Journal of
Marine Science and Engineering

MDPI

Editorial

Distribution and Metabolic Activities of Marine Microbes in Response to Natural and Anthropogenic Stressors

Gabriella Caruso [1,*] and Kai Ziervogel [2,*]

[1] Institute of Polar Sciences, National Research Council (CNR-ISP), Spianata S. Raineri 86, 98122 Messina, Italy
[2] Ocean Process Analysis Laboratory, University of New Hampshire, Morse Hall Rm 136A, Durham, NH 03824, USA
* Correspondence: gabriella.caruso@cnr.it (G.C.); kai.ziervogel@unh.edu (K.Z.); Tel.: +39-090-6015423 (G.C.); +1-603-862-5478 (K.Z.)

check for updates

Citation: Caruso, G.; Ziervogel, K. Distribution and Metabolic Activities of Marine Microbes in Response to Natural and Anthropogenic Stressors. *J. Mar. Sci. Eng.* **2022**, *10*, 1119. https://doi.org/10.3390/jmse10081119

Received: 2 August 2022
Accepted: 9 August 2022
Published: 14 August 2022

As a consequence of climate change and increased human pressure, aquatic ecosystems are experiencing natural and anthropogenic stressors and events such as temperature warming, acidification, biodiversity loss, and degraded water quality [1]. To understand the response of marine ecosystems to environmental and anthropogenic pressures, integration of long-term and large-scale monitoring of marine communities with mapping of habitats and ecosystem services was previously recommended [2].

In a global climate change scenario, the IPCC (Intergovernmental Panel on Climate Change) in its most recent Assessment Report (AR6 [3] has confirmed a projection of amplification by the second half of the 21st century of sea surface temperature (SST) variability, with potential dramatic changes on the marine food webs and fishing resources. Moreover, ocean acidification caused by the burning of fossil fuels and the increased absorption of carbon dioxide into the ocean from the atmosphere is proceeding at very high rates according to NOAA estimates [4], by the end of this century ocean's surface could reach a pH of about 7.8 compared to 8.1 recorded in 2020. Ocean acidification may result in biodiversity losses, death of calcifying organisms, and impairment in marine-related activities. On the other hand, massive development of algae and blooms of harmful species due to excessive nutrient loading from anthropogenic activities (i.e., about 64 Tg N year^{-1} and 9 Tg P year^{-1}, respectively [5]) have been recorded in several marine water bodies worldwide (see [6] for reference), with consequent threats for water security and ecosystem services and increased numbers of dead zones, where the life of marine organisms cannot be supported [7].

In addition, marine systems suffer from chronic and point source contaminations such as plastic contamination and oil spills, just to name two of the major sources of chemical contaminants in the aquatic environments. Impacts of marine pollution are heavy, especially in semi-enclosed areas showing limited exchanges with the ocean, such as the Mediterranean Sea and the Baltic Sea [8].

Recognizing the gravity of the decline in ocean health, the United Nations has also proclaimed the Decade of Ocean Science for Sustainable Development (2021–2030) to put in action efforts to reverse this trend and address stakeholders toward a common framework for improving environmental health and favouring sustainable development.

Knowledge about ecosystem responses to environmental forcing factors such as the ones mentioned above is the key to developing mitigation strategies that address climate impacts on inland waters and oceans [9].

An updated state of currently observed changes and trends in marine conditions is periodically provided by the Ocean State Report of the Copernicus Marine Service [10]; nevertheless, the impacts of multiple stressors and cumulative effects upon marine and coastal organisms, as well as habitats and ecosystems, are not fully predictable.

