

Carbon Sequestration Capacity of Cold Seep Bivalves in the South China Sea

Chaofeng Cai ^{1,2,3}, Jing-Chun Feng ^{1,2,3*}, Xiaochun Zhang ^{1,2,3}, Si Zhang ^{1,2}, Yufan Zhou ^{1,2,3}

1 Research Centre of Ecology & Environment for Coastal Area and Deep Sea, Guangdong University of Technology & Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 510006, China

2 Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 511458, China

3 School of Ecology, Environment and Resources, Guangdong University of Technology, Guangzhou, 510006, China

(* Corresponding Author: fengjc@gdut.edu.cn)

ABSTRACT

The bivalves in cold seep ecosystem can assimilate dissolved inorganic carbon (DIC) into their shell through extrapallial fluid (EPF) and methane through endosymbionts on their gills. Therefore, the DIC and methane released from cold seep can be decreased by the biological metabolism process of the bivalves, which can reduce such carbon enter into the atmosphere. Yet the contribution of cold seep bivalves are not considered hitherto. We choose three common bivalves from cold seep of South China Sea, *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* and measure the carbon content as well as wet weight and dry weight. Comparing to five shellfish species the cold seep bivalves can store more carbon into their tissue. Combining the fact they feed on methane-oxidizing bacteria and sulfide-oxidizing bacteria, they can reduce the methane possibly released to the air. The *Archivesica marissinica* bivalves have the best carbon sequestration capacity for higher tissue weight.

Keywords: Cold Seep Bivalves, Carbon Content, Carbon Sequestration Capacity

NONMENCLATURE

Abbreviations

MOX	Methane-oxidizing bacteria
SOX	Sulfide-oxidizing bacteria
AOM	Anaerobic oxidation of methane
DIC	Dissolved inorganic carbon
OSR	organoclastic sulfate reduction

1. INTRODUCTION

Human being endeavors a lot to control the global warming, from United Nations Framework Convention on Climate Change, to Kyoto Protocol, as well as Paris Agreement, which aims to control the global average temperature rise in the 21st century to within 2 °C, and control the global temperature rise to within 1.5 °C above pre-industrial levels.

Great attention has been paid to negative carbon emission technologies, such as reforestation, afforestation, bioenergy with carbon capture and storage [1], ocean fertilization [2], etc. Overall, over 50% of carbon is sequestered by ocean. This part of carbon is called blue carbon. Macroalgae, microbial carbon pump as well as biological metabolism process of sea animals, including bivalves, contribute a lot to this part of sequestered carbon.

Generally coastal ecosystems meet the Blue Carbon criteria by having a long-term storage of fixed CO₂ to significantly remove greenhouse gas [3]. In coastal and marine animal aquaculture, the biological carbon sequestration bivalve shellfish species (primarily clams, mussels and oysters) have the high capacity of fixing carbon because when they reach their economic carrying capacity 29.23 t of CO₂ can be sequestered by bivalve shellfish [4]. Beside their food production (tissue) worthy of \$23.92 billion, the shell can serve for poultry grit, fertilizer, lime and so on, with a worldwide non-food services worthy of \$6.47 billion [5]. The Global total carbon sequestration of deep sea ecosystem is 6.0 Tg·C·a⁻¹ and comparatively the coastal ecosystem is 237.6 Tg·C a⁻¹, yet the contribution of deep sea is not clear and the estimation is based on mathematical model and experimental parameters.

Cold seeps are seafloor ecosystem, where methane-rich fluid migrates from the sedimentary subsurface to the seabed and into the water column, and ultimately some of

the methane may even reach the atmosphere [6]. Study reveals the fact that the methane emitted from cold seep may not reach the sea surface due to the dilution effect [7] yet the DIC contribution is not clear. DIC from cold seep can reach the sea surface through upwelling and emit old carbon to the air in South China Sea [8]. As far as we know, the research is dedicated to study the dilution effect of methane released from cold seep so the metabolism effects of cold seep bivalves (calcification and assimilation) are not considered. Also the study focuses only on methane but methane can be transformed to DIC through anaerobic oxidation of methane (AOM) and DIC is also an important part of greenhouse gases emission. Furthermore, the contribution part of bivalves to decrease DIC on the seabed, including cold seep, is not considered.

