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国家重点研发计划课题 中期执行情况报告

项目名称：海洋生态系统储碳过程的多尺度调控
及其对全球变化的响应

项目牵头单位：厦门大学

课题名称：海洋典型生态系统储碳过程的调控机制

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编报要求

一、内容说明

课题中期执行情况报告着重围绕课题任务书的内容，报告课题中期重要进展情况，具体包括课题的总体目标及考核指标实现程度，人员、资金等支撑条件落实情况，课题经费使用情况等，并报告中期执行过程中的重大事项及突出进展。

二、格式要求

文字简练；报告的密级一般与任务书规定的密级相同；报告文本统一用 A4 幅面纸，文字内容一律通过“国家科技管理信息系统公共服务平台”在线填报；报告文本第一次出现外文名称时要写清全称和缩写，再出现时可以使用缩写。

三、编制程序及时间要求

项目中期总结前，由课题承担单位组织课题参与单位编制课题中期执行情况报告，经课题负责人及课题牵头单位审核后，提交项目牵头单位。

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一、总体进展情况

1. 课题中期总体进展情况

对照课题任务书的计划目标和各项主要指标要求，简要阐明课题中期进展情况，评述课题中期任务的实施进展状态。

本课题的目标是，聚焦海洋储碳这一海洋碳循环的关键环节，针对项目的第一个关键科学问题——“海洋生态系统固碳和储碳过程的主要调控机制是什么？”，利用多组学技术（宏转录组学、宏蛋白组学等）以及传统生态学方法、原位培养实验等，研究典型海洋生态系统中浮游生物关键功能类群对颗粒碳（POC）输出通量的影响，解析颗粒有机碳代谢的关键分子机制；揭示微型生物关键功能类群在溶解有机碳（DOC）代谢中的作用，阐释微型生物群落组成及有机碳代谢通路之间的关联。通过揭示海洋生态系统储碳的多时空调控机制，为海洋生态系统碳循环及其对气候的调节作用研究提供科学依据。

课题考核指标包括：（1）海洋生态系统 POC 代谢的关键分子机制；（2）关键微型生物功能群在 DOC 代谢中的调控作用。

中期考核指标包括：（1）揭示浮游生物关键功能类群在全水柱的分布特征及其参与有机碳代谢的机制；（2）发表论文 5-8 篇。

根据实施方案，课题组积极开展现场航次观测与实验研究，同时进行历史数据的整合分析，在生物泵（BP）和微型生物碳泵（MCP）关键生物类群时空分布特征及其调控有机碳代谢分子机制、浮游生物群落结构对 POC 输出的调控及 POC 动力学过程、细菌对 POC 输入的响应和矿化等方面取得重要进展，达到中期目标。主要研究进展如下：

- 1) 课题组参加了项目组织的 2016 年南海东北部（台湾海峡南部）夏季预研航次、2017 年南海中部夏季航次、2017 年南海东北部-吕宋海峡航次、2017 年南海东北部-西太冬季航次，搭载 2016-2017 年福建九龙江河口-厦门湾冬、春、夏航次。航次期间，采集包括不同季节、不同生态系统、不同水层及不同粒径浮游生物样本超过 800 个，其中用于 DNA 条形码分析生物多样性样本 650 个，环境组学（宏基因组、宏转录组及宏蛋白质组）样本 97 个，浮游生物形态鉴定样本 81 个。现已完成所有 DNA 条形码测序、74 个环境组学样本测序及 21 个样本的形态鉴定及种类计数，共计获得序列

数据超过 1.6Tb。已完成大部分样本的生物信息学分析，包括 DNA 条形码的聚类分析、种类注释，宏转录组组装及功能注释，正在进行与环境因子的关联分析、表达量定量分析等。

- 2) 基于上述航次数据及历史数据，揭示了南海夏季浮游生物群落组成及昼夜分布特征，鉴定得到了一系列与碳产生、转运、分泌、代谢等途径相关的基因及蛋白质，并初步解析其不同生态系统、不同水层及不同粒径生物中的表达差异。
- 3) 通过室内培养实验、组学分析以及对目标基因转录水平和蛋白表达水平的定量分析，揭示了米氏凯伦藻、东海原甲藻、赫氏颗石藻等多种浮游植物利用有机磷的分子机制，发现了东海原甲藻应对低光条件的重要应答基因并解析其表达模式，为进一步利用环境组学手段研究浮游生物固碳和储碳过程的分子调控机制奠定了数据及方法基础。
- 4) 在南海边缘海陆架-陆坡-海盆、东海藻华暴发区和非暴发区开展 POC 输出通量、浮游植物和浮游动物粪球沉降速率研究，阐释了浮游生物群落结构对 POC 组成、分布和输出通量的影响；结合原位观测数据和历史数据，建立了定量南海中层水体颗粒物输出的 ^{210}Po - ^{210}Pb 不平衡方法，并基于该方法揭示了南海中层水体活跃的颗粒动力学过程，发现南海陆架沉积物向海盆中深层输送颗粒物的现象广泛存在。
- 5) 搭载中国大洋科考第 45 航次南海海试及第 I 航段，于南海北部海盆、西太平洋牛郎平顶海山各布放了一套深海微生物原位培养系统。基于原位样品，开展 POC 衍生物降解微生物种群的实验室模拟培养和菌群结构解析，构建 POC 降解菌群 12 个，发现 2 株新型烃类降解菌，并初步揭示其系统进化地位、POC 降解特性和机理。
- 6) 已发表论文 11 篇，其中 SCI 论文 9 篇，第一标注 8 篇（见附件清单）。

综上，课题实施以来，总体进展良好，按照计划顺利进行，达到中期目标，初步揭示了浮游生物关键功能类群在全水柱的分布特征及其参与有机碳代谢的机制，为进一步深入揭示海洋典型生态系统储碳过程的调控机制奠定了良好的基础。

2. 课题调整情况

如课题出现超前/迟滞等情况，请详细说明原因、措施及履行相关审批管理制度的情况。

无。

二、取得的重要进展及成果

1. 课题中期重要进展及成果

简要介绍课题研究工作的重要进展、阶段性成果（一般不超过3项）及前景。

课题组积极开展现场航次观测与实验研究，同时进行历史数据的整合分析，在BP和MCP关键生物类群时空分布特征及其调控有机碳代谢分子机制、浮游生物群落结构对POC输出的影响及POC动力学过程、细菌对POC输入的响应和矿化等方面取得重要进展。

1.1. 夏季南海BP和MCP关键生物类群多样性及时空分布特征

(1) 2016年夏季南海东北部浮游生物多样性及时空分布

“2016年南海东北部夏季预研航次”期间，在两个时间序列站位——陆架区的C6站及陆坡区的C9站进行了原位海水样品采集及浮游生物样品拖网采集，并投放了沉积物捕获器。综合考量样本来源及提取质量，选取了72个浮游生物样品及4个trap样品，利用高通量测序获得其DNA条形码序列，开展浮游生物多样性及时空分布分析。测序均已完成，利用标准化分析流程得到初步结果显示：C6、C9站浮游生物群落结构有显著差异，各粒径生物垂直分布存在明显的昼夜变动模式，其中，浮游动物的昼夜垂直迁移最为显著；且不同站位、不同粒径生物昼夜变动模式存在差异。目前，正在进行个性化分析，包括完善种类注释数据库、优化比对及最佳注释选择方法、群落结构与环境因子相关性分析等。

以下分别介绍原核浮游生物、微微型/微型/小型真核浮游生物、小型浮游动物及中型浮游生物物多样性及时空分布特征分析进展。

• 原核浮游生物多样性分析

C6、C9站真光层原核生物中有一半以上的未知类群，已知类群中以变形菌（Proteobacteria）为主，其次为蓝细菌（Cyanobacteria）。其中，蓝细菌以原绿球藻（*Prochlorococcus*）和聚球藻（*Synechococcus*）为主（图1）。基于OTU丰度的主成分分析（PCA）显示（图2），C6站表层与DCM的3-200 μm 样品中的原核生物群落结构存在明显差异，但0.2-3 μm 群落结构在不同水层差异不明显。C9站粒径-水层的关系与C6站相反，小粒径受水层影响明显，而大粒径在不同水层间差异较小。此外，C9站检出的DOM降解菌，在种类丰富度和每个水层中的丰度上，均显著高于C6站，且菌株

多为颗粒附着生态型，暗示了它们可能参与了水体中 POM 的降解过程。

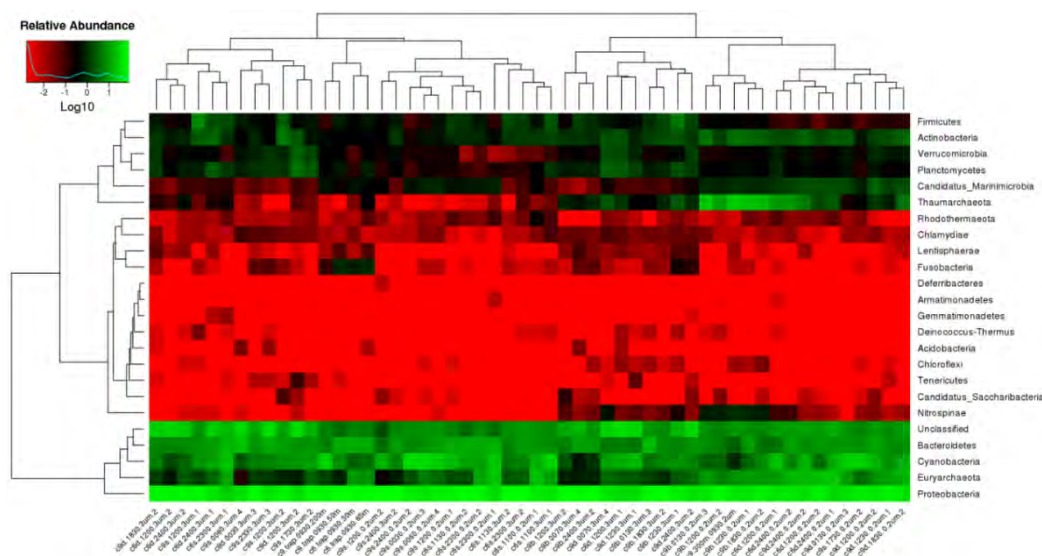


图 1. C6、C9 站原核生物相对丰度热图

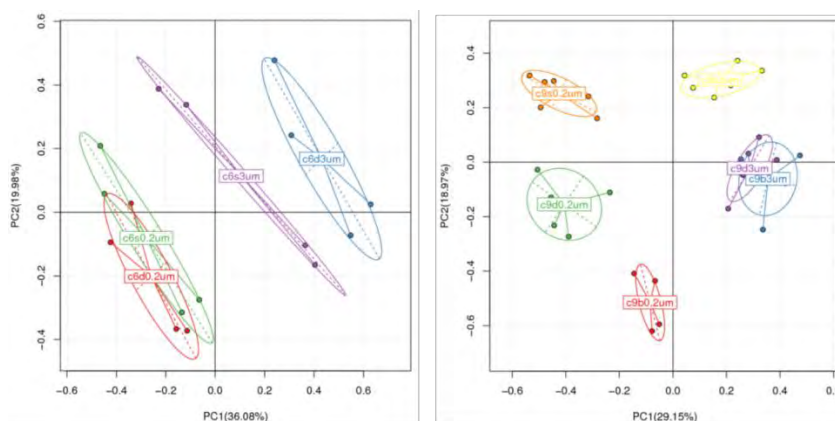


图 2. C6、C9 站原核生物基于 OTU 丰度的 PCA 分析

• 微型/中型/小型真核浮游生物（0.22-200 μm）多样性分析

种类注释结果显示，粒径为 0.22-200μm 的真核浮游生物可归为 Rhizaria, Stramenopiles, Opisthokonta, Alveolata, Archaeplastida, Excavata 及 Incertae_Sedis 7 个超级类群，包含 40 余个主要类群，其中 Dinophyceae, Syndiniales 和 Metazoa 平均相对丰度最高（图 3）。聚类分析和 Alpha 多样性分析显示，深度对粒径为 3-200μm 类群的分布影响较大，对 0.22-3μm 类群的分布影响较小（图 4）。说明深度对大粒径的生物的分选作用明显，小粒径生物的分布较大粒径生物更为广泛。主要浮游生物类群间的相关性分析显示（图 5），Dinophyceae 与 Syndiniales、Haptophyte 等多个类群呈显著负相关，C9 站位生物间的相关关系随深度增加而减少，相关性可能与类群间的寄生、共生或摄食等关系有关。

