Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.43379

Integrating zooplankton biomarkers within the European Marine Strategy Framework Directive (MSFD) assessment

Maria PROTOPAPA[1], Inma HERRERA[2], Wilhelm HAGEN[3], Rolf KOPPELMANN[4], Jonna ENGSTRÖM-ÖST[5], Catherine TSANGARIS[1], Soultana ZERVOUDAKI[1], and Lidia YEBRA[6]

[1] Institute of Oceanography, Hellenic Centre for Marine Research, 46.7 km Athens-Sounio ave., 19013 Anavyssos, Attiki, Greece
[2] Research Group in Biodiversity & Conservation, Research Institute ECOAQUA, University of Las Palmas de Gran Canaria, Scientific & Technological Marine Park, Crta. Taliarte s/n, 35214, Telde, Spain
[3] Universität Bremen, BreMarE – Bremen Marine Ecology, Marine Zoology, P.O. Box 330440, 28334 Bremen, Germany
[4] University of Hamburg, Institute of Marine Ecosystem and Fishery Science, Große Elbstraße 133, 22767 Hamburg, Germany
[5] Novia University of Applied Sciences, Raseborgsvägen 11, FI-10600 Ekenäs, Finland
[6] Centro Oceanográfico de Málaga (IEO, CSIC), 29002, Málaga, Spain

Corresponding author: Maria PROTOPAPA; mariaprot@hcmr.gr

Contributing Editor: Maria Grazia MAZZOCCHI

Received: 21 November 2025; Accepted: 10 March 2026; Published online: 29 May 2026

Abstract

Biomarkers are increasingly recognized as sensitive indicators of physiological and ecological responses in marine organisms, offering new perspectives for monitoring and ecosystem status assessment. Traditional indices of species composition and biomass, though valuable, often fail to capture early sublethal or functional changes in zooplankton communities. To explore the potential of biomarkers as complementary tools for environmental monitoring within the European Marine Strategy Framework Directive (MSFD), the EuroMarine workshop BIOZOO gathered European experts to evaluate the use of enzyme activities, oxidative stress markers, fatty acids, and stable isotopes for zooplankton-based assessments. Participants identified methodological priorities, including protocol standardization, inter-laboratory calibration, and baseline development, to ensure comparability across regions. This synthesis highlights how integrating biochemical and physiological biomarkers into existing monitoring frameworks can improve the detection of ecosystem responses to environmental pressures such as warming, pollution, and eutrophication. Strengthening biomarker applications in European Seas represents a step toward a more functional, harmonized, and responsive approach to pelagic habitats assessment.

Keywords: zooplankton monitoring; biomarkers; food webs; Marine Strategy Framework Directive; pelagic habitats.

Introduction

To address anthropogenic pressures—including eutrophication, pollution, and underwater noise—the European Union has implemented two key legislative frameworks. The Water Framework Directive (WFD; 2000/60/EC) aims to prevent further deterioration and to achieve good ecological and chemical status of inland surface waters, transitional waters, coastal waters, and groundwater. Complementing this, the Marine Strategy Framework Directive (MSFD; 2008/56/EC) seeks to protect the marine environment across Europe, promoting the sustainable use of marine resources while ensuring the maintenance or restoration of marine biodiversity. To operationalize this goal, the MSFD outlines 11 qualitative Descriptors (D1–D11; Table 1) that define Good Environmental Status (GES), addressing elements such as biodiversity, food web structure, and the presence of contaminants.

Phytoplankton have long been used as indicators of nutrient status in aquatic ecosystems (Garmendia *et al*., 2013). In contrast, zooplankton have historically been underrepresented in biomonitoring programs. However, the systematic development, coordination, and application of zooplankton indicators have recently expanded (Caroppo *et al*., 2013), driven largely by the MSFD's mandate to incorporate plankton into GES assessments, particularly for descriptors related to biodiversity, food webs, and eutrophication (Gorokhova *et al*., 2013, 2016; HELCOM, 2018; McQuatters-Gollop *et al*., 2019; Magliozzi *et al*., 2021).

To enhance marine protection and monitoring efforts, scientists have developed index-based methods to assess community richness, abundance, and biomass (Desrosiers *et al*., 2013). Biomarkers, however, offer complementary advantages by providing early-warning signals

Table 1. Marine Strategy Framework Directive (MSFD) Descriptors: full names and classification as indicators of environmental pressure or ecosystem state.

Descriptor	Name	Type
D1	Marine biodiversity	State
D2	Non-indigenous species	Pressure
D3	Commercial fish and shellfish	Pressure
D4	Food webs	State
D5	Eutrophication	Pressure
D6	Seabed integrity	State
D7	Hydrographical conditions	Pressure
D8	Contaminants	Pressure
D9	Contaminants in seafood	Pressure
D10	Marine litter	Pressure
D11	Energy including underwater noise	Pressure
Climate change	It is not a specific descriptor under the Marine Directive; however, its impacts on hydrographical conditions are fully covered by Descriptor 7.	Pressure

and long-term data on cellular, molecular, and tissue-level responses to environmental stressors (Herrera *et al*., 2024a). Various biomarkers, either individual or in combination, offer insights into biodiversity shifts, changes in food quality and quantity, pollution effects in various taxa (e.g., mussels, fish) and species-specific vulnerability to environmental stressors, such as heatwaves (Lam, 2009). Biomarkers also offer the advantage of enabling site-specific assessments of ecosystem health.