This study shed light on the potential carbon sequestration capacity of cold seep bivalves with their shell and tissue by measuring the wet weight, dry weight and carbon content of the shell and tissue of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica*.

2. GEOLOGICAL SETTING

In this work, cold seep bivalves were obtained from two well-explored cold seep of the South China Sea, F cold seep and Haima cold seep. Site F, with an approximate estimate area of 100 m × 140 m, is located at the water depth of 1120m on the northeastern continental slope of the SCS and Site Haima is located at 1370-1390 m in the southwestern part of Qiongdongnan Basin. Haima is a more active cold seep with bigger bubble diameter and faster release rate (For F 3.35 mm and 6.17 bubbles s⁻¹ and for Haima 6.17 mm and 22.6 bubbles s⁻¹) [9]. Also a number of dead shells seen in F site is another evidence of less activity [10]. What's more, thiotrophic species like tubeworms and bivalves *Archivesica marissinica*, indicating a higher concentration of sulfide, are found in Haima cold seep but such species are not found hitherto in F cold seep.

3. MATERIALS AND METHODS

As shown in Fig.1, the bivalves were captured alive in three locations respectively. The cold seep bivalves were captured alive and frozen under 80 °C. In laboratory the shell and tissue are departed and cleaned carefully. The wet weight and dry weight are measured according to Wetzel, M.A. [11]. In short, the wet weight was estimated after blotting the live animals on tissue paper for 1 min and samples were placed for 24 h in an oven at 100 °C and then the dry weight was estimated. The dry tissue and shell

were delivered to elemental analyzer (EA Isolink CNHO) to measure carbon content. The carbon content of shell or tissue is calculated from the following equation:

$$\text{carbon storage/g} = \text{dry weight} \times \text{carbon content}$$

And the DIC fixed by shell and the methane fixed by tissue is simply evaluated according to moles of carbon from carbon content.

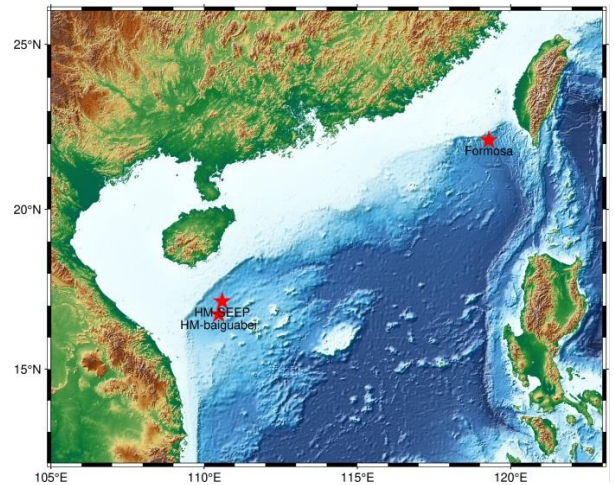


Figure.1 The sampling location (Red star) of bivalves *Bathymodiolus platifrons* (Formosa) from F cold seep, *Gigantidas haimaensis* (HM-SEEP) *Archivesica marissinica* (HM-baiguabei) from Haima cold seep.

4. RESULTS AND DISCUSSION

4.1 Characteristics of carbon storage of cold seep bivalves

As shown in Fig.2 and Table.1, the stored carbon via shell of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* is 2.35g±1.61, 2.89g±1.05, 8.60g±1.29. The carbon storage of tissue of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* 2.58g±1.86, 2.09g±1.40, 11.24g±2.03. The average shell carbon content of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* is 14.05%±0.88, 13.59%±1.15, 13.22%±0.96. The average tissue carbon content of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* is 42.18%±0.79, 39.36%±1.69, 40.16%±2.14. The dry weight ratio of *Bathymodiolus platifrons*, *Gigantidas haimaensis*, *Archivesica marissinica* is 0.70±0.08, 0.57±0.05, 0.71±0.05.

Table 1. The carbon content of shell and tissue of cold seep bivalves (dry weight).

