

Editorial: Applying Metabolomics to Questions in Marine Ecology and Ecophysiology

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High biodiversity and deep evolutionary histories have led to the extreme diversification of molecules in the ocean, explaining why some of humanity's most potent drugs have been discovered in marine organisms. Historical challenges in sampling and extracting molecules from marine life and seawater, along with the sparsity of molecular reference libraries, leaves much to be explored in the marine chemosphere (Steen et al. 2020). With the ongoing global loss of marine biodiversity due to anthropogenic pressures (O'Hara et al. 2021), opportunities to identify new marine molecules are declining. Here, we identify advances in metabolomics that can rapidly generate new knowledge about the ecology and evolution of marine molecules, while aiding the pursuit of new drugs to fight emerging diseases.

1. Metabolomics offers a high sensitivity tool in the toolkit of ecologists

a. Apply database-adjacent and database-independent methods: Marine metabolomes often yield extremely low molecular identification rates (da Silva et al. 2015),

routinely falling below 2% (e.g., Quinn et al. 2016, Hartmann et al. 2017). New chemi- and bioinformatic approaches can improve annotation rates beyond searching reference libraries, while others avoid reference libraries by characterizing specific aspects of molecular structures. Bioinformatic approaches to improve the annotation of marine molecules include *in silico* structure prediction tools from tandem mass spectra, often based on fragmentation trees, such as SIRIUS and DeepEI (Dührkop et al. 2019, Ji et al. 2020, respectively). Despite these advances, high error rates and uncertainty of molecular predictions remain a challenge in the field. Molecular networking of tandem mass spectrometry data groups structurally related molecules with putatively similar functions without the need for molecular identifications. These networks expand the frame of inference from changes in individual molecules to chemosphere-wide shifts in the marine environment over time (Petras et al. 2021a). Networking can also illuminate molecular modifications based on known mass shifts between related molecules (e.g., 2.016 Da = H₂). This approach has identified specific biochemistries activated in response to environmental change (Hartmann et al. 2017, Quinn et al. 2020, Petras et al. 2021b, Gamba et al. 2022). Li et al. (2021) took this approach a step further by combining hardware modifications and new data analysis algorithms to improve the quantification of trace metabolites in seawater and classify them with novel elemental mass search algorithms for bound nutrients. Their approach expands our understanding of a large group of ecologically important molecules in the sea, siderophores and other trace metal organic ligands produced by marine microalgae, without the need for complete structural elucidations.

b. Combine -omics: Multi-omics refers to approaches that combine -omics tools and datasets. While metabolomics provides a desired level of resolution—effector molecules—it rarely offers the full story due to the lack of molecular annotations. To identify the source of

molecules and the environmental conditions that stimulate their production, it is necessary to connect molecules back to genes or organisms. These connections are being made with recent tools that use co-occurrence networks, machine learning, and neural networks (e.g., Sogin et al. 2017, Little et al. 2021, Roach et al. 2020). For example, mmvec (Morton et al. 2019), a neural network approach, was used to identify an association between a specific bacterial strain and two coral molecules, demonstrating specific links between the coral microbiome and its metabolome (Little et al. 2021).

c. Use metabolomics for hypothesis testing: The application of metabolomics to hypothesis testing is limited by inconsistent detection of target metabolites, the inaccuracy of quantifications, and the dearth of molecular annotations (Steen et al. 2020). The approaches described above demonstrate that expanding analyses beyond known molecules to whole networks or shared chemical transformations (i.e., across many molecules) can smooth out single-molecule variation, increasing potential for hypothesis testing. As an example, networking was used to find hydration/dehydration reactions throughout metabolomes of mostly unknown molecules in coral holobionts and demonstrated that coral mitochondria and algal symbiont cells adjust membrane fluidity in opposite directions in response to elevated temperatures (Gamba et al. 2022).

II. Apply metabolomics across scales of biological and environmental organization

The fundamental role of molecules in carrying out physiological functions makes them useful across scales of biological organization. For example, sampling organelles and cells can be used to characterize biochemical pathways, sampling holobionts (as individuals and host species) can be used to profile the molecular phenotypes of individuals and species, and sampling

communities and ecosystems can quantify the chemical diversity of entire systems. Applications of sampling at scales reveal that distinct organelles and cell types have disparate physiological responses to the same stimulus (Gamba et al. 2022), individual holobionts of the same host species have different molecular profiles (Sogin et al. 2016, Hartmann et al. 2017, Lohr et al 2019), and holobionts of closely related host species show large differences in their molecular profiles and responses to stress (Quinn et al. 2016, Sweet et al. 2021).