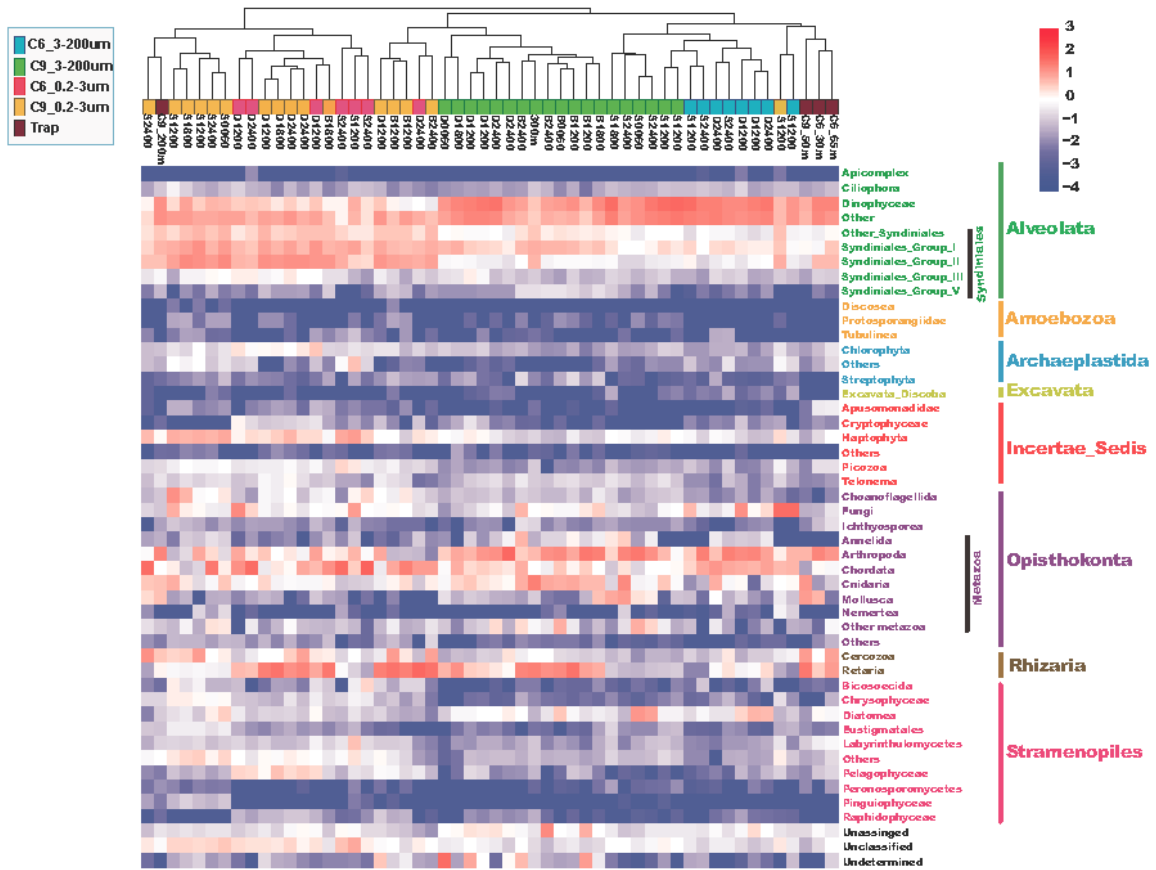


图 3. C6、C9 站 0.22-200 μm 真核浮游生物主要类群 rDNA 相对丰度

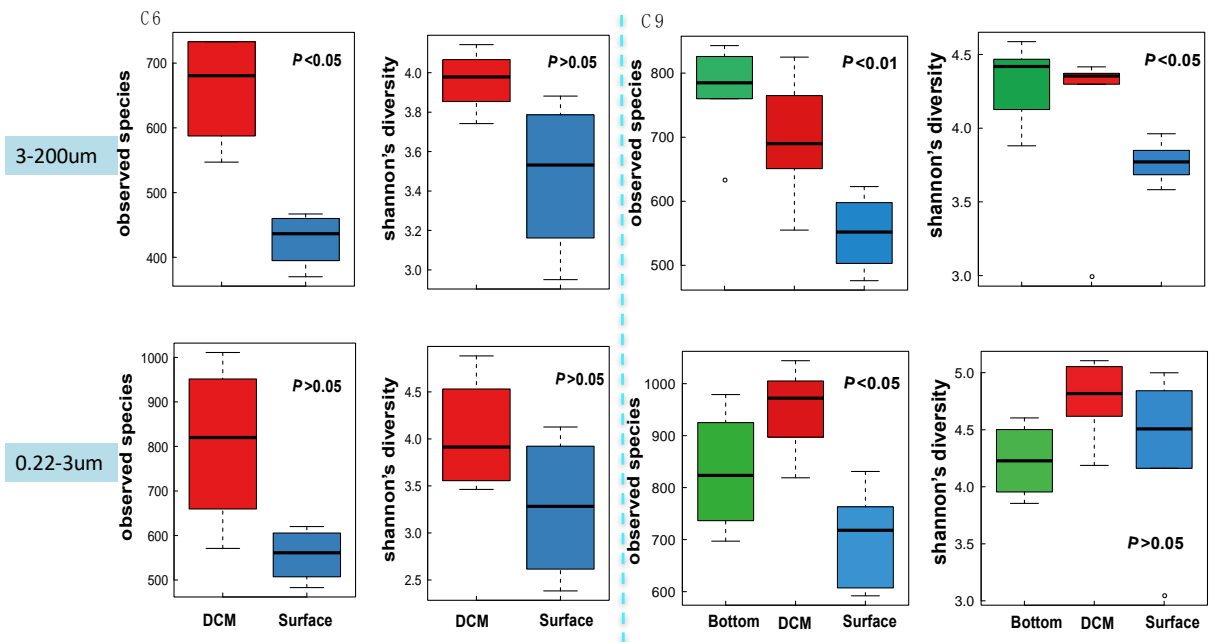


图 4. C6、C9 站 0.22-200 μm 真核浮游生物群落 Alpha 多样性分析

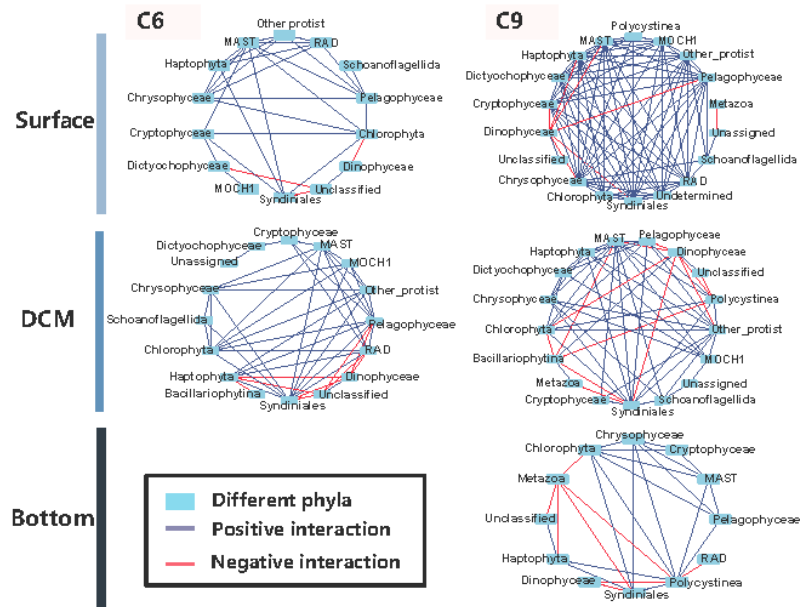


图 5. C6、C9 站 0.22-200 μm 真核浮游生物网络互作图

• 小型浮游动物的沿环境梯度分布特征

2016 年夏季除了对 C6、C9 两个时间序列站进行浮游生物多样性分析，还着重对台湾海峡大面观测站的小型浮游动物群落结构进行了研究。台湾海峡具有复杂水系环境，采样水域可划分为冲淡水、混合水、上升流和外海水。海峡内纤毛虫分别由旋毛纲（Spirotrichea）、叶咽纲（Phyllopharyngea）、寡膜纲（Oligohymenophorea）等 7 纲组成（图 6）。其中，旋毛纲纤毛虫为最优势类群，其次是叶咽纲。不同水团纤毛虫的多样性存在显著差异，其中冲淡水和外海水中纤毛虫多样性较高，上升流区域内纤毛虫多样性最低（图 6）。海峡内纤毛虫群落分布模式受水团影响显著；在各个水团内部，环境因子对群落变动的解释度较高，且不同水团内，调控纤毛虫群落结构的环境因子不同。

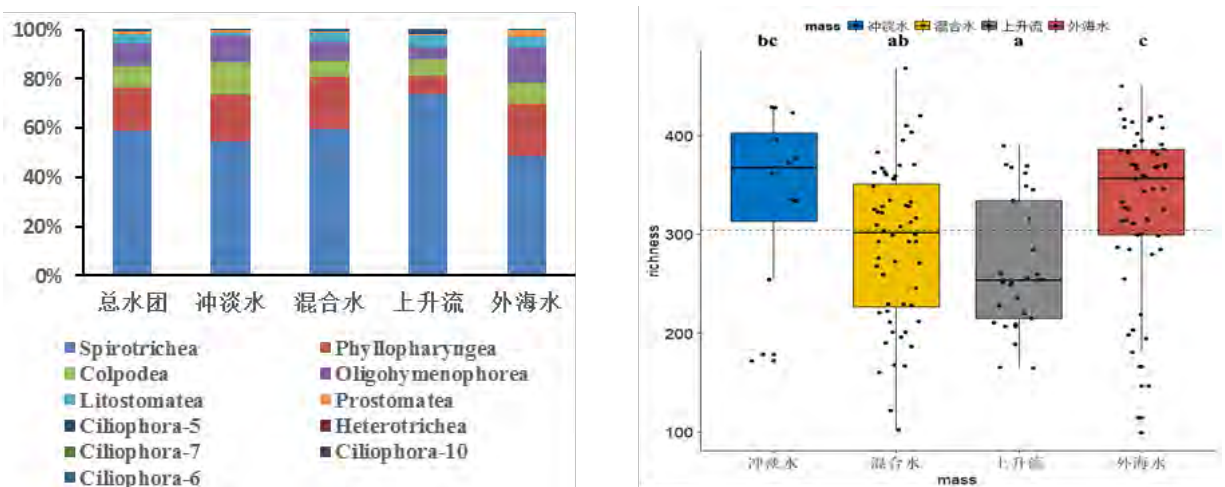


图 6. 2016 夏季台湾海峡不同水团间纤毛虫的纲级物种组成和 OTU 丰富度差异

此外，使用 18S rRNA 高通量测序与定量蛋白银染色两种方法，对春、夏两季采自南海东北部 C 断面的样品进行分析，两种方法均揭示了表层与弱光层中纤毛虫的 alpha 多样性相当，表明了以往对深海纤毛虫多样性的一个低估。镜检结果显示，C 断面纤毛虫群落主要由小于 60 μm 虫体组成，其中 20-40 μm 为优势组（图 7）。结合前述基于 RNA 的分析结果可发现：弱光层中多样性被低估的纤毛虫主要来自于活跃的、粒径较小的虫体。基于形态、分子以及统计学方法所得到的 beta 多样性分析结果均表明：与水平空间变化、季节与年际变化相比，深度为调控纤毛虫 beta 多样性的主要因素。本研究首次采用形态与分子相结合方法，对沿多环境梯度、全类群纤毛虫群落的时空变化进行研究，其结果显示了基于多来源信息探讨小型浮游动物多样性、地理分布等生态学问题的重要性。

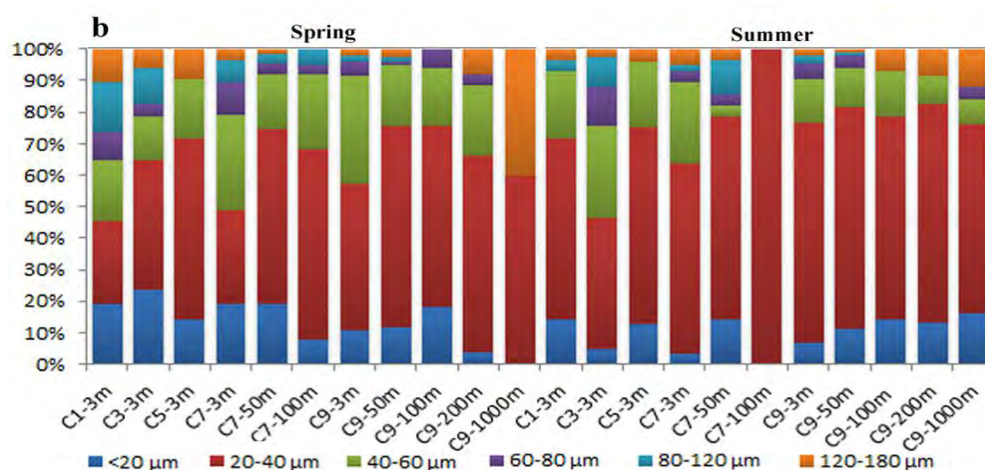


图 7. 南海东北部 C 断面纤毛虫群落粒径大小组成

• 中型浮游生物 (>200 μm) 多样性分析

对 C6、C9 站中型浮游生物样品分别进行形态鉴定和 18S rDNA、18S rRNA 测序分析。形态鉴定显示，C6 站中型浮游动物的平均种类数为 57，C9 站为 66；C6 站浮游动物平均丰度为 $780.6 \pm 393.6 \text{ ind/m}^3$ ，C9 站 $282.9 \pm 206.7 \text{ ind/m}^3$ ；C6、C9 站浮游动物的优势类群均为桡足类和被囊类（图 8A、B）。18S rDNA 测序共获得 10110 个 OTU，各样品中 OTU 数量不等（ 620 ± 132 个），在门、纲或亚纲水平将所有物种归类到 11 个主要类群，各样品中桡足类均为相对丰度（65.1-96.3%）及 OTU 丰富度最高的类群，C6 站被囊类丰度次之（0.6-19.5%），C9 站水螅虫总纲生物丰度次之（0.1-28%）。C6 与 C9 站中型浮游生物群落结构具有显著差异，Bray Curtis 相似度在 40 以下。C6 站内各样品相似度较高（相似度 >60），而 C9 站不同时间及深度群落组成差异较大。在一个昼夜

闭合周期中，桡足类、被囊类及水螅水母等中型浮游生物存在昼夜垂直移动，不同类群垂直迁移模式可能存在差异，迁移主要发生在昼夜交替时，在白天或夜间全水柱群落结构趋于相对稳定状态，且在一昼夜周期内保持相对稳定的交替节律。利用 rRNA 及 rDNA 分别表征各类群代谢活性和相对丰度，比较分析显示相对丰度低的类群 (<0.01%) 代谢活性总体高于丰度高的类群 (>1%)。其中，代谢活性最高的 3 个类群分别为等辐骨虫、纤毛虫和环节动物 (图 9)。

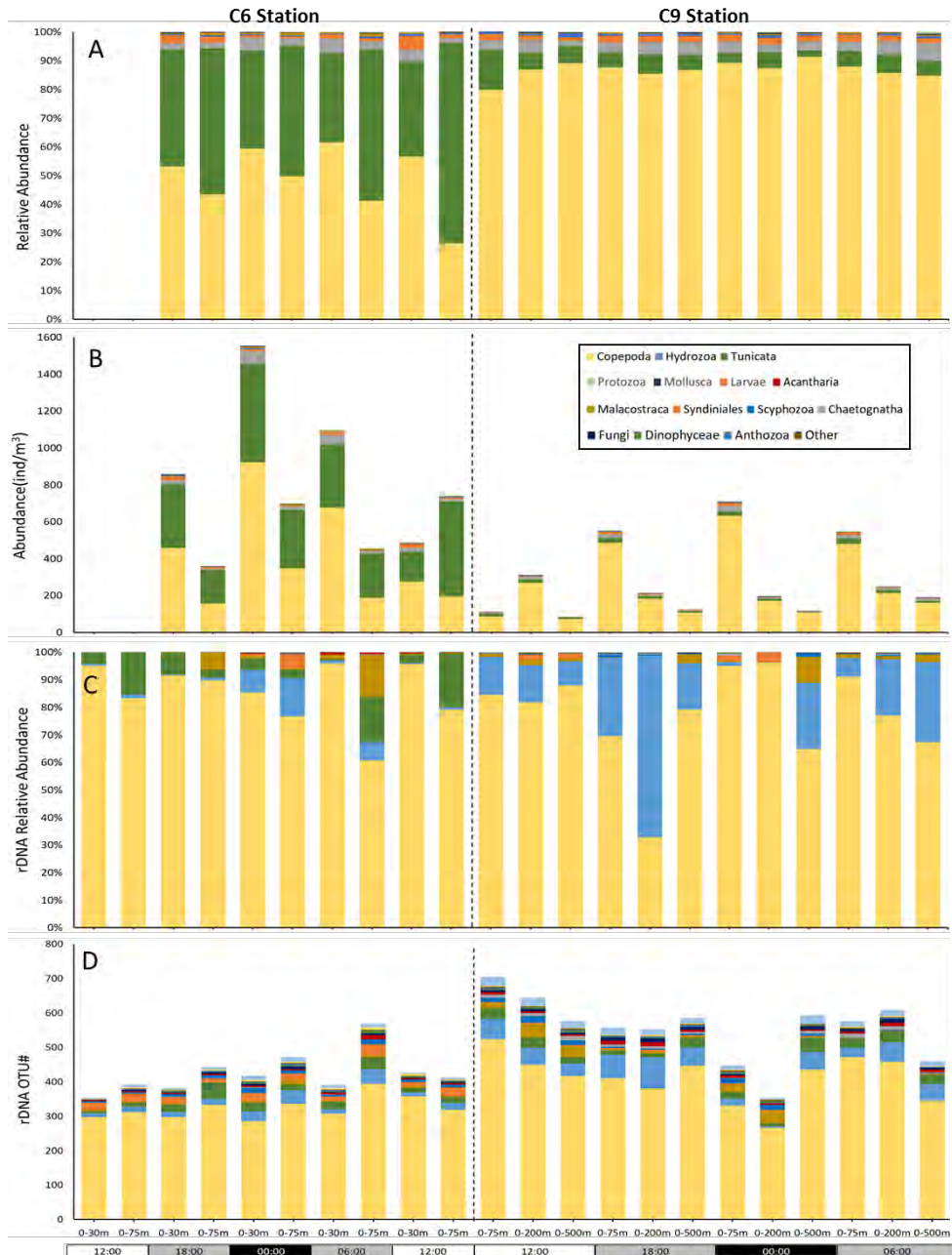


图 8. C6、C9 站中型浮游生物群落结构
通过形态鉴定及计数获得的各类群相对丰度 (A) 及丰度 (B)；通过 18S rDNA 测序获得的各类群相对丰度 (C) 及 OTU 数 (D)

