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Marine specialized metabolites: Unveiling Nature's chemical treasures from the deep blue

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ABSTRACT

Marine specialized metabolites (MSM) represent a fascinating realm of chemical diversity with multifaceted functions across the spectrum of life on Earth. These metabolites serve as weapons, metal transporters, regulatory agents, and more. The conservation of genes responsible for their production over extensive evolutionary timescales underscores their selective advantage. Recent decades have witnessed an upsurge in MSM studies, driven by advancements in analytical techniques and the ever-growing accessibility of the aquatic environment. Marine macro and microorganisms offer a rich tapestry of specialized metabolites, some exhibiting potent activities in diverse domains, including medicine. The study of MSM presents several challenges, reflecting the need to separate complex mixtures into individual bioactive metabolites and utilize state-of-the-art extraction methods. Comprehensive structural analysis relies on advanced spectroscopic approaches, including nuclear magnetic resonance and mass spectrometry. These tools are instrumental in unravelling the chemical diversity of MSM and understanding their potential applications. While bioprospecting offers enormous potential, it raises critical challenges concerning sustainability, conservation, and equitable benefit-sharing. International protocols like the Nagoya Protocol seeks to regulate access to and share benefits from genetic resources, with considerable implications for marine bioprospecting. The convergence of advanced metabolomics, metagenomics, and synthetic biology offers promising avenues for accelerating the discovery and sustainable production of MSM, shaping the future of this field. This comprehensive review provides a deep dive into the challenges, methodologies, and emerging trends in studying marine-derived natural products, underscoring the immense potential of MSM for advancing chemical sciences and their transformative applications in diverse areas such as food, medicine, biotechnology, and environmental conservation. By bridging multiple disciplines, the continued exploration and sustainable utilization of these metabolites hold the promise of unlocking new innovations for society's benefit.

1. Introduction

The ocean, covering over 70 % of our planet, is a vast and largely unexplored frontier teeming with life. Among its myriad inhabitants, marine organisms have evolved to produce a remarkable array of specialized metabolites. These unique chemical compounds, often displaying potent biological activities, hold immense potential for biotechnological and pharmaceutical applications. This review discusses the fascinating world of marine specialized metabolites (MSM), unveiling the invaluable chemical compounds hidden within the deep blue and highlighting their significance in nature and potential benefits for humanity.

The diversity and abundance of specialized metabolites (known as secondary metabolites, natural products or phytochemicals) have captivated the scientific community for decades, encompassing the multitude of chemical compounds and the wide-ranging sources from which they originate. This diversity extends to the potential functions these molecules serve. Specialized metabolites are ubiquitous in the realm of living organisms on Earth; virtually every organism either produces specialized metabolites or participates in some form of specialized metabolism. Moreover, it is crucial to acknowledge the multifaceted roles that these molecules can play [1]. Specialized

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metabolites serve a plethora of functions, including acting as defense, attack, or competitive weapons to compete for resources or deter competitors such as bacteria, fungi, plants, amoebae, and insects (a form of self-protection/exclusion) [2]. They also function as metal-transporting agents, play roles in symbiosis, regulate sexual hormones, influence cellular differentiation within and between cells, facilitate the excretion of unwanted products, and serve as a reserve pool for new metabolic pathways [3].

Primary metabolism follows a linear trajectory in which its products remain within the organism, ensuring that the organism has sufficient nutrients and resources for reproduction. In contrast, specialized metabolism can be conceptualized as a form of "lateral thinking" employed by microorganisms and multicellular organisms. Lateral thinking refers to a creative and unconventional approach to problem-solving. It involves the production of specialized metabolites that may not have an immediate role in basic life processes. Instead, these metabolites often serve specialized functions, allowing organisms to navigate and interact with their environments in novel ways, such as a plant producing specialized metabolites as a defence strategy against herbivores. The plant engages in lateral thinking by synthesizing compounds that deter herbivores, attract predators of herbivores, or signal distress to neighboring plants. This allows the plant to enhance its survival chances beyond the basic requirements of primary metabolism. Specialized metabolites are typically derived from universal precursors, such as acetyl-CoA, amino acids, or shikimate. The genes encoding the enzymes responsible for specialized metabolite production likely evolved through gene duplication and divergence from those involved in primary metabolism [4]. These duplications exhibit varying degrees of antiquity, some dating back millions or even billions of years, and occurred independently multiple times across the evolutionary tree of life. In this context, specialized metabolites (SM) likely hold greater importance and diversity than macromolecular toxins due to their small molecule nature, facilitating their diffusion either into target cells or storage in producing cells [2].

Over the past two decades, marine specialized metabolites (MSM) studies have experienced a surge in interest and research activity. This phenomenon can be attributed to advancements in physicochemical techniques, such as nuclear magnetic resonance (NMR) correlations and tandem mass spectrometry (MS), which enable the analysis of complex structures and minute quantities of metabolites. Additionally, the exploration of MSM has been fueled by humanity's curiosity about the aquatic environment, now more accessible than ever. Observations of pharmacological activity, functional food/nutraceutical substitution, biotechnological application, and global population growth have further driven the investigation of MSM [5]. Both marine macro- and micro-organisms, akin to terrestrial species, produce a wide array of SMs, including terpenoids, alkaloids, and phenolic compounds. Unequivocally, numerous captivating MSM exhibit potent activities, mainly unrelated to their *in situ* functions. These metabolites have been identified as antitumor, antiviral, immunosuppressive, antimicrobial agents, neurotoxins, hepatotoxins, and cardiac stimulants.

The objectives of this perspective review article were to explore MSM, describing the current methods to unravel their chemical diversity and ecological roles (reviewed in Refs. [6,7]) while discussing the challenges specific to this field of research. From the detailed exploration of analytical techniques to discussions on sustainability and bioengineering applications, this review aims to cover the field's current landscape and future directions. An exploratory search was carried out on Scopus (from Elsevier, https://www.scopus.com/), Pubmed (from the National Center for Biotechnology Information of the United States of America (available at https://pubmed.ncbi.nlm.nih.gov/), and Google Scholar (https://scholar.google.com/) databases using marine specialized metabolites, algae metabolite or marine natural products as key words and no specific restrictions of time was applied. This review provides insights into marine biochemistry and chemistry, highlighting key gaps where further integration of biochemical, ecological, and technological approaches could enhance our understanding of marine specialized metabolites and their applications.

2. Isolation and extraction techniques

2.1. Challenges in isolating specialized metabolites

Collecting MSMs poses inherent challenges in sample preparation due to the complexity of seawater matrices, biofouling, and the stability of metabolites under oceanic conditions. MSM are typically synthesized in conjunction with, or connected to, other substances. Hence, the recurrent separation of complex extracts into individual bioactive metabolites is required for purification and concentration (Fig. 1). Chemists are constantly challenged with detecting, isolating, purifying, characterizing, and evaluating MSM across various biosynthetic classes, including those exclusive to the marine environment, such as pseudopterosins—a group of marine diterpene glycosides (alkaloid)—and halichondrin B, a complex marine polyether macrolide (terpenoid). A significant shift in this field involved transitioning from combustion analysis to high-resolution MS for precise molecular formula assignment and an increased reliance on high-field NMR, utilizing specialized probe assemblies and pulse sequences. Despite the routine nature of structure determination, isolation remains a crucial and often underestimated step in natural product chemistry. The success or failure of research projects frequently hinges on the effectiveness of isolating and purifying bioactive metabolites, as it serves as a dividing point between structural investigations and further explorations into the mode of action.