Species	Carbon Content/%	
	Shell	tissue

<i>Bathymodiolus platifrons</i>	14.05±0.88	42.18±0.79
<i>Gigantidas haimaensis</i>	13.59±1.15	39.36±1.69
<i>Archivesica marissinica</i>	13.22±0.96	40.16±2.14

Compared to five main bivalve aquaculture species (oyster, mussel, scallop, clam and razor clam), except for *Gigantidas haimaensis*, another two bivalves have the nearly same dry weight ratio, carbon content of both shell and tissue but three cold seep have higher tissue ratio. For shellfish species the tissue accounts for 0.34 but cold seep bivalves account for 0.50 [12]. Since the tissue have higher carbon content than shell, the cold seep bivalves can store more carbon by fixing it into tissue. Cold seep bivalves live with chemoautotrophic symbionts, which use reduced compounds to fix carbon dioxide and transport the organic carbon to the host [13]. That would be methane-oxidising bacteria (MOX) for *Bathymodiolus platifrons* and *Gigantidas haimaensis* and sulfur-oxidising bacteria (SOX) for *Archivesica marissinica* [14]. It should be noted that the above species living with MOX contain SOX as well but MOX are the main bacteria. MOX convert methane to carbon dioxide and the energy is used to increase its biomass. Simultaneously the host can control the population of endosymbionts by feeding on a certain part of endosymbionts. In cold seep, the digestive system degrades or even disappears and endosymbionts become the main source of cold seep bivalves. So they can indirectly reduce methane and hydrogen sulfur by feeding on endosymbionts. The shell can sequester dissolved inorganic carbon from the ambient water. It is estimated that major diffusive methane-powered carbon fluxes with 8.7 Tmol·year⁻¹ DIC (range 6.4–10.2 Tmol·year⁻¹) entering the shallow sediments and 6.5 Tmol·year⁻¹ (range 3.2–9.2 Tmol·year⁻¹) of the this DIC pool flows toward the water column [15]. The major part of the shell is biogenic carbonates so the shell can reduce DIC in the water flux and therefore reduce a certain part emitted to the atmosphere through upwelling.

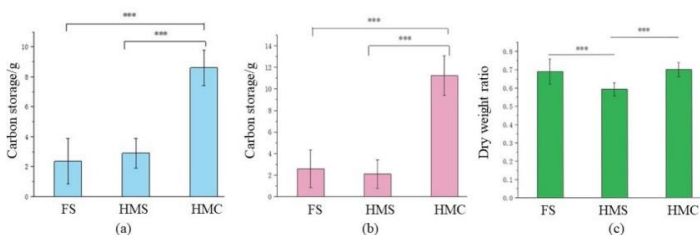


Figure 2. carbon storage of a) shell b) tissue (dry weight) and c) dry weight ratio of three cold seep bivalves. FS, HMS, HMC represent *Bathymodiolus platifrons*, *Gigantidas haimaensis* and *Archivesica marissinica* respectively.

*** means the two group are significantly different (P<0.05).

4.2 Different carbon sequestration capacity between three cold seep bivalves

Calculated from carbon content, one *Bathymodiolus platifrons* is equivalent to 1.67 g DIC and 1.44 g methane. One *Gigantidas haimaensis* and one *Archivesica marissinica* would be 2.06 g DIC as well as 1.09 g methane and 5.68 g DIC as well as 5.99 g methane. The three cold bivalves have the nearly the same carbon content but *Bathymodiolus platifrons* and *Archivesica marissinica* have higher dry weight ratio. Yet the carbon content of tissue are slightly lower so the two mussels have the same carbon sequestration capacity. *Archivesica marissinica* have seemingly highest capacity for its highest weight in three bivalves. However *Archivesica marissinica* lives on SOX so its carbon source of tissue is not indirectly from methane. Study shows that the $\delta^{13}\text{C}$ of *Archivesica marissinica* is $-36.3\text{‰} \pm 0.5$, which is similar to that of sediment organic matter (-35.17‰ for average) [16]. This indicates that the carbon source of *Archivesica marissinica* tissue is from sediment organic matter. The carbon in the sediment organic matter may not be easily released so the carbon fixed by *Archivesica marissinica* tissue seems to circulate between sediments and tissue and this part of carbon is stable in each form. From what has been discussed above, even though *Archivesica marissinica* may adsorbed more carbon into tissue, the main carbon sequestration capacity lies in its shell. But still DIC fixed by clam shell is five times as high as that of mussle shell.