To ensure the effective application of biomarkers across European marine waters, it is essential to establish standardized quality assurance protocols, including inter-laboratory calibrations and the development of baseline data to distinguish natural variability from human-induced stress (Desrosiers *et al*., 2013). In this context, we highlight the need to integrate innovative tools, such as biomarkers, within the MSFD. Expanding their use in marine monitoring will enhance environmental assessments and significantly improve our capacity to detect and respond to anthropogenic impacts on marine ecosystems.

To explore the integration of biomarkers into MSFD monitoring frameworks, the EuroMarine-funded workshop BIOZOO was held in November 2022. The primary aim of the workshop was to evaluate the feasibility, standardization, and applicability of biomarker-based approaches —such as enzyme activities, oxidative stress markers, fatty acid profiles, and stable isotope ratios— for routine zooplankton monitoring across European waters. Here we synthesize the expert perspectives, highlight current methodological advances, identify existing challenges, and propose a roadmap for harmonized biomarker implementation within the MSFD. As the third MSFD implementation cycle takes off, there is growing momentum to incorporate functional, process-based indicators—such as zooplankton biomarkers for holo- and meroplankton—to enhance pelagic habitats and food web assessments, complementing traditional abundance and biomass metrics.

Historical and current application of biomarkers in marine environment

The application of biomarkers in environmental assessment originated in pharmacology and medical toxicology before being adapted to marine ecosystem monitoring (McCarty *et al*., 1993). Biomarker approaches applied to zooplankton communities in marine and brackish environments serve as valuable tools for evaluating ecotoxicological risks and providing early warnings about ecosystem health (Minutoli *et al*., 2007). Planktonic organisms are particularly suitable for marine ecological assessments due to their crucial role in biogeochemical cycles and ecosystem functioning. They form the base of the food web and exhibit rapid responses (abundance and/or diversity) to environmental changes or pressures. Zooplankton hold significant potential as an indicator of environmental changes and stressors (Beaugrand *et al*., 2008). Additionally, unlike species affected by fishing activities, zooplankton are not commercially harvested in Europe (Zervoudaki *et al*., 2020). As a result, variations in their abundance and diversity can be attributed exclusively to environmental factors.

Biomarkers help shift from *taxonomy-based* to *functionally informative* indicators. A range of biomarkers, including those assessing respiratory metabolism (electron transport system activity, ETS; Packard *et al*., 1971), growth rates (aminoacyl t-RNA synthetases activity, AARS; Yebra & Hernández-León, 2004), oxidative stress responses (Halliwell & Gutteridge, 2007), stable isotopes (Peterson & Fry, 1987), and fatty acids (Dalsgaard *et al*., 2003; Parrish, 2013), can be applied to field-collected zooplankton with reasonably affordable laboratory preparation (Table 2). Integrating these approaches enhances our understanding of zooplankton ecology, predator-prey relationships, and ecosystem dynamics.

In the 1970s, Packard *et al*. pioneered the use of enzyme activities, such as the ETS method, as biomarkers to

Table 2. Key biomarkers for zooplankton-based marine ecosystem monitoring and assessment.

Biomarker	Ecological Indicator	Primary Application	Advantages	Limitations	Original description	Descriptors
ETS (Electron Transport System) activity	Respiratory metabolism	Estimates potential respiration rates and oxygen demand	Sensitive to environmental stress	Requires freezing samples in liquid nitrogen	Packard *et al.*, 1971	D1, D4, D8
AARS (Aminoacyl-tRNA synthetases) activity	Growth rate	Measures protein synthesis rates as a proxy for growth	Assessment of field zooplankton production; responsive to food and temperature	Requires freezing samples in liquid nitrogen	Yebra & Hernández-León, 2004	D1, D4, D8
Stable Isotopes ($\delta^{13}C$, $\delta^{15}N$)	Trophic position and feeding ecology	Traces carbon sources and quantifies trophic levels	Long-term dietary integration; minimal sample preparation	High cost; requires specialized instrumentation: variety in sample preparation and analytical protocols	McConnaughey & McRoy, 1979	D1, D4
Fatty Acids (FA)	Diet quality, trophic interactions	Identifies food sources based on lipid profiles	Effective for trophic transfer studies; reflects long-term feeding habits	Affected by preservation method and seasonal food availability	Graeve *et al.*, 1994	D1, D4
Oxidative Stress Markers	Sublethal physiological stress	Assesses cellular responses to environmental stressors	Early-warning signals of ecosystem health deterioration	Lack of standard markers and baselines; requires freezing samples in liquid nitrogen	Monaghan *et al.*, 2009	D1, D4, D8

