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Spatiotemporal Dynamic of *Ostreococcus lucimarinus* in IMTA System at Enclosed Sea (Hangzhou Bay) East China Sea Using Environmental DNA (eDNA)

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Abstract: Integrated Multi-Trophic Aquaculture (IMTA) is growing fast in China, in order for cultivation with this system to continue. Through eDNA approach in able to detect *Ostreococcus lucimarinus* which include picoeukaryotic in IMTA system at enclosed sea (Hangzhou Bay). Information about this species and their ecological placement in the IMTA system is still very limited. eDNA is an ecological approach that can detect supply down to the species level in monitoring aquatic ecology in the IMTA system. The purpose of this study was to determine the taxonomy and guarantees of *Ostreococcus lucimarinus* and the role of this species in the IMTA system descriptively. Through high throughput sequencing, the taxonomic results of *Ostreococcus lucimarinus* and confinement of this picoekaryotic species were highest in winter with a total of 599,632 ind. Based on the sampling location, the highest abundance were in aquaculture areas of 337,165 ind. The approach using eDNA has proven to be capable of detecting up to the species level as well as spatiotemporal abundance dynamics of *Ostreococcus lucimarinus*.

Keywords: Abundance, eDNA, IMTA, Ostreococcus lucimarinus, Taxonomy

1. Introduction

Aquaculture with emphasis on the concept of sustainable fisheries and a more environmentally friendly approach is currently being developed in China for many decades and rapidly growing, because China responsible for more than 60% of global fisheries farm production [1], [2], [3]. This cultivation is known as IMTA (Integrated Multi-trophic Aquaculture) which has many advantages that are integrated between ecology, economy and society [4], [5]. The term IMTA refers to the integrity of cultivation using various species at each tropic level [6]. The dominant aquaculture industry in China, such as net cages, ropes, other structures in water columns that have impacted aquaculture waste on the surrounding environment [7]. IMTA activities in coastal areas must receive special attention because of the impact of offshore transfer of cultivation waste and transfer of waste from the mainland to the cultivation area [4]. Monitoring, management and evaluation of both organic and inorganic waste recycling can be carried out using several methods, one of which is using the eDNA approach [8], [9], [10], [11].

eDNA is defined genetic material from a wide range of organisms obtained directly from environmental samples (soil, sediment, water, ice) [12], [13]. eDNA provide key information from using high-throughput sequencing, it can be effective and sensitive assay for detecting single or multiple species (biodiversity), monitoring, exploiting, management, and conservation in wide range of ecosystem, include marine ecosystem [14], [15], [16].One of the eDNA studies in the IMTA area is the role of algae in controlling nutrient content in waters and their potential in the fields of biotechnology, food industries, biofuels, and reducing carbon emissions [17], [18]. Marine algae range in size from picoplanktonic cells to macroalgal kelps [19].

Ostreococcus is a marine algae which is one of the genera of Mamiellales which has a major role as primary production in the food chain in marine ecosystems [20], [21], [22]. *Ostreococcus* is a photosynthetic picophytoplankton and is included in the smallest group of eukaryotes because their size is less than 2µm [23], [24]. The identified algae groups, *Ostreococcus lucimarinus* is the most dominant species of Eukaryota found in the IMTA area. Further

information regarding the abundance of this species is still very limited and it is a current status that can be further investigated regarding the role of specific species in environmental change in the IMTA area.

The purpose of this research is to know the taxonomy, the abundance of *Ostreococcus lucimarinus* based on different sampling points and seasons, and to describe their role in the marine environment.

2. Material and Methods

2.1. Materials

Water sampling is carried out in the enclosed sea, Hangzhou Bay, East China Sea in January (Winter), April (Spring), July (Summer), and October (Autumn) 2019. The enclosed sea located in Hangzhou Bay, Jinshan City Beach (121°34‘73.57‖ E, 30°70‘86‖ N).

