup>-1</sup> and 0.3 mmol.m<sup>2</sup>.d<sup>-1</sup>) (Table 3).

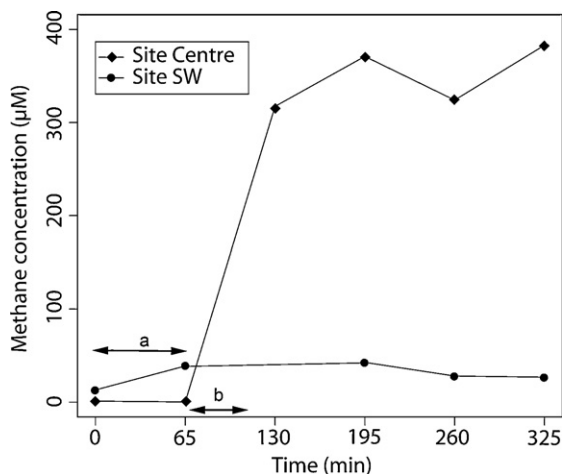
**Table 3**  
Total oxygen uptake, methane flux and respiratory rates estimates at each site.

Site	1-Centre	2-South-West
Total oxygen uptake ( $\text{mmol.m}^{-2}.\text{d}^{-1}$ )	332	492
Methane flux ( $\text{mmol.m}^{-2}.\text{d}^{-1}$ )	14.6	0.3
Mean oxygen consumption rate (SD) ( $\mu\text{mol.g tissue wet mass}^{-1}.\text{h}^{-1}$ )	2.2 (0.4)	n.d.
Min	1.4	n.d.
Max	3.7	n.d.
Mean oxygen consumption rate (SD) ( $\mu\text{mol.g tissue dry mass}^{-1}.\text{h}^{-1}$ )	10.9 (6.1)	12.8 (2.8)
Min	6.8	10.2
Max	17.9	16.2
Mean oxygen consumption rate (SD) ( $\mu\text{mol.g total dry mass}^{-1}.\text{h}^{-1}$ )	2.5 (1.4)	2.3 (0.5)
Min	1.5	1.9
Max	4.0	3.0
Mean oxygen consumption rate (SD) ( $\mu\text{mol.g AFDM}^{-1}.\text{h}^{-1}$ )	12.5 (7.0)	13.6 (2.9)
Min	7.8	10.8
Max	20.6	17.2

### 3.2. Density, biomass and condition index

Densities estimated from images were lower than those estimated from blade cores. Combining both estimates provides upper and lower estimates of density for each site. From image and blade core data, vesicomid density ranged from 444 to 1277  $\text{ind.m}^{-2}$  with highest mean value observed at Site 2 ( $1056 \pm 207 \text{ ind.m}^{-2}$ ) and the lowest at Site 1 ( $681 \pm 119 \text{ ind.m}^{-2}$ ) (Table 2).

At Site 1, individual total mass (dry weight + shell) was significantly higher than at Site 2: respectively  $9.8 \pm 2.3 \text{ g}$  and  $8.6 \pm 2.1 \text{ g}$  ( $t$ -test:  $t = 1.97$ ,  $P < 0.053$ ). Similar results were observed for AFDM ( $t = 3.61$ ,  $P < 0.001$ ) (Table 2). However, due to higher density, and despite lower individual weights, total biomass was higher at Site 2 ( $9.1 \pm 1.9 \text{ kg total mass.m}^{-2}$  and  $1.6 \pm 0.3 \text{ kg AFDM.m}^{-2}$ ) than at Site 1 ( $6.7 \pm 2.9 \text{ kg total mass.m}^{-2}$  and  $1.3 \pm 0.6 \text{ kg AFDM.m}^{-2}$ ), but this difference was not significant (Wilcoxon-Mann-Whitney test, total dry mass:  $W = 3$ ,  $P = 0.28$ ; AFDM:  $W = 5$ ,  $P = 0.72$ ). Significantly higher condition indexes were observed for individuals at Site 1 compared to Site 2 (Wilcoxon-Mann-Whitney test:  $W = 658.5$ ,  $P < 0.001$ ).