4.3 Carbon sequestration mechanism of cold seep bivalves

As shown in Fig.3, *Bathymodiolus platifrons*, and *Gigantidas haimaensis* lies on the seafloor (exposed to water almost entirely) but *Archivesica marissinica* are half buried. This is because the thiotrophic bivalves would reach their foot deep into sediments to seek sulfide for energy. Combined with similar carbon signature between sediment organic matter and their tissue, it is indicated that the organic matter is adsorbed through organoclastic sulfate reduction (OSR) and the clam *Archivesica marissinica* digest related symbionts for carbon source. For *Bathymodiolus platifrons* and *Gigantidas haimaensis*, their main carbon source is the most abundant symbionts on their gills, MOX. Besides symbiosis, the host digests a proportion of symbionts to extract nutrients for its own growth. The carbon signature of three cold seep bivalves is similar to water DIC, which indicates the carbon source of

all comes directly from DIC of ambient water like other land snails, intertidal and aquatic mollusks. Environmental DIC can be calcified through fluid exchange. Fluid exchange is realized through pericellular pathways of mantle, gaps between shell and the periostracum [17]. It is suggested that with higher CO_2/O_2 (especially for deep sea), more respired CO_2 is released into ambient environment [18]. But in deep sea, it would turn into DIC instead of releasing into the air and therefore reach calcification site again.

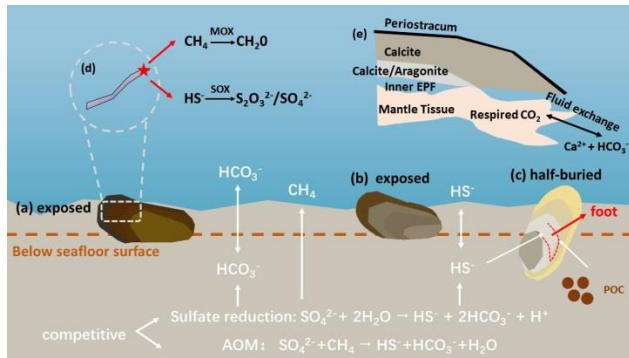


Figure.3 The carbon sequestration mechanism of shell and tissue of (a) *Bathymodiolus platifrons* (b) *Gigantidas haimaensis* and (c) *Archivesica marissinica*. (d) The methane-oxidation and sulfide-oxidation symbionts on the dissected gill filaments. (e) The carbon sequestration mechanism of shell.(modified from Ted A.M. et, 2008).

5. CONCLUSION

This study tests the carbon content and measures the wet weight as well as dry weight from bivalves of three cold seep sites in the South China Sea. Based on these results, we evaluate the DIC adsorbed by shell and the methane adsorbed by tissue of three cold seep bivalves. The main conclusions can be drawn as follows:

- (1) Despite the similar carbon content and dry weight ratio compared to five main shellfish bivalves, cold seep bivalves with higher tissue to shell ratio can store more carbon into the tissue by feeding on endosymbionts.
- (2) For *Bathymodiolus platifrons* and *Gigantidas haimaensis* the carbon source of tissue is from methane-oxidising bacteria so they can fix the methane carbon by endosymbionts. For *Archivesica marissinica* the carbon source of tissue is from sediment organic matter so the carbon sequestration capacity should be considered carefully.
- (3) *Archivesica marissinica* shell contain higher carbon sequestration capacity for higher weight and the tissue of two other bivalves can fix carbon

indirectly because they live on methane-oxidising bacteria.

- (4) The carbon source of cold seep bivalves comes from digested symbionts. They exploit methane or sediment organic matter and transport it to hosts through digestion or symbiosis. The carbon source of shell is the same to other mollusks, which comes directly from DIC of ambient water transported to calcification site through fluid exchange.

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DECLARATION OF INTEREST STATEMENT

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors read and approved the final manuscript.

