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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Stoichiometric dynamics of carbon and nitrogen in two major hypoxia zones of Chinese coastal water

中國近海兩個主要缺氧區碳和氮的化學計量動力學

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Professor Hongbin Liu 劉紅斌教授	Professor Shuh-Ji Kao 高樹基博士
Post		
Unit / Department / Institution	Department of Ocean Science/ HKUST	State Key Laboratory of Marine Environmental Science/Xiamen University
Contact Information	5005 CYT Building, HKUST, Clear Water Bay, Kowloon	
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	1/1/2016		
Project Completion date	31/12/2019		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31/12/2020		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. *To determine the rates of major oxygen consumption processes (organic matter respiration and nitrification rates in both water column and sediment) and the origins of oxygen-consuming substances in Changjiang and Pearl estuaries.*
2. *To quantify the rates of key nitrogen and carbon biogeochemical processes at different interfaces of hypoxic systems (oxic-suboxic-anoxic) and to establish a*

stoichiometric linkage between nitrogen and carbon in the Changjing and Pearl River estuaries.

3. *To study the structure, abundance and activity of N-cycle-related microorganisms under various oxygenation status at the molecular level.*
4. *To explore the controlling factors of N and C cycles and the climatic effect of these hypoxic systems.*

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____
_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

1. Ammonia oxidizing archaea (AOA) outnumbered ammonia oxidizing bacteria (AOB) in PRE, indicated AOA is the dominant nitrifier in the estuary. Moreover, AOA were found mostly in the free-living form, while AOB were in the particle-attached fraction (Lu et al. 2020). Intense nitrification contributed significantly to hypoxia conditions (nitrification contributed averaged 12.2% of oxygen consumption) in the Pearl River Estuary. A marked disagreement of AOA community at DNA and RNA level was revealed, with WCA sublineages dominating at DNA level, while SCM1-like sublineages dominating at RNA level, indicating different level of ammonia oxidizing activity among different phylogenetic groups (Lu et al. 2020).

3. Seven genomes of ammonia oxidation archaea (AOA) were reconstructed from the metagenome collected from Pearl River estuary. These genomes added crucial new

genomic information of AOA to the dataset. Comparing with previous reported AOA genomes, the AOA identified in Pearl River estuary showed additional genes encoding amino acid synthesis, xenobiotic biodegradation metabolism and transportation of inorganic phosphate and heavy metals. These finding extended our understanding of AOA related genomic adaption to anthropogenic pollution in Pearl River estuary. Combining with the dramatic high ammonia oxidation rate in the hypoxia region of Pearl River estuary, the 99% contribution of AOA for ammonia oxidation gene at the hypoxia region illustrates the importance of AOA in biological nitrification process in Pearl River estuary (Li et al. 2019).

4. Bioinformatics analysis of the metagenomics dataset reveal low denitrification and anammox activities. Instead, genes encoding sulfate reduction prevailed. This could be explained by the factor that, compared with nitrate reduction bacteria, sulfate reducing bacteria are more tolerant to low pH and fluctuating oxygen concentration of the highly dynamic Pearl River estuarine waters. Our results suggest that nitrate reduction is minimal and sulfate reducing bacteria play a very important role in the microbial nutrient cycling in the estuarine and coastal waters with short-lived hypoxia (Li et al. 2020).

5. AOA sublineages co-occurred with heterotrophic bacteria (e.g., Gamma proteobacteria and Bacteroidetes), indicating reciprocal interactions via dissolved organic matter transformations. The consistent occurrence of AOA sublineages and NOB at RNA level indicated the potential interaction in active nitrification activities in the dynamic Pearl River estuary (Lu et al. in preparation).

6. Under dark condition, ammonium transformation presents a bilayer structure in the PRE, with NH_4^+ assimilation > NH_4^+ oxidation in almost all the surface waters and vice versa in all bottom waters, suggesting bacteria and phytoplankton (mainly bacteria) control NH_4^+ consumption in surface during the night while nitrifiers are the major NH_4^+ consumer in the bottom waters. Through redundancy analysis, we found that both processes are mainly driven by NH4+ in the PRE during summer (Chen et al. 2020).

