University of Genoa & Stazione Zoologica Anton Dohrn of Naples

XXXVIII PhD Cycle in Marine Ecosystem Science

# Plankton food webs: new tools for management and assessment of the marine ecological status

Ph.D. Thesis

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XXXVIII PhD Cycle in Marine Ecosystem Science – University of Genoa (Department of Earth, Environment and Life Sciences, DISTAV) and Stazione Zoologica "A. Dohrn" of Naples (Department of Integrative Marine Ecology, EMI)

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## List of papers:

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# **ABSTRACT** (Italiano)

Il plancton rappresenta una componente fondamentale degli ecosistemi marini, comprendendo organismi unicellulari (come batteri e protisti) e multicellulari (come i copepodi e altri metazoi). Questi organismi svolgono un ruolo chiave nei cicli biogeochimici e nella struttura delle reti trofiche marine, in quanto costituiscono il principale canale di trasferimento dell’energia prodotta nella colonna d’acqua verso i livelli trofici superiori. Le comunità planctoniche si caratterizzano per un’elevata diversità tassonomica, funzionale e trofica, che include fotoautotrofi, eterotrofi e mixotrofi. Questa eterogeneità consente la formazione di reti trofiche complesse e dinamiche, la cui struttura può variare significativamente in risposta a fattori ambientali come la temperatura, gli apporti terrestri, la disponibilità di nutrienti e le condizioni idrodinamiche.

Nonostante il loro ruolo centrale negli ecosistemi marini, le comunità planctoniche sono ancora poco considerate nei programmi di monitoraggio ambientale previsti dalla Direttiva Quadro sulla Strategia Marina (MSFD), specialmente all’interno del Descrittore 4 relativo alle reti trofiche. In particolare, la dicotomia tradizionale tra fitoplancton e zooplancton tende a semplificare eccessivamente la reale complessità ecologica del comparto planctonico, limitando così l’efficacia degli strumenti di valutazione dello stato ecologico marino.

Questo progetto di dottorato si è basato sull’analisi di un ampio insieme di dati raccolti nel corso di due campagne di campionamento condotte in ambienti marini costieri caratterizzati da differenti condizioni ecologiche e livelli di pressione antropica. Le attività di raccolta si sono svolte lungo le coste della regione Campania, durante le stagioni autunno 2020 ed estate 2021, nell’ambito del progetto FEAMP-ISSPA, e successivamente nel Mare del Nord, lungo le coste del Belgio, nel luglio 2022, durante una crociera oceanografica inserita nel progetto internazionale S4GES – JPI Oceans.

L’obiettivo principale della tesi è stato quello di comprendere come la struttura e il funzionamento delle reti trofiche planctoniche rispondano a diversi gradienti ambientali, utilizzando un approccio integrato e multidisciplinare. In primo luogo, sono stati sviluppati modelli ecologici basati su *Ecopath with Ecosim* (EwE), al fine di quantificare i flussi di biomassa tra gruppi funzionali planctonici e individuare le principali variazioni strutturali nelle reti trofiche in risposta a

differenti condizioni ambientali. In secondo luogo, sono state applicate tecniche molecolari di DNA ambientale (*eDNA metabarcoding*) per ottenere una descrizione ad alta risoluzione della biodiversità planctonica, con particolare attenzione alle dinamiche spazio-temporali delle comunità e alla loro connettività influenzata dai processi di dispersione costiera. Infine, è stato utilizzato l'approccio emergy, uno strumento di contabilità ambientale in grado di fornire una valutazione biofisica del capitale naturale planctonico, stimando in termini energetici il contributo delle comunità planctoniche ai processi ecosistemici.

I risultati ottenuti hanno evidenziato come la struttura delle reti trofiche planctoniche sia fortemente modulata da fattori ambientali locali, quali gli apporti fluviali, la stratificazione della colonna d'acqua e le condizioni di marea. L'applicazione dei modelli trofici ha permesso di identificare specifici indicatori ecologici sensibili a tali pressioni, con potenziale utilità per la valutazione dello stato ecologico in ambito MSFD. I dati molecolari da eDNA, pur non essendo utilizzabili direttamente per la costruzione di indicatori, hanno fornito un quadro dettagliato della composizione e della dinamica delle comunità planctoniche, arricchendo l'interpretazione ecologica dei modelli. L'approccio emergy ha infine permesso di stimare, il valore funzionale del plancton in differenti contesti ambientali, contribuendo a una visione più completa del ruolo ecologico di questo comparto all'interno dei processi di sviluppo del capitale naturale marino.