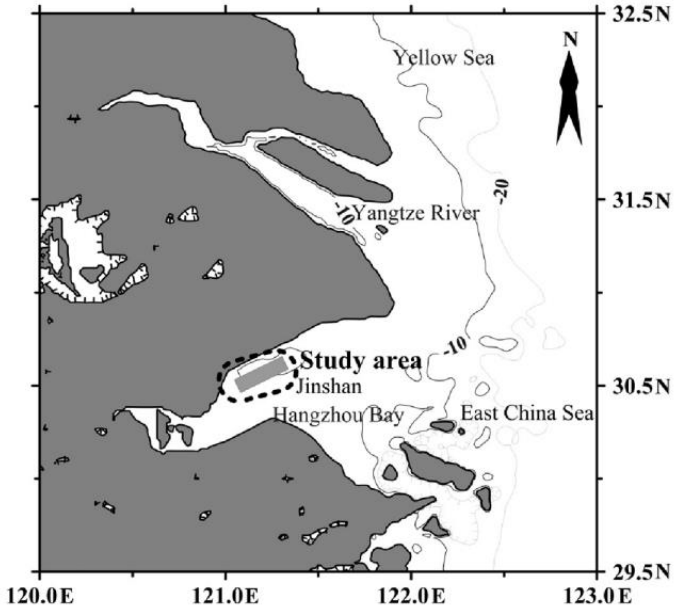

Figure 1. Sampling location in the enclosed sea, Hangzhou Bay (red circle) (25).

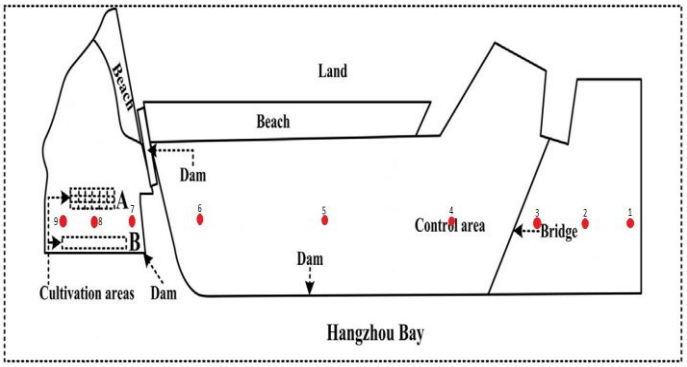

Figure 2. Location of eDNA sampling points, where a) aquaculture area (1,2,3), b) control area (4,5,6), c) algae cultivation (7,8,9) (25)

Water samples were taken at three (3) different sampling points, the right (sites 1, 2, 3) are locations representing aquaculture areas (black sea bream, sea bass, and blue crab), the middle area (sites 4, 5 , 6) is the control area, and to the left (site 7, 8, 9) is the algae (*Myriophyllum spicatum*) cultivation area. The depth of each pond is between 6-7 meters.

The materials used in this study were membrane polycarbonate filters (0.22 µm pore size), gloves, masks, and 70% ethanol. The tools used in this study were sterile 5L polyethylene bottles, Kemmerer water sampler, 1 set of eDNA filtering (filter tunnel and electric pump), and eDNA vial.

2.2. Methods

2.2.1. eDNA sample collection and filtering

Water samples were collected using a Kemmerer water sampler (depth 2.5 m) and placed in sterile 5L polyethylene bottles (for eDNA analysis). Then eDNA water was immediately filtered in the laboratory and stored at -80°C.

Water from the polyethylene bottles was poured slowly into the filter funnel with an engaged electric pump. During filtering, the wearing of gloves and disinfecting of forceps using ethanol for at least 1 min was ensured, followed by rinsing thoroughly in distilled water. Membrane *polycarbonate filters* (0.22 µm pore size) (Millipore Corporation, Billerica, MA, USA) were folded in half four times, this membrane is proven to be able to capture the most DNA compared to other membrane sizes (26). After the filter funnel was removed from the rubber stopper, the vials were stored in a cool place away from light exposure [27], [28]. Samples (eDNA membrane and soil) were stored at -80°C and sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for DNA extraction.