The application of metabolomics is particularly powerful in microbiology. These tools can be used to identify the unique chemicals and ecophysologies of bacteria, such as surprisingly high sugar production in the seagrass rhizosphere or the production of novel bile salts that aid digestion in the human gut (Sogin et al. 2022, Quinn et al. 2020, respectively). In marine ecology, metabolites mediate interactions between bacteria and between bacteria and metazoans (Hay 2009). For example, chemical signals produced by surface-associated bacteria (e.g., on the seafloor) attract or deter invertebrate larvae and algal spores from settling (Hadfield and Paul 2001). Settlement is often accompanied by metamorphosis to an adult stage, which in turn dictates local community composition, community succession, and biodiversity. The chemicals that attract or deter settlement can be leveraged for valuable applications including ecosystem restoration and the development of antifouling compounds. Advancements in mass spectrometry are being used to produce two-dimensional maps of metabolites across space, providing extremely highly resolved chemical landscapes and pinpointing new chemical signals with ecological functions (Watrous and Dorrestein 2011).

III. Study extracellular metabolites in seawater to identify key physiological processes

Marine organisms release molecules into seawater for many reasons, including acquiring mutualistic symbionts, attracting or killing bacteria, defending against predators and competitors, and preying upon other organisms (Hay 2009). The evolution of these behaviors and associated molecules is likely influenced by the predictable direction and protracted residence time of molecules in seawater in contrast to air. Few of these exometabolites, or molecules produced by organisms in the environment, have been characterized (summarized in Kelly et al. 2021). This dissolved organic matter can be extracted from seawater using solid phase extraction (SPE) columns (Dittmar et al. 2008) and analyzed using traditional tools, including approaches that overcome salt contamination (Sogin et al. 2019). Sampling the exometabolome is particularly informative because it isolates the molecules that holobionts release in response to changes in their environment, rather than all the molecules in the holobiont's tissues. Isolating the exometabolome thus allows researchers to identify molecular indicators of the stress or the molecules acting against it (e.g., antibacterial compounds), focusing data outputs on those most relevant to the ecological interaction being studied (Roach et al. 2020, Kelly et al. 2021). As an example, riboflavin was enriched in benthic seawater across numerous reefs relative to surface seawater (Weber et al. 2020). This vitamin plays an intracellular role in bacterial oxidative metabolism and extracellular roles in bacterial quorum-sensing and plant-microbe interactions (summarized in Weber et al. 2020). Finding riboflavin in the exometabolome suggests that its extracellular roles are important in these communities, a conclusion that would have been missed or ambiguous had riboflavin been detected in bacterial isolates or tissue samples, the more common sampling approach.

Summary:

1. New tools that enhance or bypass reference libraries and combine -omics approaches are enhancing our understanding of complex marine metabolomes and our ability to use metabolomics for hypothesis testing.
2. Metabolomics is a scalable tool that can identify the ultimate causes of physiological changes, be they in organelles or across ecosystems.
3. Studying the exometabolome, molecules released by organisms into their environment, simplifies molecular complexity to the most ecologically relevant components of the chemosphere.

Literature cited

- da Silva, R. R., Dorrestein, P. C., & Quinn, R. A. (2015). Illuminating the dark matter in metabolomics. *Proceedings of the National Academy of Sciences*, 112(41), 12549-12550.
- Ji, H., Deng, H., Lu, H., & Zhang, Z. (2020). Predicting a molecular fingerprint from an electron ionization mass spectrum with deep neural networks. *Analytical Chemistry*, 92(13), 8649-8653.
- Dittmar, T., Koch, B., Hertkorn, N., & Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods*, 6(6), 230-235.
- Dührkop, K., Fleischauer, M., Ludwig, M., Aksenov, A. A., Melnik, A. V., Meusel, M., et al. (2019). SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. *Nature Methods*, 16(4), 299-302.
- Gamba, A., Petras, D., Little, M., White, B., Dorrestein, P. C., Rohwer, F., et al. (2022). Applying tissue separation and untargeted metabolomics to understanding lipid saturation