A literature analysis [8] reveals that the isolation of specialized metabolites using various adsorptive resins, such as phenyl, aminopropyl, cyanopropyl, or diol-derivatized silica-gel, represents 3 % of articles published between 1996 and 2009 in J. Nat. Prod. HP-20 resin emerges as a favoured choice, being four times more prevalent than Amberlite XAD resins. C8, polyamide, and other adsorptive or ion-exchange resins were merely used. Water-soluble specialized metabolites received even less attention, with less than 10 % of articles reporting their isolation during the same period. A subsequent analysis of articles published between 2017 and 2019 in J. Nat. Prod. reveals several trends: plant metabolites were the most investigated (51 %), followed by microbial metabolites (35 %) and marine specialized metabolites (11 %). Organic-soluble extracts dominated (67 %), while highly polar and water-soluble extracts constituted 33 %. Approximately 12 % of isolation reports utilized adsorptive resins, mainly HP-20 and XAD-16. Silica gel remained the preferred stationary phase (used alone or in combination), followed by C18 reversed-phase silica gel and Sephadex LH-20. HPLC with C18 reversed-phase silica gel columns was the primary high-resolution purification method. Evaporating organic solvents is notably quicker and does not necessitate specialized high-vacuum equipment. Additionally, when isolating specialized metabolites from water-soluble extracts, it becomes imperative to eliminate tannins, polar pigments, salts, primary metabolites, and microbial media components. However, it is worth noting that the slow evaporation of high-boiling-point solvents is a critical obstacle that often deters the exploration of water-soluble metabolites. Isolating bioactive SM typically involves monitoring through bioactivity assays, such as antioxidant and cytotoxicity tests, which reveal the active metabolites. It is crucial to tailor isolation procedures to the physical and chemical properties of the metabolites, particularly their lipophilic or hydrophilic nature. The focus is exploring low- and medium-polar fractions from marine extracts, yielding numerous new metabolites. This preference arises from the relative ease and cost-effectiveness of isolating and purifying lipophilic metabolites compared to hydrophilic ones. Furthermore, with their lower boiling points, nonpolar solvents enable faster distillation compared to polar solvents, which require equipment like SpeedVacs [9].

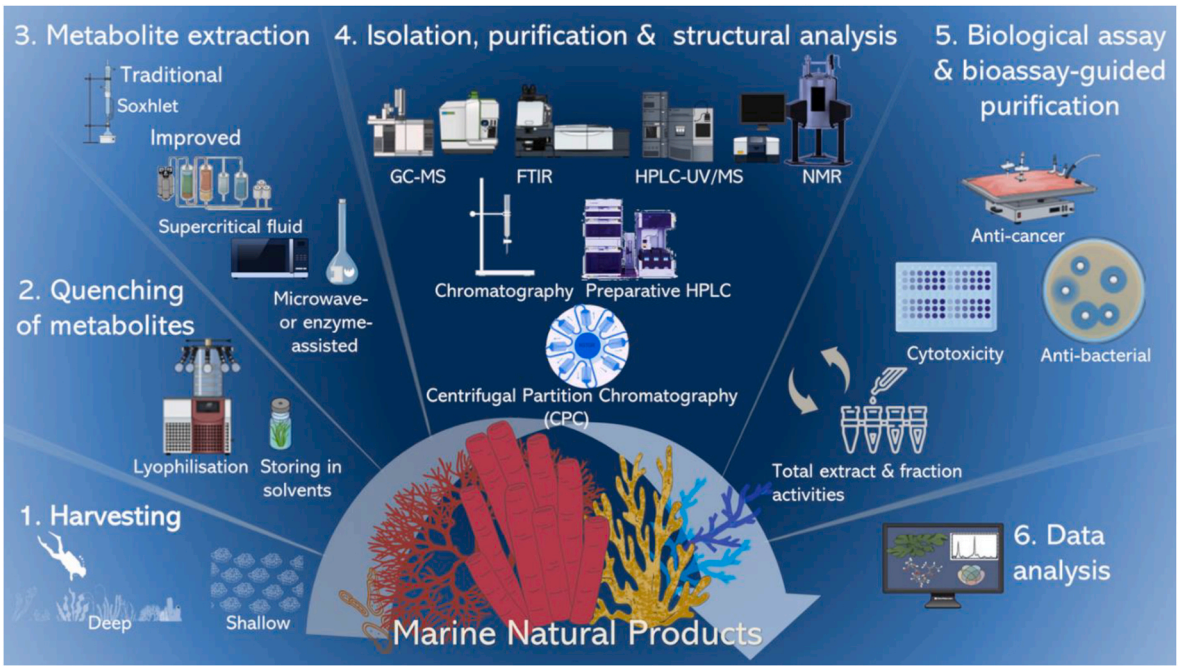

Fig. 1. Schematic representation of the marine specialized metabolites unravelling process highlights the milestones. 1. Harvesting of material, 2. Quench the metabolites for further analysis, 3. Extraction of metabolites using continuously improving methods, 4. Isolation, purification and structural analysis including determination of stereocenters, 5. Guidance through biological assays, 6. Data analysis.

2.2. State-of-the-art extraction methods

The characteristics of isolated biologically active SM significantly depend on the choice of extraction procedure, emphasizing the pivotal role of a well-selected methodology in ensuring high-quality products. Various factors, including the physicochemical properties of the source material, matrix characteristics, solvent type and concentration, pH, temperature, pressure, and time, must be considered, as they can profoundly impact overall effectiveness of the targeted SM extraction. However, many traditional techniques heavily depend on solvent strength, sample size, and concentration parameters. For example, soxhlet extraction relies on harsh solvents, requires pre-digestion with acids, and is time-consuming, limiting its modern applicability. Traditional methods often involve preliminary fractionation or purification steps, such as solvent fractionation or partition, and can be resource-intensive, consuming substantial volumes of solvents and energy. Other drawbacks of conventional techniques include extended extraction periods, the need for high-purity solvents, significant solvent evaporation, low yield, selectivity issues, and the risk of thermolabile ingredient decomposition. To address these limitations, contemporary non-conventional (modern) extraction techniques, like enzyme-assisted extraction (EAE), supercritical-fluid extraction (SFE), microwave-assisted extraction (MAE), and pressurized-liquid extraction (PLF) have emerged as promising environmentally friendly alternatives [10]. Compared to traditional methods, these techniques offer numerous advantages, including more sustainable processing conditions, reduced reliance on hazardous chemicals, safer auxiliary solvents, water use, improved energy efficiency, reduced derivative formation, support on renewable feedstocks, cost-effectiveness, more straightforward preparatory steps, higher efficacy, prevention of degradation, and avoidance of protection and deprotection steps [11]. These methods use green solvents like supercritical CO_2 and deep eutectic solvents (DES), which offer a safer, eco-friendly alternative to traditional organic solvents and reduce environmental impact. For example, DES and ionic liquids improved the extraction of phenolic compounds and bioactive pigments while enhancing the selectivity and yield of valuable MSM [12,13]. Sequential extraction approaches, such as coupling SFE with pressurized liquid extraction (PLE), also optimize compound recovery by harnessing multiple mechanisms in one process. Additionally, biorefineries are using marine biomass sources like microalgae and food waste to produce bioactive compounds efficiently, supporting a circular bioeconomy. These methods align with green chemistry principles by minimizing waste and energy consumption, offering scalable options for sustainable MSM extraction [12,14].

3. Structural characterization, spectroscopic approaches

Natural product SM research heavily relies on advanced analytical approaches, such as MS or NMR (Table 1). These tools are invaluable for the identification and authentication of specialized metabolites and find applications in metabolic profiling. A comprehensive qualitative and quantitative assessment of starting materials is essential in pharmaceutical and academic settings when working with specialized metabolites. When the specialized metabolites are intended for *in vitro* and *in vivo* testing, their identity and purity must be confirmed through physicochemical characterization and spectroscopic and spectrometric analysis (UV/VIS, IR, Raman, NMR, MS/MS) [15]. To achieve comprehensive structure elucidation of SM in solution, researchers heavily rely on the exhaustive utilization of 1D and 2D homo- and heteronuclear NMR measurements, complemented by other spectroscopic methods and high-resolution MS. Various detectors, such as diode array detectors (DAD), are commonly employed, allowing real-time UV/VIS spectra recording. However, their application is limited to analytes possessing conjugated double-bond systems (chromophores). Furthermore, UV/VIS detection is constrained by mobile phases and additives that do not absorb light within the detection wavelength ranges. Fourier transform infrared (FTIR) spectroscopy offers advantages like spectra acquisition from diverse environments and minimal sample requirements. It allows both qualitative and quantitative analysis. Vibrational spectroscopy, including FTIR, surpasses NMR spectroscopy in terms of shorter analysis times and surface-level *in situ* analysis of specialized metabolites despite providing less structural detail (Table 1). It also serves as a unique compound fingerprint. Raman spectroscopy provides insights into chemical structures, electronic environments, and molecular bonding,

Table 1
Comparison of Methods for Specialized Metabolite Characterization. These methods are often combined to enhance the overall characterization of specialized metabolites. The most commonly used techniques for the specific metabolites of interest are included.