2.2.2 DNA extraction, library contruction, and sequencing

The extracted DNA samples were dissolved in 30.0 L of Tris-EDTA buffer and quantified. Dilute the experimental DNA solution in TE to a final volume of 1.0 mL in disposable cuvettes or test tubes. Add 1.0 mL of the aqueous working solution of the Quant-iT™ PicoGreen® reagent in the presence of 500 ng/mL calf thymus DNA. Purity of DNA extraction was determined with NanoDrop2000. The extracted DNA was then confirmed by 1% agarose gel electrophoresis, then library preparation was performed by Shanghai Majorbio Bio-Pharm Technology Co. The primers that used for detection *Ostreococcus lucimarinus* is V4 region of the 18S rRNA [29].

2.3. Data Analysis

Abundance of *Ostreococcus lucimarinus* using high throughput sequencing data were assembled using Illumina MiSeq (Illumina, San Diego, CA, USA) and interpretation itu using PAST 4.03 software (32-bit and 64-bit PCs, Oyvind Hammer).

3. Results and Discussion

3.1. Taxonomy of Ostreococcus lucimarinus

The results of reading the eDNA sequence, *Ostreococcus lucimarinus* was identified by matching taxonomy based on www.algabase.org. The taxonomy consists of 9 classifications. Part of the calcification of this species in detail can be seen in Table 1.

Table 1. Taxonomy classification of *Ostreococcus lucimarinus*

Taxonomy of *Ostreococcus lucimarinus*	
Empire	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Chlorophyta infrakingdom
Phylum	Chlorophyta
Class	Mamiellophyceae
Order	Mamiellales
Family	Bathycoccaceae
Genus	*Ostreococcus*

The contribution of knowledge about the taxonomy of marine algae is very much needed in relation to a thorough understanding of their role in environmental changes in the sea [30], [21]. Knowing the taxonomy of *Ostreococcus lucimarinus* can provide relevant information regarding its role in marine waters, especially the IMTA area.

3.2 Spatiotemporal abundance of Ostreococcus lucimarinus

Ostreococcus lucimarinus was found in abundance in all seasons and research areas in the enclosed sea, because this species is cosmopolitan and can survive in different temperature [31], [21]. However, the highest abundance occurred in winter (total 599,632 ind.) followed by spring (total 288,593 ind.). The discovery of species abundance in this study is also the same as the research conducted by research by [21], *Ostreococcus lucimarinus* is one of the species found in abundance in winter. In winter, the most abundance of this species is found in IMTA areas of 337,165 ind. (Figure 3). In this study, the abundance of *Ostreococcus lucimarinus* in winter and also in aquaculture area is due to the abundance of micronutrients and macronutrients from the excretions of fish and crab farming in aquaculture area at enclosed sea. The abundance of eukaryotic picophytoplankton is due to the availability of essential micronutrients, this supports its role as primary productivity and carbon transfer at other trophic levels [32].

Winter in the East China Sea and The Yellow blows winds from the north intensively which contributes to causing heat loss to the atmosphere, thus impacting the mixed layer which affects physical and biogeochemical processes [33], [34].