- kinetics of host mitochondria and symbiotic algae in corals under high temperature stress. *Frontiers in Marine Science*, 406.
- Hartmann, A. C., Petras, D., Quinn, R. A., Protsyuk, I., Archer, F. I., Ransome, E., et al. (2017). Meta-mass shift chemical profiling of metabolomes from coral reefs. *Proceedings of the National Academy of Sciences*, 114(44), 11685-11690.
- Hay, M. E. (2009). Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. *Annual review of marine science*, 1, 193.
- Kelly, L. W., Nelson, C. E., Aluwihare, L. I., Arts, M., Dorrestein, P. D., Koester, I., et al. (2021). Molecular commerce on coral reefs: Using metabolomics to reveal biochemical exchanges underlying holobiont biology and the ecology of coastal ecosystems.
- Li, J., Boiteau, R. M., Babcock-Adams, L., Acker, M., Song, Z., McIlvin, M. R., & Repeta, D. J. (2021). Element-selective targeting of nutrient metabolites in environmental samples by inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry. *Frontiers in Marine Science*, 8, 630494.
- Little, M., George, E. E., Arts, M. G., Shivak, J., Benler, S., Huckleba, J., et al. (2021). Three-dimensional molecular cartography of the Caribbean reef-building coral *Orbicella faveolata*. *Frontiers in Marine Science*, 8, 627724.
- Matthews, J. L., Cunning, R., Witson-Williams, R., Oakley, C. A., Lutz, A., Roessner, U., et al. (2022). The metabolic significance of symbiont community composition in the coral-algal symbiosis. In *Applied Environmental Metabolomics* (pp. 211-229). Academic Press.
- Morton, J. T., Aksenov, A. A., Nothias, L. F., Foulds, J. R., Quinn, R. A., Badri, M. H., et al. (2019). Learning representations of microbe–metabolite interactions. *Nature Methods*, 16(12), 1306-1314.

- O'Hara, C. C., Frazier, M., & Halpern, B. S. (2021). At-risk marine biodiversity faces extensive, expanding, and intensifying human impacts. *Science*, 372(6537), 84-87.
- Petras, D., Minich, J. J., Cancelada, L. B., Torres, R. R., Kunselman, E., Wang, M., et al. (2021a). Non-targeted tandem mass spectrometry enables the visualization of organic matter chemotype shifts in coastal seawater. *Chemosphere*, 271, 129450.
- Petras, D., Caraballo-Rodríguez, A. M., Jarmusch, A. K., Molina-Santiago, C., Gauglitz, J. M., Gentry, E. C., et al. (2021b). Chemical proportionality within molecular networks. *Analytical Chemistry*, 93(38), 12833-12839.
- Quinn, R. A., Melnik, A. V., Vrbanc, A., Fu, T., Patras, K. A., Christy, M. P., et al. (2020). Global chemical effects of the microbiome include new bile-acid conjugations. *Nature*, 579(7797), 123-129.
- Roach, T. N., Little, M., Arts, M. G., Huckeba, J., Haas, A. F., George, E. E., et al. (2020). A multiomic analysis of in situ coral–turf algal interactions. *Proceedings of the National Academy of Sciences*, 117(24), 13588-13595.
- Sogin, E. M., Putnam, H. M., Anderson, P. E., & Gates, R. D. (2016). Metabolomic signatures of increases in temperature and ocean acidification from the reef-building coral, *Pocillopora damicornis*. *Metabolomics*, 12(4), 1-12.
- Sogin, E. M., Putnam, H. M., Nelson, C. E., Anderson, P., & Gates, R. D. (2017). Correspondence of coral holobiont metabolome with symbiotic bacteria, archaea and *Symbiodinium* communities. *Environmental microbiology reports*, 9(3), 310-315.
- Sogin, E. M., Puskás, E., Dubilier, N., & Liebeke, M. (2019). Marine metabolomics: a method for nontargeted measurement of metabolites in seawater by gas chromatography–mass spectrometry. *Msystems*, 4(6), e00638-19.

Steen, A. D., Kusch, S., Abdulla, H. A., Cakić, N., Coffinet, S., Dittmar, T., et al. (2020).

Analytical and computational advances, opportunities, and challenges in marine organic biogeochemistry in an era of “omics”. *Frontiers in Marine Science*, 7, 718.

Weber, L., Armenteros, M., Kido Soule, M., Longnecker, K., Kujawinski, E. B., & Apprill, A. (2020). Extracellular reef metabolites across the protected Jardines de la Reina, Cuba reef system. *Frontiers in Marine Science*, 7, 582161.