Nel complesso, questo lavoro dimostra come l'integrazione di dati biologici, modellazione ecologica e strumenti di contabilità biofisica rappresenti un approccio efficace per colmare le attuali lacune nella valutazione del Buono Stato Ambientale. Tale prospettiva integrata può supportare la gestione sostenibile delle aree costiere e contribuire alla valorizzazione sistemica del capitale naturale marino.

## **ABSTRACT** (English)

Plankton represents a fundamental component of marine ecosystems, encompassing both unicellular organisms (such as bacteria, and protists) and multicellular organisms (such as copepods and other metazoans). These organisms play a key role in biogeochemical cycles and in the structure of marine food webs, acting as the primary channel for transferring energy produced in the water column to higher trophic levels. Planktonic communities are characterized by high taxonomic, functional, and trophic diversity, including photoautotrophs, heterotrophs, and mixotrophs. This heterogeneity supports the formation of complex and dynamic trophic networks, whose structure can vary significantly in response to environmental factors such as temperature, terrestrial inputs, nutrient availability, and hydrodynamic conditions.

Despite their central role in marine ecosystems, planktonic communities remain underrepresented in environmental monitoring programs established under the Marine Strategy Framework Directive (MSFD), particularly within Descriptor 4, which addresses trophic networks. In particular, the traditional dichotomy between phytoplankton and zooplankton tends to oversimplify the ecological complexity of the planktonic compartment, limiting the effectiveness of tools used to assess marine ecological status.

This PhD project was based on the analysis of a large dataset collected during two sampling surveys conducted in coastal marine environments characterized by different ecological conditions and levels of anthropogenic pressure. Sampling activities took place along the coasts of the Campania region (during autumn 2020 and summer 2021), within the framework of the FEAMP-ISSPA project, and in the North Sea, off the Belgian coast, in July 2022 during an oceanographic cruise as part of the international S4GES – JPI Oceans project.

The main objective of this thesis was to understand how the structure and functioning of planktonic trophic networks respond to different environmental gradients, using an integrated and multidisciplinary approach. Firstly, ecological models based on *Ecopath with Ecosim* (EwE) were developed to quantify biomass flows among planktonic functional groups and identify major structural changes in trophic networks in response to varying environmental conditions. Secondly, molecular techniques based on environmental DNA (*eDNA*

*metabarcoding*) were applied to obtain a high-resolution description of plankton biodiversity, with particular attention to the spatiotemporal dynamics of communities and their connectivity influenced by coastal dispersion processes. Finally, the *emergy* approach (*emergy accounting*) was employed as a tool for environmental accounting, providing a biophysical assessment of planktonic natural capital by estimating, in energetic terms, the contribution of plankton communities to ecosystem processes.

The results showed that the structure of planktonic trophic networks is strongly influenced by local environmental factors, such as riverine inputs, water column stratification, and tidal conditions. The application of food web models allowed the identification of specific ecological indicators sensitive to these pressures, with potential relevance for MSFD ecological assessments. Although eDNA molecular data are not directly usable for indicator development, they offered a detailed picture of plankton community composition and dynamics, enriching the ecological interpretation of model outcomes. The emergy approach, finally, enabled a functional valuation of plankton in different environmental contexts, contributing to a more comprehensive understanding of its ecological role within marine natural capital.

Overall, this work demonstrates that integrating biological data, ecological modelling, and biophysical accounting tools represents an effective strategy for addressing current gaps in the assessment of Good Environmental Status. This integrated perspective can support the sustainable management of coastal areas and enhance the systemic valuation of marine natural capital.

# 1. INTRODUCTION

Since the onset of the industrial era, marine ecosystems have been increasingly subjected to anthropogenic pressures, including population growth, climate change, and pollution (Halpern et al., 2007). Human activities have intensified stress on marine environments, leading to significant alterations in the structure and dynamics of marine ecosystems. In response to these challenges, the European Union has implemented a series of directives aimed at protecting the marine environment, such as the Water Framework Directive (2000/60/EC), the Habitats Directive (92/43/EEC), and the Marine Strategy Framework Directive (MSFD; 2008/56/EC). Among these, the MSFD represents one of the key legislative instruments for marine environmental protection. Its primary objective is to achieve “Good Environmental Status” (GES) through an ecosystem-based approach, structured around 11 different descriptors, each comprising various indicators to allow for an integrated assessment of ecosystem status (Piroddi et al., 2015; de Jonge et al., 2021 (Figure 1). The outcomes derived from these descriptors are communicated through specific ecological indicators, which are essential for conveying complex information not only within scientific contexts but also to a wide range of stakeholders (Saint-Béat et al., 2015; Piroddi et al., 2015; Fath et al., 2019).