Method	Output	Advantages	Disadvantages	Complementary Technique(s)	Specialized Metabolite/ Class
Nuclear Magnetic Resonance (NMR)	Detailed structural information	Non-destructive, high specificity	Requires pure samples, sensitivity limits	LC-NMR, LC-HR MS	Alkaloids, Flavonoids, Terpenoids
High-Resolution Mass Spectrometry (HR-MS)	Accurate mass measurements, molecular formulae	High sensitivity, rapid analysis	Limited structural info, ionization variation	NMR, LC-HR MS	Polyphenols, Glycosides, Saponins
Liquid Chromatography-Mass Spectrometry (LC-MS)	Compound identification via separation	Wide applicability, high sensitivity	Matrix effects, sample prep, ion suppression	LC-NMR, LC-HR MS	Phenolic acids, Tannins, Coumarins
Gas Chromatography-Mass Spectrometry (GC-MS)	Mass spectra for volatile compounds	Excellent for volatiles, high resolution	Requires derivatization, destructive	GC-MS, GC-O	Essential oils, Monoterpenes, Sesquiterpenes
GC-FID (Flame Ionization Detector)	Quantitative data based on ionization	Quantitative, suitable for volatiles	Limited to volatiles	GC-MS, GC-O	Fatty acids, Steroids, Carotenoids
GC-O (Gas Chromatography-Olfactometry)	Odor profiles (olfactory perception)	Reveals aroma-active compounds	Subjective (human perception)	GC-MS, GC-FID	Aromatic compounds, Volatile terpenes
Triple Quadrupole Mass Spectrometry (QqQ MS)	Quantitative analysis of targeted compounds	High sensitivity, specific for analytes	Limited structural info, prior knowledge	HR MS, NMR	Amino acids, Lipids, Nucleosides

particularly for nonpolar bonds like C–C modes in ring structures. In natural product research, IR spectroscopy primarily determines functional groups by deriving structural information from characteristic vibrational bands. The presence of multiple functional groups within a specific frequency range underscores the need to examine the complete spectrum. Identifying an unknown substance's structure often necessitates combined complementary techniques like NMR and MS.

Due to its numerous advantages, NMR spectroscopy is the preferred choice for large-scale metabolomic studies. It offers ease of sample preparation, quantification of metabolite levels, excellent experimental reproducibility, and a non-destructive nature. Furthermore, NMR allows researchers to record spectra for various nuclei (i.e., ^{1}H, ^{13}C, ^{15}N, and ^{31}P) separately or simultaneously, facilitating the study of different metabolite classes. Multidimensional NMR methods enable the correlation between two or even three different nuclei. NMR spectroscopy can assess protein-bound metabolites like lipoprotein particles and measure certain inorganic metabolites or ions (i.e., metal ions and H+ ions via pH), which cannot be accomplished using LC-MS or GC-MS. The field of NMR continues to evolve, with exciting techniques such as high-resolution magic-angle sample spinning (HRMAS), hyperpolarization methods, ultrafast 2D NMR methods, pure-shift NMR techniques, and hybrid NMR approaches emerging and enhancing NMR-based metabolomics [16,17].

Mass spectrometry is generally recognized for its role in providing structural information. However, it is crucial to emphasize that this data alone, even when using advanced tandem mass spectrometers with high-resolution mass selectors, often offers no more than the molecular formula. Detailed structural aspects, such as geometrical isomerism, positional isomerism, and 3D structures, still need to be discovered. Electrospray ionization (ESI) facilitates the transition of analyte molecules from the liquid phase to gas phase ions, enabling the coupling of mass spectrometers with liquid chromatography systems (Table 1). Diverse types of mass spectrometers, including triple quadrupole mass spectrometers (QqQ), ion trap mass spectrometers (IT-MS), hybrid mass spectrometers like QqTOF instruments, and Fourier-Transform mass spectrometers (FT-MS), can be integrated with separation techniques such as HPLC and capillary electrophoresis (CE) [18]. The selected reaction monitoring (SRM) experiments achievable with QqQ tandem mass spectrometers offer unmatched selectivity, reproducibility, sensitivity, and a wide linear range. When incorporated into mass spectrometers, time-of-flight (ToF) mass analyzers are specifically designed for recording high-resolution mass data, often revealing intricate molecular scaffold information. While Fourier transform mass spectrometry (FT-MS) employing magnetic field ion traps (FT-ICR-MS) or electrostatic ion traps (*e.g.*, the "orbitrap") delivers high mass resolution, it necessitates longer observation times compared to QqQ or ToF instruments [18, 19].

Other ground-breaking methods such as X-ray crystallography require large quantity of pure metabolites. This pre-requisite, added to other technical limitations, makes of structure elucidation a bottleneck step in natural product discovery.

4. Ecological roles and biosynthesis pathway discovery

4.1. Challenges in MSM biosynthesis

Marine-produced infochemicals encompass various ecologically significant allelochemicals phytohormones, and volatile organic metabolites, such as fatty acids, peptides, alcohols, esters, aldehydes, ketones, terpenoids, furans, sulfur containing and halogenated molecules, hydrocarbons, polyphenols and derivatives metabolites, which are essential for information transfer within and between individuals, playing crucial roles in interactions among marine organisms and with predators [20]. These chemically mediated interactions play a substantial role in shaping community structure, population dynamics, and ecosystem function, with metabolites acting as chemical defenses against predators and pathogens. Dimethyl sulfide (DMS) is a key sulfur compound released by marine algae, implicated in global climate regulation and in tritrophic mutualism between primary producers (algae) and top predators. This mutualism occurs through the accumulation of DMS in herbivorous zooplankton that grazes on phytoplankton, which in turn attracts carnivorous predator species [21]. The extent to which MSMs shape ecological interactions in marine environments remains an open question [22]. Undoubtedly important, their ecological roles are multifaceted, and sometimes overstated. The impact of SMs on the competitive dynamics of marine communities is often context-dependent, making it difficult to generalize their ecological significance [23]. The study of these metabolites present challenges compared to terrestrial ecosystems but innovative analytical techniques have been developed to map surface metabolites and track metabolite movement within marine holobionts [22].

In addition to their ecological relevance, many possess biotechnological, agricultural, nutraceutical and therapeutical properties. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), known as marine omega-3s are produced by microalgae and primarily sourced from cold-water fish [24]. Halichondrin B, an intricate polyether macrolide derived from the marine sponge *Halichondria okadai*, exhibits exceptional antitumor potency and a high therapeutic index [25]. Bryostatin 1 from marine organism Bugula neritina is employed in treating various cancers, including melanoma, lymphomas, sarcoma, leukemias, breast, colon, and lung cancer [26]. Didemnins, cyclic depsipeptide compounds isolated from Caribbean tunicates of the genus

Tridemnum, possesses notable pharmacological activities [27]. Pseudopterosin A, a diterpene glycoside from the gorgonian sea whip *Antillogorgia elisabethae*, found in the Bahamas and Florida Keys, along with its derivatives pseudopterosins A-D, with varying degrees of acetylation at the sugar ring, exemplify the rich diversity of therapeutic compounds sourced from marine organisms [28]. These MSMs of different class illustrate the diverse and significant therapeutic potential of marine-derived metabolites. This diversity underscores the importance of marine biochemistry in drug discovery and development.

