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PERSPECTIVE

Interrogating Metabolic Plasticity in Marine Organisms: A Framework for Best Practices Using Metabolomic and Lipidomic Approaches

Yaamini R. Venkataraman [*,1] and Ariana S. Huffmyer[†]

[*]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA ; [†]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA

Both authors contributed equally to this manuscript.

[1]E-mail: yaamini.venkataraman@gmail.com

Synopsis Understanding the mechanisms that underlie resilience in marine invertebrates is critical as climate change and human impacts transform coastal ecosystems. Metabolic plasticity, or an organism's capacity to modulate energy production, allocation, and use, plays a central role in mediating resilience under environmental stress. While research on marine invertebrate stress responses has grown, integrative studies that examine metabolic plasticity by connecting molecular, physiological, and organismal scales remain limited. In this Perspective, we advocate for the rigorous and thoughtful use of metabolomic and lipidomic approaches to understand resilience in marine systems through the lens of metabolic plasticity. We provide recommendations for experimental design, summarize current methodologies, and provide an overview of commonly used data analysis approaches. Advances in other molecular approaches such as genomics, epigenomics, and transcriptomics can be harnessed to further explore stress responses through multi-omic integrative analyses. As quantitative integrative analysis remains limited in marine fields, we call for a stronger integration of molecular, metabolomic, physiological, and organismal data sets to link mechanisms to phenotypes. We explore the use of these approaches in studies of marine invertebrates and highlight promising areas of multi-omic research that deserve exploration. By embracing metabolic complexity and scaling from molecules to phenotypes, we suggest that the marine invertebrate research community will be better equipped to understand, anticipate, and mitigate the impacts of environmental change on marine ecosystems.

Introduction

Energy metabolism is a central component of organismal responses to environmental stressors. Under a particular environmental condition, the Oxygen- and Capacity-Limitation of Thermal Tolerance (OCLTT) hypothesis suggests that the optimal temperature for an organism is one that maximizes its aerobic scope (Pörtner 2001, 2002, 2010). Within the upper and lower bounds of tolerance, or "pejus" temperatures, organisms must respond to environmental stressors using metabolic compensation (Pörtner 2010; Pörtner et al. 2017). Metabolic compensation can occur prior to the onset of physiological or organismal manifestations of stress. Specifically, once in the "pessimum" range, organisms shift to anaerobic processes, prioritizing energy conservation and essential function maintenance at the expense of growth or reproduction (Pörtner 2010; Pörtner et al. 2017). In order to understand transitions between active and passive tolerance (sensu [Pörtner et al. 2017]), it is essential to examine metabolic responses, as whole-organism physiological metrics alone offer an incomplete view of how organisms respond to stress.

Examinations of metabolic responses using metabolomics and lipidomics (see definitions in Box 1) are increasingly used to investigate plasticity in response to environmental stressors in marine invertebrates (Fig. 1A). These fields emerged in the 20th century allowing for increased high-throughput

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Box 1: Glossary.

Metabolomics: The study of chemical processes involving the small molecules that are the direct and indirect products of metabolic pathways. Metabolites are often classified into primary metabolites involved in growth, development, and reproduction, and secondary metabolites that are more important for ecological function.

Lipidomics: The study of complete fatty acid and lipid profiles in a sample. Lipid molecules are crucial for short- and long-term energy storage.

Plasticity: Ability of one genotype to produce more than one phenotype.

Metabolic plasticity: An organism's capacity to modulate energy production, allocation, and use.

Compound: A distinct chemical substance that is measured and analyzed within a biological sample such as a metabolite or lipid.

Steady state: Absolute or relative concentrations of a compound at a particular point in time.

Metabolic flux: Rate at which compounds pass through a metabolic pathway.

Liquid chromatography mass spectrometry (LC-MS): Combines physical separation capability of liquid chromatography with mass analysis capability of mass spectrometry. Can characterize a wide range of molecule types, and is considered more robust for lipidomic applications.

Gas chromatography mass spectrometry (GC-MS): Combines features of gas chromatography with mass spectrometry. Best suited for volatile molecules, and is considered more robust for metabolomic applications.

Nuclear magnetic resonance (NMR): Quantify compounds by placing a sample in a magnetic field and using the inherent magnetic properties to identify the compounds. 1H-NMR is most commonly used with metabolomics data.

Targeted: Predefined list of compounds quantified using standards.

Semi-targeted: Screening for a broad set of known compounds without necessarily having all standards.

Untargeted: Profiling as many compounds as possible including unknown compounds.

Permutational analysis of variance (PERMANOVA): Unsupervised multivariate approach used to test if centroids of groups are significantly different from each other. Often paired with permutational analyses of dispersion (PERMDISP).

Weighted gene co-expression network analysis (WGCNA): Correlation-based approach originally developed to identify groups of genes that shared expression patterns. Can be applied to identify metabolites or lipids with shared abundance patterns.

ANOVA-simultaneous components analysis (ASCA): Decomposes multivariate data according to variables of interest. Useful for examining multivariate responses across time or multiple additional factors.

Principal components analysis (PCA): Unsupervised molecular approach generally used for exploratory analysis. Reduces the number of dimensions in large datasets to principal components that retain most of the original information.

Partial least squares discriminant analysis (PLS-DA): Supervised approach that uses group labels to reduce dimensionality by identifying variables that explain relationships between predictors and responses. Orthogonal PLS-DA (OPLS-DA) is a variation commonly used with metabolomic and lipidomic data.

Variable Importance in Projection (VIP): Quantifies the discriminatory power of a compound from a PLS-DA model.

Significance Analysis of Microarray (SAM): Feature-specific modified t-tests combined with permutation analysis used to determine if a compound has differential abundance between experimental conditions.

Machine learning (ML): Computer systems that learn or adapt using statistical models to mimic human behavior and recognize patterns. Useful when datasets are complex, large, or require automation.

KEGG: Kyoto Encyclopedia of Genes and Genomes. Publicly-available database for pathway annotation.

HMDB: Human Metabolome Database. Publicly-available database for small molecule metabolites present in humans.

Overrepresentation-based enrichment: Determines if specific pathways or functions are observed in a target dataset more than expected by chance in comparison to a background dataset.

Network topology-based enrichment: Incorporates additional factors that impact pathway activity, such as feature position in a pathway or feature-feature interactions, into an enrichment analysis.