What are the information and the relative contribution provided by studies about microbial dynamics (in terms of structure and function) in this context? Microbial communities-including bacteria, archaea, microalgae, and protists-are the major drivers of biogeochemical cycles and food chain interactions in aquatic environments [11,12]. Their role at the base of the food chain makes them ideal sentinels for understanding the effects of environmental stressors on aquatic ecosystems. Microbes respond on relatively short timescales to environmental change given their short generation times (relative to higher organisms) and wide "metabolic plasticity" [13], that is the adaptive plasticity in metabolism shown by microorganisms both at single species and community level. Spatial scales of microbial responses to perturbations range from system-wide harmful algal blooms to responses at the microscale in which microbes operate. For example, microbial interactions with pollutants from spilled oil and microplastics involve complex biochemical processes and metabolic pathways on the molecular and single-cell scale.

This Special Issue comprises eight studies on microbial distribution patterns, community structure, metabolic pathways, and activities as well as interactions between microbial community components in response to natural and anthropogenic stressors in temperate and polar environments or laboratory trials simulating in-situ conditions.

Regarding phytoplankton responses to stressors including nutrients, light, and salinity, Prairie et al. [14] studied the effects of nutrient stress on aspects of diatom metabolism and cell aggregation during a controlled culture experiment. Marine diatoms often form aggregates (also known as "marine snow") during the latter part of their growth cycle when nutrients are depleted. As a response to nutrient stress, diatoms produce copious amounts of exopolymeric substances (EPS) as metabolic waste. A major form of EPS is carbohydrate-rich transparent exopolymer particles (TEP), which often form the glue for marine snow. Prairie et al. [14] determined relative levels of TEP during phytoplankton growth phases and their role in aggregate formation and settling ex-situ. Cultures of the diatom *Thalassiosira weissflogii* were grown to four different growth phases and incubated in rotating cylindrical tanks to form marine snow. Compared to the first growth phase, later phytoplankton growth phases were found to have higher relative TEP concentrations and aggregates with lower excess densities and longer delayed settling times. These findings suggest that marine snow that forms during the later stages of phytoplankton blooms in surface waters may be less dense and sink slower compared to those formed during initial growth phases with consequences for the efficiency of carbon export and cycling during algal blooms.

Li et al. [15] present field observations on benthic-pelagic interactions and nutrient cycling with consequences for the metabolism of harmful phytoplankton in Taiwan Strait. Although nutrients as a driving force for harmful algal blooms (red tides) have been intensively studied, the spatial patterns of the phytoplankton community and its metabolic responses to benthic nutrient cycling are poorly constrained. A total of 30 phytoplankton genera were identified in the field survey, including the red tide genera *Prorocentrum* and *Trichodesmium*. Their abundance and relative levels of extracellular alkaline phosphatase by means of enzyme-labelled fluorescence (ELF) were negatively correlated with the ratios of dissolved inorganic N (DIN) and ammonium (NH_4) to soluble reactive P (DIN/SRP, NH_4+/SRP) in bottom waters. Furthermore, the ELF-labelling percentage in *Trichodesmium* was negatively correlated with total P and SRP but positively correlated with TN/TP, DIN/SRP, and NH_4+/SRP in waters overlying the sediment. In contrast to high DIN/SRP in surface waters, lower DIN/SRP in bottom waters was explained by the elevated release of P from the sediment coupled with a weak sequestration of P as evidenced by the distribution of P solubilizing bacteria and P content. Li et al.'s findings indicate that the benthic nutrient regime shapes the structure of the pelagic phytoplankton community.

Hotos [16] conducted culture experiments with a strain of the filamentous non-N-fixing cyanobacterium *Phormidium* sp. isolated from the Messolonghi (W. Greece) saltworks to study the effects of salinity and light regimes on the content of cyanobacterial pigments, including phycocyanin which is used as a potent antioxidant for human health and a natural dye in the food industry. At salinities of 60 and 40 ppt and in high illumination (8000 lux), *Phormidium* growth rates were double those at 20 ppt. At all salinities, the higher illumination resulted in the highest growth rates and shorter cell doubling times compared to lower light levels. Phycocyanin, phycoerythrin, and allophycocyanin production was elevated in the lower illumination and in all salinities compared to higher illumination levels. Chlorophyll-a content was also higher in lower illumination at all salinities compared with total carotenoids that followed the opposite trend. Given the results on growth and pigment dynamics, Hotos [16] suggests that *Phormidium* species are promising candidates for mass culture to effectively harvest phycocyanin for industrial purposes.