estimate plankton community potential respiration in the Pacific and Atlantic Oceans (Packard *et al.*, 1971, 1974). In the early 2000s, Koppelmann *et al.* (2000) applied this approach in the Indian Ocean, measuring the potential oxygen demand of mesozooplankton and estimating the organic carbon demand using conversion factors from literature; the importance of further research and of standardizing the conversion rates currently in use should therefore be highlighted. Also, Hernández-León *et al.* (2000) used ETS to assess respiration rates in size-fractionated zooplankton along the Antarctic Peninsula, a region known for its high biological variability. Later, Yebra & Hernández-León (2004) developed the AARS method to estimate field zooplankton growth rates, and designed a combined AARS and ETS enzymatic assay protocol (Yebra *et al.*, 2004), expanding the application of these biomarkers in the subtropical eastern Atlantic Ocean (Herrera *et al.*, 2017), Antarctic Ocean (Yebra *et al.*, 2009), and western Mediterranean Sea (Yebra *et al.*, 2017, 2020). Their subsequent research examined the impacts of oxygen minimum zones in the Pacific Ocean using these biomarkers (Herrera *et al.*, 2019). Similarly, McKinnon *et al.* (2015) applied AARS and ETS to assess the zooplankton pelagic production and food web dynamics in Australian waters, while Protopapa *et al.* (2019a, 2025) and Drakopoulou *et al.* (2024) explored their use in the eastern Mediterranean Sea. The latest study by Hernández-León *et al.* (2025) further advances this research by applying AARS and ETS combined in the subtropical eastern Atlantic Ocean.

The application of stable isotopes to zooplankton ecology began in the late 1970s and early 1980s, marking a significant advancement in understanding trophic relationships and energy flow in marine ecosystems. One of the earliest and most influential studies was conducted by McConnaughey & McRoy (1979), who utilized carbon isotopes ($\delta^{13}C$) to trace the origins of primary production in marine food webs, including zooplankton. Minagawa & Wada (1984) demonstrated the utility of nitrogen isotopes ($\delta^{15}N$) in delineating trophic levels, providing an essential framework for studying zooplankton feeding ecology. These pioneering studies established stable isotope analysis as a robust tool to infer dietary sources, trophic position, and habitat use of zooplankton, offering insights into their ecological roles that were previously inaccessible through traditional gut content analysis. Since then, the use of stable isotopes in zooplankton research has expanded dramatically, both methodologically and conceptually. Through the 1990s and 2000s, studies increasingly incorporated both $\delta^{13}C$ and $\delta^{15}N$ to investigate spatial and temporal variability in zooplankton diets, migratory behavior, and trophic interactions within increasingly complex food webs (e.g., Koppelmann *et al*., 2003, 2009; Hannides *et al*., 2014; Protopapa *et al*., 2019b; Massing *et al*., 2022). Recent advancements in marine zooplankton ecology have integrated stable isotope analysis with biochemical and molecular tools to assess environmental stressors, food web dynamics under climate change, and biogeochemical cycling (Garcia *et al*., 2024). These studies provide insights into how anthropogenic influences and natural variability affect marine ecosystems.

The use of fatty acids (FA) in zooplankton ecology commenced in the early 1970s (Lee *et al*., 1971), as researchers recognized their value as dietary biomarkers and indicators of trophic interactions. One of the early influential studies, by Sargent & Henderson (1986), highlighted the importance of essential fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in marine food webs and their selective retention and transfer by zooplankton. In an experimental study, Graeve *et al*. (1994) clearly showed that the fatty acid marker profile of dominant Arctic *Calanus* species can be changed from a diatom signature (major marker FA 16:1(n-7)) to a flagellate signature (major marker FA 18:4(n-3)) and vice versa, if fed for several weeks with flagellates and diatoms, respectively. The FA trophic marker method has been applied widely in polar research, focusing on lipids of larger calanoid copepods and euphausiids (krill), which dominate the zooplankton biomass and are particularly important in upwelling, northern temperate and high-latitude pelagic food webs (Sargent & Henderson, 1986; Dalsgaard *et al*., 2003). On the contrary, knowledge on zooplankton FA is still scarce in the Mediterranean Sea (Najdek *et al*., 1994; Serrazanetti *et al*., 1994; Mayzaud *et al*., 1999; Rossi *et al*., 2006; Protopapa *et al*., 2019b). These applications demonstrated that fatty acids could serve as trophic markers, enabling the reconstruction of feeding relationships and energy pathways on a longer-term basis than traditional ‘snap-shot’ gut content analyses alone.