**Fig. 5.** Time evolution of methane concentration under the CALMAR benthic chamber on both beds, obtained using the Winkler chemical method. a: linear regression used for flux calculation for SW site; b: linear regression used for flux calculation for Centre site.

### 3.3. Oxygen consumption rates

At the centre of the pockmark (Site 1), the oxygen consumption rate estimated using the upper and lower biomass estimates and the TOU ranged from 1.5  $\mu\text{mol.g total mass}^{-1}.\text{h}^{-1}$  to 4.0  $\mu\text{mol.g total mass}^{-1}.\text{h}^{-1}$  (Table 3). On the bed at the south-western part of the pockmark (Site 2), oxygen consumption rates were slightly lower: 1.9  $\mu\text{mol.g total mass}^{-1}.\text{h}^{-1}$  to 3.0  $\mu\text{mol.g total mass}^{-1}.\text{h}^{-1}$  (Table 3), but the difference between the two sites was not significant (Wilcoxon-Mann-Whitney test,  $W = 4$ ,  $P = 1$ ).

## 4. Discussion

### 4.1. Total oxygen uptake in deep-sea environment

Our values of TOU (from 332 to 492  $\text{mmol.m}^{-2}.\text{d}^{-1}$ ) are the highest ever reported for deep-sea species from chemosynthetic environments. To date, the highest TOUs from deep-sea species have been measured at the Håkon Mosby mud volcano (HMMV) in siboglinid tubeworm fields ( $161 \text{ mmol.m}^{-2}.\text{d}^{-1}$ ) [22] and at the Hikurangi margin, in ampharetid polychaete ones ( $117.7 \text{ mmol.m}^{-2}.\text{d}^{-1}$ ) [23]. However, polychaete biomass is probably lower than vesicomid biomass. For example, on HMMV, biomass of siboglinid tubeworms ranges from 350 to 435  $\text{g.m}^{-2}$  [33], which is 10 times lower than the vesicomid value on Regab (Table 2). Other TOU measurements performed with benthic chambers are much lower. For example, at the Hikurangi margin (New Zealand), benthic chamber deployment provided a TOU of 65.2  $\text{mmol.m}^{-2}.\text{d}$  for siboglinid polychaetes [23]. At the Napoli mud volcano (Mediterranean Sea), the CALMAR deployed on *Lamellibracchia* sp. and small mytilid bivalves *Idas* sp. revealed a TOU of 35  $\text{mmol.m}^{-2}.\text{d}^{-1}$  [21]. Measurements performed on a vesicomid bed at Hydrate Ridge [20] also show lower TOUs (from 2.0 to 5.1  $\text{mmol.m}^{-2}.\text{d}$ ).

### 4.2. Estimates of respiration rate of vesicomid bivalve from TOU

Vesicomid bivalves are the most pervasive of symbiont-bearing taxa in deep cold-seep ecosystems, and occur at most known cold-seep sites. Their density can reach up

**Table 4**

Total oxygen uptake from sulphide oxidation (83.4% of TOU) without endofauna respiration (5% of TOU) and lower estimates of respiration rate from these values of TOU from sulphide oxidation.

Site	1-Centre	2-South-West
Total oxygen uptake from sulphide oxidation (mmol.m <sup>-2</sup> .d <sup>-1</sup> )	259	384
Mean oxygen consumption rate (SD) (μmol.g tissue wet mass <sup>-1</sup> .h <sup>-1</sup> )	1.7 (0.3)	n.d.
Min	1.1	n.d.
Max	2.8	n.d.
Mean oxygen consumption rate (SD) (μmol.g tissue dry mass <sup>-1</sup> .h <sup>-1</sup> )	8.5 (1.7)	10.0 (2.1)
Min	5.3	7.9
Max	14.0	12.6
Mean oxygen consumption rate (SD) (μmol.g total dry mass <sup>-1</sup> .h <sup>-1</sup> )	1.9 (0.4)	1.8 (0.4)
Min	1.2	1.4
Max	3.1	2.3
Mean oxygen consumption rate (SD) (μmol.g AFDM <sup>-1</sup> .h <sup>-1</sup> )	9.8 (1.9)	10.6 (2.2)
Min	6.1	8.5
Max	16.1	13.4