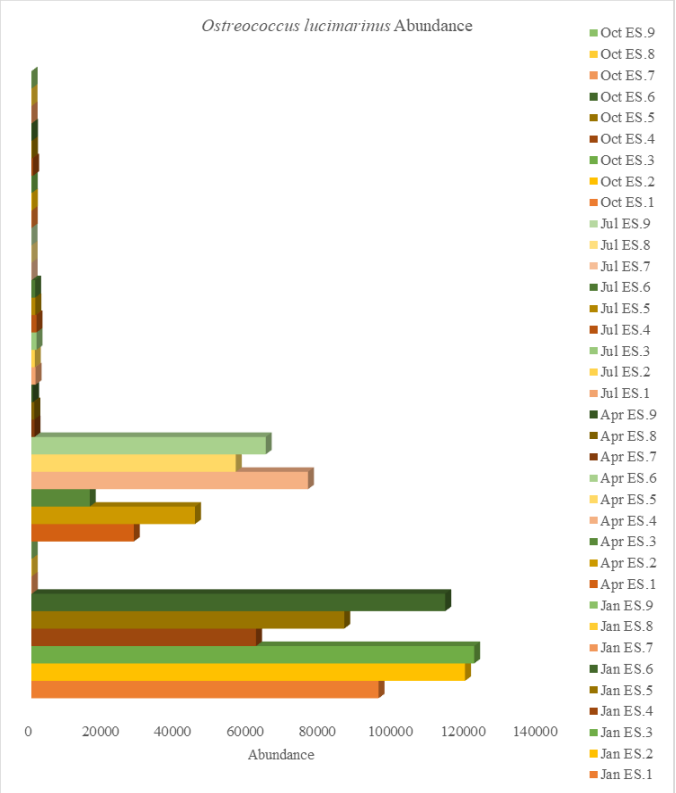

Figure 3. Dynamics of *Ostreococcus lucimarinus* abundance based on different sampling points and seasons.

Iron (Fe) is one of the important micronutrients which is a limiting factor for the growth of phytoplankton at sea level which affects the ability to photosynthesize, fix and transfer nitrogen, carbon fixation, and the dynamics of phytoplankton. [35], [36], [37]. Besides that, there is also manganese, molybdenum, and nickel [38], cobalt [39], dan copper [40] those become micronutrient for phytoplankton. Based on research by [24] by culturing *Ostreococcus lucimarinus*, this species was able to reduce the Fe content by up to 68%. Waste products from pelagic vertebrates can thus contain high concentrations of Fe released through reduction of Fe from microbial activity [41], [42], because more than 99% of iron is bound to complex organic ligands [43]. In winter, iron bound to organic ligands will be in a stable condition and precipitate in sediments (large iron deposits) and release dissolved Fe in the water column which will be utilized for phytoplankton productivity [44], [45], [46], [43]. Waters with low temperatures can enrich the dissolved Fe content on the surface of the water through remineralization of Fe [47], [48].

4. Conclusion

Taxonomy of *Ostreococcus lucimarinus* are consists of 9 classification that the abundance of this species is highest in winter and at aquaculture areas. Based on the study description, *Ostreococcus lucimarinus* is able to bioindicator and absorb iron for their growth.

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References

[1] Qi, Z., Shi, R., Yu, Z., Han, T., Li, C., Xu, S., Xu, S., Liang, Q., Yu, W., Lin, H., Huang, H. Nutrient Release From Fish Cage Aquaculture And Mitigation Strategies In Daya Bay, Southern China. *Marine Pollution Bulletin*, Vol. 146, 2019, pp 399-407.https://doi.org/10.1016/j.marpolbul.2019.06.079

[2] Wang, Q., Li, Z., Gui, J.F., Liu, J., Ya, S., Yuan, J. De Silva, S.S. Paradigm Changes In Freshwater Aquaculture Practices In China: Moving Towards Achieving Environmental Integrity And Sustainability. *Ambio*, Vol. 47, 2018, pp 410–426. https://doi.org/10.1007/s13280-017-0985-8.

[3] Zhou, X., Zhao, X., Zhang, S., Lin, J. Marine Ranching Construction and Management in East China Sea: Programs for Sustainable Fishery and Aquaculture. *Water*, Vol. 11, No. 6, 2019, pp 1237. https://doi.org/10.3390/w11061237.

[4] Buck, B.H., Troel, M.F., Krause, G., Angel, D.L., Grote, B., Chopin, T. State of the Art and Challenges for Offshore Integrated Multi-Trophic Aquaculture (IMTA). *Frontiers in Marine Science*, Vol. 5, No. 165, 2018. https://doi.org/10.3389/fmars.2018.00165.