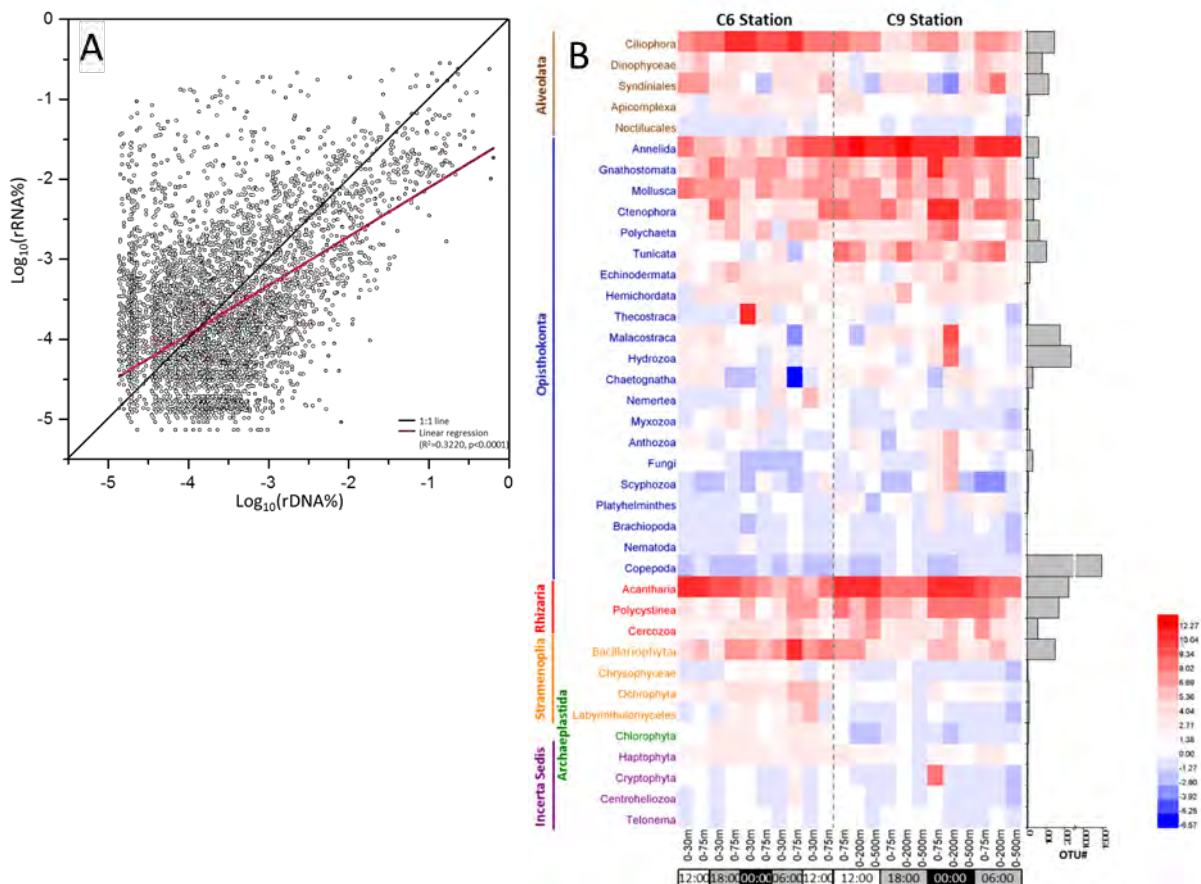


图 9. C6、C9 站中型浮游生物代谢活性 (rRNA) 与相对丰度 (rDNA) 关系。(A)所有 OTU rRNA 及 rDNA 相对丰度相关性分析; (B) 主要类群 rRNA:rDNA 比值及 OTU 数

(2) 2017 年夏季南海北部-吕宋海峡浮游生物多样性及时空分布

本航次调查在南海北部-吕宋海峡水域的 F2、E1、SEATS、DC2、DC6 五个时间序列站 (图 12) 分粒径采集浮游生物, 以期解析浮游生物的多样性及时空分布特征。现已在固氮微生物及中型浮游动物多样性分析方面取得一定进展, 其余粒径样本正在分析中。

• 固氮微生物多样性

在五个主要时间序列站经分粒径过滤表层与 DCM 海水, 开展固氮微生物的多样性与时空分布研究, 并探索其对不同环境的生态位适应机制。目前已完成部分 DNA 样品的高通量测序, DNA 样品的 qPCR、RNA 样品的后续分析正在进行中。

基于固氮酶基因 *nifH* PCR 扩增子高通量测序的原始数据, 经质量过滤后共获得 362,665 条序列, 与 *nifH* 数据库比对得到 258 个可操作分类单元 (OTU)。测序结果显示, 中国南海的 *nifH* 系统进化类型 (phylotype) 主要包括系群 I 的固氮蓝藻和变形菌 (Proteobacterio)、系群 III 和系群 IV, 其中固氮蓝藻中的丝状束毛藻 *Trichodesmium*, 单

细胞固氮蓝藻 UCYN-A、UCYN-B、UCYN-C，以及分别与硅藻半管藻 (*Hemiaulus*)、根管藻 (*Rhizosolenia*) 共生的具异形胞的植生藻 (*Richelia* Het-1、Het-2) 在南海五个站点均能检测到 (图 10)。在所调查的五个站点中，蓝藻和变形菌是主要的固氮微生物 (图 11A)。各样品间的主坐标典型分析 (Canonical Analysis of Principal coordinates, CAP) 结果显示，每个站位的固氮微生物大体聚在一起，可见固氮微生物的分布主要由其所在的生态位决定，但同时也受外界环境的影响。如 F2 站点由于受黑潮入侵带来高温与高盐度海水的影响，其微生物群落结构与其它站点有较大不同 (图 11B)。

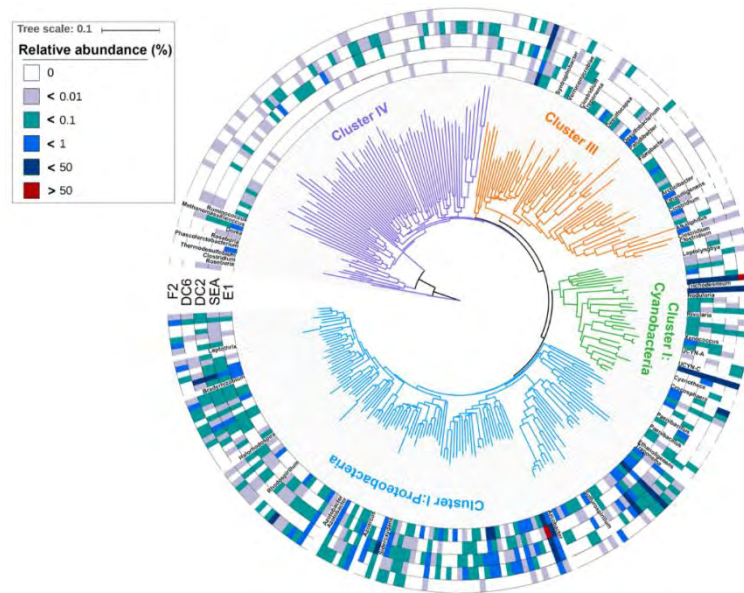


图 10. 中国南海 *nifH* 生态型 (ecotype) 进化树。系统进化树基于对南海北部五个站点海水样品中国氮酶基因 *nifH* 进行高通量测序所获得的 OTU 构建，每个 OTU 在不同站位的相对丰度根据标准化后的序列数计算。

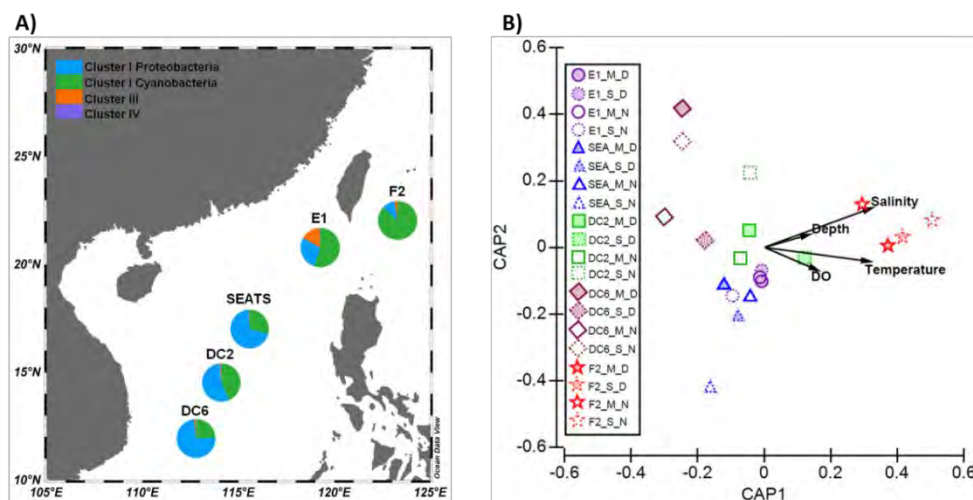


图 11. 中国南海主要固氮微生物群落结构及其与环境因子相关性分析。(A) 主要固氮微生物类群及其丰度。该结果通过整合表层和叶绿素最大层的 *nifH* OTU 获得。B) 各样品间的主坐标典型分析 (CAP) 结果及其与环境因子的相关性。

• 中型浮游动物多样性与昼夜垂直分布特征

根据代表性 OTU 对应的各样品中的序列数量计算类群相对丰度 (图 12), 根据同一水层昼夜相对丰度的差值分析其可能的垂直迁移趋势 (图 13)。尽管 5 个站位群落结构差异明显, 但相对丰度 >1% 的类群均集中在以下几类, 包括放射虫门等辐骨虫目 (Acantharia)、放射虫 Collodaria、软甲类 (Malacostraca)、桡足类 (Copepoda)、环节动物 (Annelida)、软体动物 (Mollusca)、丝足虫 (Cercozoa)、水螅虫总纲 (Hydrozoa)、罩笼虫亚纲 (Nassellaria)、被囊动物 (Tunicata)、甲藻纲 (Dinophyceae) 和夜光藻 (Noctilucales)。其中, 放射虫门等辐骨虫目 (Acantharia) 和放射虫 Collodaria 在某些站位的某些深度形成绝对优势, 相对丰度达到 60-80%。各个站位在同一水层昼夜相对丰度差值 >1% 的类群不同, 且昼夜迁移模式不同。例如, 放射虫 Collodaria 在 E1 站日间集中在 0~30m 水层和 200-300m 水层, 而夜间集中在 0-30m。放射虫、软体动物及软甲类的昼夜相对丰度差异较为明显。

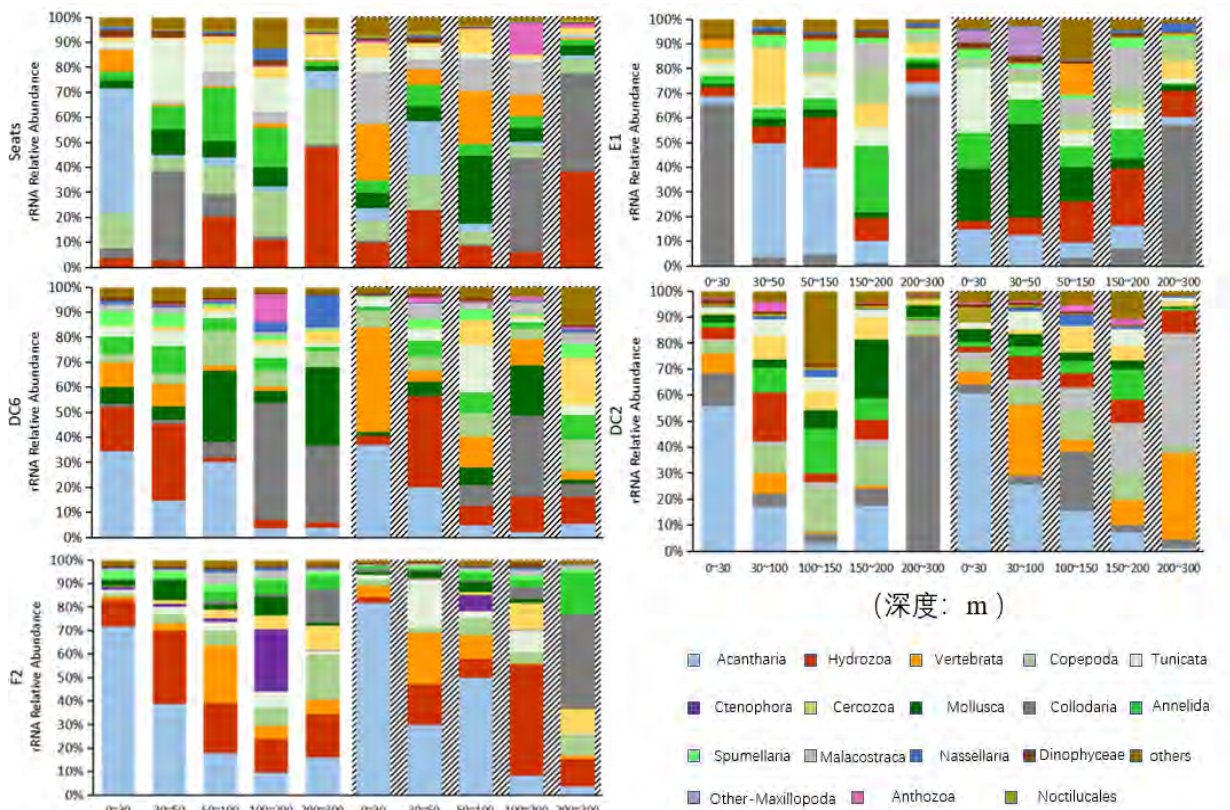


图 12. 各类群 rRNA 相对丰度 (阴影代表夜间采集的样品)

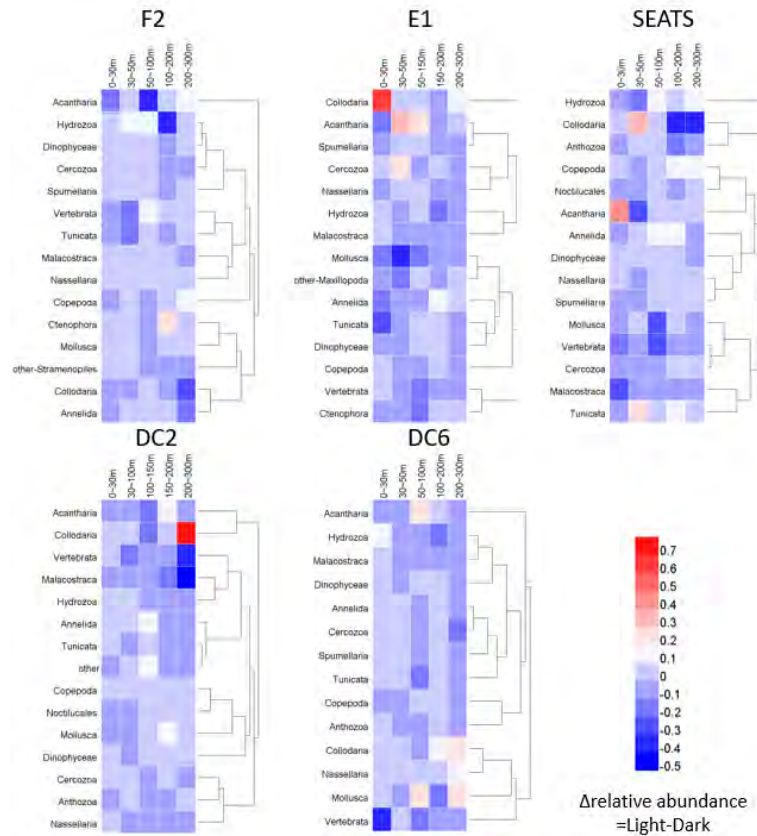


图 13. 各站位同一水层浮游动物昼夜相对丰度差值热图
 红色代表该类群在该水层日间相对丰度高于夜间，蓝色代表日间相对丰度低于夜间。

1.2. 初步揭示了 BP 和 MCP 关键生物类群调控有机碳代谢的分子机制

(1) 真核浮游植物对有机磷利用的分子机制研究以及天线蛋白的表达模式

通过室内培养实验、组学分析以及对目标基因转录水平和蛋白表达水平的定量分析，揭示了不同种浮游植物利用有机磷的分子机制：（1）米氏凯伦藻（*Karenia mikimotoi*）对 ATP 的利用机制并非由碱性磷酸酶介导水解，而是通过另一种胞外酶 5'-核苷酸酶水解 ATP，大量的 ADP/AMP 进入细胞后改变内部的核苷酸、糖酵解等代谢机制（图 14A，Luo et al., 2017, *Environmental Microbiology*, 第二标注）；（2）东海原甲藻（*Prorocentrum donghaiense*）自身并不能利用草甘膦（C-P 键有机磷化合物）作为唯一的磷源促进种群生长，但环境中共存的细菌可以介导 C-P 键类有机磷的水解并促进环境中真核浮游植物的细胞生长（图 14B，Wang et al., 2018, *Frontier in Microbiology*, 第一标注）；（3）赫氏颗石藻（*Emiliania huxleyi*）可表达产生两种不同的碱性磷酸酶，在细胞处于磷限制条件下时，两种碱性磷酸酶呈现出不同的表达模式并可能具有不同的亚细胞分布。非典型性碱性磷酸酶在细胞内磷循环过程中起着重要的作用，而当胞内磷源紧张的时候分泌型