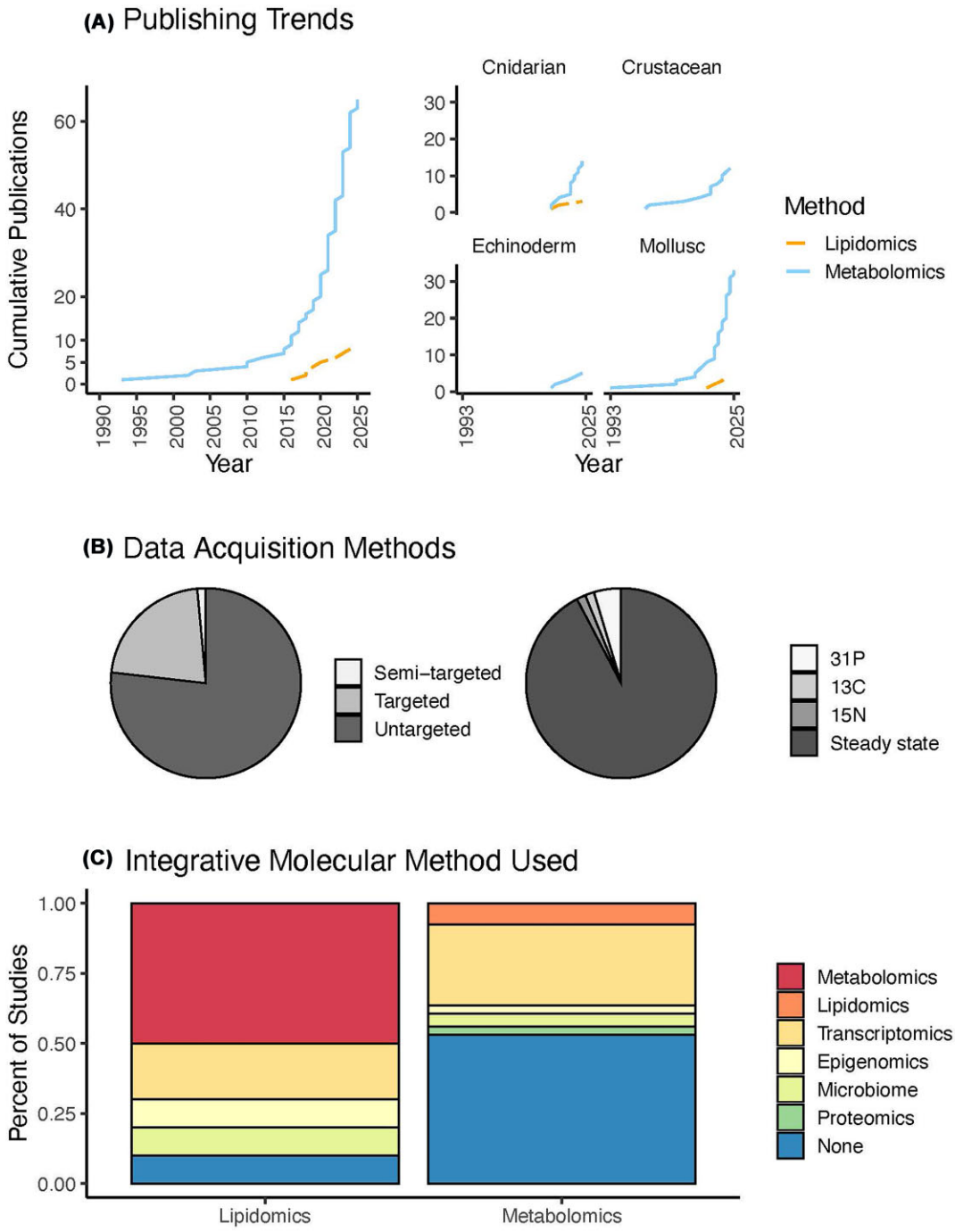

Fig. 1 Metadata for 68 published studies examining metabolic plasticity. Studies were papers examining metabolome and/or lipidome responses to environmental stress in marine invertebrates. See **Appendix A** for search terms and results. (**A**) Cumulative metabolomic and lipidomics papers published between 1993 and 2025. Published research papers were identified through Web of Science and ProQuest searches and supplemented with manual searches through Google Scholar. In this timeframe, 65 used metabolomics and eight studies used lipidomics. Publishing trends are also shown by phylum for cnidarian (17), crustacean (13), echinoderm (5), and molluscs (37). Two or fewer papers were published for annelids, brachiopods, and bryozoans each over this time frame, and therefore are not visualized separately. (**B**) Data acquisition methods of papers in **Appendix A**. Metabolomics and lipidomics data were collected primarily using untargeted experiments (77.9%), followed by targeted studies (22.1%), then semi-targeted studies (1.5%). One study used both targeted and untargeted methods. The majority of studies (92.6%) examined steady state responses, while 7.4% used ^{31}P, ^{13}C, or ^{15}N labeling methods to understand metabolic flux. (**C**). Other molecular methods used to integratively study metabolic plasticity with either lipidomics or metabolomics. A total of 30 of 65 metabolomics studies integrated an additional molecular method, while 7 of 8 lipidomics studies used an additional molecular method. Only two studies used more than two molecular methods (Rodriguez-Casariego: (2023): lipidomics, epigenomics, transcriptomics, microbiome; Wei et al. (2015): metabolomics, transcriptomics, proteomics).

profiling through advances in mass spectrometry and nuclear magnetic resonance (NMR) techniques (Viant 2008; Lindon and Wilson 2016; Beale et al. 2018). However, use of these approaches in non-model systems was not more prevalent until the 2000s due to challenges in protocol development and compound identification in non-model systems (Viant 2008; Williams et al. 2011; Schock et al. 2014; Carriot et al. 2021). In recent years, increased instrument sensitivity, expanded databases and libraries, reduced costs, and improved computational approaches have made metabolomics and lipidomics more accessible (Putri et al. 2013; Beale et al. 2018; Munjal et al. 2022).

Recent work has investigated how energetic constraints lead to susceptibility or resilience in response to various stressors, such as temperature, ocean acidification, toxin exposure, salinity, and hypoxia (see Appendix A for additional examples). For example, American lobster (*Homarus americanus*) exposure to ocean acidification resulted in broad metabolic reprogramming, demonstrating plasticity in energy usage (Noisette et al. 2021). Increased diversity of lipid classes suggested deepwater corals (*Acropora cervicornis*) employ heterotrophy more than shallow reef counterparts to meet energetic demands in stressful conditions (Rodriguez-Casariego et al. 2023). The power of metabolomic and lipidomic approaches lies in their ability to reveal sublethal impacts that are not detectable at the whole-organism level. Coral larvae exposed to elevated temperatures demonstrated metabolic reprogramming under elevated temperature without a decrease in survival (Huffmyer et al. 2024). In the blue mussel (*Mytilus edulis*), 1H-NMR metabolomics revealed differential energetic responses to OA stress in males as compared to females (Ellis et al. 2014). These studies demonstrate that metabolic and lipidomic tools can uncover subtle, yet critical shifts in energy allocation that underpin organismal resilience.

Due to their direct connection to energy metabolism, metabolomic and lipidomic approaches can be used to quantify metabolic plasticity. Metabolic plasticity can be achieved by processing the same compounds in different pathways to achieve similar results for cellular metabolism (Fendt et al. 2020), or through wholesale shifts in metabolic pathways, particularly in changing conditions or hostile environments (Jia et al. 2019). While the concept of metabolic plasticity has its origin in cancer biology, we encourage its use in organismal biology to guide proper use of metabolomic and lipidomic approaches. Placing metabolic plasticity at the center of investigations provides critical insight into mechanisms of organismal response to stress.

This perspective highlights the applications of these technologies to the interrogation of metabolic plasticity. We propose a framework to navigate experimental and analytical decisions centering these concepts. We also demonstrate the power of combining these methods with the study of other molecular mechanisms, such as gene expression. Finally, we highlight the importance of understanding these molecular mechanisms in the context of whole-organism physiological metrics. As metabolomics and lipidomics technologies become widely-used in organismal biology, establishing consensus around these practices will allow for rigorous, reproducible, and biologically meaningful analyses to examine plasticity in important ecosystems.

Experimental design choices influence the capacity to characterize metabolic plasticity

Sampling considerations

As is the case with any molecular tool, experimental design choices will influence data interpretation and robustness. We encourage readers to make appropriate choices in the context of experimental hypotheses and budget, and refer to published literature when determining the appropriate number of technical and biological replicates (Blaise et al. 2016; Jacyna et al. 2019; Lee et al. 2022). Mass spectrometry labs can also provide guidance on sample sizes, with most facilities recommending at least six biological replicates per treatment for adequate analytical power. Samples used should be chosen with the scientific question in mind, especially if sampling involves tissue isolation (Fig. 2). For example, studies in soft shell clams (Beaudreau et al. 2024) and abalone (Nguyen et al. 2021) show that metabolomic responses to thermal stress vary by tissue type, with greater effects observed in gills and hemolymph compared to muscle. In corals, single polyp approaches allow for examination of spatial biochemical structuring in complex holobiont systems (Roach et al. 2021) with separate host and symbiont analyses showing distinct metabolic responses between the partners (Gamba et al. 2022).