Proportion of TOU used for sulphide oxidation from Sommer et al. [20] and proportion used for endofauna respiration: 5%. Standard deviations (SD) are given in parentheses.

to 1000 ind.m<sup>-2</sup> and their biomass ranges from 10 to 30 kg wet weight m<sup>-2</sup> [34–36]. They occur in a wide range of sulphide and methane concentrations: from 0 to 25 mM [37] and from 0 to 300 μM in pore waters [38]. While mytilids and siboglinids are limited to the central, most active area of the Regab pockmark, vesicomysids are observed in soft sediments in areas affected by methane seeps, covering about 2% of the central part where methane and carbonate concretions are abundant, and 14% of the periphery area, with lower methane concentrations (0.5 to 4.4 μM at the sediment-water interface) and only on soft bottoms [26]. Via their symbiotic bacteria, vesicomysids contribute to sulphide oxidation and oxygen consumption, also needed for their own respiration. The intensity of these gas exchanges depends on the density and biomass of organisms and their metabolism. Although vesicomysids dominated the biomass in clusters, small endofauna and microbes were associated with bivalve assemblages [39–41]. According to differences in individual sizes, and therefore in biomass, associated fauna likely contribute relatively little to oxygen uptake. In Regab vesicomysid clusters, associated macrofauna densities were estimated but not biomass, therefore we used data from another cold-seep site [37] to assess the contribution of macrofaunal biomass: at the Cascadia margin vesicomysids accounted for more than 90% of the total macrofaunal biomass in vesicomysid beds. In the Regab pockmark, Site 1 showed higher macrofaunal densities (excluding vesicomysids) of 15,583 ind.m<sup>-2</sup> (unpublished data) than those observed at the Cascadia margin (4968 ind.m<sup>-2</sup>), but vesicomysid densities were higher at Regab (this study, Table 2). Taking into account the macrofaunal biomass/density relation of Sahling et al. [37], macrofauna at Site 1 accounted for less than 5% of total biomass. At Site 2, macrofaunal density was similar to that observed on the Cascadia margin but vesicomysid density was higher: respectively, 3370 ind.m<sup>-2</sup> (unpublished data) and 1056 ind.m<sup>-2</sup> (Table 2). The bivalve therefore accounted for more than 95% of total biomass. The TOUs measured using the CALMAR chamber on vesicomysid beds (332–492 mmol.m<sup>-2</sup>.d<sup>-1</sup>) were more than 20 times higher than

typical sediment just outside Regab (3.6 mmol.m<sup>-2</sup>.d<sup>-1</sup> at 250 m from the Regab pockmark [42]) and in the eastern Mediterranean mud volcano cold-seep sediment beside siboglinids (13.5 mmol.m<sup>-2</sup>.d<sup>-1</sup>) [21]. These TOU measured are likely mainly due to endofauna which densities were less than 2000 ind.m<sup>-2</sup> outside Regab [39] and 2900 ind.m<sup>-2</sup> in the mud volcano sediments [43]. We therefore assumed that the TOU used for endofauna respiratory was negligible compared to the bulk used for vesicomysid oxygen consumption.