The concept of oxidative stress was used rather loosely in earlier research until Monaghan *et al*. (2009) published a mini-review that highlighted key knowledge gaps and outlined directions for future studies, particularly in relation to life-history trade-offs. Since then, oxidative stress has been increasingly applied to investigate the eco-physiological responses of marine organisms to environmental change. For example, it has been used to examine the effects of ocean warming and acidification on copepods and pteropods (Engström-Öst *et al*., 2019), oxidative stress and antioxidant defense mechanisms in two copepod species under elevated CO_2 conditions (Engström-Öst *et al*., 2020), and the impact of heatwaves on zooplankton communities in mesocosm experiments (Zervoudaki *et al*., 2024). Recent work has studied the role of reactive oxygen species during large-scale hypoxia (Frederick *et al*., 2025), the role of diet for the antioxidant response (Gorokhova & El-Shehawy, 2022), and pollutants shaping the species life-history during oxidative stress (Soloperto *et al*., 2022).

Towards a biomarker-based approach for zooplankton monitoring in the MSFD

According to the MSFD, assessments of marine environmental status must account for various pressures and their impacts on species, habitats, and ecological communities. These evaluations provide direct insights into the ecological condition of a given area, thereby enhancing the comprehensiveness of holistic, state-based assessments (Ndah *et al*., 2022).

Zooplankton-based indices serve as valuable tools for identifying specific ecological challenges and detecting physico-chemical changes that significantly affect marine ecosystem health, including climate change. These changes are often driven by large-scale human pressures, including eutrophication, chemical pollution, contamination, hydrographic modifications due to multiple marine activities, as well as low-frequency ocean-climatic variability and ocean warming, deoxygenation and acidification. Such pressures are relevant to multiple MSFD Descriptors (D5–D11, Table 1) and their associated indicators. However, compared to state indices, pressure indices remain underdeveloped for quantifying the seven GES qualitative pressure indicators outlined in the MSFD (Cardoso *et al*., 2010; Ndah *et al*., 2022).

Among the aforementioned biomarkers, only ETS and AARS have so far been incorporated into routine monitoring programs. AARS was applied in 2007–2008 as part of the Stonehaven zooplankton time series in the UK (Cook *et al*., unpublished data), while ETS has been used in the Atlantic Ocean, specifically in the Guadalquivir estuary (Gulf of Cádiz), to assess the metabolic status of marine-estuarine species (Herrera *et al*., 2024b). Within the framework of the MSFD, AARS and ETS have been tested in Spanish Mediterranean waters since 2010 (Yebra *et al*., 2017, 2020), and in Greek waters since 2018 (Protopapa *et al*., unpublished data). Moreover, the application of ETS and AARS in marine and estuarine environments is expected to gain increasing relevance in the context of climate change, as extreme weather events become more frequent and intense. The resulting anomalous inputs of freshwater and sediments are likely to further disrupt estuarine, coastal, and transitional waters, reinforcing the need for sensitive metabolic indicators to assess ecosystem responses.

During the BIOZOO workshop, key challenges and limitations of current approaches, along with potential solutions for zooplankton monitoring (Fig. 1) were discussed. Participants highlighted the critical need for data consistency, comparability, transparency, and broader accessibility, aligning with the FAIR principles (Findability, Accessibility, Interoperability, and Reusability). Additionally, they emphasized the importance of effectively communicating the significance of plankton – not only to scientists from diverse fields, such as modelers, socio-economists, and policymakers, but also to the gen-