Samples should be processed and preserved in a way that minimizes enzyme activity during metabolite extraction (Liu and Locasale 2017). In order to quench metabolic activity and prevent degradation of compounds, samples should be isolated (e.g., seawater removed) and immediately snap-frozen in liquid nitrogen, stored at -80°C, and transported using dry ice or liquid nitrogen. Samples should not be stored in reagents such as RNALater to avoid any alterations to metabolic state during preservation. Avoid freeze thaw cycles and perform any necessary processing or extraction steps on dry ice as required for specific protocols.

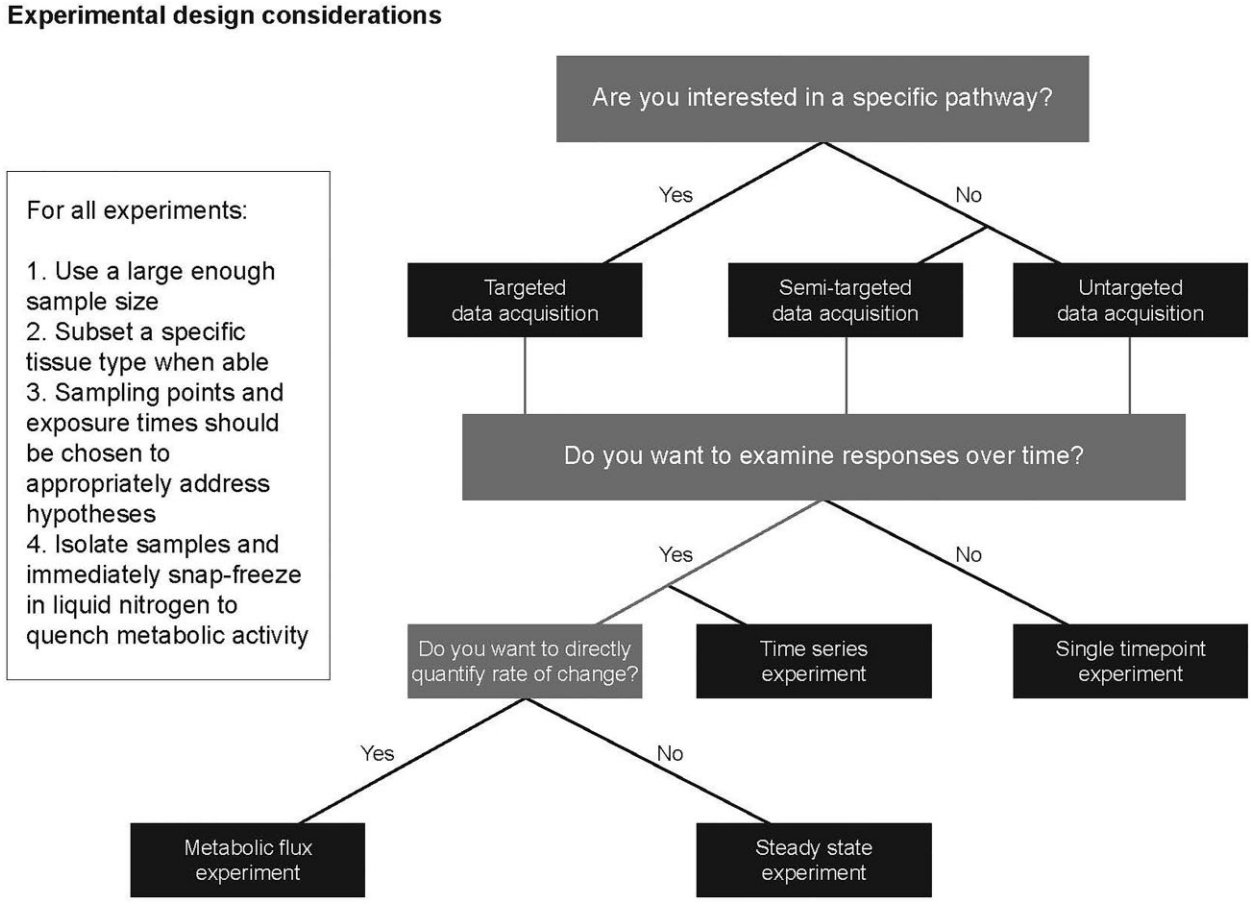

Fig. 2 Decision tree for metabolomic and lipidomic experimental design. Researchers should consider if they are interested in a specific pathway, if they want to examine changes in the metabolome or lipidome over time, or quantify rate of change of compounds. For all experiments, researchers should use appropriate sample sizes, tissue types, and sampling points to address hypotheses. Samples should be isolated (e.g., seawater removed) and immediately snap-frozen in liquid nitrogen, stored at -80°C, and transported using dry ice or liquid nitrogen.

Refer to Liu and Locasale (2017) for a review of compound extraction protocols.

Time series, or time course, experiments can provide key insights into metabolic plasticity by examining the dynamic nature of the metabolome and facilitating complete mapping of relationships between metabolites or pathways of interest (Sriyudthsak et al. 2016). Time series experiments need to be designed with prior knowledge of the turnover rate of target metabolites, and/or the time scale of physiological responses of interest. Previous work has conducted metabolomics time series studies on the order of seconds to minutes to characterize compound synthesis (Sekar et al. 2018) and metabolic responses to starvation in microbial systems (Link et al. 2015). Longer time scales such as weekly to monthly sampling are better suited to capture seasonal changes (Angelcheva et al. 2014; Rathore et al. 2021). We direct readers to previous work that discuss considerations for time series and dynamic metabolomic studies (Smilde et al. 2010; Nägele et al. 2016; Sriyudthsak et al. 2016).

Analytical considerations for data acquisition

Analytical platform choice is an important methodological consideration and should be selected based on the target compounds of interest, their chemical composition, desired output data format, and the robustness of the databases used for compound identification (Fig. 2). Data can be acquired in three different formats: targeted, semi-targeted, or untargeted (see definitions in Box 1). Targeted experiments provide specific concentrations of molecules (i.e., absolute quantitation), allowing researchers to investigate specific pathways or compounds of interest (Bennett et al. 2008; Cajka and Fiehn 2016; Park et al. 2016; Liu and Locasale 2017; Lee and Yokomizo 2018; Georgoulis et al. 2022), such as documenting how membrane remod-

eling was associated with physiological tipping points in response to low pH in the Pacific oyster (*Crassostrea gigas*) (Lutier et al. 2021). Since targeted experiments require intimate knowledge of an organism's metabolic pathways, researchers, especially those working in non-model marine systems, should consider if compound nomenclature is conserved between their organisms and those used to generate compound databases. Untargeted experiments provide relative feature abundance differences between experimental conditions or populations (Doroghazi et al. 2014; Cajka and Fiehn 2016; Liu and Locasale 2017; Lee and Yokomizo 2018). The majority of studies identified in Appendix A used untargeted data acquisition approaches (Fig. 1B). This approach may be useful in non-model systems, where several molecules are likely uncharacterized by existing databases. For example, a study in reef-building corals used untargeted metabolomics and compound identification to identify lipid classes (e.g., betaine lipids) that distinguished between thermally resilient and sensitive colonies (Roach et al. 2021), and identify dipeptides that were important in heat stress responses (Williams, Chiles, et al. 2021). However, novel compound identification and feature annotation are time consuming and require comprehensive reference databases and organismal knowledge (Liu and Locasale 2017). Identification of unknown compounds may be easier for lipidomics due to conserved nomenclature conventions based on compound structure. Semi-targeted data acquisition may be a sufficient alternative to targeted or untargeted assays (Breitling et al. 2006; Gika et al. 2016; Liu and Locasale 2017; Reisz et al. 2019). These experiments identify and absolutely quantify a large number of known compounds, without requiring standards for every compound. Diversity of waxy ester and triglyceride compounds detected with semi-targeted lipidomics in the coral *A. cervicornis* highlight how outplanting in deep environments promotes heterotrophy (Rodriguez-Casariego et al. 2023).