碱性磷酸酶被细胞释放到水体中进而分解有机磷同时释放无机磷元素供细胞生长发育。两种碱性磷酸酶的发现表明颗石藻有机磷利用的复杂性和对海洋磷循环的重要生态意义（图 14C, D, Li et al., 2018, *Frontier in Microbiology*, 第一标注）。

PCP 和 *acpPC* 是含多甲藻素的甲藻中两个重要的天线蛋白,以东海原甲藻为目标种,在昼夜节律变动以及不同光照强度下,将天线蛋白编码基因的转录水平与细胞中的色素含量结合进行分析,结果显示 PCP 和 *acpPC* 的基因表达模式不同,是东海原甲藻对低光条件的重要应答基因（Shi et al., 2018, *Harmful Algae*, 第一标注）

对浮游植物利用有机磷的分子机制和天线蛋白表达模式的研究结果大大提升了对浮游植物营养盐吸收、同化和代谢机制、对环境胁迫（营养盐限制、低光等）的响应机制的认识,并为进一步研究海洋生态系统固碳和储碳过程的分子调控机制奠定基础。

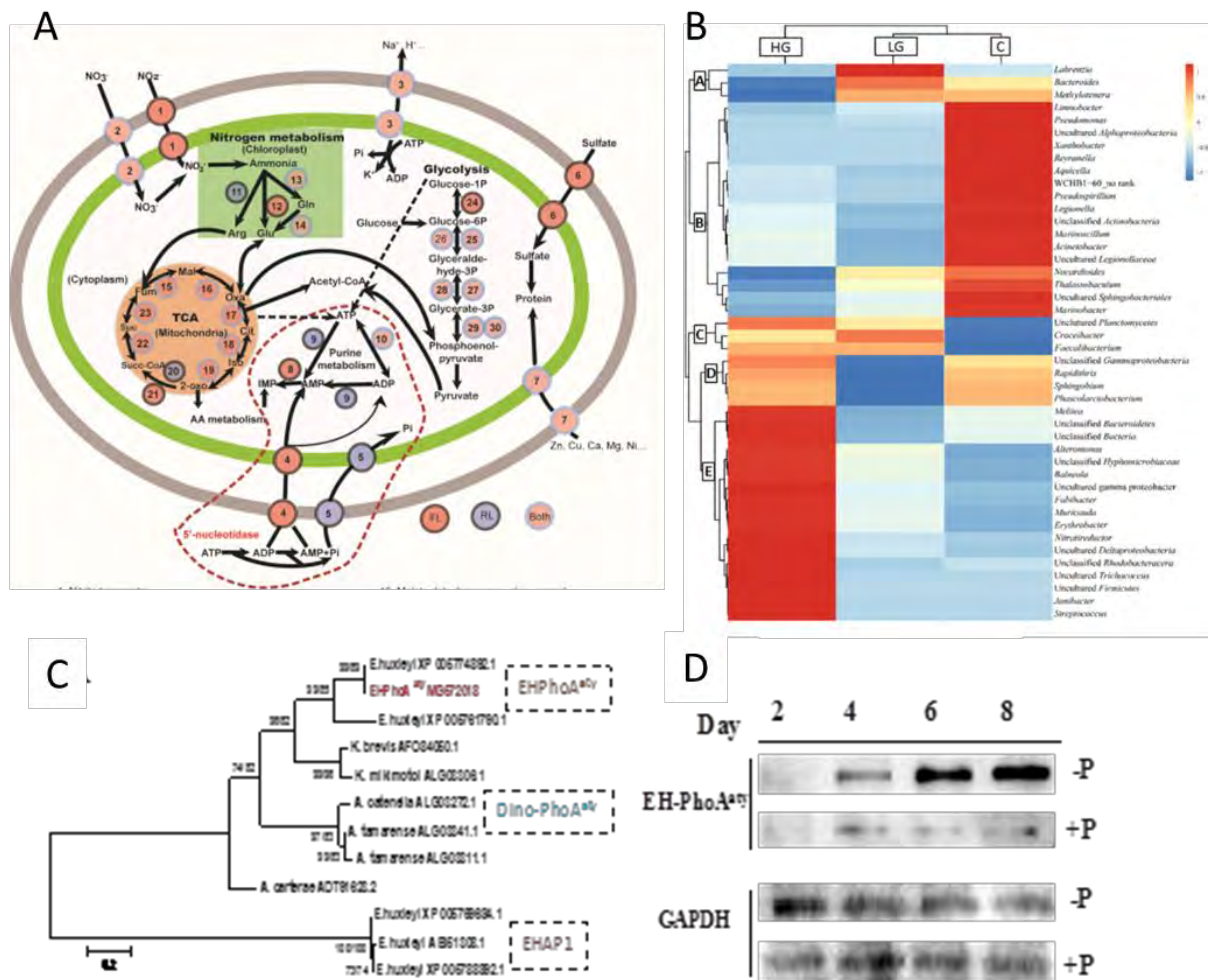


图 14.不同种浮游植物利用有机磷的分子机制。

(A) ATP 利用对米氏凯伦藻细胞代谢的影响模式图；(B) 草甘膦对东海原甲藻培养基中共存细菌的群落结构影响；(C) 颗石藻碱性磷酸酶系统进化分析；(D) 颗石藻非典型性碱性磷酸酶在不同磷浓度下的表达差异

(2) 南海寡营养海区有机物宏蛋白质组学研究

通过多个航次，于南海寡营养海区 SEATS 站不同深度（DCM、200m 和 3000 m）采集样品，用以分析大粒径颗粒有机物、小粒径颗粒有机物和溶解有机物宏蛋白质组（图 15）。结果表明，蛋白总鉴定数和来源多样性随水深增加而减少，群落中的主要代谢种群为浮游植物中的原绿球藻、聚球藻及青绿藻，异养细菌中以 SAR11 为代表的 α -变形菌，病毒种群中的蓝细菌噬菌体和 SAR11 噬菌体。病毒和非病毒蛋白的水层分布具有特异性，深海病毒群落主要以表层病毒为主，如聚球藻病毒及未培养的地中海 DCM 病毒的类似种群。代谢活动中，蓝细菌与微微型浮游植物的固碳、甲基化化合物氧化，古菌和细菌硝化作用以及多种多样的底物转运是较活跃的生物过程。有关甲基化化合物如甲酸、甜菜碱和甲醇的转运和氧化相关的谱图常被检测到，暗示它们是深海微生物能量供应的重要有机碳源。同时，浮游植物如原绿球藻的翻译相关蛋白的发现暗示南海深层水中可能存在仍具细胞活力的浮游植物细胞，可能通过尿素和甲酸代谢供能而维持细胞活力。溶解有机物组分中存在大量的非病毒蛋白，如比例较高的细胞质蛋白和转运蛋白，暗示深海微生物细胞的生理状态以及它们与周围严峻的环境之间的相互作用，在细胞分泌、泄露或裂解后维持一段时间的稳定而逐渐累积足以被检测到的量。该分析首次利用宏蛋白质组表征了南海全粒径范畴的微生物代谢活动，为详细阐释海洋生物地球化学循环过程提供了部分理论依据。

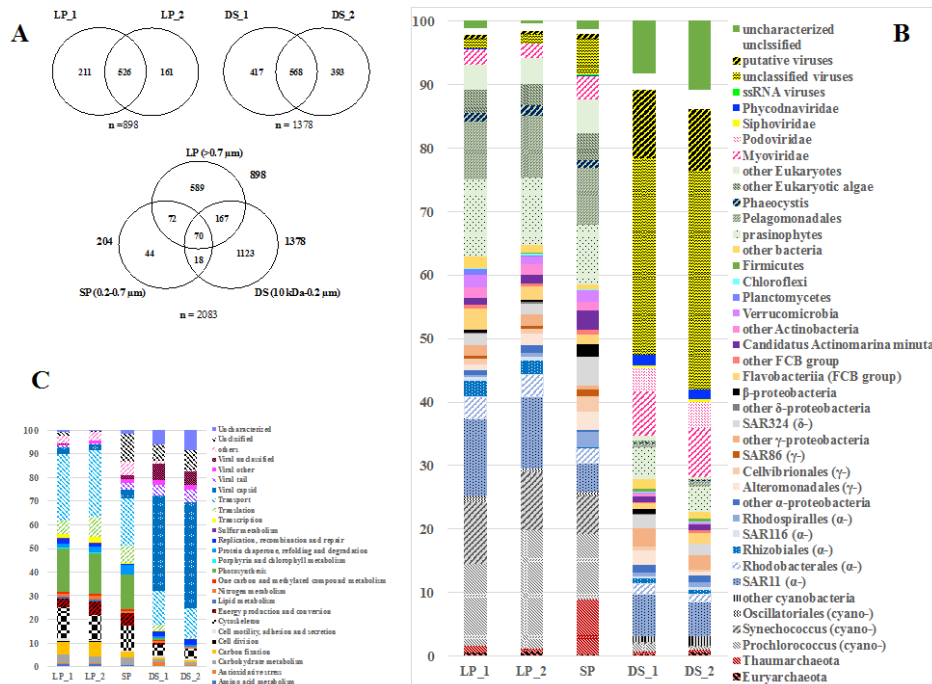


图 15. 南海全粒径有机物宏蛋白质组不同组分间蛋白鉴定韦恩图 (A)、蛋白物种 (B) 和功能分布 (C)

1.3. 阐明了浮游生物群落结构对 POC 输出的影响及 POC 动力学过程

(1) 浮游生物群落结构对 POC 组成、分布和通量的调控

采用漂浮式沉积物捕获器、甲板模拟沉降等方法，在南海边缘海陆架-陆坡-海盆、东海藻华暴发区和非暴发区开展POC输出通量、浮游植物和浮游动物粪球沉降速率研究，探讨浮游生物群落结构与POC组成及输出通量的关系。

结果显示，陆架-陆坡-海盆POC通量的主要贡献者不同。在陆架区浮游植物及其聚集体是主要POC通量贡献者；陆坡区上层浮游植物及其聚集体和粪球颗粒均为POC通量的主要贡献者，而在深层，粪球颗粒为POC通量主要贡献者；海盆区粪球颗粒为POC通量的主要贡献者（图16）。初级生产力随着近岸到远岸叶绿素a的降低而减小，生物泵效率ThE范围是10%~50%。从陆架到海盆虽然桡足类的丰度降低，但是桡足类的圆柱形粪球在边缘海陆架-陆坡-海盆生态系统的粪球POC通量中均占优势，桡足类是边缘海粪球POC通量的主要贡献者。

浮游植物细胞直接沉降POC通量虽然比率不高，但是其群落结构组成变化对POC的输出具有重要影响，特别是硅藻，由于自身含有硅质壳的原因，硅藻细胞不仅更容易输出到深海，还会影响粪球和聚集体的沉降速率，调控生物泵效率。通过相关性分析和GAMs模型分析表明（图17），水柱中浮游动物粪球POC含量与优势浮游植物类群生物量呈显著正相关；浮游动物粪球沉降速率与硅藻和甲藻的生物量显著正相关，二者生物量可以解释60%的沉降速率变化，是影响粪球沉降速率的主要浮游植物类群。因此，浮游植物群落结构变化影响浮游动物粪球POC通量及其对总POC的贡献，对调控生物泵效率具有重要意义。