7. From the biological parameters measured during the 2017 summer cruise, we demonstrated that the mechanisms underlying the formation of the bottom hypoxia in PRE were spatially heterogeneous. In the western area near Huangmaohai, continuous supply of nutrients from the western tributaries of the Pearl River fueled high primary production in the upper layer and high microbial activity in bottom layer. On the contrary, the eastern area near Hong Kong Island was sustained by regenerated nutrients (Liu et al. in prep.). High correlation between cyanobacteria bloom in surface layer and enrichment of microbial organic degradation genes in the bottom hypoxic layer support such hypothesis (Li et al. 2020).

8. During the summer but not during the winter, protist communities, identified from their DNA or RNA signatures, could be better explained by mass effects and species sorting, respectively. Moreover, protest diversity in the DNA and RNA surveys exhibited similar trends along the transect, that is, a linear upstream-to-downstream decrease during the summer and a weakly U-shaped curve during the winter. In contrast, the taxonomic compositions in the DNA- and RNA-derived communities were remarkably different during either the summer or the winter (Wu et al. 2018).

Potential for further development of the research and the proposed course of action *(maximum half a page)*

1) We are currently conduct research on the effect of warming on the microbial metabolic rates using Metabolic Theory of Ecology (MTE), with several major breakthrough (*Liu, K., Chen, B., Zhang, S., Sato, M., Shi, Z.,* ***Liu, H****.* (2019) Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton. Limnol. Oceanogr. 64(3): 1103-1119. doi: 10.1002/lno.11101; Liu, K., Suzuki, K., Chen, B****., Liu, H****.* (2020) Are temperature sensitivities of Prochlorococcus and Synechococcus suppressed by resource availability in the western North Pacific Ocean? Limnol. Ocenogr. DOI: 10.1002/lno.11629*). We are now expand our research to rates related to microbial carbon and nitrogen metabolisms.

2) Dramatic high abundance of antibiotic and metal resistance related genes were detected in bottom and surface layers, respectively, indicating the microbial adaptation to anthropogenic pollution in Pearl River estuary (Li et al. 2020). Also, the close genetic distance between reconstructed genomes and pathogens may further support that the anthropogenic input in PRE is making the microorganisms more drug-resistant and pathogenic. We will continue data-mining from our metagenomics results and other database to expand the scope of our study on the adaption strategy of microorganisms in highly polluted estuarine environments.

3) We will expand our study to other important nutrients and biological processes. We have already done a substantial amount of work on nitrogen fixation in various coastal and oceanic regions. We have also done work on the effect of phosphate availability on nitrogen assimilation in PRE (manuscript in preparation). Recently, a pre-proposal for studying the control mechanism and developing an early warning system of eutrophication in reservoirs has been submitted, of which I am a co-PI.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

Coastal eutrophication, fuelled by increasing anthropogenic nutrient input, results in oxygen depletion in bottom water (called hypoxia or "dead zone"), which has a detrimental impact on living organisms and biogeochemical cycling of carbon, nitrogen and other biogenic elements in the affected waters. Because the hypoxic condition promotes anaerobic metabolic processes, the hypoxic zones may play an important role in global nitrogen balance, but the relative roles of aerobic and anaerobic nitrogen metabolic pathways in eutrophic estuarine water is not clear.

In this project, we use two powerful techniques, stable isotope to track and quantify different metabolic rates and next generation high-throughput sequencing to identify the genes and microbial organisms that are performing the functions, to study the microbial nitrogen metabolism in Pearl River and Changjiang estuaries. We found that despite sporadic hypoxia event in summer, denitrification is not widespread in water column of the Pearl River estuary. Nitrification is mostly done by ammonia oxidizing archaea, and they can contribution between 10 to 20% of the oxygen consumption. Our study provides new insights on nitrogen cycling in oxygen-depleted eutrophic estuarine and coastal waters, which could have significant implications on the global nitrogen cycle and climate change.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*