[5] Khanjani, M.H., Zahedi, S., Mohammadi, A. Integrated Multitrophic Aquaculture (IMTA) As An Environmentally Friendly System For Sustainable Aquaculture: Functionality, Species, And Application Of Biofloc Technology (BFT). *Environmental Science and Pollution Research*, Vol. 29, No. 45, 2022, pp 67513-67531. https://doi.org/10.1007/s11356-022-22371-8

[6] Kleitou, P., Kletou, D., David, J. Is Europe Ready For Integrated Multi-Trophic Aquaculture? A Survey On The Perspectives Of European Farmers And Scientists With IMTA Experience. *Aquaculture*, Vol. 490, 2018, pp 136-148. https://doi.org/10.1016/j.aquaculture.2018.02.035

[7] Ying, C., Chang, M. J., Hu, C. H., Chang, Y. T., Chao, W. L., Yeh, S. L., Chang, S. J., Hsu, J. T. (2018). The Effects Of Marine Farm-Scale Sequentially Integrated Multi-Trophic Aquaculture Systems On Microbial Community Composition, Prevalence Of Sulfonamide Resistant Bacteria And Sulfonamide Resistance Gene Sul1. *Science of the Total Environment*, 643, 2018, pp 681–691. https://doi.org/10.1016/j.scitotenv.2018.06.204.

[8] Gold, Z., Sprague, J., Kushner, D.J., Marin, E.Z., Barber, P.H. eDNA Metabarcoding as a Biomonitoring Tool For Marine Protected Areas. *PLoS ONE*, Vol. 16, No. 2, 2021, pp e0238557. https://doi.org/10.1371/journal.pone.0238557.

[9] Hinz, S., Coston-Guarini, J.. Marnane, M., Guarini, J.M. Evaluating eDNA for Use within Marine Environmental Impact Assessments. *Journal of Marine Science and Engineering*, Vol. 10, No. 3, 2022, pp 375. https://doi.org/10.3390/jmse10030375.

[10] Jensen, M.R., Sigsgaard, E.E., Ávila, M.P., Agersnap, S., Brenner-Larsen, W., Sengupta, M.E., Xing, Y., Krag, M.A., Knudsen, S.W., Carl, H., Møller, P.R., Thomsen, P.F. Short-term Temporal Variation Of Coastal Marine eDNA. *Environmental DNA*, Vol. 4, No. 4, 2022, pp 747-762. https://doi.org/10.1002/edn3.285.

[11] Jumah, Y. U. A Review On Waste Absorption Efficiency Of Different Extractive Integrated Multi-Trophic Aquaculture (IMTA) Species: Implications In Coastal And Offshore Aquaculture Waste Management. *GSC Biological Pharmaceutical Sciences*, Vol. 11, No. 2, 2020, pp 257–264. https://doi.org/10.30574/gscbps.2020.11.2.0141.

[12] Pedersen, M. W., Overballe-Petersen, S., Ermini, L., Der Sarkissian, C., Haile, J., Hellstrom, M., Spens, J., Thomsen, P. F., Bohmann, K., Cappellini, E., Schnell, I. B., Wales, N. A., Carøe, C., Campos, P. F., Schmidt, A. M. Z., Gilbert, M. T. P., Hansen, A. J., Orlando, L., & Willerslev, E. (2015). Ancient And Modern Environmental DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol. 370, No. 1660, 2015, pp 1-11. https://doi.org/10.1098/rstb.2013.0383.

[13] Thomsen, P.F., Willerslev, E. Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183, 2015, pp. 4–18. https://doi.org/10.1016/j.biocon.2014.11.019.

[14] Cristescu, M.E., Hebert, P.D.N. Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation. *Annual Review of Ecology, Evolution, and Systematics*, Vol. 49, 2018, pp 209-230. https://doi.org/10.1146/annurev-ecolsys-110617-062306.

[15] Liu, Q., Zhang, Y., Wu, H., Liu, F., Peng, W., Zhang, X., Chang, F., Xie, P., Zhang, H. A Review and Perspective of eDNA Application to Eutrophication and HAB Control in Freshwater and Marine Ecosystems. *Microorganisms*, Vol. 8, No. 3, 2020, pp 417. https://doi.org/10.3390/microorganisms8030417.