Metabolomic and lipidomic analyses are most commonly conducted to estimate metabolite absolute or relative concentrations at a particular point in time, known as "steady-state" measurements (Fig. 1B). Although characterizing shifts in metabolite concentration with steady-state metabolomics can inform researchers of relative differences in concentration of metabolites, concentration alone does not directly relate to metabolic flux (see definitions in Box 1), or the rate at which metabolites pass through a metabolic pathway (Jang et al. 2018), which can provide rich information on metabolic plasticity. It is important to consider that increased pool size of a metabolite may be the result of either increased production or decreased downstream metabolism, resulting in accumulation (Jang et al. 2018; Huffmyer et al. 2024). Stable isotope tracing can quantify metabolic flux of pathways of interest by tracking the incorporation of labeled atoms from stable isotope tracers into metabolites, providing insight into pathway activity and regulation that cannot be obtained with steady-state metabolomics alone (Jang et al. 2018). We direct the reader to previous literature that describes stable isotope tracing methods in detail (Creek et al. 2012; Fan et al. 2012; Chokkathukalam et al. 2014; Jang et al. 2018; Balcells et al. 2019). Although stable isotope tracing is less utilized in marine invertebrates (Fig. 1B), one promising use is the investigation of symbiotic nutritional exchange and nutrient metabolism in reef-building corals (Hillyer et al. 2018; Chiles et al. 2022; Huffmyer et al. 2024). These methods have also be applied to other organisms like the blue crab (*Callinectes sapidus*) (Holt and Kinsey 2002; Kinsey and Lee 2003) and red abalone (Haliotis rufescens) (Tjeerdema et al. 1993), to track flux through central energy metabolism reactions and provide insights into energetic state under environmental stress. However, isotopic tracing studies are more expensive and researchers should consider advantages and limitations prior to use.

Metabolomic analyses are commonly performed using NMR, mass spectrometry (MS), or a combination of the two (Ren et al. 2015). Most MS analyses are conducted as gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS), the latter of which is commonly analyzed using high performance (HPLC) or ultra high performance liquid chromatography (UHPLC). GC-MS platforms are well suited for volatile molecules (Ren et al. 2015), LC-MS is commonly used for lipidomic applications quantifying polar, non-volatile compounds (Cajka and Fiehn 2014; Ren et al. 2015), and NMR (commonly, 1H-NMR) is used to quantify metabolites using inherent magnetic properties (Markley et al. 2017; Bingol 2018; Emwas et al. 2019). Platform specifications and technical descriptions of the analytical pipelines have been described elsewhere (Naz et al. 2014; Beale et al. 2018) and are defined in Box 1. While both MS and NMR present limitations and challenges, recent efforts have emphasized the advantages of using both methods for complete characterization of the metabolome (Nagana Gowda and Raftery 2015). Previous work has discussed considerations for compound identification datasets and potential challenges in dataset nomenclature (Kind et al. 2009; Neumann and Böcker 2010; Blaženović et al. 2018; Sindelar and Patti 2020; Misra 2021; de Jonge et al. 2022). We recommend that researchers determine whether targeted, semi-targeted, untargeted analyses are required, then select the platform(s) best suited for the size and nature of com-

Analytical considerations

	Purpose	Considerations	Single metabolite tests: ANOVA	Single metabolite tests: Linear models	Unsupervised analyses: PERMANOVA	Unsupervised analyses: WGCNA	Unsupervised analyses: ASCA	Supervised analyses: PLS-DA + VIP	Supervised analyses: SAM	Supervised analyses: ML
How does concentration or composition change between treatments?	• Identify differences in metabolomic or lipidomic response between groups • Identify differences in specific compounds between groups of interest	• Violations of normality, heteroskedasticity, collinearity (Vinaixa et al. 2012) • Multiple comparison and false discovery rate corrections (Broadhurst and Kell 2007; Vinaixa et al. 2012) • Assess contribution of centroid location and dispersion by pairing PERMANOVA and PERMDISP tests (Anderson 2017) • Sensitive to low or unbalanced sample sizes • Assess appropriateness of distance metric used in PERMANOVA tests	✓	✓				✓	✓	✓
How do compounds correlate with quantitative responses or time?	• Correlate groups of compounds with other responses or time • Identify compounds that exhibit similar patterns across groups or change over time	• Consider effects of collinearity, autocorrelation, and missing values • Limitations of correlations vs causation and careful interpretation • Sensitivity to small sample sizes and unbalanced design (Bertinetto et al. 2020) • Careful evaluation of user-defined parameters			✓	✓	✓			✓
What compounds drive differences between treatments?	• Identify metabolites or lipids that are important in driving differences between groups • Biomarker selection	• Caution in interpretation of biological vs statistical significance • Sensitivity to sample size and overfitting (Gromski et al. 2015; Nadon and Shoemaker 2002) • Pair supervised methods with unsupervised methods to validate results (Gromski et al. 2015) • Assess appropriateness of ML and deep learning for biological question and use careful selection and training of models						✓	✓	✓

Fig. 3 Analytical considerations for metabolomic and lipidomic experiments. This table indicates suitable analytical options (*ie*., single metabolite tests, unsupervised analysis, or supervised analyses) for different experimental objectives (*ie*., examining changes to concentration or composition between treatments; examining correlations between compounds and either quantitative responses or time; or identifying compounds that drive differences between treatments), and provides an example of what the output visualization may look like. Researchers should determine which methods are most appropriate for their data and hypotheses.

pounds of interest while considering robustness of reference databases.

Data analysis to effectively address questions on marine invertebrate plasticity

Using appropriate analytical methods to address hypotheses and challenges with commonly used approaches

The choice of analytical method to address questions and hypotheses is a critical decision when analyzing metabolomic or lipidomic data (Fig. 3). Here, we provide an overview of analytical approaches to answer commonly asked questions and provide specific considerations in Fig. 3.

Prior to conducting statistical analyses, it is critical to conduct biologically appropriate normalization, assess quality controls (i.e., pooled biological quality control samples), and control for batch and confounding effects. The statistical approaches we discuss should be used with data that has been combined from negative and positive ion modes, and previously undergone necessary peak alignment and quantification, spectral deconvolution, and corrections (Ren et al. 2015). We point readers to previous discussions of these aspects of data analysis (Issaq et al. 2009; Li et al. 2014; Smith et al. 2014; Zhao et al. 2019) and reviews that discuss analytical approaches for more details (Worley and Powers 2013; Checa et al. 2015; Ren et al. 2015; Zhao et al. 2019).

How does the concentration of a metabolite/lipid of interest or the composition of the metabolome/lipidome change across groups or treatments?

Some lines of questioning may require testing the concentrations of particular metabolites. If single metabolite tests are necessary, analysis of variance (ANOVAs) or linear models (general and generalized linear models) are widely-used and robust methods useful for testing specific hypotheses. When identification of a single metabolite is desired, laboratory assays may be more appropriate than whole metabolome characterization (e.g., succinate quantification in (Zittier et al. 2018) and glycogen quantification in (Chen et al. 2022)).