Climate change is known to affect directly and indirectly the structure and diversity of microbial communities [17]. In this issue, Caruso et al. [18] studied the microbial abundance and extracellular enzyme activities in the Arctic waters of the Svalbard Archipelago, which is one of the most vulnerable regions to the effects of climate changes. This field survey reports total bacterial abundance and the fraction of actively respiring cells (labelled by cyanotetrazolium chloride, CTC), cultivable heterotrophic bacterial abundance, and extracellular enzymatic activities [leucine aminopeptidase (LAP), beta-glucosidase (GLU), and alkaline phosphatase (AP)] along with abiotic water properties in waters affected by melting glaciers. Chromophoric Dissolved Organic Matter (CDOM) absorption slope values (275–295 nm) varied from 0.0077 to 0.0109 nm^{-1}, and total bacterial cell count and cultivable heterotrophic bacterial abundance were in the order of 10^6 cells mL^{-1} and 10^3 colony forming units mL^{-1}, respectively. Enzymatic rates <1.78, 1.25, and 0.25 nmol $\text{L}^{-1}\text{h}^{-1}$ were recorded for AP, LAP, and GLU, respectively. Inorganic compounds, TSM, and turbidity correlated inversely with temperature; AP was significantly related to CDOM absorption spectra and heterotrophic bacteria (r = 0.59, 0.71, $p < 0.05$); and LAP with Chl-a, Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON) (0.97, 0.780, 0.734, $p < 0.01$), suggesting that fresh material from ice melting stimulated the metabolism of the cultivable fraction of heterotrophic microbial communities.

As one example of microbial response to anthropogenic forcings, Rughöft et al. [19] studied the metabolic responses of marine hydrocarbon-degrading bacteria to oil pollution including chemical dispersants, under controlled laboratory conditions. Cells of *Marinobacter* sp. TT1 were exposed to the dispersant Corexit EC9500A when pre-adapted to either low *n*-hexadecane concentrations (starved culture) or high *n*-hexadecane concentrations (well-fed culture). The growth of previously starved cells was inhibited when exposed to the dispersant, as evidenced by 55% lower cell numbers and 30% lower *n*-hexadecane biodegradation efficiency compared to cells grown on *n*-hexadecane alone. Cultures that were well-fed did not exhibit dispersant-induced inhibition of growth or *n*-hexadecane degradation. In addition, fluorescence microscopy revealed amorphous cell aggregate structures when the starved culture was exposed to dispersants, suggesting that Corexit affected the biofilm formation behavior of starved cells. This study indicates that (previous) substrate limitation, resembling oligotrophic open ocean conditions, can impact the response and hydrocarbon-degrading activities of oil-degrading organisms when exposed to Corexit, and highlight the need for further work to better understand the implications of environmental stressors on oil biodegradation and microbial community dynamics.

In their perspective study, Ziervogel et al. [20] discussed the use of methylumbeliferyl substrate proxies for esterase activities as indicators for microbial oil degradation in the ocean. The authors synthesize previously published results from studies conducted in the aftermath of the 2010 Deepwater Horizon (DwH) oil spill in the northern Gulf of Mexico (nGoM), to test the hypothesis that fluorescence assays of esterases, including lipase activity, are sensitive indicators for microbial oil degradation in the ocean. In agreement with the rates and patterns of enzyme activity in oil-contaminated seawater and sediments in the nGoM, Ziervogel et al. [20] found close correlations between esterase activity measured by means of methylumbeliferyl (MUF) oleate and MUF butyrate hydrolysis, and the concentration of petroleum hydrocarbons in two separate laboratory incubations using surface (<1 m) and deep nGoM waters (>1200 m). Correlations between esterase activities and oil were driven by the presence of chemical dispersants, suggesting a connection to the degree of oil dissolution in the medium. The results of this study clearly show that esterase activities measured with fluorogenic substrate proxies are a good indicator for oil biodegradation in the ocean; however, there are certain factors as discussed in this study that need to be taken into consideration while utilizing this approach.