[16] Rahayu, D.M., He, Peimin. Analysis of Species Diversity using eDNA Technology in Different IMTA System at Enclosed Sea (Hangzhou Bay) and Island Reef Area East China Sea. Disertation Thesis [Unpublished], 2021.

[17] Gao, K., Beardall, J. Using Macroalgae to Address UN Sustainable Development Goals Through CO2 Remediation And Improvement Of The Aquaculture Environment. *Applied Phycology*, Vol. 3, No. 1, 2022, pp 360-367. https://doi.org/10.1080/26388081.2022.2025617.

[18] Rey, F., Cartaxana, P., Melo, T., Calado, R., Pereira, R., Abreu, H., Domingues, P., Cruz, Sónia, M., Domingues, R. Domesticated Populations of Codium tomentosum Display Lipid Extracts with Lower Seasonal Shifts than Conspecifics from the Wild—Relevance for Biotechnological Applications of this Green Seaweed. *Marine Drugs*, Vol. 18, No. 4, 2020, pp 188; https://doi.org/10.3390/md18040188.

[19] Park, B. S., Li, Z. Taxonomy and Ecology of Marine Algae. *Journal of Marine Science and Engineering*, Vol. 10, No.), 2022, pp 105. https://doi.org/10.3390/jmse10010105.

[20] Blanc-Mathieu, R., Krasovec, M., Hebrard, M., Yau, S., Desgranges, E., Martin, J., Schackwitz, W., Kuo, A., Salin, G., Donnadieu, C., Desdevises, Y., Sanchez-Ferandin, S., Moreau, H., Rivals, E., Grigoriev, I. V., Grimsley, N., Eyre-Walker, A., Piganeau, G. Population Genomics Of Picophytoplankton Unveils Novel Chromosome Hypervariability. Science Advances, Vol. 3, No. 7, 2017, pp 1-10. https://doi.org/ 10.1126/sciadv.1700239.

[21] Leconte, J., Benites, L.S., Vannier, T., Wincker, P., Piganeau, G., Jaillon, O. Genome Resolved Biogeography of Mamiellales. *Genes*, Vol. 11, No. 1, 2020, pp 66. https://doi.org/10.3390/genes11010066.

[22] Limardo, A.J., Sudek, S., Choi, C.J., Poirier, C., Rii, Y.M., Blum, M., Roth, R., Goodenough, U., Church, M.J., Worden, A.Z. Quantitative Biogeography of Picoprasinophytes Establishes Ecotype Distributions And Significant Contributions to Marine Phytoplankton. Environmental *Microbiology*, Vol. 19, No. 8, 2017, pp 3219-3234. https://doi.org/10.1111/1462-2920.13812.

[23] Benites, L.F., Poulton, N., Labadie, K., Sieracki, M.E., Grimsley, N., Piganeau, G. Single Cell Ecogenomics Reveals Mating Types Of Individual Cells And Ssdna Viral Infections In The Smallest Photosynthetic Eukaryotes. *Philosophical Transactions of The Royal Society B*, Vol. 374, No. 1786, 2019, pp 1-12. https://doi.org/10.1098/rstb.2019.0089.

[24] Sofen, L.E., Antipova, O.A., Ellwood, M.J., Gilbert, N.E., LeCleir, G.R., Lohan, M.C., Mahaffey, C., Mann, E.L., Ohnemus, D.C., Wilhelm, S.W., Twining, B.S. Trace Metal Contents Of Autotrophic Flagellates From Contrasting Open-Ocean Ecosystems. *Limnology and Oceanography Letters*, Vol. 7, No. 4, 2022, pp 354-362. https://doi.org/10.1002/lol2.10258.

[25] Liu, Q., Sun, B., Huo, Y., Liu, M., Shi, J., Jiang, T., Zhang, Q., Tang, C., Bi, H., He, P. Nutrient Bioextraction And Microalgae Growth Inhibition Using Submerged Macrophyte *Myriophyllum Spicatum* In A Low Salinity Area Of East China Sea. *Marine Pollution Bulletin*, Vol. 127, 2018, pp 67-72. https://doi.org/10.1016/j.marpolbul.2017.11.031.