Oxygen is also consumed by microbes in the sediment [19,20,44,45]. Nevertheless, Sommer et al. [20] have measured that on vesicomysid beds of Hydrate Ridge, aerobic oxidation of methane was negligible. Moreover, they have found that more than 83% of TOU were used for sulphide oxidation and the rest for ammonia and organic carbon mineralization. As vesicomysid clams contain sulphide-oxidizing symbionts, we can assume that 83% of TOU was used for their metabolism. Then, oxygen can also be consumed by the chemical oxidation of sulphide. Nevertheless, as indicated by Luther et al. [46], the chemical oxidation of sulphide rate is at least three orders of magnitude lower than the microbial anaerobic or aerobic oxidation rate.

Taking into account that 17% of TOU was used for microbial processes at both sites and 5% for endofauna respiration, TOU induced to vesicomysid oxygen consumption is 259 and 384 mmol.m<sup>-2</sup>.d<sup>-1</sup> respectively at Sites 1 and 2. A lower estimation of vesicomysid respiratory rate therefore could be 1.9 ± 0.4 and 1.8 ± 0.4 μmol.g total dry mass<sup>-1</sup>.h<sup>-1</sup> respectively and 1.7 ± 0.3 μmol.g wet tissue mass<sup>-1</sup>.h<sup>-1</sup> for Site 1 (Table 4).

#### 4.3. Comparison of respiration rate estimates of vesicomysid bivalves with deep and shallow species

The *ex situ* experimentally obtained oxygen consumption rate of the vent species *C. magnifica* Boss and Turner, 1980 [12] from the Galapagos Rift ranges from 0.4 μmol.g tissue wet weight<sup>-1</sup>.h<sup>-1</sup> at 2.1 °C to 1.58 μmol.g tissue wet weight<sup>-1</sup>.h<sup>-1</sup> at 8 °C [8]. The consumption of the other vent

species *C. pacifica* Dall, 1891 and *C. elongata* Dall, 1916 is lower (average  $0.2 \mu\text{mol g tissue wet weight}^{-1} \text{ h}^{-1}$  [10,11]. In comparison, our lower estimates were about  $1.7 \mu\text{mol g tissue wet weight}^{-1} \text{ h}^{-1}$  at about  $2.6^\circ\text{C}$ . These oxygen consumption rates were higher than those of other species measured at similar temperature (e.g. *C. pacifica*) and close to the upper estimate obtained for *C. magnifica* at higher temperature.

*Ex situ* respiratory rate of the deep-sea bivalve *Acesta excavaca* is lower than for vesicomid bivalves living in the Regab pockmark (respectively  $5 \mu\text{mol.g tissue dry weight}^{-1} \text{ h}^{-1}$  and  $8.5$  to  $10.0 \mu\text{mol g tissue dry weight}^{-1} \text{ h}^{-1}$  at  $9^\circ\text{C}$ ) [47] (Table 4). For comparison with shallow-water bivalves, *ex situ* measurements of oxygen consumption at  $20^\circ\text{C}$  for *Tapes philippinarum* and *Crassostrea gigas* are higher than for deep-sea species, with values from  $30$  to  $40 \mu\text{mol g tissue dry weight}^{-1} \text{ h}^{-1}$  and  $11 \mu\text{mol.g tissue wet weight}^{-1} \text{ h}^{-1}$ , respectively [48,49]. Finally, Arp et al. [8] observed similar oxygen consumption rates between *C. magnifica* and shallow-water bivalves.

#### 4.4. Variation of TOU and respiration rate estimates of vesicomid bivalve along the Regab pockmark

Although both CALMAR measurements were performed within the same pockmark (distance 430 m) and in the same biogenic habitat, TOU was different:  $332$  vs.  $492 \text{ mmol.m}^{-2}.\text{d}^{-1}$ . Differences in TOU may likely be not attributed to microbial process as microbial community and especially the number of AOM aggregates producing sulphide do not vary between sites populated by vesicomids within Regab [40]. Small macrofauna density was higher at Site 1 than at Site 2 (unpublished data) and therefore cannot explain higher TOU at Site 2. These results can be therefore related to density differences of the two vesicomid beds:  $681 \pm 297 \text{ ind.m}^{-2}$  and  $1056 \pm 206 \text{ ind.m}^{-2}$  at Sites 1 and 2, respectively. However, taking into account mean individual vesicomid biomass, and given that individuals from Site 1 were larger than those from Site 2, and with higher dry weight for a given size, the average oxygen consumption rate was similar in both beds. These results show the importance of estimating biomass using indicators other than density to compare TOUs. Moreover, these estimations of TOU suggest that the oxygen consumption rates are relatively constant for a species within a given cold-seep. Condition indexes appeared to be related to metabolism, with individuals in good condition (Site 1) having the highest oxygen uptake.