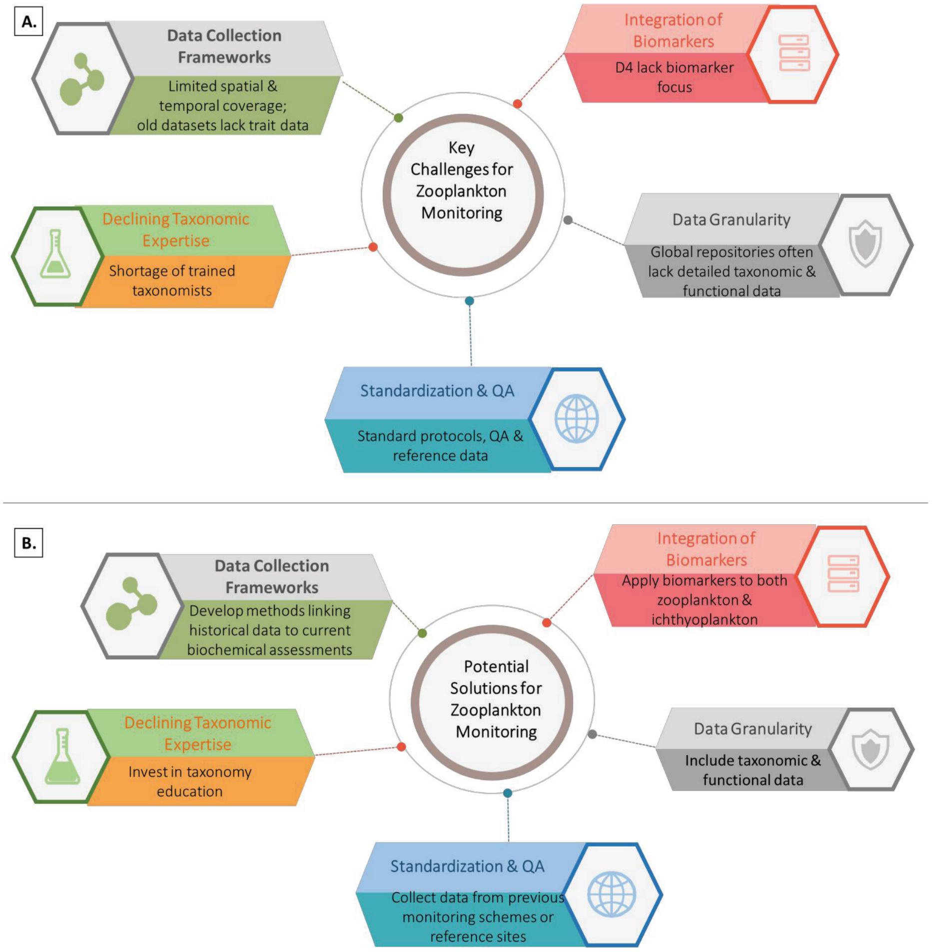

Fig. 1: (A) Key challenges and limitations of current zooplankton monitoring approaches; and (B) potential solutions to overcome these challenges.

eral public. Discussions also underscored the growing role of artificial intelligence (AI) in taxonomy and marine species identification, recognizing its rapid advancements as a valuable tool in the field (Chen *et al.*, 2023). Furthermore, the increasing demand for empirical data, particularly experimental data, was noted as essential for modelers to refine and update simulation models (Ndah *et al.*, 2022).

The key challenges and limitations of current approaches, along with potential solutions include: **Data Collection Frameworks**: There is a limited utilization of existing frameworks, such as the MSFD, for zooplankton data collection. This underuse, coupled with insufficient spatial and temporal data availability, hampers comprehensive assessments. Moreover, older datasets predominantly report aggregated measures like total abundance or biomass data, posing challenges for trait-based approaches. Therefore, developing methods to link historical data with current biochemical assessments can provide new insights into ecophysiology and species distributions.

Integration of Biomarkers: A key improvement for MSFD assessment of food webs involves incorporating specific biomarkers applicable to both zooplankton and ichthyoplankton. This integration would enable a more detailed understanding of pelagic habitat health by complementing traditional data with biochemical information.

Data Granularity: Traditional plankton datasets stored in global repositories primarily report bulk abundance (counts) or biomass (measured by mass, carbon, or nitrogen content). However, they often lack detailed trait information or precise taxonomic resolution beyond broad categories (e.g., bacteria, phytoplankton, zooplankton). To improve our understanding of biodiversity and ecosystem functioning, data collection should also include species identification, process rates (e.g., growth, respiration, ingestion), stoichiometry, and functional traits (e.g., feeding mode, body size, reproduction, resting stages).

Declining Taxonomic Expertise: Biomarkers can be applied, not only to bulk communities, but also at genus or even species level. However, the notable decline in proficient taxonomists and plankton specialists, due to inadequate funding and training opportunities, presents a significant challenge. Addressing this gap by fostering taxonomic literacy and supporting integrative morphological and molecular (DNA) analyses, is vital for a seamless integration of field observations, laboratory research, data interpretation, and synthesis efforts.

Standardization and Quality Assurance: The application of biomarkers on a pan-European scale requires standardized protocols and rigorous quality assurance (QA). Intercalibration of biomarkers among laboratories involved in monitoring programs is essential for consistency and reliability. Baseline data are essential for setting-up regular biomarker monitoring; these data can be obtained from previous monitoring schemes or from reference sites, depending on the research questions posed.

Conclusions

The integration of biomarkers into zooplankton monitoring provides a functional dimension to the assessment of marine ecosystem status, complementing traditional structural indicators used within policy frameworks such as the Marine Strategy Framework Directive. Biomarkers such as ETS and AARS capture short-term metabolic and growth dynamics, while stable isotopes and fatty acids trace long-term trophic relationships, and oxidative stress markers reveal physiological responses to sublethal pressures. Their combined use enhances our understanding of ecosystem functioning and resilience.

Further progress requires harmonized protocols, inter-laboratory quality control, and the establishment of reference baselines across European regions. By embedding these methods in ongoing monitoring initiatives, particularly in the Mediterranean and North Atlantic, scientists can generate comparable datasets that link physiological responses to ecosystem change. This approach advances the regional implementation of functional indicators, improves the sensitivity of food web assessments, and supports more comprehensive evaluations of Good Environmental Status in European marine ecosystems.

Importantly, this synthesis comes at a pivotal time, when the need for more sensitive, process-based indicators has become increasingly evident. As the third MSFD cycle (2024–2030) began, incorporating biomarker-based approaches into marine ecosystem assessment offers a timely opportunity to strengthen functional monitoring, bridge research and operational practice, and enhance the responsiveness of European marine observation systems to climate and anthropogenic pressures.

Acknowledgements

We thank all participants in the EuroMarine Foresight Workshop BIOZOO for the fruitful discussions during the workshop, and HCMR for hosting this event.