Moreover, in another study regarding bathing water quality monitoring, CDOM was applied as a tracer of faecal contamination in the Northern Tyrrhenian Sea (Latium, Italy) [21]. DOM present in natural aquatic environments is a heterogeneous mixture of allochthonous and autochthonous materials. In coastal areas vulnerable to sewage waste, its biologically active component (CDOM) is expected to change its composition and distribution in relation to anthropogenic activities, suggesting the possible use of CDOM as a proxy for faecal contamination. The parallel factor analysis (PARAFAC) applied to the excitation–emission matrices (EEMs) of CDOM allowed us to distinguish three main components: C1 ($\lambda_{Ex}/\lambda_{Em}$ = 342 nm/435 nm), C2 ($\lambda_{Ex}/\lambda_{Em}$ = 281–373 nm/460 nm), and C3 ($\lambda_{Ex}/\lambda_{Em}$ = 286 nm/360 nm). C1 and C2 corresponded to humic acids of terrestrial origin, while C3 to tryptophan, whose fluorescence peak was detected close to sewage sites, strongly related to active *Escherichia coli* cells. The comparison between spectral and microbiological methods is suggested as a suitable approach to monitoring bathing water quality for the implementation of coastal observing system capability.

Both natural and anthropic forcings are found to be responsible for variations in the distribution and metabolic activities of the microbial community, inhabiting a coastal marine environment exposed to human pressure and frequently subject to harmful algal blooms, such as the Syracuse harbour [22]. This study reports on seasonal surveys of the phytoplankton communities (diatoms, dinoflagellates, and other *taxa*), the potential microbial enzymatic activity rates (leucine aminopeptidase, beta-glucosidase, and alkaline phosphatase) and heterotrophic culturable bacterial abundance together with the thermohaline structure and trophic status in terms of nutrient concentrations, phytoplankton biomass (as Chlorophyll-a), and total suspended and particulate organic matter. A seasonal pattern of microbial abundances was observed, with the highest concentrations of phytoplankton in spring and low values in winter, whereas heterotrophic bacteria were more abundant during the autumn period. The autumn peaks of the rates of enzymatic activities suggested that not only phytoplankton-derived but also allochthonous organic polymers strongly stimulated microbial metabolism. Increased microbial response in terms of abundance and metabolic activities was detected especially at the sites directly affected by organic matter inputs related to agriculture or aquaculture activities. Nitrogen salts such as nitrate, rather than orthophosphate, were primary drivers of phytoplankton growth. This study also provides insights into the different seasonal scenarios of water quality in Syracuse Bay, which could be helpful for management plans of this Mediterranean coastal environment.

In conclusion, it is clear that extrapolation of microbial structure and function to larger-scale processes may sometimes be complicated by the unpredictability of microbial responses to environmental changes and by the fact that microbes act on the microscale. Given these uncertainties, there is a critical need for microbiological research on aspects of environmental stressors. Major bottlenecks and challenges for future studies in marine microbial ecology are:

- the lack of long-term data series related to microbial food webs.
- the lack of standardized parameters and simple methodologies for large data set acquisition.
- the acquisition of data related to microbial response in terms of functional diversity.
- the identification of key processes or biomarkers of metabolic changes that can be used as suitable descriptors of the microbial response to forcings.
- the need for a deep understanding of microbial-environmental interactions to forecast the evolutionary trends of ecosystems.

Recent advances in the analytical protocols are now available for the study of microbial processes, so providing a tool to improve current knowledge of ecosystem functioning and plasticity played by microbial communities. Combining field observations and laboratory incubations under controlled conditions can be a suitable strategy to fill some of the above-mentioned research gaps, helping to better understand the broad range of microbial responses to environmental variability.