Many studies evaluate the composition of the metabolome or lipidome as a multivariate response and examine variation in these responses between groups, treatments, or across time. The most commonly used unsupervised multivariate statistical approach is performing a permutational analysis of variance (PERMANOVA), which is non-parametric and

well-suited for highly dimensional and non-normal data (Anderson 2017) should be paired with permutational analyses of dispersion (PERMDISP) to evaluate whether multivariate differences are a product of centroid location and/or dispersion (Anderson 2017). For example, multivariate analyses were used to examine the influence of saxitoxin on metabolites and lipids in *M. edulis* immune cells (Beauclercq et al. 2023), revealing significant differences in fatty acid profiles when mussels were fed the toxin-producing *Alexandrium catenella* versus the non-toxic *Tetraselmis suecia* algae.

How do metabolomic or lipidomic features correlate with quantitative responses or time?

Examining the relationships between metabolomic and lipidomic features with quantitative responses, phenotypes, or time can be accomplished through several correlation-based and network approaches. We point the reader to work discussing the nature of dynamic metabolomic datasets and analyses in more detail (Smilde et al. 2010). Here, we discuss several approaches utilized in biological studies.

First, weighted gene co-expression network analyses (WGCNA) are commonly used in gene expression studies to identify modules, or groups, of genes that share expression patterns (i.e., co-expression) (Langfelder and Horvath 2008). This approach can be applied not only to gene expression data, but also to metabolomic and lipidomic data, which is less frequently utilized (Pei et al. 2017). For example, WGCNA has been applied to characterize metabolomic responses in tomato plants (DiLeo et al. 2011), dinoflagellate algae (Sui et al. 2014), and pathogenic fungi (Sun et al. 2024) but has rarely been applied in the study of marine invertebrate metabolomic or lipidomic analyses. WGCNA also provides a framework to correlate time, physiological responses, or survival with groups of metabolites or lipids (Langfelder and Horvath 2008; Pei et al. 2017). For example, a study in corals correlated metabolites and genes using WGCNA, but found no significant correlations (Drury et al. 2022). Exploration of the utility of WGCNA approaches in the study of marine organism responses is warranted.

ANOVA-simultaneous components analyses (ASCA) can also be useful to examine multivariate responses across time or multiple levels of factors of interest (Jansen et al. 2005; Smilde et al. 2005; Bertinetto et al. 2020). The strength of ASCA analyses is the ability to decompose multivariate data according to factors or variables of interest and visualize the effects and is particularly well suited for characterizing changes in responses across time (Jansen et al. 2005). For example, this approach has been used to identify metabolites that contributed to differences by treatment, lifestage, and their interaction in cuttlefish exposed to ocean acidification conditions (Minet et al. 2025).

Which compounds drive differences between treatments or groups?

After data exploration through unsupervised analyses and examining experimental effects such as time and treatment variables, the next step is often identification of individual metabolites or lipids (i.e., features) that drive significant differences. Partial least squares discriminant analysis (PLS-DA) can identify metabolites or lipids that distinguish between groups of interest (Kalivodová et al. 2015; Saccenti and Timmerman 2016). Conclusions and interpretations of biological importance of a particular metabolite or lipid must be made by conducting functional or pathway analyses and when contextualized with phenotypic or physiological responses (see *Enabling biological interpretation of metabolic plasticity through enrichment analyses*).

Significance Analysis of Microarray (or Metabolites; SAM) models provide an additional method to identify differential features between treatment groups of interest (Nadon and Shoemaker 2002; Xia and Wishart 2011). Use of SAM in conjunction with other multivariate methods demonstrated that heat-hardening upregulates metabolic pathways to promote homeostasis in elevated temperatures in *Mytilus galloprovincialis* mussels (Georgoulis et al. 2022). SAM methods also identified metabolites that differed by symbiont profiles, but not heat stress, in the coral *Pocillopora acuta* (Haydon et al. 2023).

Machine learning (ML) approaches are increasing in use as "big data" becomes more readily available for biological studies (Greener et al. 2022). Broadly, machine learning approaches are computer systems that learn or adapt using statistical models to mimic human behavior and recognize patterns. They are useful when datasets are too complex, too large, or require automation beyond the capacity of human analysis (Greener et al. 2022). Deep learning models are a subset of ML appropriate for large and complex datasets that utilize neural networks and include many layers to learn hierarchical representations of data (Reel et al. 2021; Greener et al. 2022). Deep learning approaches require large amounts of data—the more complex the problem, the more data is required—and are "black box" approaches that result in reduced interpretability (Reel et al. 2021). ML approaches require careful attention to choice of models (e.g., supervised or unsupervised), objectives (e.g., clustering, regression, or classification) and proper design of test and training datasets and procedures for training, validating, and testing models (Greener et al. 2022).

Best practices in quantitative analyses: contextualizing results and applying complementary approaches

Given the large number of data analysis tools available to researchers and the diverse sets of hypotheses tested using lipidomic and metabolomic data, we strongly encourage the use of multiple complementary analysis approaches to validate findings. Different statistical methods capture distinct aspects of data structure. Univariate analyses identify individual metabolites or lipids that differ significantly between conditions, while multivariate techniques (Kalivodová et al. 2015; Saccenti and Timmerman 2016) and ML approaches reveal patterns and interactions across multiple variables (Reel et al. 2021; Greener et al. 2022). Correlation network analyses can further uncover biochemical pathway relationships (Langfelder and Horvath 2008; Pei et al. 2017), while time series approaches track dynamic shifts over experimental conditions of interest (Jansen et al. 2005; Smilde et al. 2005; Bertinetto et al. 2020). Applying multiple statistical approaches to the same metabolomic or lipidomic dataset enhances the reliability, depth, and interpretability of findings. For example, a combination of PCA, PLS-DA, and pairwise tests showed that the bryozoan *Bugula neritina* metabolome was largely unchanged after heat stress, demonstrating this species' resilience to high temperature (Gauff et al. 2025). Sensitivity of *Mya arenaria* and *Mya truncata* clams to marine heat waves was examined using PERMANOVA, linear mixed effects models, and PLS-DA analysis to examine differential use of metabolic pathways under stress (Beaudreau et al. 2024).

Metabolomic and lipidomic data provide valuable insights into the biochemical state of an organism, but these data are effectively interpreted only when contextualized with phenotypic or physiological data. A total of 41 studies of the 68 represented in Fig. 1 paired molecular data with whole-organism physiology or phenotypic data (Appendix A). Without integrating physiological responses and phenotypes such as metabolic rate, growth, reproduction, or survival, it is difficult to determine whether observed molecular shifts correspond to metabolic plasticity and result in either adaptive or maladaptive responses. For example, clams (*Sinonovacula constricta*) had an increase in Arrhenius breakpoint temperature after heat hardening, and increased glycerophospholipid abundance suggests homeoviscous adaptation at higher temperatures (Zhang and Dong 2021). Marine copepods (*Apocyclops royi*) reared in hyposaline conditions for multiple generations demonstrated reproductive resilience, but metabolomics analysis showed that an increase in anaerobic stress is a "cost" to this resilience (Winding Hansen et al. 2022). The addition of physiological or phenotypic data enables researchers to move beyond descriptive metabolomic or lipidomic profiles and instead contextualize -omic data with organismal function, helping to uncover the mechanistic basis of metabolic changes.