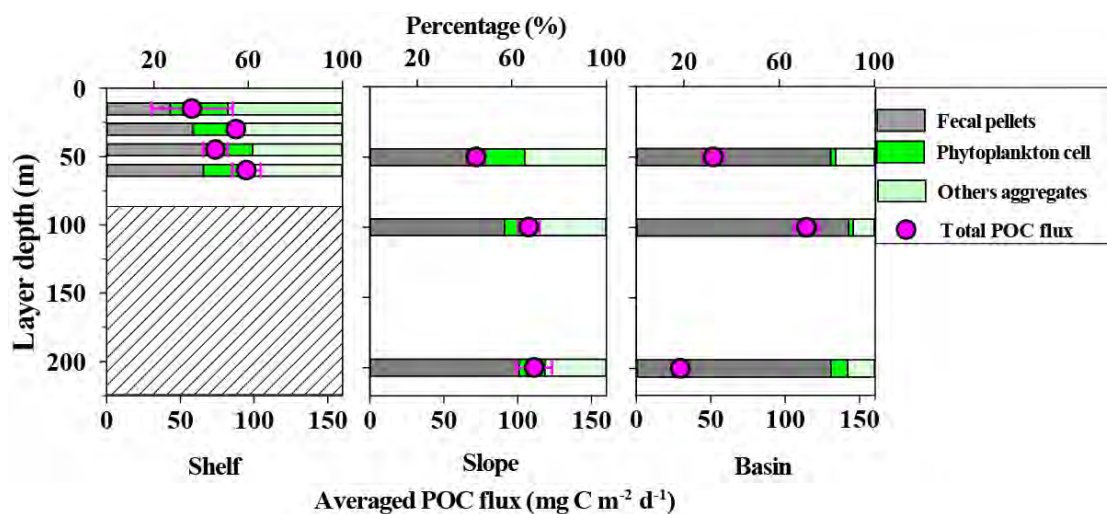


图 16. 陆架-陆坡-海盆不同生态系统各水层平均 POC 通量及其组成

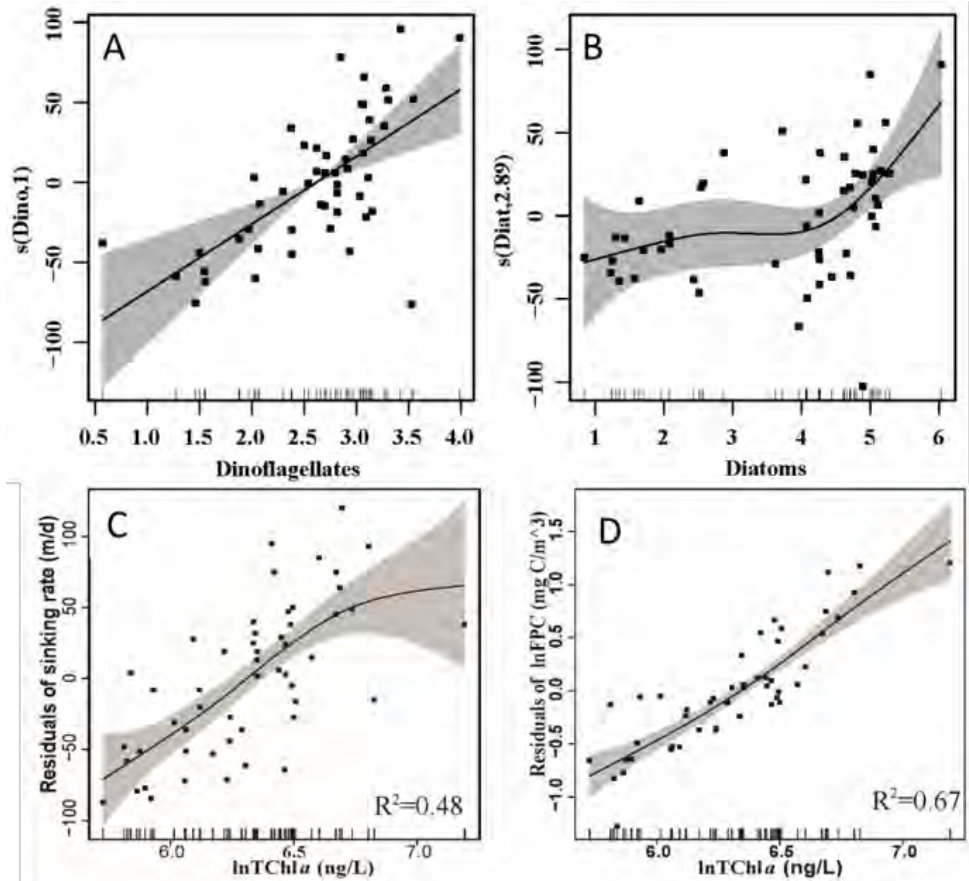


图 17. 水柱中浮游动物粪球 POC 含量与优势浮游植物类群生物量相关性

对比研究东海原甲藻藻华站位和附近未发生藻华站位发现，在藻华发生站，浮游植物的平均沉降速率约为非藻华站的 10 倍，浮游植物 POC 通量约是非藻华站的 100 倍，浮游动物粪球 POC 通量是非藻华站的 5-6 倍（图 18）。这是由于藻华期间浮游植物生物量急剧增加，大多聚集成聚合物后以聚合物的形式加快沉降，进而使 POC 通量急剧增加。与此同时，藻华站浮游植物生物量的聚集增加改变了浮游动物在藻华过程中的摄食，浮游动物的摄食率和产粪率显著提高，促使水柱中的粪球 POC 含量显著增加进而使浮游动物粪球 POC 含量增加。按照藻华事件发生在 1000 公里范围内并持续 7 天估算，这一偶然事件的 POC 通量约为全年东海近岸海域固定空气中二氧化碳量的 17%。因此，藻华这类短暂的偶然事件对年度 POC 输出通量作出了非常重要的贡献，对海洋碳输出和埋藏具有十分重要意义。

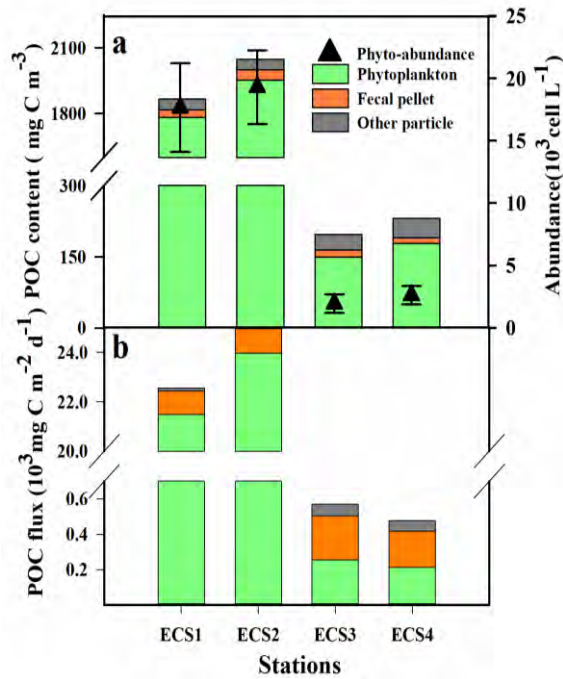


图 18. 比较藻华站和非藻华对照站位的 POC 含量和 POC 通量组成。

(2) 南海中层水体活跃的颗粒动力学过程

海洋生物泵将真光层产生的 POC 向中深层输送的过程中, POC 经历显著的再矿化, 最终到达深海的 POC 仅为表层海洋的一小部分。如何定量 POC 下行过程中通量的变化及再矿化过程是在年际-百年时间尺度上认识生物泵对海洋储碳能力贡献的关键问题。由于传统 POC 输出通量方法在真光层以深水体应用的局限性, 当前对中、深层水体 POC 通量的研究极其稀少。基于南海现有的中层水体 ^{210}Po 和 ^{210}Pb 研究结果, 研究了南海西部不同海域中层水体 ^{210}Po 与 ^{210}Pb 之间的平衡状况, 建立了定量南海中层水体颗粒物输出的 ^{210}Po - ^{210}Pb 不平衡方法。结果表明, 在 ^{210}Po 与 ^{210}Pb 平衡的真光层底部 200 m 以深中层水体, 普遍存在 ^{210}Po 相对于 ^{210}Pb 的亏损现象 (图 28), 揭示了南海西部中层 ^{210}Po 的亏损并非由真光层颗粒物沉降所致, 极有可能源自陆架沉积物的水平输送。 ^{210}Po 和 ^{210}Pb 之分馏因子与颗粒物浓度之间的关系亦为此观点提供了佐证。基于 ^{210}Po - ^{210}Pb 不平衡法发现南海西部中层水体颗粒物输出通量均显著高于同站位真光层颗粒物的输出通量 (图 19), 进一步证实该海域存在显著的陆架沉积物水平输送现象。通过综合研究南海已有的沉积物捕集器结果和 SEATS 站不同季节 ^{210}Po - ^{210}Pb 特征, 发现南海陆架沉积物向海盆中深层输送颗粒物的现象广泛存在 (图 20, Ma et al., 2017, *Geochemistry, Geophysics, Geosystems*, 第一标注)。该研究为深入开展南海不同水层生物泵效率奠定了基础。

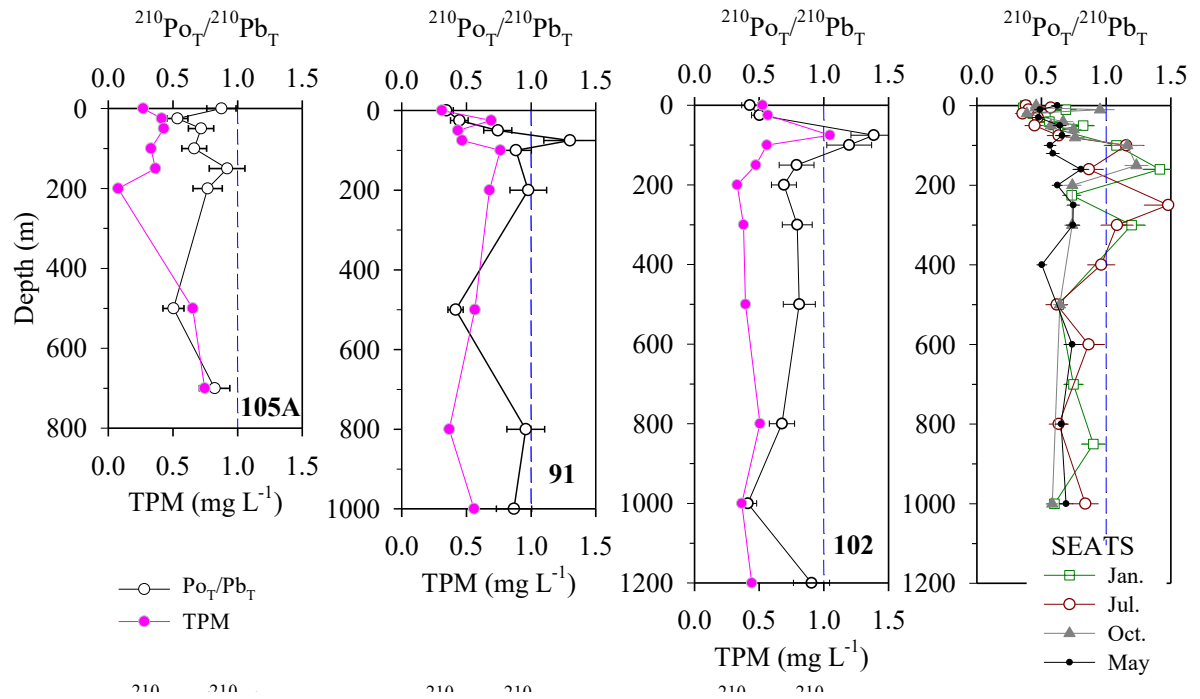


图 19. 南海西部及 SEATS 站 ^{210}Po - ^{210}Pb 不平衡特征。

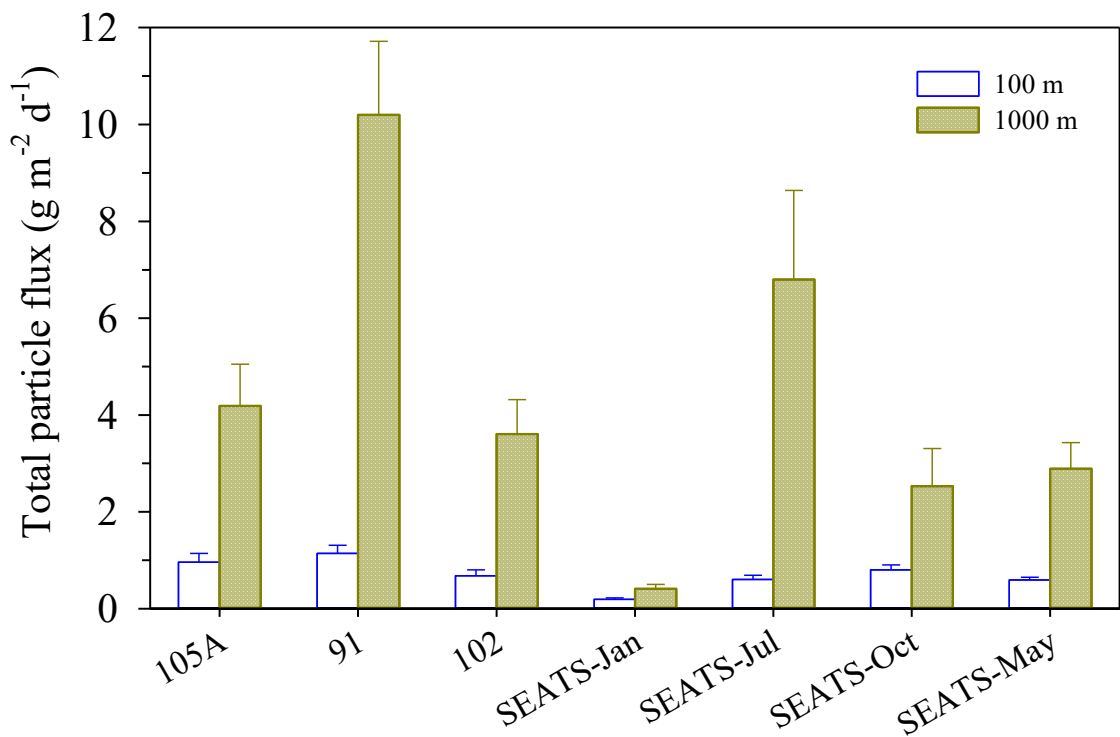


图 20. 南海西部真光层和弱光层不同深度颗粒物输出通量。

1.4. 深海微生物原位富集培养及新型颗粒附着烃类降解菌的发现

于 2017 年 6 月、8 月分别在南海北部海盆 MIES03 站（116° 16'E，18°30'N，水深 3758m）、西太平洋牛郎平顶海山 DY45I-NLG-S03-MIES01 站（20°24' N，160°47' E，水深 1617m）各布放了一套深海微生物原位培养系统（图 21），进行了深海近底微生物种群对海洋表层颗粒有机物（POM）及其衍生物响应与矿化作用研究等 8 类现场实验。布放于南海北部海盆 MIES03 站的系统已于 2018 年 7 月 8 日成功回收，在深海原位实际进行了近 13 个月的原位培养，并获取了无污染高保真的原位富集样品。该装备在南海大洋科考航次中的应用，将为深入研究南海细菌对颗粒有机碳降解特性和机理提供一个不可替代的海底实验平台。

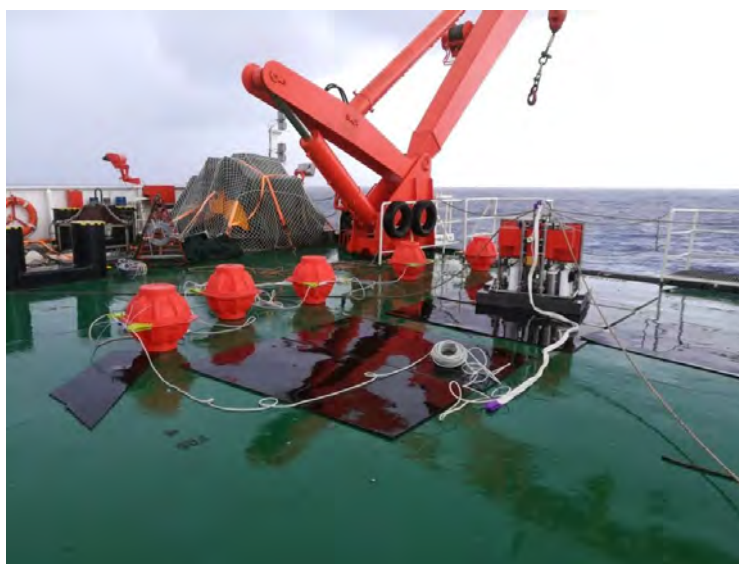


图 21.深海微生物原位富集系统布放前状态

(1) 颗粒附着烃类降解菌富集培养及菌群结构分析

基于南海北部深海海盆 3 个站位和陆架区 3 个站位采集的表层海水样品，利用 DNA 条形码分析原位颗粒附着微生物群落结构，开展颗粒有机碳衍生物（烷烃和芳烃）降解微生物种群的实验室模拟培养和菌群结构解析，基于原位样品构建了 12 个颗粒有机碳降解菌群。

DNA 条形码测序及分析结果显示，无论是颗粒附着还是自由生物的微生物种群， α - γ -变形菌纲、黄杆菌纲、放线菌纲和蓝细菌纲的细菌都是丰度最高的 5 个类群。原位海水中颗粒附着和自由生活的烃类降解菌中包括多种烷烃、芳烃降解菌（图 22A）。基于原位颗粒附着微生物种群，用烃作为唯一碳源富集培养后，原本低丰度的烃类降解菌迅速成为菌群中的优势类群。比如，在十六烷富集菌群中，已知的烷烃降解菌 *Alcanivorax*，

Thalassospira, *Marinobacter* 和 *Acinetobacter* 迅速成为优势菌 (图 22B); 在萘菲混合物富集菌群中, 不具备芳烃降解能力的 *Thalassospira* 是最优势的类群 (图 22C)。各富集菌群中还存在多个丰度>1%的次优势类群, 它们均可能是南海表层海水颗粒物中潜在的新型烷烃或芳香烃降解菌。