Funding sources: The Foresight Workshop (FSW) BIOZOO: Ecological Applications of Biomarkers in Zooplankton was funded by EuroMarine. This work was partially supported by the Spanish Ministry for the Ecological Transition under project ESMARES3 (20243TE020), and by the program ‘Monitoring and recording the status of the marine sub-regions of Greece’ (MIS 6017388), funded by national Greek and EU funds under the Ministry of National Economy and Finance, National Strategic Reference Framework (NSRF 2021–2027).

References

Beaugrand, G., Edwards, M., Brander, K., Luczak, C., Ibanez, F., 2008. Causes and projections of abrupt climate-driven ecosystem shifts in the North Atlantic. *Ecology Letters*, 11 (11), 1157–1168.

Cardoso, A.C., Cochrane, S., Doerner, H., Ferreira, J.G., Galbani, F. *et al*., 2010. *Scientific support to the European Commission on the Marine Strategy Framework Directive: Management Group Report.* Joint report prepared under the administrative arrangement between JRC and DG ENV no 31210 – 2009/2010, the European Commission Joint Research and ICES Centre, 57 pp.

Caroppo, C., Buttino, I., Camatti, E., Caruso, G., De Angelis, R. *et al*., 2013. State of the art and perspectives on the use of planktonic communities as indicators of environmental status in relation to the EU Marine Strategy Framework Directive. *Biologia Marina Mediterranea*, 20, 65–73.

Chen, H., Barton, S., Yang, M., Rickaby, R.E., Bouman, H.A. *et al*., 2023. AI facilitated fluoro-electrochemical phytoplankton classification. *Chemical Science*, 14 (22), 5872–5879.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology*, 46, 225–340.

Drakopoulou, I., Protopapa, M., Tsangaris, C., Zervoudaki, S., 2024. Mesozooplankton production and respiration across a trophic gradient in a coastal Eastern Mediterranean ecosystem. *Marine Environmental Research*, 198, 106551.

Desrosiers, C., Leflaive, J., Eulin, A., Ten-Hage, L., 2013. Bioindicators in marine waters: Benthic diatoms as a tool to assess water quality from eutrophic to oligotrophic coastal ecosystems. *Ecological Indicators*, 32, 25–34.

Engström-Öst, J., Kanerva, M., Vuori, K., Riebesell, U., Spisla, C. *et al.,* 2020. Oxidative stress and antioxidant defence responses in two marine copepods in a high CO_2 experiment. *Science of the Total Environment*, 745, 140600.

Engström-Öst, J., Glippa, O., Feely, R.A., Kanerva, M., Keister, J.E. *et al*., 2019. Eco-physiological responses of copepods and pteropods to ocean warming and acidification. *Scientific Reports*, 9 (1), 4748.

Frederick, L., Urbina, M.A., Escribano, R., 2025. Reviews and syntheses: On increasing hypoxia in eastern boundary upwelling systems–zooplankton under metabolic stress. *Biogeosciences,* 22, 1839–1852.

Garcia, T., Carlotti, F., Lepoint, G., Guilloux, L., Tesán-Onrubia, J.A. *et al*., 2024. The decadal variability of elemental, isotopic, and biochemical compositions of coastal Mediterranean zooplankton responds to environmental changes. *Marine Biology*, 171 (5).

Garmendia, M., Borja, Á., Franco, J., Revilla, M., 2013. Phytoplankton composition indicators for the assessment of eutrophication in marine waters: Present state and challenges within the European directives. *Marine Pollution Bulletin*, 66 (1–2), 7–16.

Gorokhova, E., Lehtiniemi, M., Lesutiene, J., Strake, S., Uusitalo, L. *et al*., 2013. *Zooplankton mean size and total abundance*. HELCOM Core Indicator Report, 14pp.

Gorokhova, E., Lehtiniemi, M., Postel, L., Rubene, G., Amid, C. *et al*., 2016. Indicator properties of Baltic zooplankton for classification of environmental status within marine strategy framework directive. *PLoS ONE*, 11 (7), e0158326.

Gorokhova, E., El-Shehawy, R., 2022. Antioxidant responses in copepods are driven primarily by food intake, not by toxin-producing cyanobacteria in the diet. *Frontiers in Physiol*ogy, 12, 805646.

Graeve, M., Kattner, G., Hagen, W., 1994. Diet-induced changes of the lipid composition of herbivorous Arctic copepods: Experimental evidence of fatty acids as trophic markers. *Journal of Experimental Marine Biology & Ecology*, 182, 97–110.

Halliwell, B., Gutteridge, J.M.C., 2007. *Free radicals in biology and medicine*. 4th ed. Oxford University Press, Oxford, United Kingdom, 346 pp.

Hannides, C.S., Zervoudaki, S., Frangoulis, C., Lange, M.A., 2014. Mesozooplankton stable isotope composition in Cyprus coastal waters and comparison with the Aegean Sea (eastern Mediterranean). *Estuarine Coastal and Shelf Science,* 154, 12–18.

HELCOM, 2018. *Zooplankton mean size and total stock*. HELCOM Core Indicator Report, 24 pp.