Enabling biological interpretation of metabolic plasticity through enrichment analyses

One approach to interpret complex metabolomic and lipidomic datasets is to manually map compounds of interest to known pathways. This approach is best used when metabolite and lipid pathways are well characterized and conserved across organisms and when researchers have a hypothesis regarding a specific pathway using a targeted approach. Of the 68 studies included in Fig. 1, 47 manually mapped compounds to known pathways. Many studies use publicly available databases (e.g., KEGG [Kanehisa and Goto 2000] and HMDB [Wishart et al. 2007]) to obtain pathway information. For example, in reef-building corals, researchers examined shifts in glycolysis (Huffmyer et al. 2024) and amino acid metabolism under stress (Chiles et al. 2022). Wanamaker et al. (2019) used MetaMapp (Barupal et al. 2012) and Cytoscape (Shannon et al. 2003) to visualize affected metabolic networks in Dungeness crab (*Cancer magister*) juveniles under low pH and oxygen conditions, identifying disrupted amino acid metabolism. This specific examination of pathways of interests provides a method for testing hypotheses regarding a particular pathway or function.

Enrichment analysis, originally developed for transcriptomics (Khatri et al. 2012; Zhao and Rhee 2023), facilitates biological interpretation of molecular datasets by linking changes in individual compound responses with large-scale shifts in biological processes. There are three types of enrichment analyses—ranking-based enrichment, overrepresentation-based enrichment, and network topology-based enrichment (Wright et al. 2015; Ihnatova et al. 2018; Nguyen et al. 2019; Geistlinger et al. 2021; Zhao and Rhee 2023)—with the latter two being more common in metabolomic and lipidomic studies and most appropriate for semi-targeted and untargeted approaches (Zhao and Rhee 2023). Overrepresentation-based enrichment methods determine if specific pathways or functions are observed in a target dataset more than expected by chance in comparison to a background dataset (Das et al. 2020; Maleki et al. 2020). Network-topology based enrichment incorporates additional factors that impact pathway activity, such as feature position in a pathway or feature-feature interactions (Bayerlová et al. 2015; Ihnatova et al. 2018; Yang et al. 2019). Both methods

Enrichment with MetaboAnalyst

(A) Pros and cons of MetaboAnalyst for enrichment

Pros	Cons
• Tools available to analyze data from raw spectra through enrichment and visualization • Interactive platform with no coding required • Provides the foundation for other analytical packages	• Extended capabilities for untargeted data, but better suited for targeted data • Issues with shared nomenclature or inability to identify molecules • Limited available reference databases

(B) Different KEGG databases impact output

Human Pathway	Match Status	FDR	Impact
Starch and sucrose metabolism	6/17	0.003	0.62
Nitrogen metabolism	3/6	0.053	0
Galactose metabolism	4/15	0.059	0.05
Valine, leucine, and isoleucine biosynthesis	3/8	0.059	0

C. elegans Pathway	Match Status	FDR	Impact
Starch and sucrose metabolism	6/16	0.005	0.54
Phenylalanine metabolism	3/6	0.07	0.5
Amino sugar and nucleotide sugar metabolism	6/31	0.07	0.31
One carbon pool by folate	5/23	0.07	0.11

(C) Nomenclature inconsistencies impact enrichment results

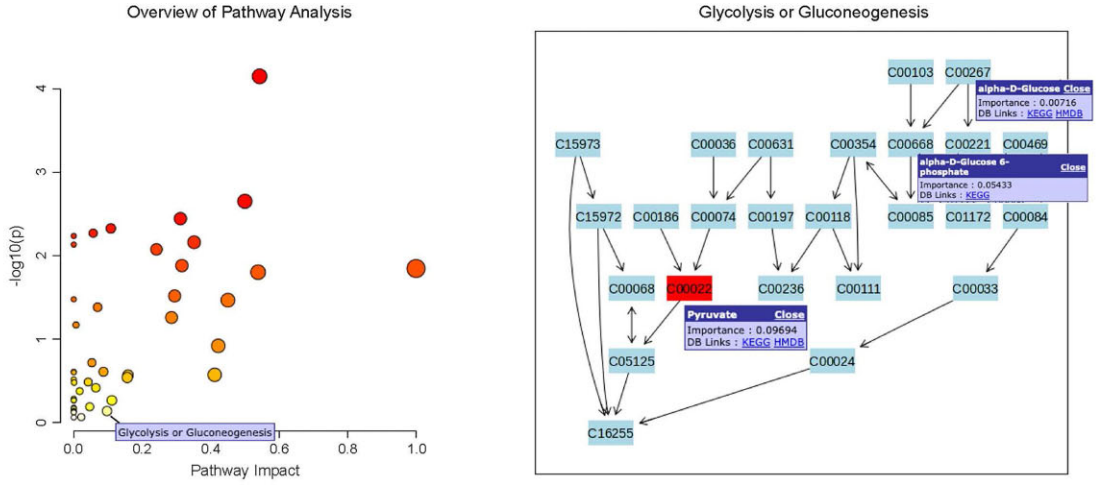

Fig. 4 Enrichment with MetaboAnalyst. (**A**) Pros and cons of using MetaboAnalyst. Panels (**B**) and (**C**) show MetaboAnalyst (v6.0) Pathway Analysis output for an example differential metabolite dataset generated in a previous study of reef-building coral early life stages (Huffmyer et al. (2025)) to highlight potential challenges in using MetaboAnalyst with non-model marine invertebrate species. This dataset is available in **Appendix B**. (**B**) Differences in pathway analysis output based on reference KEGG database. Pathway analysis was conducted using either humans (*Homo sapiens*) or *Caenorhabditis elegans* as KEGG references. The top four pathway results are shown. While the top pathway did not change based on the database, there are differences in the remaining pathways identified, the number of metabolites in the dataset that match the database (Match Status), FDR, and pathway impact values. (**C**) KEGG pathway analysis results using *C. elegans* as a reference. Arrow and label box indicate the enrichment of glycolysis or gluconeogenesis pathways. FDR *P*-value is indicated by color. These pathways were not significantly enriched (*P*-value = 1.0) and were considered low impact (impact = 0.10). Details of the glycolysis and gluconeogenesis KEGG pathway are shown, with red boxes indicating metabolites in the test set that matched to the KEGG pathway. While the dataset included core metabolites in the glycolysis and gluconeogenesis pathways such as "glucose," "glucose-6-phosphate," and "pyruvate," only pyruvate was recognized as a hit by MetaboAnalyst. This is due to differences in the nomenclature of glucose and glucose-6-phosphate required by Metaboanalyst to match to pathways ("alpha-D-Glucose" and "alpha-D-Glucose 6-phosphate," respectively, as indicated in text label boxes). These results demonstrate that nomenclature and specificity of nomenclature can limit pathway analysis results in databases that rely on particular nomenclature. Results were not different when running against the *C. elegans* (nematode), *Strongylocentrotus purpuratus* (urchin), *Mus musculus* (mouse), or human KEGG databases. Note that in the study (Huffmyer et al. (2025)) acknowledged this limitation and additionally examined glycolytic metabolic pathways through individual metabolite abundance.

rely on input data selection and background choice, which critically influence results. Input datasets can include metabolite modules linked to physiology or experimental variables (e.g, WGCNA) or differential features identified in multivariate analyses (e.g., VIP scores from PLS-DA), while using unfiltered data may yield misleading results (Chicco and Agapito 2022; Zhao and Rhee 2023). Background sets often include all detected compounds above noise thresholds, but more targeted backgrounds may be needed depending on the study design (Zhao and Rhee 2023). For example, using all detected compounds as a background may be appropriate for an untargeted analysis, but not for a targeted assay focusing on a specific pathway.