We hope that this selection of scientific contributions is helpful to extrapolate microbial processes to environmental scales and thus improve our current knowledge of microbial dynamics and their response to stressors to predict future scenarios and guide decision makers.

Author Contributions: Conceptualization, G.C. and K.Z.; formal analysis, K.Z.; resources, G.C. and K.Z.; writing—original draft preparation, G.C. and K.Z.; writing—review and editing, G.C. and K.Z.; supervision, G.C. and K.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. The APC was funded by MDPI, being G.C. and K.Z. Co-Guest Editors of this Special Issue.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Halpern, B.S.; Frazier, M.; Potapenko, J.; Casey, K.S.; Koenig, K.; Longo, C.; Lowndes, J.S.; Rockwood, R.C.; Selig, E.R.; Selkoe, K.A.; et al. Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nat. Commun.* **2015**, *6*, 7615. [CrossRef] [PubMed]
2. Borja, A. Grand challenges in marine ecosystems ecology. *Front. Mar. Sci.* **2014**, *1*, 1. [CrossRef]
3. Intergovernmental Panel on Climate Change (IPCC). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V.P., Zhai, A., Pirani, S.L., Connors, C., Péan, S., Berger, N., Caud, Y., Chen, L., Goldfarb, M.I., Gomis, M., et al., Eds.; Cambridge University Press: Cambridge, UK, 2021; pp. 1–3949.
4. National Oceanic and Atmospheric Administration (NOAA). Ocean Acidification. Available online: https://www.noaa.gov/education/resource-collections/ocean-coasts/ocean-acidification (accessed on 26 July 2022).
5. Le Moal, M.; Gascuel-Odoux, C.; Ménesguen, A.; Souchon, Y.; Étrillard, C.; Levain, A.; Moatar, F.; Pannard, A.; Souchu, P.; Lefebvre, A.; et al. Eutrophication: A new wine in an old bottle? *Sci. Total Environ.* **2019**, *651*, 1–11. [CrossRef] [PubMed]
6. Eutrophication & Hypoxia Map Data Set. Available online: https://www.wri.org/resources/data-sets/eutrophication-hypoxia-map-data-set (accessed on 26 July 2022).
7. Breitburg, D.; Levin, L.A.; Oschlies, A.; Grégoire, M.; Chavez, F.P.; Conley, D.J.; Garçon, V.; Gilbert, D.; Gutiérrez, D.; Isensee, K.; et al. Declining Oxygen in the Global Ocean and Coastal Waters. *Science* **2018**, *359*, 6371. [CrossRef] [PubMed]
8. Karydis, M.; Kitsiou, D. (Eds.) *Marine Eutrophication: A Global Perspective*; CRC Press: Boca Raton, FL, USA, 2019; pp. 1–206. [CrossRef]
9. Gattuso, J.-P.; Magnan, A.K.; Bopp, L.; Cheung, W.W.L.; Duarte, C.M.; Hinkel, J.; Mcleod, E.; Micheli, F.; Oschlies, A.; Williamson, P.; et al. Ocean Solutions to Address Climate Change and Its Effects on Marine Ecosystems. *Front. Mar. Sci.* **2018**, *5*, 337. [CrossRef]
10. von Schuckmann, K.; Le Traon, P.-Y.; Smith, N.; Pascual, A.; Djavidnia, S.; Gattuso, J.-P.; Grégoire, M. (Eds.) Copernicus Marine Service Ocean State Report, Issue 5. *J. Oper. Oceanogr.* **2021**, *14* (Suppl. S1), s1–s185. [CrossRef]
11. Pomeroy, L.R. The Ocean's Food Web, A Changing Paradigm. *BioScience* **1974**, *24*, 499–504. [CrossRef]
12. Azam, F.; Fenchel, T.; Field, J.G.; Gray, J.S.; Meyer-Reil, L.A.; Thingstad, F. The Ecological Role of Water-Column Microbes in the Sea. *Mar. Ecol. Prog. Ser.* **1983**, *10*, 257–262. [CrossRef]
13. Sarmento, H.; Montoya, J.M.; Vazquez-Dominguez, E.; Vaqué, D.; Gasol, J.M. Warming effects on marine microbial food web processes: How far can we go when it comes to predictions? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 2137–2149. [CrossRef] [PubMed]
14. Prairie, J.C.; Montgomery, Q.W.; Proctor, K.W.; Ghiorso, K.S. Effects of Phytoplankton Growth Phase on Settling Properties of Marine Aggregates. *J. Mar. Sci. Eng.* **2019**, *7*, 265. [CrossRef]
15. Li, X.; Chai, X.; Zheng, L.; Deng, Q.; Chen, X.; Zhang, Q.; Wan, L.; Song, C.; Song, L.; Zhou, Y.; et al. Coupling between Benthic Nutrient Cycling and Pelagic Phytoplankton Community in Taiwan Strait in Spring 2018. *J. Mar. Sci. Eng.* **2020**, *8*, 807. [CrossRef]
16. Hotos, G.N. Culture Growth of the Cyanobacterium *Phormidium* sp. in Various Salinity and Light Regimes and Their Influence on Its Phycocyanin and Other Pigments Content. *J. Mar. Sci. Eng.* **2021**, *9*, 798. [CrossRef]
17. Cavicchioli, R.; Ripple, W.J.; Timmis, K.N.; Azam, F.; Bakken, L.R.; Baylis, M.; Behrenfeld, M.J.; Boetius, A.; Boyd, P.W.; Classen, A.T.; et al. Scientists' warning to humanity: Microorganisms and climate change. *Nat. Rev. Microbiol.* **2019**, *17*, 569–586. [CrossRef] [PubMed]