The study of biosynthetic pathways involved in producing MSMs has thus garnered significant interest. However, a critical perspective in this area of research reveals several challenges and limitations that must be considered. One of the foremost challenges in studying biosynthetic pathways of MSMs is their immense complexity and diversity. Marine organisms employ a wide range of biosynthetic pathways for terpenoids, fatty acids, phenolics, and nitrogen-containing metabolites, each with its unique set of enzymes and intermediates involving specific enzymes such as oxidoreductase, acyl-transferase, polyketide synthase, and methyltransferase. This diversity complicates efforts to generalize findings and to develop a comprehensive understanding of these pathways. Even when biosynthetic pathways are partially understood, substantial gaps in knowledge often persist. Many marine organisms produce complex molecules through hybrid biosynthetic pathways that combine elements from multiple pathways, such as non-ribosomal peptide synthetase (NRPS)-derived SMs produced by marine bacteria, whose synthesis involves a hybrid polyketide synthase-NRPS [29,30]. MSMs sometimes possess specific chemical moieties such as macro rings with nitrogen and halogen atoms, and stereocenters, which are difficult to analyze [31]. This added complexity challenges our ability to predict their chemical structures metabolites solely based on genomic data, hindering the targeted discovery of new bioactive molecules. Computational and omics studies present a unique opportunity to tackle these issues.

4.2. Metabolomics

Metabolomics is used to decipher the metabolite profile of an organism to various stimuli, providing insight of their metabolism. Such experimental scheme also enables the discovery of cryptic pathways, which remain dormant under standard conditions, but are activated in response to specific triggers, providing an alternative avenue for obtaining novel specialized metabolites [32]. Metabolomics often relies on MS-based techniques. UPLC-ESI-MS/MS was recently used to unravel astaxanthin pathway and increase its yield in the microalgae *Chromochloris zofingiensis* [33]. Each analytical platform (*i.e.,* targeted to detect defined groups of previously characterized and annotated metabolites; or untargeted to perform a comprehensive analysis of all measurable metabolites; using LC- or GC-MS/MS.) has its limitations, and a combination of methods should be used to provide a broader picture. Other analytical tools such as supercritical fluid chromatography coupled to MS [34], capillary electrophoresis coupled to MS [35], infrared spectroscopy [36], and high-performance thin-layer chromatography [37] complement current LC- or GC-MS techniques, although they are scarcely used right now [38]. Alternatively, cryoelectron microscopy (cryoEM) method microcrystal electron diffraction (MicroED) is a sensitive techniques that could help to detect structures of biosynthetic pathways intermediate metabolites that are produced in low amounts [39]. MicroED involves frozen hydrated samples analyzed in a transmission electron microscope at liquid nitrogen temperatures in diffraction mode. It benefits from easier sample preparation compared to X-ray crystallography, requiring submicron-sized crystals of metabolites, and can resolve the atomic structure of a natural product from a mixture of metabolites [40]. Recently, MicroED helped to elucidate the structure of cryptic algaecides alkaloids in marine-algal-bacterial symbioses [41]. Its broader use in marine organisms could accelerate the characterization of new marine specialized metabolites. While cryoEM and MicroED are not traditional metabolomics techniques, their emerging role in the structural elucidation of biosynthetic pathway intermediates and specialized metabolites suggests that their integration in the metabolomics workflow would complement traditional MS-based techniques.

Recent advances in metabolomics are reshaping approaches to understanding bioactive compounds derived from marine environments. Modern studies emphasize non-targeted and functional metabolomics, which uncover bioactivity by associating specific metabolites with genetic and environmental factors, aiding in the discovery of novel therapeutic agents. Techniques like LC-MS/MS-based untargeted metabolomics and molecular networking are integral for cataloging diverse compounds, particularly specialized metabolites in marine microbiomes and holobiont systems such as algae, sea sponges, and corals [42,43]. Emerging methods in functional metabolomics, including CRISPR-based gene editing, enable the assessment of metabolic responses to genetic changes, identifying metabolite-gene interactions that support drug discovery. For example, comprehensive metabolite profiling is applied to map spatial metabolomes within marine ecosystems, revealing interactions between organisms that have pharmaceutical potential, particularly for anticancer and antimicrobial applications [43]. This metabolomic shift from purely discovery-driven work to function-focused studies is helping to overcome challenges related to sample complexity and environmental variation, thereby enhancing marine chemical ecology and drug development pipelines.

4.3. Genomic, transcriptomic, and multi-omics

In marine specialized product research, multi-omics approaches—including genomics, transcriptomics, and metabolomics—have emerged as transformative methods for discovering novel bioactive compounds. By integrating these datasets, researchers can target specialized metabolites and their biosynthetic pathways, advancing beyond traditional activity-guided approaches. Genomic and transcriptomic analyses are instrumental in mining biosynthetic gene clusters (BGCs) that govern the production of complex marine natural products. These clusters can be identified and linked to specific compounds, significantly accelerating natural product discovery from marine microorganisms and algae, which possess unique metabolic pathways and specialized metabolites with potential therapeutic benefits [44].

Unravelling biosynthetic pathways involves collecting information on biosynthetic genes in addition to metabolite content. Concomitantly to the enrichment of metabolite databases, the ever-decreasing cost of sequencing technology has enabled the identification of genes closely associated with natural product pathways. Genomic has been especially successful in simple organisms such as marine bacteria and has led to the identification of biosynthetic gene cluster regions responsible for metabolites production, but this technique remains expensive for more complex and less characterized genomes. Transcriptomics is a more affordable technique that unravels transcripts' identity and modulation in their expression levels. Comparative genomics and/or transcriptomics studies performed on the same tissues as metabolomics correlate metabolites and transcripts levels, and the presence of associated candidate genes, leading to the identification of enzyme candidates and gene clusters. Omics science relies on rigorous experimental designs and powerful bioinformatics tools, that ease the analysis of large databases and integrate multiple types of information (genetic, epigenetic, transcriptomic, proteomic and metabolomic) to reveal interaction networks and biosynthetic pathways.

The multi-omics integration allows for a deeper understanding of organismal responses and adaptability in marine ecosystems, enabling researchers to explore chemical diversity and identify bioactive compounds with biomedical potential. For example, high-throughput sequencing techniques now allow for the mapping of genetic information and gene expression changes under different environmental conditions, revealing insights into adaptive metabolite production in

marine species. Coupled with advancements in bioinformatics, multi-omics frameworks support comprehensive metabolic profiling, offering valuable insights into the relationship between gene expression, metabolite production, and ecological roles in marine organisms [46].

In MSMs research as in other fields, genome-scale metabolic reconstructions have proven invaluable, providing insight into fucoxanthin biosynthesis pathway in *Isochrysis galbana,* for example [45]. This knowledge nourishes synthetic biology with stronger basis by pinpointing remote genetic targets and preventing unintended consequences that may not be evident through local pathway analysis alone.

4.4. Computational chemistry

Computational chemistry in marine natural products research has seen significant advancements recently, with various methods enhancing both the discovery and optimization of bioactive compounds. Key trends include the use of molecular docking, pharmacophore modeling, and molecular dynamics simulations to predict interactions between marine specialized metabolites and biological targets. Such techniques are invaluable for elucidating structure-activity relationships (SAR), which help scientists better understand how specific chemical modifications in marine compounds may alter their therapeutic potential. This has implications in drug discovery for antibiotics, antivirals, and anticancer agents, where marine compounds often exhibit unique structural characteristics developed under oceanic environmental pressures. Databases like the Comprehensive Marine Natural Products Database (CMNPD) now offer curated datasets with bioactivity, taxonomy, and structural data that support computational screening efforts by allowing researchers to efficiently explore chemical space, prioritize compounds for synthesis, and assess compound novelty through dereplication (comparing new compounds to known ones). This has streamlined the search for novel marine compounds with desirable pharmacokinetic and pharmacodynamic profiles, especially when paired with machine learning algorithms that can further refine compound selection based on previous successes and structure-property relationships. Moreover, platforms such as the “Marine Drugs” journal are supporting open-access research on synthetic strategies for marine natural products, which often integrates computational tools for molecular design and optimization. These efforts make the development of analogs more sustainable by allowing *in silico* assessments before laboratory synthesis and testing. Consequently, computational chemistry remains a central pillar in the evolving field of marine natural products, fostering rapid, targeted discovery of novel bioactives for pharmaceutical and biotechnological applications [47].