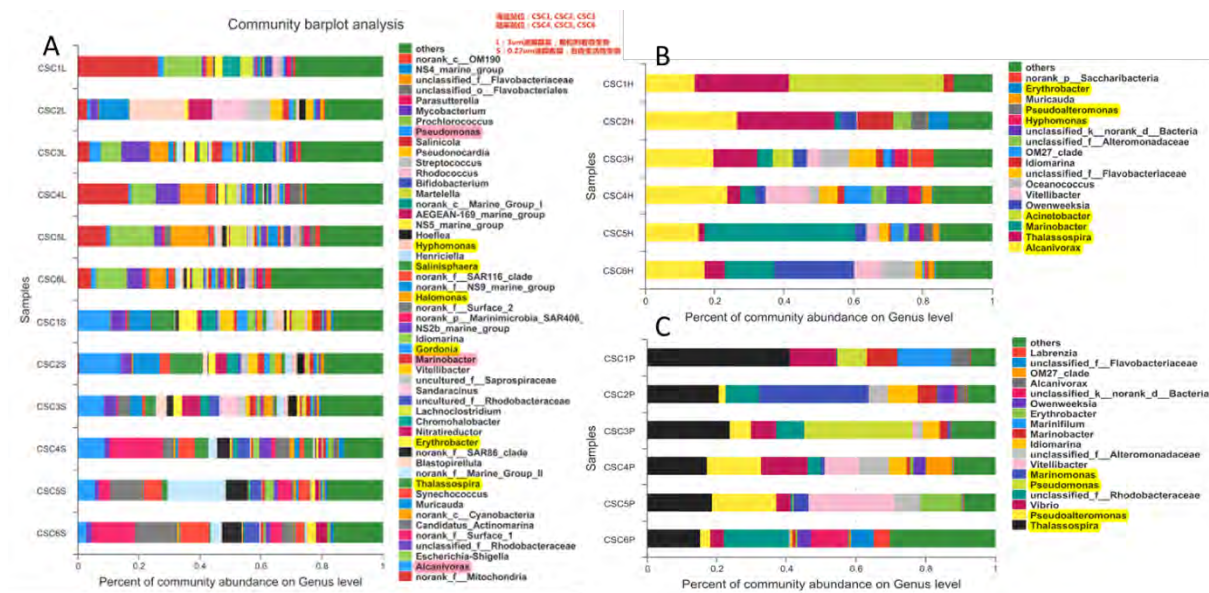


图 22. 南海原位表层海水颗粒附着和自由生活烃类降解菌分析

(A) 十六烷富集菌群结构分析 (B) 和芳烃富集菌群结构分析 (C)。

(2) 新型颗粒附着烃类降解菌生理生化特性及系统进化地位研究

在对南海表层海水中颗粒附着的可培养烃类降解菌的研究过程中, 发现了一类 *Thalassospira* 属的菌株为潜在新类群 (图 23)。以分别来自烷烃和芳烃富集菌群的 CSC3H3^T 和 CSC1P2 为代表菌株, 解析了它们的生理生化特性、系统进化地位和基因组。这两个菌株严格好氧, 能够还原硝酸盐和亚硝酸盐, 均能缓慢利用正烷烃和低环的芳香烃, 以及多种溶解性有机物。

通过测序并分析菌株 CSC3H3^T 基因组 (图 24), 在其质粒和染色体上均含有烷烃和芳烃的降解基因, 暗示了他们具有转化海洋碳的潜能。基因组中负责链烷烃降解的羟化酶主要有 Alkane-1 monooxygenase (AlkB) 和细胞色素 P450 CYP153 家族, 两者均通过烃的末端加氧羟基化, 进而通过醇醛脱氢酶形成脂肪酸, 最终进入脂肪酸的 β 氧化, 从而实现通过代谢烷烃来提供自身的能量与生长需要。对于某些降解微生物来说, 由于其缺乏膜结合的 AlkB 羟化酶基因, 而细胞色素 P450 成为其降解中链烷烃的关键。通过基因组分析发现, 菌株 CSC3H3 质粒中有 2 个 P450 基因, 它们可能参与了烷烃的降解。菌株 CSC3H3 染色体中, 注释到 2 个参与到芳香烃降解的双加氧酶 (extradiol dioxygenase),

此外，在质粒上有 2 个芳香环羟化的双加氧酶亚基也可能参与了芳香烃的初步氧化。CSC3H3 染色体中包含一个完整的原儿茶酸降解基因簇，一个调控该途径的调控子基因 *pcaQ*，说明其具有利用芳香烃的能力。

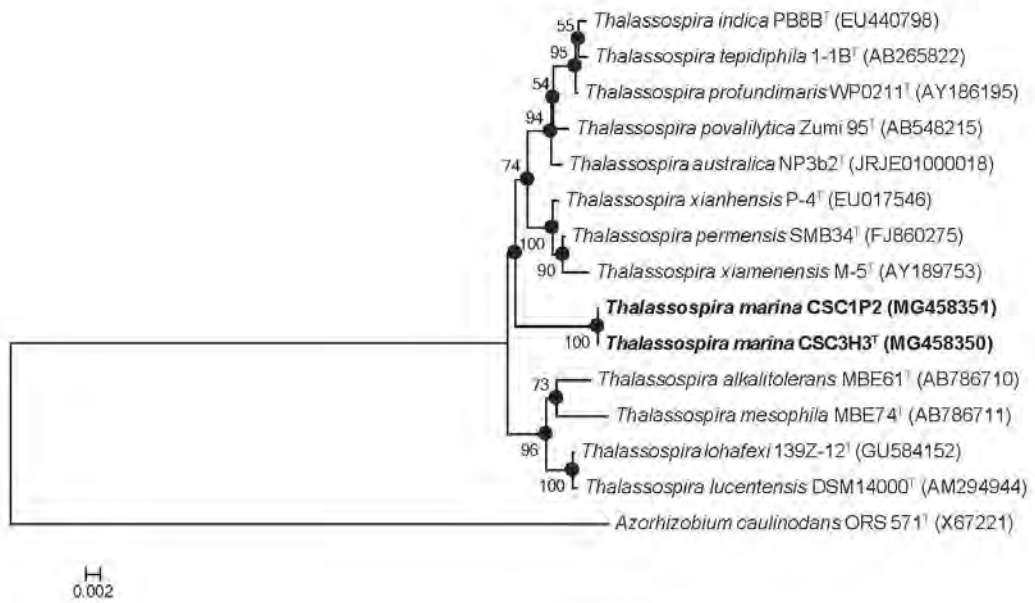


图 23. 菌株 *Thalassospira marina* CSC3H3T 和 CSC1P2 系统进化分析

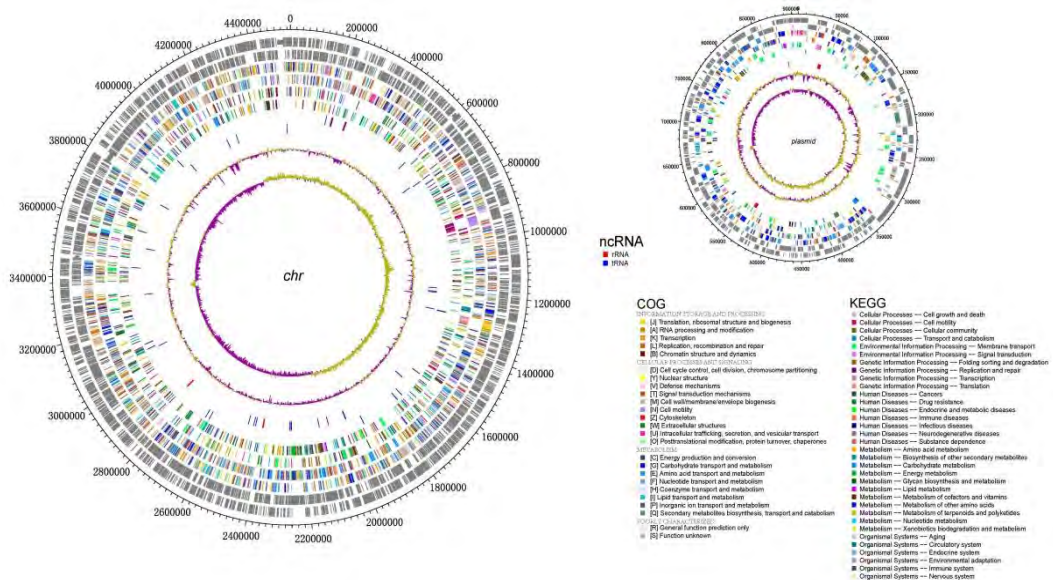


图 24. 菌株 *Thalassospira marina* CSC3H3T 基因组完成图

2. 预期社会经济效益

重点阐明对学科/行业产生的重要影响，对社会民生、生态环境、国家安全等的作用，以及研究成果的合作交流、转移转化和示范推广情况，人才、专利、技术标准战略在课题中的实施情况等。

2.1 科学研究和学术交流

课题的实施为利用 DNA 条形码解析浮游生物多样性、利用环境组学研究浮游生物原位基因表达奠定数据和方法基础，将提升对浮游生物关键功能群在颗粒有机碳输出通量调控作用中的认识。课题成果以撰写、发表学术研究报告、论文和参加学术会议交流等形式呈现。

(1) 科学研究

已构建南海北部浮游生物 DNA 条形码数据库 1 个，环境组学（宏转录组、宏基因组和宏蛋白质组）数据库 1 个，包括不同季节、不同生态系统、不同水层及不同粒径浮游生物样本超过 800 个，序列数据总量超过 1.6Tb。发表 SCI 论文 11 篇，详见附件清单。

(2) 学术交流

组织国际学术会议 2 次，参加国内外学术会议 18 个，参与学术交流超过 80 人次。

- 2017 年 7 月，林森杰、石拓共同发起组织厦门大学凌峰论坛“珊瑚的环境变化响应”
- 2018 年 6 月，刘光兴作为专题召集人共同发起组织第 15 届亚洲地球科学学会年会海洋科学专题“Carbon Sequestration in Marginal Seas: Regulation and Response to Global Change (Ocean Sciences, OS25)”
- 2016 年 7 月，石拓参加第 13 届亚洲地球科学学会年会（北京），作口头报告
- 2017 年 6 月，林森杰、石拓参加第七届海峡两岸珊瑚礁研讨会（垦丁），作特邀报告
- 2017 年 7 月，林森杰参加第 19 届国际植物学大会（深圳），作特邀报告
- 2017 年 8 月，谢彰先参加第十一届海峡两岸海洋科学研讨会（泰安），作口头报告
- 2018 年 4 月，林昕参加 The Changing Marine Ecosystem: A TARA-TKK Science Symposium（厦门），作口头报告
- 2018 年 5 月，林森杰参加全球变化下海洋有害藻华与缺氧研讨会（杭州），作口头报告
- 2018 年 6 月，林森杰参加第 15 届亚洲地球科学学会年会（火奴鲁鲁），作口头报告

此外，课题组成员还参加了第 25 届国际动植物基因组会议、首届国际海洋基因组会议、第 4 届硅藻分子生物学、联合国教科文组织政府间海洋学委员会西太平洋分委会第十届国际科学大会等 11 个国际、国内学术会议，展示并交流课题学术成果。

2.2 队伍建设和人才培养

- (1) 2017 年 8 月，庄昀筠受聘为中国海洋大学“青年英才工程”岗位、环境科学与工程学院副教授
- (2) 2017 年 11 月，孙萍获第二届中国原生动物学会青年科技奖
- (3) 2018 年 5 月，林昕受聘为近海海洋环境科学国家重点实验室（厦门大学）、厦门大学海洋与地球学院副教授
- (4) 出站博士后 2 人，毕业博士研究生 3 人，毕业硕士研究生 5 人

2.3 公众科普传播

刘光兴团队于 2017 年 4 月参加联合国教科文组织政府间海洋学委员会（IOC）西太平洋分委会（WESTPAC）第十届国际科学大会“海洋科学进校园”的科普活动。活动期间，为青岛市实验高级中学的一千余名师生做了题为“DNA、浮游生物与海洋的”的主题讲座，结合课题研究介绍了海洋浮游生物及其生态地位，浮游生物与人类的关系，通过实例分析，着重介绍了分子生物学技术在浮游生物研究中的应用。介绍了 DNA 提取的基本原理和简要步骤，并现场邀请学生参与提取草莓 DNA（图 25）。



图 25. 刘光兴团队参加 IOC-WESTPAC“海洋科学进校园”的科普活动

三、课题人员及经费投入使用情况

1.人员及经费投入情况

对照课题任务书阐述课题及课题资金（包括专项经费、自筹经费等）到位情况、课题资金单独核算情况、预算调剂情况、支出情况和经费使用监督管理情况、人员投入情况等。

课题中央财政资金专项经费预算批复数 468.0 万元，已到位数 140.4 万元，资金到位率 30%，课题累计支出 107.44 万元，实际支出占预算 22.96%，实际支出占已到位经费 76.52%。其中设备费 0.62 万元、材料费 17.17 万元、测试化验加工费 36.68 万元、差旅费 13.24 万元、出版/文献/信息传播/知识产权事务费 0.78 万元、劳务费 17.52 万元和间接费用 21.43 万元。课题无自筹经费，未作预算调剂。

国发【2014】11 号文及中办发【2016】50 号文发布后，课题承担单位中国海洋大学陆续发布了《中国海洋大学差旅费管理办法》（海大财字〔2016〕22 号，2016 年 8 月 31 日发布）、《中国海洋大学会议费管理办法》（海大财字〔2016〕23 号，2016 年 8 月 31 日发布）、《中国海洋大学纵向科研项目（课题）结余经费管理办法》（海大字〔2016〕24 号，2016 年 12 月 30 日发布）、《中国海洋大学国家科技计划（专项、基金）经费预算调整实施办法》（海大字〔2016〕25 号,2016 年 12 月 30 日发布）及《中国海洋大学纵向科研项目间接费用管理办法》（海大字〔2016〕26 号,2016 年 12 月 30 日发布）等文件对经费规范化管理。

课题参与人员共计 33 人，其中高级职称 10 人，中级职称 1 人，初级职称 3 人，其他人员 19 人，工作时间共计 29 人年。

2.课题经费拨付情况

课题牵头单位向课题承担单位、课题承担单位向课题参与单位拨付中央财政资金情况。

项目牵头单位厦门大学向课题承担单位中国海洋大学拨付中央财政资金 140.0 万元，课题承担单位按计划向课题参与单位厦门大学拨付中央财政资金 86.7 万元，国家海洋局第三海洋研究所 19.2 万元。

3.人员及经费实际调整情况

如出现课题人员的调整，以及经费未及时到位、停拨、迟拨等特殊情况，请详细说明原因、措施、履行相关审批管理制度以及整改等情况。

无。

四、课题配套支撑条件情况

阐述各主要研究任务的配套支撑条件落实及调整变化情况。如有调整变化，请说明调整变化对完成课题目标的影响和作用。

课题承担单位中国海洋大学、厦门大学、国家海洋局第三海洋研究所等，为该研究提供了海上调查与实验、室内研究与分析所需要的仪器设备等条件，为本课题的实施提供了有力支撑。其中，“东方红 2”、“嘉庚号”、“向阳红 03”等大型海洋科考船，为本课题提供航次保障和海上观测支持。近海海洋环境科学国家重点实验室（厦门大学），海洋环境与生态教育部重点实验室（中国海洋大学）拥有一系列先进的生物海洋学、海洋生态学、分子生物学及细胞生物学研究平台，以上平台仪器已全部投入使用。国家海洋局第三海洋研究所研制的深海沉积物和水体微生物的原位富集系统能作为实验平台，在海洋原位条件下开展与本项目相关的 POM 和 DOM 降解实验，为本课题中南海洋微生物原位培养关键技术提供设备支撑。