Hernández-León, S., Torreblanca, M.L., Herrera, I., Armengol, L., Franchy, G. *et al*., 2025. Variability of plankton communities in relation to the lunar cycle in oceanic waters. *Frontiers in Marine Science*, 12, 1476524.

Hernández-León, S., Almeida, C., Portillo-Hahnefeld, A., Montero, I., 2000. Biomass and potential feeding, respiration and growth of zooplankton in the Bransfield Strait (Antarctic Peninsula) during austral summer. *Polar Biology*, 23, 679–690.

Herrera, I., Fraile-Nuez, E., González-Ortegón, E., 2024a. Exploring marine zooplankton dynamics through carbon stable isotope signatures in a recently formed submarine volcano. *Estuarine, Coastal and Shelf Science*, 310, 109005.

Herrera, I., de Carvalho-Souza, G.F., González-Ortegón, E., 2024b. Physiological responses of the invasive blue crab *Callinectes sapidus* to salinity variations: Implications for adaptability and invasive success. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 297, 111709.

Herrera, I., López-Cancio, J., Yebra, L., Hernández-Léon, S., 2017. The effect of a strong warm winter on subtropical zooplankton biomass and metabolism. *Journal of Marine Research*, 75, 557–577.

Herrera, I., Yebra, L., Antezana, T., Giraldo, A., Färber Lorda, J. *et al*., 2019. Vertical variability of *Euphausia distinguenda* rates during diel migration into the oxygen minimum zone of the Eastern Tropical Pacific off Mexico. *Journal of Plankton Research*, 41, 165–176.

Koppelmann, R., Böttger-Schnack, R., Möbius, J., Weikert, H., 2009. Trophic relationships of zooplankton in the eastern Mediterranean based on stable isotope measurements. *Journal of Plankton Research*, 31 (6), 669–686.

Koppelmann, R., Weikert, H., Lahajnar, N., 2003. Vertical distribution of mesozooplankton and its δ^{15}N signature at a deep-sea site in the Levantine Sea (eastern Mediterranean) in April 1999. *Journal of Geophysical Research*, 108 (C9), 8118.

Koppelmann, R., Schäfer, P., Schiebel, R., 2000. Organic carbon losses measured by heterotrophic activity of mesozooplankton and $CaCO_3$ flux in the bathypelagic zone of the Arabian Sea. *Deep-Sea Research*, 47, 169-187.

Lam, P.K., 2009. Use of biomarkers in environmental monitoring. *Ocean & Coastal Management,* 52 (7), 348–354.

Lee, R.F., Nevenzel, J.C., Paffenhöfer, G.A., 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Marine Biology*, 9, 99-108.

Magliozzi, C., Druon, J., Palialexis, A., Aguzzi, L., Alexande, B. *et al*., 2021. *Pelagic habitats under the MSFD D1: scientific advice of policy relevance*. JRC Science for Policy Report, Publications Office of the European Union, Luxembourg, 45 pp.

Massing J.C., Schukat A., Auel H., Auch D., Kittu L. *et al*., 2022. Towards a solution of the "Peruvian Puzzle": Pelagic food-web structure and trophic interactions in the northern Humboldt Current upwelling system off Peru. *Frontiers in Marine Science,* 8, 759603.

Mayzaud, P., Virtue, P., Albessard, E., 1999. Seasonal variations in the lipid and fatty acid composition of the euphausiid *Meganyctiphanes norvegica* from the Ligurian Sea. *Marine Ecology Progress Series*, 186, 199–210.

McCarty L. S., Mackay, D., 1993. Enhancing ecotoxicological modeling and assessment. Body residues and modes of toxic action. *Environmental Science & Technology*, 27 (9), 1718–1728.

McConnaughey, T., McRoy, C.P., 1979. Food web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology*, 53 (3), 257–262.

McKinnon, D., Doyle, J., Duggan, S., Murray, L., Lønborg, C. *et al*., 2015. Zooplankton growth, respiration and grazing on the Australian margins of the tropical Indian and Pacific Oceans. *PLoS ONE*, 10 (10), e0140012.

McQuatters-Gollop, A., Atkinson, A., Aubert, A., Bedford, J., Best, M. *et al*., 2019. Plankton lifeforms as a biodiversity indicator for regional-scale assessment of pelagic habitats for policy. *Ecological Indicators*, 101, 913–925.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. *Geochimica et Cosmochimica Acta*, 48 (5), 1135–1140.

Minutoli, R., Fossi, M., Granata, A., Casini, S., Guglielmo, L., 2007. Use of biomarkers in zooplankton for assessment of the 'health status' of marine and brackish environments: A short overview. *Chemistry and Ecology,* 23, 471–477.

Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters*, 12 (1), 75–92.

Najdek, M., PuSkaric, S., Bochdansky, A.B., 1994. Contribution of zooplankton lipids to the flux of organic matter in the northern Adriatic Sea. *Marine Ecology Progress Series,* 111, 241–249.