On the structural side, computational chemistry has revolutionized our knowledge of metabolites and biosynthetic enzyme functions. *In silico* prediction tools can resolve MSM pharmacophores (functional groups essential for their function) and anticipate the impact of structural modification to accelerate structure-based drug design. Using this approach, the potential of microcolin B lipopeptide isolated from algae *Lyngbya majuscule* as anticancer agent was optimized, and its molecular target was uncovered [48]. Concomitantly, the spectacular ongoing progress in accuracy of *ab initio* and homology-based protein modeling artificial intelligence system, such as AlphaFold developed by DeepMind [49], has facilitated the resolution of enzyme structures and of their functions, providing evidence of their most probable ligands through virtual screening. Such approach remains at an early age for the MSMs field, but some promising potential antiviral candidates were identified in the marine seaweed database [50]. Even though experimental data remains necessary to confirm any *in silico*-based hypothesis, these tools will deepen our understanding of metabolic pathways and accelerate drug design.

Advancements in bioinformatics, molecular biology, and an improved understanding of metabolic pathways with combined omics analysis have afforded researchers unprecedented control over the intricate biosynthetic processes. In recent years, all this knowledge culminated to enrich metabolic engineering strategies, emerging as a promising approach to enhance natural product synthesis and create novel metabolites.

5. Biotechnological applications

5.1. Marine specialized metabolites in biotechnology

Marine organisms offer a rich reservoir of specialized metabolites with diverse biological activities, including cytotoxic, antioxidant, antimicrobial, and more; holding great promise for applications in nutraceuticals, pharmaceuticals, cosmeceuticals, and various bio-based products. The vast potential of marine biomass as a source material for biofuels, bioplastics, and biomaterials further underscores the burgeoning field of marine biotechnology. Despite having characterized over 35,000 specialized metabolites from marine organisms [51], there is a wealth of untapped biodiversity within marine systems (Fig. 2). Yet, only a small fraction of marine species can be cultivated under laboratory conditions. As marine biotechnology is still in its nascent stages, the establishment of collaborative, transnational networks is imperative for knowledge sharing, training, best practice dissemination, and identifying emerging technological trends. Such collaboration is essential to drive innovation, facilitate commercialization, and contribute to developing a circular bioeconomy [51]. Specialized metabolites derived from marine sources have become a cornerstone of therapeutic agents, with the World Health Organization reporting that roughly 80 % of the global population relies on natural remedies for basic health concerns. The discovery of MSM has grown exponentially since the 1990s [52,53]. These metabolites exhibit remarkable structural diversity and a wide range of activities, making them a focal point for research programs promoting innovation, industrial applications, and the growth of biotechnology-oriented enterprises. The unique properties of marine specialized metabolites have ignited scientific interest, fostering a competitive environment for the biotechnology sector while contributing to job creation and economic development.

5.2. Bioengineering strategies

Specialized metabolites confer a selective advantage upon their producing organisms, albeit at a metabolic cost. They are produced just enough to maintain host’s edge, limiting our ability to harvest them, and consequently/possibly slowing commercial interest. In addition, extraction of metabolites directly from marine hosts that grow in remote areas is impractical and may endanger species or ecosystems. In recent years, metabolic engineering has emerged as a promising approach to enhance natural product synthesis and create novel metabolite molecules with enhanced pharmacological properties [54]. Bioengineering to synthetize *de novo* a MSM of interest or increase its yield requires prior information on its structure and biosynthetic pathway, a knowledge that relies on the multiple analytical techniques detailed above. Metabolic engineering involves the deliberate genetic modification of organisms to bolster their metabolic capabilities. Several approaches can be employed to direct or redirect metabolic flux towards the desired metabolite. These include increasing the availability of precursor molecules, optimizing the efficiency of bottleneck enzymes, modifying gene expression regulation, reducing the diversion of resources towards unwanted by-products or competing pathways, and even reconstituting entire biosynthetic pathways within a heterologous host [55]. Innovations in protein engineering has resulted in improved enzyme efficiency and the ability to customize enzyme functions to a remarkable degree [56,57]. Microalgae have been directly engineered to increase endogenous metabolites production or to produce *de novo* exogeneous metabolites [58,59]. Boosting macroalgae potential rather uses an indirect approach that targets the genetic modification of the interacting microbiome instead [60]. In a few microalgae species, episomal expression or nuclear integration of transgenes delivered through

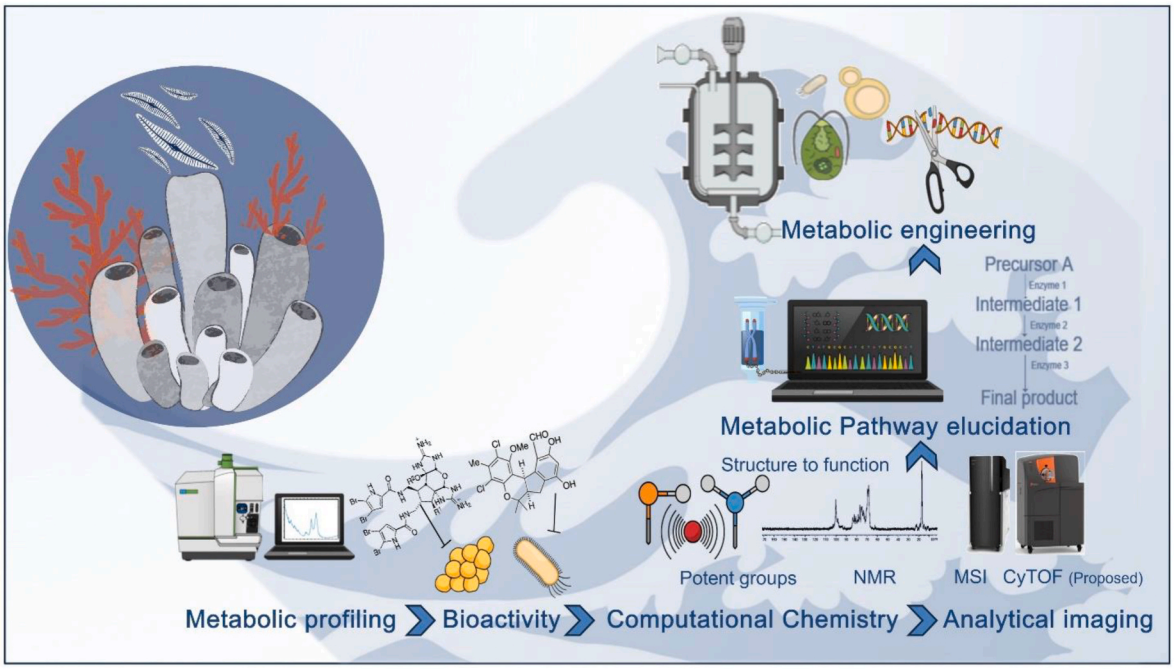

Fig. 2. Pipeline illustrating the integration of marine specialized metabolite isolation, biosynthetic pathways and biotechnological applications.

electroporation, conjugation, or biolistic with the help of CRISPR nucleases or CRISPR-related interference or activation are used to introduce new genes or modulate gene expression [61,62]. Through these means, metabolic engineering promises to unlock the full potential of MSM biosynthesis for diverse industrial applications in a sustainable manner.

5.3. *Contribution of analytical imaging techniques to bioengineering*

Imaging techniques have also greatly contributed to our comprehension of metabolic pathways and paved the way for metabolic engineering. This has been made possible through the expression of transgene of interest fused of various fluorescent proteins encoding genes, and because of high endogenous levels of autofluorescent substances such as pigments (chlorophyll *a and b*, carotenoids, fucoxanthin, astaxanthin [63]) or lipids [64]. Indeed, many SMs that contain benzene or triazole rings have the ability to release absorbed light energy in the form of fluorescence. SMs can also be directly tagged to fluorescence probes, or modified to fluoresce more, enabling spatio-temporal studies that unwind their subcellular localization and cellular uptake [65]. The impact of the modification on the metabolite properties (permeability) should be considered in this kind of analyses.