五、组织实施风险及应对情况

阐述课题在组织实施过程中，面对外部政策、组织管理、研发变化和知识产权等方面的风险以及应对措施。

无。

六、课题组织实施中的重大问题及建议

无。

七、任务书中有特殊约定或其他需要说明的事项

无。

附件：发表论文清单和首页

共发表论文 11 篇，SCI 收录 9 篇；其中第一标注 8 篇，第二标注 2 篇，其他标注 1 篇。

1. Cong Wang, Senjie Lin*, Xin Lin, Ling Li, Lingxiao Lin, 2017, Glyphosate shapes a dinoflagellate-associated bacterial community while supporting algal growth as sole phosphorus source. *Frontier in Microbiology*, 8, 2530. | 2 区, 4.076, 第一标注
2. Tangcheng Li, Chentao Guo, Yaquin Zhang, Cong Wang, Xin Lin, Senjie Lin*, 2018, Identification and expression analysis of an atypical alkaline phosphatase in *Emiliana huxleyi*. *Frontier in Microbiology* | 已接收, 2 区, 4.076, 第一标注
3. Haoyang Ma, Weifeng Yang*, Lihao Zhang, Run Zhang, Min Chen, Yusheng Qiu and Minfang Zheng, 2017, Utilizing ²¹⁰Po deficit to constrain particle dynamics in mesopelagic water, western South China Sea. *Geochemistry, Geophysics, Geosystems*, 18(4): 1594-1607. | 2 区, 3.201, 第一标注
4. Ping Sun, Liying Huang, Dapeng Xu, Bangqin Huang, Nengwang Chen, Alan Warren , 2017, Marked seasonality and high spatial variation in estuarine ciliates are driven by exchanges between the ‘abundant’ and ‘intermediate’ biospheres. *Scientific Reports*, 7: 9494. DOI:10.1038/s41598-017-10308-y. | 3 区, 4.259, 第一标注
5. Xinguo Shi, Ling Li, Senjie Lin*, 2018, Circadian and irradiance effects on expression of antenna protein genes and pigment contents in dinoflagellate *Prorocentrum donghaiense* (Dinophyceae). *Harmful algae*, 75, 27-34. | 3 区, 4.138 , 第一标注
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Glyphosate Shapes a Dinoflagellate-Associated Bacterial Community While Supporting Algal Growth as Sole Phosphorus Source

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Glyphosate is a widely used herbicide that can potentially be a phosphorus (P) source for phytoplankton and microbes when discharged into the coastal ocean. In contrast to bacteria, few eukaryotic phytoplankton species appear capable of directly utilizing glyphosate. In this study, we observed, after a long delay (>60 days), *Prorocentrum donghaiense*, a dinoflagellate known to cause major harmful algal blooms in the East China Sea, could grow in a medium with glyphosate as the sole P source; suggesting that *P. donghaiense* growth was through bacterial mediation. To understand how the bacteria community might respond to glyphosate, we analyzed the 16S rRNA genes of the microbial community present in *P. donghaiense* cultures when grown under lower (36 μM) and higher (360 μM) glyphosate concentrations. Based on both Sanger and Illumina high throughput sequencing, we obtained more than 55,323 good-quality sequences, which were classified into six phyla. As the concentration of glyphosate rose, our results showed a significant increase in the phyla Proteobacteria and Firmicutes and a decrease in the phylum Bacteroidetes. Further qPCR (Quantitative PCR) analysis showed higher abundances of two specific phylotypes in the higher-glyphosate *P. donghaiense* cultures when compared to the lower-glyphosate and no-glyphosate cultures. Correspondingly, qPCR displayed the same trend for the abundance of a gammaproteobacterial type of *phnJ*, a gene encoding Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P lyase, which is responsible for phosphonate degradation. In addition, Tax4Fun analysis based on our 16S rRNA gene sequences results in higher predicted abundances of phosphonate metabolizing genes in glyphosate-treated cultures. This study demonstrates that glyphosate could selectively promote the growth of particular groups of bacteria within an algal culture and in glyphosate enriched coastal waters, this interaction may potentially further facilitate the growth of alga.

Keywords: glyphosate, P-source, *Prorocentrum donghaiense*, microbial community, 16s rRNA gene profiling, *phnJ*

Identification and expression analysis of an atypical alkaline phosphatase in *Emiliana huxleyi*

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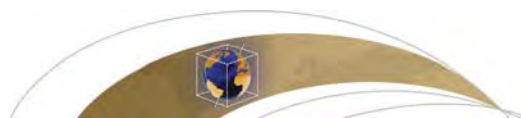
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Key Points:

- Large deficiencies of ^{210}Po were observed in mesopelagic water in the South China Sea
- Residence times and export fluxes of ^{210}Po indicate active particle cycling in mesopelagic water in the SCS
- The ^{210}Po - ^{210}Pb pair was used to constrain the particle cycling in mesopelagic water

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Utilizing ^{210}Po deficit to constrain particle dynamics in mesopelagic water, western South China Sea

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Abstract The ^{210}Po - ^{210}Pb pair is increasingly used as a proxy of quantifying organic carbon export from the euphotic zone. However, disequilibria between ^{210}Po and ^{210}Pb in mesopelagic water have been poorly studied. Here we present unusual deficiencies of ^{210}Po with respect to ^{210}Pb in mesopelagic water (200–1000 m) in the South China Sea (SCS). The total particulate matter (TPM) increased by up to 32% in the mesopelagic layer comparing with the euphotic zone. The total $^{210}\text{Po}/^{210}\text{Pb}$ ratio varied from 0.41 to 0.98 with an average of 0.72 ± 0.19 , showing an enhanced removal of ^{210}Po in mesopelagic water. On average, particulate ^{210}Po and ^{210}Pb increased by 23% and 32% at the slope stations, respectively. These results indicated that the ^{210}Po deficits result from lateral transport, probably via benthic nepheloid layer. Based on the deficiency of ^{210}Po , the residence times of particulate ^{210}Po were estimated to range from 0.11 to 0.25 year (avg. 0.17 ± 0.07 year), allowing resuspended sediment to disperse over a long range. The export fluxes of ^{210}Po varied from 68 to 121 dpm $\text{m}^{-2} \text{d}^{-1}$ with an average of 96 ± 27 dpm $\text{m}^{-2} \text{d}^{-1}$, which was 6 times that out of the euphotic zone. Using the ^{210}Po deficits, the export fluxes of TPM out of the mesopelagic layer were quantified to vary from 4.19 to 10.20 g $\text{m}^{-2} \text{d}^{-1}$, revealing a large amount of particles from the shelf to the SCS basin. This study suggests that ^{210}Po - ^{210}Pb could be an effective tracer of tracking particle cycling in mesopelagic water.

1. Introduction

In seawater, ^{210}Po ($T_{1/2} = 138.4$ days) is mainly generated by its grandparent ^{210}Pb ($T_{1/2} = 22.3$ years) via radioactive decay. Both ^{210}Po and ^{210}Pb have very strong particle-reactive natures in seawater [Chuang *et al.*, 2013; Yang *et al.*, 2013]. In the euphotic zone, abundant biogenic particles usually introduce a quick sorption and sinking of ^{210}Po and ^{210}Pb on a short time scale, leading to insufficient time for ^{210}Po accumulation and resulting in an observable deficiency of ^{210}Po with respect to ^{210}Pb [Bacon *et al.*, 1976; Nozaki and Tsunogai, 1976; Cochran *et al.*, 1983]. Due to the tight link between particulate organic components and ^{210}Po or ^{210}Pb adsorption [Stewart *et al.*, 2007; Yang *et al.*, 2013, 2015a; Chuang *et al.*, 2014; Rigaud *et al.*, 2015], the disequilibria between ^{210}Po and ^{210}Pb have increasingly been used to trace the export flux of particulate organic carbon (POC) [Buesseler *et al.*, 2008; Verdery *et al.*, 2009; Stewart *et al.*, 2010; Roca-Martí *et al.*, 2016; Su *et al.*, 2017], biogenic silica [Friedrich and Rutgers van der Loeff, 2002], and nitrogen [Yang *et al.*, 2011] out of the euphotic zone in the last decade. The ^{210}Po - ^{210}Pb pair is also listed as one of the important proxies for investigating the oceanic particle dynamics in the ongoing GEOTRACES program [Church *et al.*, 2012; Rigaud *et al.*, 2013, 2015]. Since the half-life of ^{210}Po enables ^{210}Po - ^{210}Pb pair to record information on particle cycling over time scales from days to seasons, this pair, together with sediment trap and ^{234}Th - ^{238}U approach, is used to better decipher the POC export from the euphotic zone on different time scales [Hong *et al.*, 2013; Ceballos-Romero *et al.*, 2016; Maiti *et al.*, 2016].

Although the application of ^{210}Po - ^{210}Pb in the euphotic zone has been intensively studied recently, the cycling of ^{210}Po and its utilization in the mesopelagic (200–1000 m) and bathypelagic (1000–4000 m) zones are poorly understood to date [Kim, 2001; Hu *et al.*, 2014]. It is generally accepted that ^{210}Po is in equilibrium with ^{210}Pb in deep water because the scarcity of particle allows ^{210}Po to reside longer and reach equilibrium as reported before, such as in North Atlantic [Bacon *et al.*, 1976, 1988], Pacific [Turekian and Nozaki, 1980], and eastern and central Indian Ocean [Cochran *et al.*, 1983]. However, the deficiencies of ^{210}Po with respect to ^{210}Pb have increasingly been found in mesopelagic water in the past years, e.g., in the Sargasso Sea

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Marked seasonality and high spatial variation in estuarine ciliates are driven by exchanges between the 'abundant' and 'intermediate' biospheres

Ping Sun¹, Liying Huang¹, Dapeng Xu², Bangqin Huang¹, Nengwang Chen¹ & Alan Warren³

We examined the spatial and temporal variability of ciliate community in a subtropical estuary by rRNA and rDNA-based high throughput sequencing of 97 samples collected along the entire salinity gradient at two-month intervals in 2014. Community divided statistically into three groups: freshwater (salinity < 0.5‰), oligohaline and mesohaline (0.5‰ < salinity < 18‰), and polyhaline and euhaline (18‰ < salinity < 40‰). Across all three groups, salinity explained most of the community variability. Within each group, seasonal shifts in community formed cool (spring and winter) and warm (summer and autumn) subgroups, indicating that spatial variability overrode seasonal changes in determining community composition. Cool and warm groups showed opposite associations with temperature and prey proxies, suggesting distinct seasonal niche separation. The community reassembly of cool and warm groups was essentially due to transitions between intermediate (with relative abundance of 0.01–1%) and abundant (with relative abundance > 1%) OTUs. Further analyses demonstrated that the intermediate group not only encompassed comparable OTU richness to that of the total community and maintained high metabolic activity but also had the highest proportion in transition, either to abundance or rarity, thus offering a first view on how it varies across space and time and revealing the essential role it played in maintaining stability and functionality within the community.

Microzooplankton are heterotrophic organisms with body size between 20 and 200 µm. They can feed on pico- and nanoplankton unavailable to most meso- and macrozooplankton. They therefore occupy a key position in aquatic foodwebs as their grazing significantly affects primary producers and bacteria^{1,2}. However, our knowledge of the spatial and temporal distribution patterns of microzooplankton across a wide variety of environments and their shaping factors is still limited. Ciliates are single-celled eukaryotes (protists) that dominate many microzooplankton communities in terms of both species diversity and abundance³. By virtue of their short generation times, ciliates can quickly respond to environmental fluctuations⁴. Therefore, understanding the community dynamics of ciliates is increasingly important in an era of rapid environmental change, particularly in habitats where such changes are likely to be sufficiently pronounced to affect ecosystem function. Estuaries are highly dynamic ecosystems that undergo constant change due to both natural, e.g. daily (tidal) and seasonal variations, and anthropogenic factors. These variations in space and time make estuaries ideal ecosystems to perform investigations targeting the community dynamics of ciliates.

Estuarine ciliates have been included as part of investigations on wider taxonomic scales, e.g. protozoa⁵ or protists⁶, or alongside studies of various metazoans groups, e.g. copepods, fishes, molluscs and crustaceans⁷. Other studies have focused on specific groups of ciliates e.g. tintinnids⁸ or aloricate ciliates⁹. Studies on the full spectrum of ciliates along entire salinity gradients are limited, and those dealing with both temporal and spatial variations

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Circadian and irradiance effects on expression of antenna protein genes and pigment contents in dinoflagellate *Prorocentrum donghaiense* (Dinophyceae)



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ABSTRACT

PCP and acpPC are the two major antennae proteins that bind pigments in peridinin-containing dinoflagellates. The relationship between antennae proteins and cellular pigments at molecular level is still poorly understood. Here we identified and characterized the two antennae protein genes in dinoflagellate *Prorocentrum donghaiense* under different light conditions. The mature PCP protein was 32 kDa, while acpPC was a polyprotein each of 19 kDa. Both genes showed higher expression under low light than under high light, suggesting their possible role in a low light adaptation mechanism. The two genes showed differential diel expression rhythm, with PCP being more highly expressed in the dark than in the light period and acpPC the other way around. HPLC analysis of cellular pigments indicated a diel change of chlorophyll *c2*, but invariability of other pigments. A stable peridinin: chlorophyll *a* pigment ratio was detected under different light intensities and over the diel cycle, although the diadinoxanthin: chlorophyll *a* ratio increased significantly with light intensity. The results suggest that 1) PCP and acpPC genes are functionally distinct, 2) PCP and acpPC can function under low light as an adaptive mechanism in *P. donghaiense*, 3) the ratios of diadinoxanthin:chlorophyll *a* and peridinin: chlorophyll *a* can potentially be used as an indicator of algal photophysiological status and a pigment signature respectively under different light conditions in *P. donghaiense*.

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1. Introduction

Dinoflagellates and other phytoplankton live in a variable light environment and during photoacclimation may modulate their pigment abundance and distribution. Cellular concentrations of photosynthetic pigments are expected to correlate with the expression of individual chlorophyll-binding proteins (Iglesias-Prieto and Trench, 1997). Changes in pigments under variable light intensities experienced by dinoflagellates and other phytoplankton are usually associated with antenna remodeling (Peers et al., 2009). Each phytoplankton species has a specific distribution of pigment in its pigment-protein complexes and the distribution is rearranged during photoacclimation (Iglesias-Prieto and Trench, 1997). For example, high light can induce loss

of photosynthetic pigments and lead to photobleaching and photoinhibition, as documented in *Symbiodinium* (Takahashi et al., 2008), a genus of dinoflagellates mostly endosymbiotic with corals and other marine invertebrates. The loss of photosynthetic pigments through the loss of antenna protein, particularly acpPC is believed to be responsible for coral bleaching (Takahashi et al., 2008). Thus, understanding the responses of the two antennae proteins to the variable ambient light environment will provide valuable information regarding the functional significance of changes in cellular pigment concentration (Iglesias-Prieto and Trench, 1997).

PCP (peridinin- chlorophyll *a*-binding protein) and acpPC (chlorophyll *a*-chlorophyll *c2*-peridinin-protein) are two major light harvesting components in peridinin-containing (i.e. “typical”) dinoflagellates (Michael and Miller, 1998; Takahashi et al., 2008), which form two antennae with associated pigments in the photosynthesis system (Iglesias-Prieto and Trench, 1997; Takahashi et al., 2008). PCP is a water-soluble protein, which only occurs in light harvesting complex in dinoflagellates, usually located in

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Thalassospira marina sp. nov., isolated from surface seawater

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Abstract

Two novel marine bacteria, designated strains CSC3H3^T and CSC1P2, were isolated from surface seawater of the South China Sea. Both strains were Gram-negative, oxidase-positive, catalase-positive, curved rods and motile. They grew at 10–40 °C, pH 5–10 and in the presence of 0–15% (w/v) NaCl. Their 16S rRNA gene sequences were identical to each other. Phylogenetic analysis based on 16S rRNA gene sequences indicated that they belong to the genus *Thalassospira*, and shared 97.5–98.3% sequence similarity to all other validly type strains of the genus *Thalassospira*, and the highest similarity was to the type strain *Thalassospira povalilytica* Zumi 95^T (98.3%), followed by *Thalassospira australica* NP3b2^T (98.2%). The digital DNA–DNA hybridization value between the two strains was 80.4%, while the values with *T. povalilytica* Zumi 95^T and *T. australica* NP3b2^T were only 20.5–20.7% and 20.4–20.5%, respectively. The two strains possess similar major cellular fatty acids including C_{18:1}ω7c, C_{16:0}, C_{19:0}ω8c cyclo, C_{18:1} 2-OH and C_{17:0} cyclo. The G+C contents of the chromosomal DNA of strains CSC3H3^T and CSC1P2 were 54.6 and 54.5 mol%, respectively. The major respiratory quinone was ubiquinone 10. Phosphatidylethanolamine, phosphatidylglycerol and several unidentified phospholipids, aminolipid and lipids were present in both strains. Based on phenotypic and genotypic characteristics, the two strains represent a novel species within the genus *Thalassospira*, for which the name *Thalassospira marina* sp. nov. is proposed. The type strain is CSC3H3^T (=MCCC 1A11786^T =KCTC 62333^T).