[26] Majaneva, M., Diserud, O.H., Eagle, S.H.C., Boström, E., Hajibabaei, M., Ekrem, T. Environmental DNA Filtration Techniques Affect Recovered Biodiversity. *Scientific Reports*, Vol. 8, No. 4682, 2018. https://doi.org/10.1038/s41598-018-23052-8

[27] Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M.A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., Taberlet, P. Critical Considerations For The Application Of Environmental DNA Methods To Detect Aquatic Species. *Methods in Ecology and Evolution*, Vol. 7, No. 1, 2016, pp 1299–1307. https://doi.org/10.1111/2041-210X.12595.

[28] Laramie, M. B., Pilliod, D. S., Goldberg, C. S. Characterizing The Distribution Of An Endangered Salmonid Using Environmental DNA Analysis. *Biological Conservation*, Vol. 183, 2015, pp 29–37. https://doi.org/10.1016/j.biocon.2014.11.025.

[29] Tragin, M., Vaulot, D. Novel diversity within marine Mamiellophyceae (Chlorophyta) Unveiled by Metabarcoding. *Scientific Reports*, Vol. 9, No. 5190, 2019, pp 1-14. https://doi.org/10.1038/s41598-019-41680-6.

[30] Neto, A.I. Contribution To The Taxonomy And Ecology of The Azorean Benthic Marine Algae. *Biological Journal of the Linnean Society*, Vol. 46, No. 1-2, 1992, pp 163–176. https://doi.org/10.1111/j.1095-8312.1992.tb00858.x.

[31] Castillo, Y.M., Forn, I., Yau, S., Morán, X.A.G., Alonso-Sáez, L., Arandia-Gorostidi, N., Vaqué, D., Sebastián, M. Seasonal Dynamics Of Natural Ostreococcus Viral Infection At The Single Cell

Level Using VirusFISH. *Environmental Microbiology*, Vol. 23, No. 6, 2021, pp 3009-3019. https://doi.org/10.1111/1462-2920.15504.

[32] Botebol, H., Lelandais, G., Six, C., Lesuisse, E., Meng, A., Bittner, L., Lecrom, S., Sutak, R., Lozano, J.C., Schatt, P., Vergé, V., Blain, S., Bouget, F.Y. Acclimation Of A Low Iron Adapted Ostreococcus Strain To Iron Limitation Through Cell Biomass Lowering. *Scientific Reports*, 7: 327, 2017, pp. http://10.1038/s41598-017-00216-6.

[33] Kim, Y. S., Jang, C. J., Yeh, S.W. Recent Surface Cooling In The Yellow And East China Seas And The Associated North Pacific Climate Regime Shift. Continental Shelf Research, Vol. 156, 2018, pp 43-54. https://doi.org/10.1016/j.csr.2018.01.009.

[34] Yu, J., Gan, B., Jing, Z., Wu, L. Winter Extreme Mixed Layer Depth South of the Kuroshio Extension. Journal of Climate, Vol. 33, No. 24, 2020, pp 10419–10436. https://doi.org/10.1175/JCLI-D-20-0119.1.

[35] Chinni, V., Singh, S. K. Dissolved Iron Cycling In The Arabian Sea And Sub-Tropical Gyre Region Of The Indian Ocean. *Geochimica et Cosmochimica Acta*,Vol. 317, No. 15, 2022, pp 325-348. https://doi.org/10.1016/j.gca.2021.10.026.

[36] Hutchins, D.A., Boyd, P.W. Marine Phytoplankton And The Changing Ocean Iron Cycle. *Nature Climate Change*, Vol. 6, 2016, pp 1072–1079. https://doi.org/10.1038/nclimate3147.

[37] Nickelsen, L., Keller, D.P., Oschlies, A. A Dynamic Marine Iron Cycle Module Coupled To The University Of Victoria Earth System Model: The Kiel Marine Biogeochemical Model 2 For Uvic 2.9. *Geoscientific Model Development*, Vol. 8, 2015, pp 1357–1381.https://doi.org/10.5194/gmd-8-1357-2015.