Ndah, A.B., Meunier, C.L., Kirstein, I.V., Göbel, J., Rönn, L. *et al*., 2022. A systematic study of zooplankton-based indices of marine ecological change and water quality: Application to the European Marine Strategy Framework Directive (MSFD). *Ecological Indicators*, 135, 108587.

Packard, T., Harmon, D., Boucher, J., 1974. Respiratory electron transport activity in plankton from upwelled waters. *Tethys*, 6, 213-222.

Packard, T.T., Healy, M.L., Richards, F.A., 1971. Vertical distribution of the activity of the respiratory electron transport system in marine plankton. *Limnology and Oceanography*, 16 (1), 60–70.

Parrish, C.C., 2013. Lipids in marine ecosystems. *International Scholarly Research Notices*, 604045.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293–320.

Protopapa, M., Yebra, L., Koppelmann, R., Zervoudaki, S., 2025. Ecological application of biomarkers to mesozooplankton communities in the Mediterranean Sea. Chapter 2.2. p. 107-123. In: *Zooplankton Challenges in a Changing World: Volume 1: A Worldwide Perspective and Research Approach (1st ed.)*. Vieira, L.R., Raposo Morgado, F.M. (Eds.). CRC Press.

Protopapa, M., Zervoudaki, S., Tsangaris, C., Velaoras, D., Koppelmann, R., *et al.,* 2019a. Zooplankton distribution, growth and respiration in the Cretan Passage, Eastern Mediterranean. *Deep-Sea Research Part II Topical Studies in Oceanography*, 164*,* 156–169.

Protopapa, M., Koppelmann, R., Zervoudaki, S., Wunsch, C., Peters, J. *et al*., 2019b. Trophic positioning of prominent copepods in the epi- and mesopelagic zone of the ultra-oligotrophic eastern Mediterranean Sea. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 164, 144–155.

Rossi, S., Sabatés, A., Latasa, M., Reyes, E., 2006. Lipid biomarkers and trophic linkages between phytoplankton, microzooplankton and the anchovy (*Engraulis encrasicolus*) larvae in the NW Mediterranean. *Journal of Plankton Research*, 28, 551–562.

Sargent, J.R., Henderson, R.J., 1986. Lipids. p. 59-108. In: *The biological chemistry of marine copepods Vol. 1*. (Corner, E.D.S., O'Hara, S.C.M. (Eds.). Clarendon Press, Oxford.

Serrazanetti G. P., Pagnucco C., Conte L. S., Artusi R., Fonda-Umani S., *et al*. 1994. Sterols and fatty acids in zooplankton of the Gulf of Trieste. *Comparative Biochemistry & Physiology*, 107B, 443–446.

Soloperto, S., Altin, D., Hallmann, A., Skottene, E., Hansen, B. H. *et al*., 2022. Oil-mediated oxidative-stress responses in a keystone zooplanktonic species, *Calanus finmarchicus*. *Science of the Total Environment*, 806, 151365.

Yebra, L., Hernández-León, S., 2004. Aminoacyl-tRNA synthetases activity as a growth index in zooplankton. *Journal of Plankton Research*, 26 (3), 351–356.

Yebra, L., Almeida, C., Bécognée, P., Hernández-León, S., 2009. Metabolism and biomass vertical distribution of zooplankton in the Bransfield Strait (Antarctic Peninsula) during summer. *Polar Research,* 28, 415–425.

Yebra, L., Hernández-León, S., Almeida, C., Bécognée, P., Rodríguez, J.M., 2004. The effect of upwelling filaments and island-induced eddies on indices of feeding and metabolism in copepods*. Progress in Oceanography,* 62, 151–169.

Yebra, L., Putzeys, S., Cortés, D., Mercado, J.M., Gómez-Jakobsen, F. *et al*., 2017. Trophic conditions govern summer zooplankton production variability along the SE Spanish coast (SW Mediterranean). *Estuarine, Coastal and Shelf Science*, 187, 134–145.

Yebra, L., Espejo, E., Putzeys, S., Giráldez, A., Gómez-Jakobsen, F. *et al*., 2020. Zooplankton biomass depletion event reveals the importance of small pelagic fish top-down control in the Western Mediterranean coastal waters. *Frontiers in Marine Science*, 7, 608690.

Zervoudaki, S., Siakavara, A., Protopapa, M., Christou, E., Siokou, I., 2020. Mesozooplankton Dynamics in the Aegean Sea. p. 115-152. In: *The Aegean Sea Environment. The Handbook of Environmental Chemistry,* Vol. 129. Anagnostou, C.L., Kostianoy, A.G., Mariolakos, I.D., Panayotidis, P., Soilemezidou, M., Tsaltas, G. (Eds.). Springer, Cham.

Zervoudaki, S., Protopapa, M., Koutsandrea, A., Jansson, A., von Weissenberg, E. *et al*., 2024. Zooplankton responses to simulated marine heatwave in the Mediterranean Sea using *in situ* mesocosms. *PLoS ONE,* 19(8), e0308846.