The genus *Thalassospira* was proposed by López-López *et al.* [1] and emended by Liu *et al.* [2] and Tsubouchi *et al.* [3], belonging to the family *Rhodospirillaceae* within the class *Alphaproteobacteria*. At the time of writing, the genus contains 11 valid species, including *Thalassospira lucentensis* [1], *Thalassospira xiamenensis* and *Thalassospira profundimaris* [2], *Thalassospira tepidiphila* [4], *Thalassospira xianhensis* [5], *Thalassospira alkalitolerans* and *Thalassospira mesophila* [3], *Thalassospira povalilytica* [6], *Thalassospira lohafexi* [7], *Thalassospira australica* [8], and *Thalassospira indica* [9] (www.bacterio.net/thalassospira.html). In addition, ‘*Thalassospira permensis*’ [10] and ‘*Thalassospira frigidophilosprofundus*’ [11] are not validly described yet.

Previous studies showed that some *Thalassospira* species were hydrocarbon degraders [2, 4, 5, 10, 12] or the dominant member in the hydrocarbon-degrading consortia retrieved from a wide range of global oceans [13–16]. In addition, some of them can degrade other environmental

xenobiotics, such as polyvinyl-alcohol [6]. These results imply that *Thalassospira* play a certain role in the carbon cycle in marine environments, yet waiting for further characterization.

During the investigation of particle-attached alkane and aromatic-hydrocarbon-degrading bacteria in surface seawater of the South China Sea, strains CSC3H3^T and CSC1P2 were isolated as potential degraders, and formed an independent clade in both 16S rRNA and *gyrB* genes phylogenetic trees of the genus *Thalassospira*, indicating that they represent a novel species of the genus. This study aimed to determine the taxonomic status of strains CSC3H3^T and CSC1P2 by using a polyphasic approach.

Strains CSC3H3^T and CSC1P2 were isolated from seawater under the surface at 1 m depth at sites CSC3 (21° 18′ 9.43″ N, 117° 42′ 39.35″ E) and CSC1 (21° 19′ 49.59″ N, 119° 21′ 23.18″ E) in the north-east of the South China Sea in October 2016. They were maintained as aqueous glycerol

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Abbreviations: dDDH, digital DNA–DNA hybridization; JCM, Japan Collection of Microorganisms; KCTC, Korean Collection for Type Cultures; MA, marine agar; MB, marine broth; MCCC, Marine Culture Collection of China; ME, minimum-evolution; ML, maximum-likelihood; NJ, neighbour-joining.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CSC3H3^T and CSC1P2 are MG458350 and MG458351, respectively; for the chromosomal genome sequences of strains CSC3H3^T, CSC1P2, *Thalassospira povalilytica* Zumi 95^T (=JCM 18746^T) and *T. lohafexi* 139Z-12^T (=MCCC 1A01959^T) are CP024199, NWTK00000000, PGTS00000000 and NXGX00000000, respectively.

One supplementary table and two supplementary figures are available with the online version of this article.

Transcriptomic and physiological analyses of the dinoflagellate *Karenia mikimotoi* reveal non-alkaline phosphatase-based molecular machinery of ATP utilisation

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Summary

The ability to utilize dissolved organic phosphorus (DOP) is important for phytoplankton to survive the scarcity of dissolved inorganic phosphorus (DIP), and alkaline phosphatase (AP) has been the major research focus as a facilitating mechanism. Here, we employed a unique molecular ecological approach and conducted a broader search for underpinning molecular mechanisms of adenosine triphosphate (ATP) utilisation. Cultures of the dinoflagellate *Karenia mikimotoi* were set up in L1 medium (+P), DIP-depleted L1 medium (–P) and ATP-replacing-DIP medium (ATP). Differential gene expression was profiled for ATP and +P cultures using suppression subtractive hybridisation (SSH) followed by 454 pyrosequencing, and RT-qPCR methods. We found that ATP supported a similar growth rate and cell yield as L1 medium and observed DIP release from ATP into the medium, suggesting that *K. mikimotoi* cells were expressing extracellular hydrolases to hydrolyse ATP. However, our SSH, qPCR and enzymatic activity assays indicated that 5'-nucleotidase (5NT), rather than AP, was responsible for ATP hydrolysis. Further

gene expression analyses uncovered that intercellular purine metabolism was significantly changed following the utilisation of ATP. Our findings reveal a multi-faceted machinery regulating ATP utilisation and P metabolism in *K. mikimotoi*, and underscore AP activity is not the exclusive indicator of DOP utilisation.

Introduction

Phosphorus (P) is a vital element required by all forms of life and its availability constrains the phytoplankton productivity in many marine ecosystems (Karl, 2014). The major dissolved P pools in aquatic environments consist of dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP). DIP is the preferable form of P because it can be directly utilized by phytoplankton cells (Currie and Kalf, 1984), while the assimilation of DOP is completed with the assistance of different types of hydrolases produced by cells (Azam *et al.*, 1983; Cotner and Biddanda, 2002). Because DIP is rapidly consumed in the euphotic zone and replenishment from external sources is slow, it is often at growth-limiting concentrations in the ocean where DOP is relatively abundant (Paytan and McLaughlin, 2007; Lin *et al.*, 2016). For instance, the measured DIP concentration was 0.2–4 nM in surface waters of North Atlantic where DOP concentrations range from 40 to 300 nM (Wu *et al.*, 2000; Mather *et al.*, 2008). This gives a selection pressure to phytoplankton for the ability to utilize DOP. Therefore, DOP availability is a potential driver of phytoplankton species composition and may be of major importance to bloom initiation and maintenance of harmful algae (Dyhrman and Ruttenberg, 2006). Numerous physiological studies have shown that most dinoflagellates and other phytoplankters are able to assimilate various types of DOP when DIP is depleted (Oh *et al.*, 2002; Huang *et al.*, 2005; Richardson and Corcoran, 2015; Lin *et al.*, 2016). Alkaline phosphatase (AP), 5'-nucleotidase (5NT) and other enzymes can facilitate utilisation of DOP in eukaryotic phytoplankton and bacteria. In dinoflagellates, AP has

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Enhancement of Non-photochemical Quenching as an Adaptive Strategy under Phosphorus Deprivation in the Dinoflagellate *Karlodinium veneficum*

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Intensified water column stratification due to global warming has the potential to decrease nutrient availability while increasing excess light for the photosynthesis of phytoplankton in the euphotic zone, which together will increase the need for photoprotective strategies such as non-photochemical quenching (NPQ). We investigated whether NPQ is enhanced and how it is regulated molecularly under phosphorus (P) deprivation in the dinoflagellate *Karlodinium veneficum*. We grew *K. veneficum* under P-replete and P-depleted conditions, monitored their growth rates and chlorophyll fluorescence, and conducted gene expression and comparative proteomic analyses. The results were used to characterize NPQ modulation and associated gene expression dynamics under P deprivation. We found that NPQ in *K. veneficum* was elevated significantly under P deprivation. Accordingly, the abundances of three light-harvesting complex stress-related proteins increased under P-depleted condition. Besides, many proteins related to genetic information flow were down-regulated while many proteins related to energy production and conversion were up-regulated under P deprivation. Taken together, our results indicate that *K. veneficum* cells respond to P deprivation by reconfiguring the metabolic landscape and up-tuning NPQ to increase the capacity to dissipate excess light energy and maintain the fluency of energy flow, which provides a new perspective about what adaptive strategy dinoflagellates have evolved to cope with P deprivation.

Keywords: non-photochemical quenching, dinoflagellates, phosphorus deprivation, metabolic machinery reconfiguration, energy flow

INTRODUCTION

Phytoplankton live in a constantly changing light environment affected by factors such as the strong solar radiation and fluctuant waves in the surface ocean, and they often absorb too much light which exceeds their photosynthetic capacity and would potentially cause photo-oxidative damage to the chloroplast (Anderson and Barber, 1996; Niyogi, 1999; Li et al., 2009). In response, these photosynthetic organisms have developed many photoprotective strategies to protect themselves

ORIGINAL ARTICLE

Transcriptomic and microRNAomic profiling reveals multi-faceted mechanisms to cope with phosphate stress in a dinoflagellate

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Although gene regulation can occur at both transcriptional and epigenetic (microRNA) levels, combined transcriptomic and microRNAomic responses to environmental stress are still largely unexplored for marine plankton. Here, we conducted transcriptome and microRNAome sequencing for *Prorocentrum donghaiense* to understand the molecular mechanisms by which this dinoflagellate copes with phosphorus (P) deficiency. Under P-depleted conditions, G1/S specific cyclin gene was markedly **downregulated**, consistent with growth inhibition, and genes related to dissolved organic phosphorus (DOP) hydrolysis, carbon fixation, nitrate assimilation, glycolysis, and cellular motility were upregulated. The **elevated** expression of ATP-generating genes (for example, rhodopsin) and ATP-consuming genes suggests some metabolic reconfiguration towards accelerated ATP recycling under P deficiency. MicroRNAome sequencing revealed 17 microRNAs, potentially regulating 3268 protein-coding genes. Functional enrichment analysis of these microRNA-targeted genes predicted **decreases in sulfatide** (sulfolipid) catabolism under P deficiency. Strikingly, we detected a significant increase in sulfolipid sulfatide content (but not in sulphoquinovosyl diacylglycerol content) and its biosynthesis gene expression, indicating a different sulfolipid-substituting-phospholipid mechanism in this dinoflagellate than other phytoplankters studied previously. Taken together, our integrative transcriptomic and microRNAomic analyses show that enhanced DOP utilization, accelerated ATP cycling and repressed sulfolipid degradation constitute a comprehensive strategy to cope with P deficiency in a model dinoflagellate.

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Introduction

Marine phytoplankton experience environmental fluctuations in light intensity, nutrient availability, temperature and pH. In the future ocean, warming and intensified stratification may bring greater nutrient stress. Phosphorus (P) is an essential nutrient for phytoplankton growth as it is required for cellular structures (membranes, DNA, RNA), metabolism (nucleotides, NADH, NADPH), storage of energy (ATP), cell signaling (cAMP, IP3), and biochemical regulation (protein phosphorylation) (Karl, 2014; Lin *et al.*, 2016). In many parts of the ocean, dissolved inorganic phosphorus (DIP), the chemical form of P that can be taken up and assimilated directly, can be diminishingly scarce (Karl, 2014; Lin *et al.*, 2016). P can be a limiting

factor for phytoplankton growth even in eutrophic coastal waters where excess nitrogen is introduced due to human activities (Lin *et al.*, 2016). Various strategies have been identified in phytoplankton to cope with low P availability, including changing P transporters to increase uptake (Perry, 1976), switching to dissolved organic phosphorus (DOP) for P source (Dyhrman and Palenik, 1997; Dyhrman *et al.*, 2007; Karl, 2014), remodeling cell membranes (Shemi *et al.*, 2016), lowering P quota, bypassing P consuming process in glycolysis reactions (Wurch *et al.*, 2011; Dyhrman *et al.*, 2012), and recycling lipid P (Dyhrman *et al.*, 2012). The realization of these strategies may involve regulation of gene expression at multiple levels, ranging from transcriptional, post-transcriptional, to post-translational. While transcriptional regulation has been extensively studied, post-transcriptional regulation (for example, via microRNA) of phytoplankton responses to P stress has not been explored even though its existence has been reported (O'Rourke *et al.*, 2013). This study was aimed to address this gap of research by investigating genome-wide responses to P stress in a dinoflagellate.

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三、课题中期目标及考核指标完成情况

课题目标	成果名称	成果类型	考核指标				考核方式 (方法)及 评价手段	中期实际完成指标状态
			指标名称	立项时已有指标值/ 状态	中期指标值/ 状态	完成时指标值/ 状态		
本课题聚焦海洋储碳这一海洋碳循环的关键环节，针对项目的第一个关键科学问题——“海洋生态系统固碳和储碳过程的主要调控机制是什么？”，利用多组学技术（宏转录组学、宏蛋白组学等）以及传统生态学方法、原位培养实验等，研究典型海洋生态系统中浮游生物关键功能类群对颗粒碳输出通量的影响，解析颗粒有机碳代谢的关键分子机制；揭示微生物关键功能类群在溶解有机碳代谢中的作用，阐释微生物群落组成及有机碳代谢通路之间的关联。通过揭示海洋生态系统储碳的多时空调控机制，为海洋生态系统碳循环及其对气候的调节作用研究提供科学依据。	1: 海洋生态系统储碳过程的调控机制	<input checked="" type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input type="checkbox"/> 新方法 <input type="checkbox"/> 关键部件 <input checked="" type="checkbox"/> 数据库 <input type="checkbox"/> 软件 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 临床指南/规范 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 标准 <input checked="" type="checkbox"/> 论文 <input type="checkbox"/> 发明专利 <input type="checkbox"/> 其他____	指标 1.1 海洋生态系统颗粒有机碳代谢的关键分子机制 指标 1.2 关键微生物功能类群在溶解有机碳代谢中的调控作用	对全水柱颗粒有机碳代谢的途径及关键分子机制认识不清； 对微生物功能群与有机代谢通路之间的关联不够明确。	揭示浮游生物关键功能类群在全水柱的分布特征及其参与有机碳代谢的机制； 发表论文 5-8 篇	提升对浮游生物关键功能群在颗粒有机碳输出通量调控作用中的认识； 解析溶解有机碳代谢与关键微生物群落结构及代谢通路间的关联； 发表论文 15-20 篇	论文； DNA 条形码数据库和多组学数据库	揭示了南海东北部夏季浮游生物群落组成及昼夜分布特征；获得了不同特征水层浮游生物功能群组学数据，初步解析了碳产生、转运、分泌、释放等代谢途径；阐释了浮游生物群落结构对 POC 组成、分布和输出通量的影响。 构建基于原位样品的颗粒有机碳降解菌群 12 个； 发表论文 11 篇，其中第一标注 8 篇，第二标注 2 篇，其他标注 1 篇；SCI 论文 9 篇。
科技报告考核指标	序号	报告类型	数量	提交时间		公开类别及时限	是否按计划提交科技报告	
	1	年度进展报告	1	2017 年 12 月		延期 3 年公开	是	
	2	中期情况报告	1	2018 年 7 月		延期 3 年公开	是	
其他目标与考核指标完成情况								

四、课题中期实现经济社会效益情况

获得企业标准数		获得行业标准数	
获得国家标准数		获得国际标准数	
申请发明专利项数		获得授权发明专利项数	
其中国外		其中国外	
申请其他各类专利项数		获得授权其他各类专利项数	
其中国外		其中国外	
毕业研究生数	8	发表科技论文数	11
其中博士生	3	其中 SCI、EI 收录数	9
取得软件著作权数		出版专著数	
取得的新理论、新原理数		取得的新技术、新工艺、新方法数	
取得的新产品、新品种、新装置 数		示范、推广面积数（亩）	
获得新药（医疗器械）证书数、临床批件数		获得临床指南、规范数	
新建生产线数		新建示范工程数	
培训农民数		培训技术人员数	
获得国家级科技奖励数		获得省部级科技奖励数	
成果转化数（项）		成果创产值(万)	成果创税收(万)
成果转化收入(万)		成果创利润(万)	成果创出口额(万)