The most commonly used enrichment platform is Metabolomics Pathway Analysis (Xia and Wishart 2010) through the web-based GUI MetaboAnalyst (Xia et al. 2009; Pang et al. 2024) (Fig. 4), which supports both overrepresentation (e.g., Enrichment Analysis module) and network topology analyses (e.g., Path-

way Analysis module) and significance testing using Fisher's tests (Xia et al. 2009; Xia and Wishart 2010; Pang et al. 2024). Ten studies in Fig. 1 used the Pathway Analysis module of MetaboAnalyst, which requires users to select a KEGG reference library (last updated December 2024). For example, Guscelli et al. (2023) used the *Drosophila* KEGG library for Pathway Analysis and identified tricarboxylic acid cycle and amino acid metabolism as significantly impacted in northern shrimp (*Pandalus borealis*) exposed to ocean acidification and warming. Combined over-representation analysis and pathway topology analysis can also be employed to robustly identify compounds and pathways that differentiate between treatments of interest (Nguyen et al. 2021; Noisette et al. 2021). Although designed for metabolomics, enrichment tools like MetaboAnalyst can be applied to lipidomics. Alternatives such as Lipid Ontology (LION/web) offer a comparable approach for lipidomics datasets supporting overrepresentation and ranking-based enrichment analyses (Molenaar et al. 2019). While no studies in Fig. 1 conducted enrichment for lipidomics, LION/web has been applied in other organisms including in rat hepatic cells (Molenaar et al. 2023) and cetacean blubber (Bories et al. 2021). Together, these examples highlight the widespread use of MetaboAnalyst for pathway-based interpretation and underscore the importance of transparent reporting of database choices and analysis parameters to ensure reproducibility and biological relevance.

Compound nomenclature is a source of variability and inconsistency for metabolomic and lipidomic enrichment analyses, especially in non-model organisms (Fig. 4). Enrichment tools often rely on human-centric or model organisms databases, creating challenges for applications in non-model systems including inflated pathway sizes, outdated databases, and mismatched compound names (Wadi et al. 2016; Zhao and Rhee 2023). Reference databases can use different ontologies to define pathways, which can change the number of compounds in a specific pathway. Further, when there is variation between databases and the number of molecules included in a specific pathway, the number of differential compounds varies in order to identify significant enrichment, altering biological interpretations (Karp et al. 2021). In addition to potential pathway misclassification, compound nomenclature variation can lead to data not being used in enrichment. For instance, general terms like "glucose" may not map to specific isomers in enrichment databases, leading to data loss and biased interpretations (Fig. 4). Therefore, we recommend that researchers consider the type of molecules in their dataset, the annotation quality of the reference database and the availability of organism-specific pathways if required. If general biological pathways are the targets of interest, a more broad or model system based database may be appropriate. On the other hand, if organism specific pathways are of interest, researchers should identify databases from closely related organisms or create custom databases. Referencing previous work and comparative methods studies can assist in decision making (Ma et al. 2019; Chicco and Agapito 2022; Mubeen et al. 2022; Wijesooriya et al. 2022; Zhao and Rhee 2023). Regardless, researchers should explicitly report which database(s) was used in enrichment analysis.

Linking responses across different levels of biological organization through multi-omic integration

Increasing availability of large molecular datasets presents a challenge in effectively integrating these data to understand organismal responses with improved mechanistic interpretations. Analyzing data using molecular datasets at different levels of biological organization enhances the robustness and depth of scientific conclusions, bridges molecular mechanisms with functional outcomes, and reveals interactions that may be overlooked in single-omics studies. Each approach provides unique insights: genomics assesses changes or differences in allele frequencies; transcriptomics identifies gene expression patterns; metabolomics provides insights on shifts in metabolic pathways; lipidomics captures membrane dynamics and energy storage; and epigenomics reveals regulatory modifications.

Integration of two molecular approaches is common (Fig. 1C; Appendix A). Of the 68 studies in Fig. 1, 19 used gene expression and metabolomics to understand the molecular underpinnings of metabolic responses. For example, concurrent analysis of transcripts and metabolites can elucidate the relevant level of biological organization impacted by environmental stress in *C. gigas*, which exhibited an altered amino acid, carbohydrate, and fatty acid metabolite profiles in response to ocean acidification (Liu et al. 2020). These changes were not only associated with downregulation of corresponding genes, but also reductions in calcification gene expression (Liu et al. 2020). Similarly, thermal stress elicited changes to gene expression and metabolites associated with redox pathways in the rice coral *Montipora capitata* (Williams, Chiles, et al. 2021) and analysis of genes and metabolites in early developmental stages in *M. capitata* reveal developmental shifts in metabolism (Huffmyer et al. 2025). In the Pacific white shrimp (*Penaeus vannamei*), correlation and network analyses of metabolomic and transcriptomic data revealed that regulation of amino acid and

lipid metabolism increased energy availability under cold stress (Zhu et al. 2024). Combining metabolomic and lipidomic approaches is also common, with five studies doing so (Fig. 1C; Appendix A). Combined metabolomic and lipidomic analysis can reveal changes in active metabolic pathways and energy storage (e.g., Reddy et al. 2023) and provide a detailed view of lipid storage, lipid metabolism, signaling, and cellular membrane state (Imbs et al. 2021; Rey et al. 2022). For example, Costa et al. (2024) utilized lipidomic and metabolomic approaches to assess the impact of red tides on core metabolic pathways in reef building corals. Lipids and metabolites provided predictive biomarkers of *Pocillopora damicornis* performance in response to ocean acidification (Sogin et al. 2016) and combined lipid and metabolomic analyses revealed shifts in lipid metabolism during reproductive maturation in the mud crab *Scylla paramamosain* (Fu et al. 2022).

While it is clear that integrative multi-omic approaches improve our mechanistic understanding of organism responses, there are significant barriers in conducting this work. Examining organismal response to environmental stress would ideally include measurements of molecular mechanisms (e.g., epigenetics and gene expression), metabolic responses (e.g., metabolomics and lipidomics), and physiological and phenotypic measurements (e.g., respiration, growth, feeding behavior). This is often not feasible due to limitations in biological material available for sampling, time, personnel, and high cost of molecular approaches. Here, we discuss the state of multi-omic integration in the study of marine invertebrates and offer recommendations to move the study of metabolic plasticity towards integrative approaches.

Methodologies for integrative analysis

Approaches to integrate multiple -omic data sets generally fall into two categories: (1) individual analysis of each data set followed by qualitative integrative interpretation; and (2) quantitative integration of data sets in joint statistical analyses (see review in (Santiago-Rodriguez and Hollister 2021)). Here, we will highlight examples of each approach and provide recommendations for use in marine invertebrate systems. We propose that multi-omic examinations should include both individual -omic examination and quantitative integration of multi-omic data when appropriate and relevant to biological hypotheses.

Individual analysis of single-omic layers is a necessary step to identify strong signals and patterns at each level of biological organization and ensure proper quality control prior to more complex multi-omic approaches (Santiago-Rodriguez and Hollister 2021). The patterns detected in single-omic analyses can help inform biological hypotheses that may then be pursued through multi-omic integration. One approach is to conduct a qualitative comparison and then narrate a biological story using conclusions from single-omic analyses, which is more common in marine invertebrate studies (18 of the 27 studies in Appendix A with multiple molecular datasets used qualitative integrative interpretation). For example, Putnam et al. (2016) utilized metabolomics and DNA methylation to examine plasticity in response to ocean acidification and qualitatively discussed relationships between the two data types. Some studies use integrative visualizations that show molecular data layers mapped onto shared pathways (Wanamaker et al. 2019; Ren et al. 2020; Sun et al. 2021; Zhu et al. 2024), which can assist in making sense of highly dimensional data. It is important to shape these investigations using biology-driven questions and fully report the limitations of qualitative comparisons when providing evidence for mechanistic explanations. Further, it is critical to consider the interplay and interactions of multiple partners in holobiont systems (Williams 2024).