Imaging techniques to track protein and metabolite accumulation have answered fundamental questions on specialized metabolites role. Confocal microscopy enables the identification of enzyme compartmentalization in natural hosts, which generates knowledge to optimize bioengineering that targets specific enzymes and the metabolite they produce to specific cellular sites. In transgenic hosts, it can be used to validate expression in the desired compartment. Single cell marine organisms such as microalgae, or protoplasts of seaweeds can also be analyzed by flow cytometry, which measures the fluorescence levels per individual cell and provide a frequency of fluorescent cells, that can then be enriched by fluorescence activating cells sorting. These techniques have reduced the time to screen and select for strains with interesting traits, *i.e* that produce desired transgenes. Coupling fluorescence techniques with analytical techniques will help progress in these fields and harness the power of autofluorescence. Mass cytometry (CyTOF) exists, but this technique detects the heavy metal ion labeled antibodies that stain targeted proteins with a time-of-flight mass spectrometer, rather than measuring endogenous metabolites. Usually, it requires prior knowledge on the targeted enzyme, and the production of specific antibodies. Interestingly, it has been used to detect silver or gold nanoparticles accumulation in bacteria and freshwater unicellular organisms [66,67], and to profile metabolic characteristics in human cells through the measurement of metabolic proteins levels. However, to date, there is no published studies in the context of MSMs. Still, its development, and the increasing sensitivity and specificity of flow cytometer fluorescence detectors hint that direct detection methods of metabolite-producing cells based on compound mass or endogenous fluorescence will be possible in the near future (Fig. 2).

Perhaps the imaging technology that revolutionized the most our comprehension of metabolic pathway and their functional role in the last 20 years is mass spectrometry imaging (MSI) [68–70]. At the interface of chemistry and biology, high resolution MSI enables a molecular view on the location and quantification of a specific SM in a biological tissue, and up to the organelle level of a single cell (down to 0.25 μM resolution [71]. MSI includes various direct soft and ambient ionizations MS, low-temperature plasma ionization, direct analysis in real time, electrospray desorption/ionization, desorption electrospray ionization, laser desorption ionization (LDI), matrix assisted LDI (MALDI)-MSI with sophisticated laser optics and Orbitrap MS, nanotechnology-assisted laser desorption/ionization, and others. These techniques reveal the *in vivo* physiological properties of SMs. They have been used, for example, to unravel the physical location and quantities of antifungal SMs in the surface tissues of a tropical red alga [69]; to detect inorganic and organic iodine species in seaweeds, helping to better understand radioactive and stable iodine uptake mechanisms in the marine environment [72]; but also to unravel a metabolic shift towards C15:0-based lipids during viral infection of bloom-forming alga *Emiliania huxleyi* using in plaque-MSI [73]. This technology generates key information on metabolites cellular localization and fate, which is key to optimize bioengineering strategies.

Bioengineering relies on prior knowledge and on a powerful production platform. MSMs, despite their vast potential, are at an early stage in this field. To prevent endangering species and facilitating access to therapeutic metabolites, more efforts should be focused on elucidating pathway first, and then on bioengineering strategies, increasing the fundamental knowledge required upstream, and testing various

heterologous hosts. Microalgae present unique advantages for MSMs synthesis over the traditional bioengineered hosts yeast and bacteria, such as a complete photosynthetic machinery. They offer a significant opportunity to synthetize MSM that would be otherwise inaccessible.

6. Challenges and future perspectives

6.1. Sustainability and conservation

Exploring MSM holds incredible promise. However, this rapidly growing field is accompanied by pressing challenges, primarily revolving around sustainability and conservation. Striking a balance between the surging demand for these invaluable metabolites and protecting vulnerable marine ecosystems is paramount. Embracing sustainable collection practices and proactive conservation measures is essential to navigate this intricate landscape. By responsibly managing marine resources, we can safeguard the long-term availability of these invaluable chemical compounds from the deep blue. International collaborations and robust marine conservation efforts are pivotal in preserving the world's oceans' biodiversity and ecological equilibrium. In sum, sustainability and conservation represent the requirement for harnessing the potential of MSM for future generations.

Marine-based drug discovery has ushered in a new era with approved MSMs for conditions like cancer and pain, alongside ongoing clinical trials. However, persistent challenges are tied to MSMs' low bioavailability and structural complexity. Their production relies heavily on costly and unsustainable marine extraction and chemical synthesis, leading to severe environmental repercussions. The introduction of the Nagoya Protocol (https://www.cbd.int/abs/), born from the Convention on Biological Diversity (CBD) in 2014, aims to regulate access and benefit-sharing agreements for genetic resources, primarily within territorial seas and exclusive economic zones. As bioprospecting concentrates on these regions, questions arise regarding the unregulated deep-sea exploration ventures beyond national boundaries [74]. The United Nations Convention on the Law of the Sea provides some reference for sustainable marine resource use but needs to address ABS in open ocean research. Thus, a comprehensive framework for benefit-sharing from open ocean research is imperative, potentially transcending bilateral and multilateral agreements to ensure equitable distribution of benefits among nations. The policy landscape navigates complex waters as legal frameworks evolve to secure benefits for resource providers based on the knowledge gained from collected organisms. While the CBD and the Nagoya Protocol seek to establish international guidelines for resource access, equitable benefit-sharing, and sustainability, their impact on biodiversity and research collaboration remains uncertain, posing potential challenges to conservation and international cooperation, particularly between developed and biodiversity-rich nations [75].

Furthermore, while well-established in ecology and conservation, biogeographic classifications need to be more utilized in MSM research despite their potential to guide efforts from governance to resource management and conservation. Assessing the impact of sampling sites is crucial for monitoring chemical diversity, given the pressing issues of biodiversity loss resulting from over-exploitation and habitat degradation in marine conservation. Notably, regions with high biodiversity and chemical diversity must be preserved, as they may be vital to unlocking future MSMs.

6.2. Emerging trends in marine metabolite research

Our knowledge of marine organisms and ecosystems has grown considerably in recent years, unveiling an ever-increasing number of species and novel molecules. However, the vast extent of unexplored marine life remains astonishing. Marine alkaloids such as marineosin stand as a beacon of hope in the realm of specialized metabolite studies and bioengineering. Its success story offers promise and lays a sturdy foundation for future research endeavours, particularly in exploring other marine-derived compounds. By unlocking the secrets of this alkaloid, scientists pave the way for innovative approaches and methodologies to harness the potential of specialized metabolites for various applications [76]. Despite technological advances, the study of marine organisms remains challenging.

Metabolomics has emerged as a promising approach to expedite the discovery of beneficial metabolites and deepen our understanding of their functions within organisms and their interactions with the marine environment. It offers insights into interactions between organisms and their symbionts, biosynthetic pathways, and compound transfer [77]. Metabolomics offers a mean to accelerate the discovery of biologically active metabolites and explore an organism's chemical diversity changes in response to various factors. Understanding the link between environmental or biological conditions and metabolite production has profound implications for bioprospecting and upscaling. Integrating metabolomics with metagenomics provides prospects for producing metabolites, primarily through synthetic biology and bioengineering, driving advancements in the field [78].

However, a significant hurdle in metabolomics studies is the identification of specific metabolites. Many metabolites remain undetectable within complex mixtures, necessitating isolation for precise identification. Limited by small sample quantities, the isolation process has led to the development of microscale isolation protocols [79,80]. Moreover, understanding the transfer of metabolites among marine organisms and their interactions is essential. Although examining exuded metabolites poses challenges due to rapid dilution, laboratory experiments under controlled conditions and filtration systems can concentrate these metabolites for analysis [81].

The full potential of metabolomics for discovering marine bioactive metabolites is hindered by the limited coverage of marine specialized metabolites in public databases. The absence of reference spectra impedes automated dereplication, necessitating labour-intensive in-house library development. There is a pressing need for a free-access database with well-curated spectrometric data on marine specialized metabolites [56]. Simultaneously, continuous enhancements in metabolomics tools are essential to improve sensitivity and repeatability. Integrating known and newly discovered marine specialized metabolites into widely used metabolomics databases represents a practical solution that promises significant advancements in the field.