18. Caruso, G.; Madonia, A.; Bonamano, S.; Miserocchi, S.; Giglio, F.; Maimone, G.; Azzaro, F.; Decembrini, F.; La Ferla, R.; Piermattei, V.; et al. Microbial Abundance and Enzyme Activity Patterns: Response to Changing Environmental Characteristics along a Transect in Kongsfjorden (Svalbard Islands). *J. Mar. Sci. Eng.* **2020**, *8*, 824. [CrossRef]
19. Rughöft, S.; Vogel, A.L.; Joye, S.B.; Gutierrez, T.; Kleindienst, S. Starvation-Dependent Inhibition of the Hydrocarbon Degrader *Marinobacter* sp. TT1 by a Chemical Dispersant. *J. Mar. Sci. Eng.* **2020**, *8*, 925. [CrossRef]
20. Ziervogel, K.; Kamalanathan, M.; Quigg, A. Hydrolysis of Methylumbelliferyl Substrate Proxies for Esterase Activities as Indicator for Microbial Oil Degradation in the Ocean: Evidence from Observations in the Aftermath of the Deepwater Horizon Oil Spill (Gulf of Mexico). *J. Mar. Sci. Eng.* **2022**, *10*, 583. [CrossRef]
21. Madonia, A.; Caruso, G.; Piazzolla, D.; Bonamano, S.; Piermattei, V.; Zappalà, G.; Marcelli, M. Chromophoric Dissolved Organic Matter as a Tracer of Fecal Contamination for Bathing Water Quality Monitoring in the Northern Tyrrhenian Sea (Latium, Italy). *J. Mar. Sci. Eng.* **2020**, *8*, 430. [CrossRef]
22. Caruso, G.; Giacobbe, M.G.; Azzaro, F.; Decembrini, F.; Leonardi, M.; Miserocchi, S.; Cao, X.; Song, C.; Zhou, Y. All-In-One: Microbial Response to Natural and Anthropogenic Forcings in a Coastal Mediterranean Ecosystem, the Syracuse Bay (Ionian Sea, Italy). *J. Mar. Sci. Eng.* **2022**, *10*, 19. [CrossRef]