2017 J1				**Xia, X.**, Guo, W., **Liu, H.***	Basin scale variation on the composition and diversity of Archaea in Pacific Ocean. Front. Microbiol. 8:2057. Doi: 10.3389/fmicb.2017.02057	2017	No	Yes	Yes
2016 J2				Jing, H., **Cheung, S.**, Zhou, Z., Wu, C., Nagarajan S. **Liu, H**.*	Spatial variations of the methanogenic communities in the sediments of tropical mangroves. PLOS One 11(9): e0161065. Doi:10.1371/journal.pone.0161065	2017	No	Yes	Yes
2018 J3				Wu, W., **Liu, H.*** (2018)	Disentangling rotest communities identified from DNA and RNA surveys in the Pearl River-South China Sea Continuum during the wet and dry seasons. Molecular Ecology. 27:4627-4640. DOI: 10.1111/mec.14867	2020	Yes	Yes	Yes

2018 J4				Jing H, Zhu W, **Liu H**, Zheng L and Zhang Y	Particle-attached and Free-living archaeal communities in the benthic boundary layer of the Mariana Trench. Front. Microbiol. 9:2821. doi: 10.3389/fmicb.2018.02821	2020	Yes	Yes	Yes
2019 J5				Shi, Z., Liu, K., Zhang, S., Xu, H., **Liu, H.***	Spatial distributions of mesozooplankton biomass, community composition and grazing impact in association with hypoxia in the Pearl River Estuary. Estuar. Coast. Shelf Sci. 225: 106237. https://doi.org/10.1016/j.ecss.2019.05.019	2020	Yes	Yes	Yes
2019 J6				Lu, Y., Xia, X., Cheung S., Jing, H., **Liu, H.***	Differential distribution and determinants of ammonia oxidizing archaea sublineages in the oxygen minimum zone off Costa Rica. Microorganisms 7: 453; doi:10.3390/microorganisms7100453	2020	Yes	Yes	Yes
2020 J7				Li, Y., Jing, H., Kao, S.-J., Zhang, W., **Liu H.***	Metabolic response of prokaryotic microbes to sporadic hypoxia in a eutrophic subtropical estuary. Mar. Poll. Bull. 154:111064	2020	Yes	Yes	Yes
2019 J8				Li Y., Zou D., S.-J. Kao, Li M.*, **Liu H.*** (2019)	Genomic adaptation to eutrophication of ammonia-oxidizing archaea in the Pearl River estuary. Environ. Microbiol. doi:10.1111/1462-2920.14613	2020	Yes	Yes	Yes

2020 J9				Chen, L., Zhang, X., He, B., Liu, J., Lu, Y., Liu, H., et al.	Dark ammonium transformations in the Pearl River Estuary during summer. *Journal of Geophysical Research:Biogeosciences*, 125,e2019JG005596. https://doi.org/10.1029/2019JG005596	2020	Yes	No* I realize that I have forgot to add RGC grant though it is a paper directly resulted from this joint study	Yes
2020 J10				Zhang, S., Li, C., Sun, M., Cheung, S., Song, S., Guo, W., Guo, C., Wu, G. **Liu, H**.* (2020).	Snapshot of peptidomics of the red tide forming species Noctiluca scintillans. Front. Mar. Sci. doi: 10.3389/fmars.2020.569807	2020	Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

August/2017 /Tai-An, China C1	Highly diverse unicellular cyanobacterial diazotrophs in the Kuroshio Current and the North Pacific Ocean	The 11th Cross-strait Conference on Marine Science and Technology	2017	No	Yes	No
July 2018/ Shanghai China, Invited C2	Hypoxia and ecosystem consequences around Hong Kong waters: A theme-based research scheme project on the Eutrophication.	The 5th Conference on Earth System Science	2020	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Lu Yanhong (S1)	PhD	1 September 2016	31 August 2021
Shi Zhiyuan (S2)	MPhil	1 September 2016	31 May 2019
Li Yingdong (S3)	MPhil	1 September 2016	31 August 2018
Zou Dayu (S4)	PhD	1 September 2017	31 May 2021

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Prof. Hongbin Liu was elected ASLO Fellow by the Association for the Science of Limnology of Oceanography in 2019

Mr. Yingdong Li received the Best Postgraduate Research award from the School of Science, HKUST

We have developed close collaboration with scientists in the Great Bay Area and beyond, including the South China Sea Institute of Oceanology, CAS, Xiamen University and Sun Yat-sen University.

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	10	2			