7. Conclusion

Marine specialized metabolites (MSMs) represent a rich source of bioactive compounds with diverse applications in biotechnology, medicine, and environmental science. Advances in extraction, isolation, and analytical methods, coupled with multi-omics and bioengineering approaches, are rapidly enhancing our understanding and utilization of MSMs. However, sustainable bioprospecting and ethical benefit-sharing remain critical to prevent ecological impacts and ensure fair practices.

Looking forward, integrating advanced analytical techniques and sustainable practices will be essential to fully realize the potential of MSMs. With interdisciplinary collaboration, the field is poised to generate new, impactful applications, underscoring the importance of marine biodiversity as a valuable resource for innovation.

CRediT authorship contribution statement

Thilina U. Jayawardena: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Conceptualization. **Natacha Merindol:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation. **Nuwan Sameera Liyanage:** Writing – review & editing, Visualization, Validation, Investigation. **Fatima Awwad:** Writing – review & editing, Visualization, Validation. **Isabel Desgagné-Penix:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

[1] X.Q. Huang, N. Dudareva, Curr. Biol. 33 (2023) R473.
[2] D.B. Sudatti, H.M. Duarte, A.R. Soares, L.T. Salgado, R.C. Pereira, Front. Plant Sci. 11 (2020) 347.
[3] D. Thirumurugan, A. Cholarajan, S. Raja, R.J.S. m, s. Vijayakumar, applications (2018) 3.
[4] C. Fang, A.R. Fernie, J. Luo, Trends Plant Sci. 24 (2019) 83.
[5] G.S. Dos Santos, T.L. de Souza, T.R. Teixeira, J.P.C. Brandao, K.A. Santana, L.H. S. Barreto, S.S. Cunha, D. Dos Santos, C.R. Caffrey, N.S. Pereira, A. de Freitas Santos Junior, Molecules 28 (2023).
[6] A. Hussain, M.L. Bourguet-Kondracki, M. Majeed, M. Ibrahim, M. Imran, X. W. Yang, I. Ahmed, A.A. Altaf, A.A. Khalil, A. Rauf, P. Wilairatana, H.A. Hemeg, R. Ullah, I.R. Green, I. Ali, S.T.A. Shah, H. Hussain, Biomed. Pharmacother. 159 (2023) 114165.
[7] L.T. Tan, Mar. Drugs 21 (2023).
[8] R.G.S. Berlinck, C.M. Crnkovic, J.R. Gubiani, D.I. Bernardi, L.P. Ioca, J.I. Quintana-Bulla, Nat. Prod. Rep. 39 (2022) 596.
[9] S.S. Ebada, R.A. Edrada, W. Lin, P. Proksch, Nat. Protoc. 3 (2008) 1820.
[10] Q.-W. Zhang, L.-G. Lin, W.-C. Ye, Chin. Med. 13 (2018) 20.
[11] J.E. Sosa-Hernandez, Z. Escobedo-Avellaneda, H.M.N. Iqbal, J. Welti-Chanes, Molecules 23 (2018) 2953.
[12] M. Usman, M. Nakagawa, S. Cheng, 11 (2023) 3444.
[13] R. Martins, A. Barbosa, B. Advinha, H. Sales, R. Pontes, J. Nunes, 11 (2023) 2255.
[14] A. Choudhary, L.M. Naughton, I. Montánchez, A.D.W. Dobson, D.K. Rai, 15 (2017) 272.
[15] M. DiBello, A.R. Healy, H. Nikolayevskiy, Z. Xu, S.B. Herzon, Acc. Chem. Res. 56 (2023) 1656.
[16] A.-H.M. Emwas, in: J.T. Bjerrum (Ed.), The Strengths and Weaknesses of NMR Spectroscopy and Mass Spectrometry with Particular Focus on Metabolomics Research, Springer New York, New York, NY, 2015, p. 161.
[17] A.H. Emwas, R. Roy, R.T. McKay, L. Tenori, E. Saccenti, G.A.N. Gowda, D. Raftery, F. Alahmari, L. Jaremko, M. Jaremko, D.S. Wishart, Metabolites 9 (2019) 123.
[18] C. Seger, S. Sturm, H. Stuppner, Nat. Prod. Rep. 30 (2013) 970.
[19] S. Grimalt, J.V. Sancho, O.J. Pozo, F. Hernandez, J. Mass Spectrom. 45 (2010) 421.
[20] A.C. Pozzer, P.A. Gomez, J. Weiss, Sci. Total Environ. 838 (2022) 156155.
[21] M.S. Savoca, G.A. Nevitt, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 4157.
[22] J.R. Pawlik, C.D. Amsler, R. Ritson-Williams, J.B. McClintock, B.J. Baker, V.J. Paul, Marine Chemical Ecology: A Science Born of ScubaM.A. Lang, R.L. Marinelli, S. J. Roberts, P.R. Taylor (Eds.), in: Research and Discoveries: The Revolution of Science through Scuba, 39, Smithsonian Institution Scholarly Press, Washington, DC, 2013, pp. 53–69. In Smithsonian Contributions to the Marine Sciences, https://doi.org/10.5479/si.1943667X.39.53.
[23] J.R. Pawlik, Bioscience 61 (2011) 888.
[24] J. Qin, E. Kurt, L.B. T, L. Sa, D. Xie, Front. Microbiol. 14 (2023) 1280296.
[25] D.S. Dissanayake, D.P. Nagahawatta, J.S. Lee, Y.J. Jeon, Mar. Drugs 22 (2024).
[26] J. Wang, Z. Wang, Y. Sun, D. Liu, Biochem. Biophys. Res. Commun. 512 (2019) 473.
[27] J. Lee, J.N. Currano, P.J. Carroll, M.M. Joullie, Nat. Prod. Rep. 29 (2012) 404.
[28] F. Berrue, M.W. McCulloch, R.G. Kerr, Bioorg. Med. Chem. 19 (2011) 6702.
[29] R. Wang, M.R. Seyedsayamdost, Nat. Rev. Chem 1 (2017) 21.
[30] A. Miyanaga, F. Kudo, T. Eguchi, Nat. Prod. Rep. 35 (2018) 1185.
[31] J. Shang, B. Hu, J. Wang, F. Zhu, Y. Kang, D. Li, H. Sun, D.X. Kong, T. Hou, J. Chem. Inf. Model. 58 (2018) 1182.
[32] C. Pinedo-Rivilla, J. Aleu, R. Duran-Patron, Mar. Drugs 20 (2022) 84.
[33] Q. Chen, Y. Chen, Q. Hu, D. Han, Bioresour. Technol. 374 (2023) 128811.
[34] V. Shulaev, G. Isaac, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1092 (2018) 499.
[35] W. Zhang, T. Hankemeier, R. Ramautar, Curr. Opin. Biotechnol. 43 (2017) 1.
[36] F.K. Pilatti, F. Ramlov, E.C. Schmidt, C. Costa, E.R. Oliveira, C.M. Bauer, M. Rocha, Z.L. Bouzon, M. Maraschin, Mar. Pollut. Bull. 114 (2017) 831.
[37] R. Verpoorte, Encyclopedia of Analytical Science, Elsevier, Amsterdam, The Netherlands, 2005.
[38] L.M. Bayona, N.J. de Voogd, Y.H. Choi, Metabolomics 18 (2022) 17.
[39] B.L. Nannenga, T. Gonen, Nat. Methods 16 (2019) 369.
[40] L.J. Kim, M. Ohashi, Z. Zhang, D. Tan, M. Asay, D. Cascio, J.A. Rodriguez, Y. Tang, H.M. Nelson, Nat. Chem. Biol. 17 (2021) 872.