[38] Ratnarajah, L., Nicol, S., Bowie, A.R. Pelagic Iron Recycling in the Southern Ocean: Exploring the Contribution of Marine Animals. *Frontiers Marine Science*, Vol. 5, 2018, pp 1-9. https://doi.org/10.3389/fmars.2018.00109.

[39] Song, L., Qin, J.G., Su, S., Xu, J., Clarke, S., Shan, Y. Micronutrient Requirements for Growth and Hydrocarbon Production in the Oil Producing Green Alga Botryococcus braunii (Chlorophyta). *PLoS ONE* 7, No.7, 2012, e41459. https://doi.org/10.1371/journal.pone.0041459.

[40] Hu, X., Wei, X., Ling, J., Chen, J. Cobalt: An Essential Micronutrient for Plant Growth?. *Frontiers in Plant Science*, Vol. 12, 2021. https://doi.org/10.3389/fpls.2021.768523.

[41] Zhang, X., Li, B., Xu, H., Wells, M., Tefsen, B., Qin, B. Effect Of Micronutrients On Algae In Different Regions Of Taihu, A Large, Spatially Diverse, Hypereutrophic Lake. *Water Research*, Vol.151, 2019, pp 500-514. https://doi.org/10.1016/j.watres.2018.12.023.

[42] Tang, D., Ma, J., Shi, X., Lechte, M., Zhou, X. The Formation Of Marine Red Beds And Iron Cycling on the Mesoproterozoic North China Platform. *American Mineralogist*, Vol. 105, No. 9, 2020, pp 1412–1423. https://doi.org/10.2138/am-2020-7406.

[43] Whitby, H., Planquette, H., Cassar, N., Bucciarelli, E., Osburn, C.L., Janssen, D.J., Cullen, J.T., González, A.G., Völker, C., Sarthou, G. A Call For Refning The Role Of Humiclike Substances In The Oceanic Iron Cycle. *Scientific Reports*, Vol. 10, No. 1644, 2020. | https://doi.org/10.1038/s41598-020-62266-7.

[44] Böning, P., Schnetger, B., Belz, L., Ferdelman, T., Brumsack, H.J., Pahnke, K. Sedimentary Iron Cycling In The Benguela Upwelling System Off Namibia. Earth and Planetary Science Letters, Vol. 538, 2020, pp 116212. https://doi.org/10.1016/j.epsl.2020.116212.

[45] Ellwood, M.J., Strzepek, R.F., Strutton, P.G., Trull, T.W., Fourquez, M., Boyd, P.W. Distinct Iron Cycling In A Southern Ocean Eddy. *Nature Communications*, Vol. 11, No. 825, 2020, pp 1-8. https://doi.org/10.1038/s41467-020-14464-0.

[46] Hassler, Christel A.E, Damien, Cabanes A., Sonia Blanco-Ameijeiras A., Sylvia G., Sander B.D., Benner, Ronald. Importance Of Refractory Ligands And Their Photodegradation For Iron Oceanic Inventories And Cycling. *Marine and Freshwater Research*, Vol. 71, No. 3, 2020, pp 311-320. https://doi.org/10.1071/MF19213.

[47] Achterberg, E.P., Steigenberger, S., Marsay, C. M., LeMoigne, F.A.C., Painter, S.C., Baker, A.R., Connelly, D.P., Moore, C.M., Tagliabue, A., Tanhua, T. Iron Biogeochemistry in the High Latitude North Atlantic Ocean. Scientific Reports, Vol. 8, No. 1283, 2018, pp 1-15. http://doi.org/10.1038/s41598-018-19472-1.

[48] Kanna, N., Sibano, Y., Toyota, T., Nishioka, J. Winter Iron Supply Processes Fueling Spring Phytoplankton Growth In A Sub-Polar Marginal Sea, The Sea Of Okhotsk: Importance Of Sea Ice And The East Sakhalin Current. *Marine Chemistry*, Vol. 206, 2018, pp 109-120. https://doi.org/10.1016/j.marchem.2018.08.006.