REFERENCE

- [1] Babin A, Vaneeckhaute C, Iliuta MC. Potential and challenges of bioenergy with carbon capture and storage as a carbon-negative energy source: A review. *Biomass and Bioenergy*. 2021;146.
- [2] Huesemann MH. Ocean fertilization and other climate change mitigation strategies: an overview. *Marine Ecology Progress Series*. 2008;364:243-50.
- [3] Lovelock CE, Duarte CM. Dimensions of Blue Carbon and emerging perspectives. *Biol Lett*. 2019;15:20180781.
- [4] Li Z, Chen Y, Wang G, Mu J, Sun Y, Yu H, et al. Ecological carrying capacity and carbon sequestration potential of bivalve shellfish in marine ranching: A case study in Bohai Bay, China. *Frontiers in Marine Science*. 2023;10.
- [5] Schatte Olivier A, Jones L, Vay LL, Christie M, Wilson J, Malham SK. A global review of the ecosystem services provided by bivalve aquaculture. *Reviews in Aquaculture*. 2018;12:3-25.
- [6] Feng D, Qiu J-W, Hu Y, Peckmann J, Guan H, Tong H, et al. Cold seep systems in the South China Sea: An overview. *Journal of Asian Earth Sciences*. 2018;168:3-16.
- [7] Joung D, Ruppel C, Southon J, Weber TS, Kessler JD. Negligible atmospheric release of methane from decomposing hydrates in mid-latitude oceans. *Nature Geoscience*. 2022;15:885-91.
- [8] Niu Z, Zhou W, Zhao H, Feng X, Lyu M. Radiocarbon in the Atmosphere and Seawater in the South China Sea: Flux, Inventory and Air - Sea CO_2 Exchange Rate Tracing. *Journal of Geophysical Research: Biogeosciences*. 2022;127.

- [9] Di P, Feng D, Tao J, Chen D. Using Time-Series Videos to Quantify Methane Bubbles Flux from Natural Cold Seeps in the South China Sea. *Minerals*. 2020;10.
- [10] Zhao Y, Xu T, Law YS, Feng D, Li N, Xin R, et al. Ecological characterization of cold-seep epifauna in the South China Sea. *Deep Sea Research Part I: Oceanographic Research Papers*. 2020;163.
- [11] Wetzel MA, Leuchs H, Koop JHE. Preservation effects on wet weight, dry weight, and ash-free dry weight biomass estimates of four common estuarine macro-invertebrates: no difference between ethanol and formalin. *Helgoland Marine Research*. 2005;59:206-13.
- [12] Shao G L, Liu B, Li C. Evaluation of carbon dioxide capacity and the effects of decomposition and spatiotemporal differentiation of seawater in Chinas mainsea area based on panel data from 9 coastal provinces in China . *Acta Ecologica Sinica* , 2019 , 39 (7) : 2614-2625.
- [13] Dubilier N, Bergin C, Lott C. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nat Rev Microbiol*. 2008;6:725-40.
- [14] Guan H, Feng D, Birgel D, Kiel S, Peckmann J, Li S, et al. Lipid Biomarker Patterns Reflect Nutritional Strategies of Seep-Dwelling Bivalves From the South China Sea. *Frontiers in Marine Science*. 2022;9.
- [15] Akam SA, Coffin RB, Abdulla HAN, Lyons TW. Dissolved Inorganic Carbon Pump in Methane-Charged Shallow Marine Sediments: State of the Art and New Model Perspectives. *Frontiers in Marine Science*. 2020;7.
- [16] Ke Z, Li R, Chen Y, Chen D, Chen Z, Lian X, et al. A preliminary study of macrofaunal communities and their carbon and nitrogen stable isotopes in the Haima cold seeps, South China Sea. *Deep Sea Research Part I: Oceanographic Research Papers*. 2022;184.
- [17] Hickson A , Johnson ALA , Heaton THE , Balson PS. The shell of the Queen Scallop *Aequipecten opercularis* Las a promising tool for palaeoenvironmental reconstruction : evidence and reasons for equilibrium stable-isotope incorporation. *Palaeogeogr Palaeoclimatol Palaeoecol*. 1999;154 : 325-337.
- [18] McConnaughey TA, Gillikin DP. Carbon isotopes in mollusk shell carbonates. *Geo-Marine Letters*. 2008;28:287-99.