Quantitative multi-omic integration offers a powerful way to move beyond side-by-side single-omic comparisons by using statistical and computational methods to combine data layers and uncover patterns that may not be apparent when analyzing each dataset separately (Santiago-Rodriguez and Hollister 2021; Greener et al. 2022). There is underexplored potential to utilize quantitative integration through statistical analyses, which are more common in biomedical contexts (see review in (Reel et al. 2021)). Only 9 of 27 studies that employed multiple molecular methods used a quantitative or statistical approach to integrate molecular datasets and largely rely on correlations.

Several statistical approaches used for individual molecular datasets can be applied towards integrative analysis. Coexpression and correlation-based approaches, such as WGCNA and network analyses, are well suited for identifying groups of genes, lipids, or other features that change together across samples and highlight shared biological functions or coordinated pathways (Sun et al. 2022; Geng et al. 2024; Zhou et al. 2024; Zhu et al. 2024). Other methods, including DIABLO and PLS-DA analyses (Sun et al. 2022; Jing et al. 2023; Zhou et al. 2024), focus on selecting the most important features that differentiate between treatments or groups of interest, which can provide a tool for multi-omic biomarker discovery or building predictive models (Zhang et al. 2011; Young and Alfaro 2018; Sweet et al. 2021). For example, Sun et al. (2021) utilized pairwise correlations to examine metabolic responses

to salinity in the clam *R. philippinarum,* Geng et al. (2024) conducted correlation network analyses to identify genes and metabolites correlated with biomarkers in the blue mussel (*M. galloprovincialis*), and Pespeni and Lloyd (2023) used WGCNA to integrate microbiome and transcriptomic responses in sea stars.

Machine learning approaches for multi-omic integration have promising applications for the study of marine invertebrate plasticity, but are not widely employed. ML approaches can be used with a variety of integration strategies, depending on how and when the data are brought together (Reel et al. 2021; Greener et al. 2022; Manochkumar et al. 2023). Integration methods can be unsupervised (e.g., cluster analyses, factor analyses, Bayesian approaches), which are used to discover structure and patterns, or supervised (e.g., Bayesian networks, support vector machines, hierarchical classifiers, ensemble-based methods) in order to make predictions and classifications (Reel et al. 2021; Greener et al. 2022). For example, a metabolomics and transcriptomics study in corals used "MAGI," which provides a method for integration of metabolite and gene information (Erbilgin et al. 2019) to study metabolite-gene interactions in *M. capitata* under thermal stress (Williams, Panthmanathan, et al. 2021). We direct readers to previous reviews that discuss challenges in multi-omic integration in marine systems for a more detailed discussion (Manochkumar et al. 2023).

Best practices for integrative analysis

As with any analytical approach, best practices for multi-omic integration must be grounded in clear biological questions and hypotheses. Supervised methods are best suited for predictive tasks, such as classifying phenotypes or forecasting physiological outcomes; unsupervised methods are appropriate for discovering structure or patterns; and network or regression models may be useful when the goal is to infer mechanisms or relationships among layers of data (Reel et al. 2021; Greener et al. 2022; Manochkumar et al. 2023). Regardless of the approach, thoughtful preprocessing is essential as molecular data often differ in scale, distribution, and feature count and high-dimensional data can easily overfit small datasets if not properly constrained (Reel et al. 2021; Manochkumar et al. 2023).

We recommend building a foundation of single-omic analyses before layering in complexity to develop biologically relevant hypotheses and drive the responsible use of more complex integration approaches. Multi-omic integration should be used to explore biologically driven hypotheses in greater depth and uncover patterns that aren't detectable in analysis of single-omic levels. However, relying solely on single-omic approaches or one integration approach may lead to over simplification or missed deeper relationships. Correlative strategies, while informative, must be interpreted appropriately and the limitations of correlation approaches need to be acknowledged. Another challenge is that many integration platforms are designed for human or model system datasets and are not always compatible with non-model organisms or complex experimental designs (e.g., MetaboAnalyst). However, we can learn from biomedical research, where multi-omic integration has driven advances in health and medical research (Acharjee et al. 2016; Beaulieu-Jones et al. 2019; Triantafyllidis and Tsanas 2019; Ghassemi et al. 2020; Rubinger et al. 2023; Jain and Jain 2024). These approaches are beginning to be applied to ecological and evolutionary biology (Olden et al. 2008; Christin et al. 2019; Lürig et al. 2021; Greener et al. 2022; Pichler and Hartig 2023), and we argue that they are particularly needed in the study of non-model systems, such as marine invertebrates where stress responses involve complex coordination across biological levels. With careful application, these tools can help identify regulatory drivers of resilience, build predictive models of organismal health, and illuminate new layers of biological complexity in systems where mechanistic understanding has traditionally been limited.

Conclusion

Metabolomics and lipidomics are powerful tools for examining metabolic plasticity in non-model marine invertebrates. We encourage researchers to design clear, testable hypotheses and use them to guide molecular investigations. Given the complexity of these data types, appropriate statistical analysis may include complementary univariate and multivariate approaches to identify compounds of interest, and pairing manual and programmatic pathway mapping with enrichment methods to understand the biological significance of results. Pairing molecular data with physiology and/or phenotype information may elucidate sublethal impacts of stress and provide a holistic understanding of organismal resilience. When appropriate, we encourage researchers to pair metabolomic or lipidomic data with metrics at different levels of biological organization such as transcriptomics or proteomics as integrating multi-omic data can reveal mechanistics links between molecular changes and organism-level traits, providing a more comprehensive understanding of resilience.

We also identify areas of growth for the application of these methodologies to organismal biology. First and foremost, thorough reporting in manuscripts is neces-

sary to provide context and improve reproducibility. Of the 68 studies identified in Fig. 1, several were not explicit about whether or not targeted, semi-targeted, or untargeted data acquisition methods were used, or presented pathway or enrichment results without specifying methods. The lack of this necessary information makes it difficult for newer researchers to understand best practices for the field. Raw data should be housed in publicly accessible data repositories (Santiago-Rodriguez and Hollister 2021), similar to the NCBI Short Read Archive or Gene Expression Omnibus. Existing databases include Metabolomics Workbench (https://www.metabolomicsworkbench.org/) and MassIVE (https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp). Effort should be made to improve annotation databases for non-model systems to use for compound identification. More accurate databases can facilitate improved enrichment analysis or quantitative integration with other datasets.

Author contributions

Y.R.V. and A.S.H. jointly conceived, wrote the initial draft, and revised the manuscript. Y.R.V. created the Figs. with input from A.S.H.. Both authors reviewed and approved the final manuscript. We thank two anonymous reviewers for their valuable feedback on an earlier version of the manuscript.

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Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

The authors have no conflicts of interest to report.

Data availability

All appendices, figures, and metadata are available at https://github.com/yaaminiv/ICB-perspective-piece.

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Appendix

Appendix A:

Web of Science and ProQuest search terms and results for marine invertebrate studies examining metabolic plasticity in response to environmental stressors. Searches were conducted using a University of Washington login for Web of Science and ProQuest on March 24, 2025 and April 4, 2025, respectively. An additional 27 papers were added manually from Google Scholar searches.

Appendix B:

Test case metabolite dataset from Huffmyer et al. (2025) for illustrative purposes using Metaboanalyst (v6.0) platform.