#### 4.5. Influence of habitat geochemistry on the biology of vesicomid bivalves in Regab pockmark

Methane fluxes in vesicomid clusters varied along the pockmark with higher values at the centre ( $14.6 \text{ mmol.m}^{-2}.\text{d}^{-1}$ ) than at the periphery ( $0.3 \text{ mmol.m}^{-2}.\text{d}^{-1}$ ), as observed with concentrations at the REGAB pockmark in 2001 ( $3.9 \mu\text{M}$  vs  $0.65 \mu\text{M}$ ) [26]. Other measurements on vesicomid clam beds [20] show methane fluxes of  $0.2$ – $1.1 \text{ mmol.m}^{-2}.\text{d}^{-1}$ , which are similar to the lowest fluxes at Regab. Heterogeneous methane fluxes have also been observed at Napoli [21] and HMMV [22], Costa Rica

convergent margin [19] and among habitats at the Hikurangi margin [23]. In deep-sea sediment, AOM consortia oxidize methane and produce sulphide [9]. These consortia were found in sediment below vesicomid beds [40]. With the assumption that, within the same habitat type (here vesicomids) methane is a proxy of sulphide, methane flux heterogeneity may reflect sulphide flux heterogeneity.

At the Regab pockmark, methane flux heterogeneity is also observed within the same biogenic habitat (vesicomid beds) and could also explain the difference in individual biomass and condition index for *C. regab*. For example, at Site 1 located in the centre, average individual total biomass and condition indexes were higher than at the peripheral site, Site 2 ( $9.8 \pm 2.3 \text{ g}$  and  $8.6 \pm 2.1 \text{ g}$ , respectively).

To summarise, the site at the centre (Site 1) of the pockmark is populated by individuals of higher weight and in better physiological condition. Higher methane fluxes at this site support lower density of vesicomids, but almost the same amount of biomass (tissue dry weight) than at the less active SW site; oxygen demands are thus similar between both sites.

Moreover, methane may also influence the vesicomid species' distributions, as previously observed [26]. At the centre, where the methane fluxes were higher, only *C. regab* was found, whereas in the south-western part of the pockmark, *L. chuni* was sampled, in addition to *C. regab*. This distribution can be attributed to differences in physiological adaptation, e.g. sulphide-binding ability and symbiont energy turnover as demonstrated for *C. pacifica* and *C. kilmeri* inhabiting cold-seeps in Monterey Bay [50,51]. Differences in soft part morphology between the two vesicomid species at Regab, with longer siphons observed for *L. chuni* may also influence their distribution [25]. This second species was unfortunately too rarely observed to estimate its respiration rate, but it could differ from the dominant *C. regab*.

#### Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

#### Acknowledgements

We are grateful to the chief scientist of the GUINECO (MARUM) cruise (A. Boetius, MARUM); to the captain and crew of the R/V *Meteor* expedition M76/3b and the crew of the ROV *Quest 4000* (MARUM). The GUINECO cruise was done with the support of the DFG Research Center The Ocean in the Earth System – MARUM – Center for Marine Environmental Sciences at the University Bremen. We are grateful to Frank Wenzhöfer for support with work at sea and to Christian Le Gall for the oxygen and methane measurements. English usage was professionally edited by Carolyn Engel-Gautier. We thank the two anonymous reviewers for their comments that help us to improve the manuscript.



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