[41] J.D. Park, Y. Li, K. Moon, E.J. Han, S.R. Lee, M.R. Seyedsayamdost, Angew Chem. Int. Ed. Engl. 61 (2022) e202114022.
[42] T. Shi, B. Wang, D.-L. Zhao, D.J. Newman, 11 (2024).
[43] G.A. Vitale, C. Geibel, V. Minda, M. Wang, A.T. Aron, D. Petras, Nat. Prod. Rep. 41 (2024) 885.
[44] C. Lauritano, M.I. Ferrante, A. Rogato, 17 (2019) 269.
[45] D. Chen, X. Yuan, X. Zheng, J. Fang, G. Lin, R. Li, J. Chen, W. He, Z. Huang, W. Fan, L. Liang, C. Lin, J. Zhu, Y. Chen, T. Xue, Dev. Reprod. Biol. 20 (2022) 1138.
[46] L.K. Caesar, R. Montaser, N.P. Keller, N.L. Kelleher, Nat. Prod. Rep. 38 (2021) 2041.
[47] C. Lyu, T. Chen, B. Qiang, N. Liu, H. Wang, L. Zhang, Z. Liu, Nucleic Acids Res. 49 (2020) D509.
[48] F.L. Li, V. Fu, G. Liu, T. Tang, A.W. Konradi, X. Peng, E. Kemper, B.F. Cravatt, J. M. Franklin, Z. Wu, J. Mayfield, J.E. Dixon, W.H. Gerwick, K.L. Guan, Nat. Chem. Biol. 18 (2022) 1076.
[49] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Zidek, A. Potapenko, A. Bridgland, C. Meyer, S.A. A. Kohl, A.J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A.W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Nature 596 (2021) 583.
[50] S.I. Islam, S.S. Ahmed, N. Habib, M.A. Ferdous, S. Sanjida, M.J. Mou, Heliyon 9 (2023) e16383.
[51] A. Rotter, A. Bacu, M. Barbier, F. Bertoni, A.M. Bones, M.L. Cancela, J. Carlsson, M. F. Carvalho, M. Cegłowska, M.C. Dalay, T. Dailianis, I. Deniz, D. Drakulovic, A. Dubnika, H. Einarsson, A. Erdoğan, O.T. Eroldoğan, D. Ezra, S. Fazi, R. J. FitzGerald, L.M. Gargan, S.P. Gaudêncio, N. Ivošević DeNardis, D. Joksimovic, M. Kataržytė, J. Kotta, M. Mandalakis, I. Matijošytė, H. Mazur-Marzec, A. Massa-Gallucci, M. Mehiri, S.L. Nielsen, L. Novoveská, D. Overlingė, M.E. Portman, K. Pyrc, C. Rebours, T. Reinsch, F. Reyes, B. Rinkevich, J. Robbens, V. Rudovica, J. Sabotič, I. Safarik, S. Talve, D. Tasdemir, X.T. Schneider, O.P. Thomas, A. Toruńska-Sitarz, G.C. Varese, M.I. Vasquez, Front. Mar. Sci. 7 (2020).
[52] U. Lindequist, Biomol Ther (Seoul) 24 (2016) 561.
[53] A.R. Carroll, B.R. Copp, R.A. Davis, R.A. Keyzers, M.R. Prinsep, Nat. Prod. Rep. 36 (2019) 122.
[54] L.B. Pickens, Y. Tang, Y.H. Chooi, Annu. Rev. Chem. Biomol. Eng. 2 (2011) 211.
[55] K. Scherlach, C. Hertweck, Org. Biomol. Chem. 7 (2009) 1753.
[56] M. Reverter, S. Rohde, C. Parchemin, N. Tapissier-Bontemps, P.J. Schupp, Front. Mar. Sci. 7 (2020) 613471.
[57] Z. Yang, C. Liu, Y. Wang, Y. Chen, Q. Li, Y. Zhang, Q. Chen, J. Ju, J. Ma, ACS Synth. Biol. 11 (2022) 3772.
[58] E. Fantino, F. Awwad, N. Merindol, A.M. Diaz Garza, S.-E. Gélinas, G.C. Gajón Robles, A. Custeau, F. Meddeb-Mouelhi, I. Desgagné-Penix, Algal Res. 77 (2024) 103379.
[59] F. Awwad, E.I. Fantino, M. Heneault, A.M. Diaz-Garza, N. Merindol, A. Custeau, S. E. Gelinas, F. Meddeb-Mouelhi, J. Li, J.F. Lemay, B.J. Karas, I. Desgagne-Penix, Int. J. Mol. Sci. 24 (2023) 16624.
[60] Y. Sasaki, Y. Yoshikuni, Metab. Eng. 71 (2022) 42.
[61] A.P. Nievergelt, D.R. Diener, A. Bogdanova, T. Brown, G. Pigino, Cell Rep Methods 3 (2023) 100562.
[62] Y. Bai, T. Cao, O. Dautermann, P. Buschbeck, M.B. Cantrell, Y. Chen, C.D. Lein, X. Shi, M.A. Ware, F. Yang, H. Zhang, L. Zhang, G. Peers, X. Li, M. Lohr, Proc. Natl. Acad. Sci. U. S. A. 119 (2022) e2203708119.
[63] F. Perozeni, S. Cazzaniga, T. Baier, F. Zanoni, G. Zoccatelli, K.J. Lauersen, L. Wobbe, M. Ballottari, Plant Biotechnol. J. 18 (2020) 2053.
[64] M. Terashima, E.S. Freeman, R.E. Jinkerson, M.C. Jonikas, Plant J. 81 (2015) 147.
[65] D.B. Sung, J.S. Lee, RSC Med. Chem. 14 (2023) 412.
[66] Y. Guo, S. Baumgart, H.J. Stark, H. Harms, S. Muller, Front. Microbiol. 8 (2017) 1326.
[67] Q. Wu, J. Shi, X. Ji, T. Xia, L. Zeng, G. Li, Y. Wang, J. Gao, L. Yao, J. Ma, X. Liu, N. Liu, L. Hu, B. He, Y. Liang, G. Qu, G. Jiang, ACS Nano 14 (2020) 12828.
[68] L. Nyadong, E.G. Hohenstein, A. Galhena, A.L. Lane, J. Kubanek, C.D. Sherrill, F. M. Fernandez, Anal. Bioanal. Chem. 394 (2009) 245.
[69] A.L. Lane, L. Nyadong, A.S. Galhena, T.L. Shearer, E.P. Stout, R.M. Parry, M. Kwasnik, M.D. Wang, M.E. Hay, F.M. Fernandez, J. Kubanek, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 7314.
[70] E. Esquenazi, P.C. Dorrestein, W.H. Gerwick, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 7269.
[71] S. Ma, Y. Leng, X. Li, Y. Meng, Z. Yin, W. Hang, TrAC, Trends Anal. Chem. 159 (2023) 116902.
[72] D. Lebeau, N. Leroy, D. Doizi, T.D. Wu, J.L. Guerquin-Kern, L. Perrin, R. Ortega, C. Voiseux, J.B. Fournier, P. Potin, B. Fievet, C. Leblanc, J. Environ. Radioact. 231 (2021) 106552.
[73] G. Schleyer, N. Shahaf, C. Ziv, Y. Dong, R.A. Meoded, E.J.N. Helfrich, D. Schatz, S. Rosenwasser, I. Rogachev, A. Aharoni, J. Piel, A. Vardi, Nat Microbiol 4 (2019) 527.
[74] O. Heffernan, Nature 580 (2020) 20.
[75] S.o.t.C.o.B. Diversity, Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity: Text and Annex, UN, 2011.
[76] S.M. Salem, P. Kancharla, G. Florova, S. Gupta, W. Lu, K.A. Reynolds, J. Am. Chem. Soc. 136 (2014) 4565.
[77] S. Mascuch, J. Kubanek, Science 364 (2019) 1034.

[78] Y. Ahmed, Y. Rebets, M.R. Estevez, J. Zapp, M. Myronovskyi, A. Luzhetskyy, Microb. Cell Factories 19 (2020) 5.
[79] D.G. Kingston, J. Nat. Prod. 74 (2011) 496.
[80] A.G. Newsome, B.T. Murphy, R.B. van Breemen, Isolation and characterization of natural blue pigments from underexplored sources, Am. Chem. Soc. (2013) 105.
[81] L. Wegley Kelly, C.E. Nelson, D. Petras, I. Koester, Z.A. Quinlan, M.G.I. Arts, L. F. Nothias, J. Comstock, B.M. White, E.C. Hopmans, F.C. van Duyl, C.A. Carlson, L. I. Aluwihare, P.C. Dorrestein, A.F. Haas, Proc. Natl. Acad. Sci. U. S. A. 119 (2022) e2110283119.