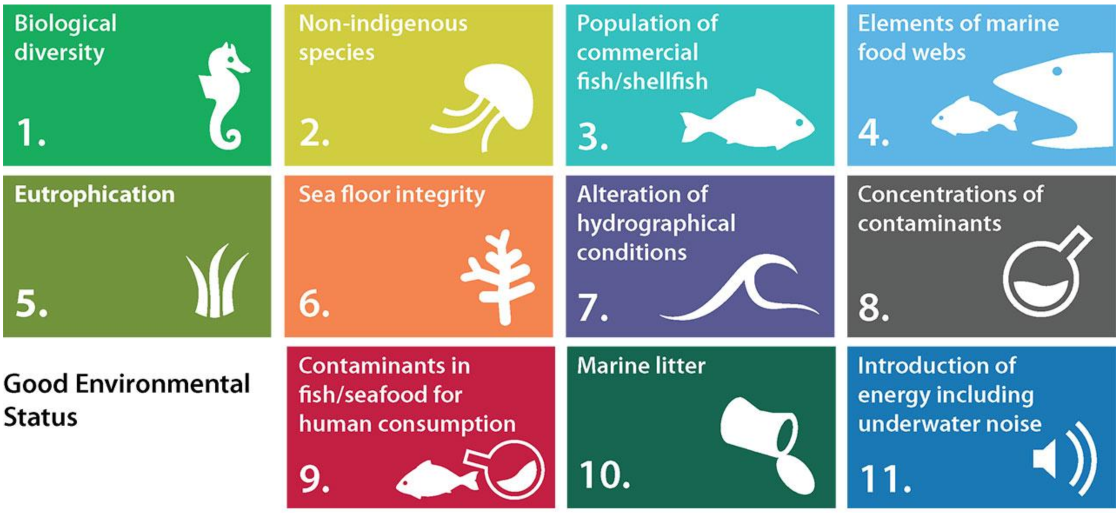


**Figure 1**. 11 Descriptors of Marine Strategy Framework Directive (MSFD). Figure from “*Sustainable Water Network*” (https://swanireland.ie/)

Among the 11 descriptors, Descriptor 4 emphasizes the importance of studying marine food webs to understand the rate of energy transfer within an ecosystem, the productivity of its components, and the ecosystem’s structure based on the identity, size, and abundance of its organisms (Cardoso et al., 2010). According to the European Commission, food-web analysis must go beyond simple species inventories and incorporate the dynamics of biomass and energy flows among organisms, allowing the study of ecological changes in the productivity of specific trophic components or in predator-prey relationships (2010/477/EU). Descriptor 4 suggests the adoption of 10 trophic guilds (see Table 1)—representing the upper, middle, and lower levels of the food chain—to estimate key indicators such as production, diversity, and community trophic level. Although this grouping addresses the practical need to translate complex scientific data into information accessible to stakeholders, it entails an inevitable simplification of food webs. This approach, while necessary, risks obscuring the complex ecological dynamics characterizing the planktonic domain, which require detailed assessments.

**Table 1**. Suggested minimum set of trophic guilds to enhance regional comparability (Table from Art. 8 MSFD Assessment Guidance, May 2022)

| ***Guild*** | ***Description*** | ***Example species/groups*** |
|---|---|---|
| *Pelagic Primary Producers* | Phytoplankton | Diatoms, dinoflagellates |
| *Benthic Primary Producers* | Macrovegetation, included where relevant for the food web assessment area | Seagrass meadows, kelp forests |
| *Secondary Producers* | Mesozooplankton (200 micron–20 mm) | Copepods, cladocerans |
| *Benthic Filter-Feeding Invertebrates* | Benthic filter-feeding invertebrates, included where relevant for the food web assessment area | Mussel, scallops, brittle stars |
| *Benthic Feeding Invertebrates* | Benthic invertebrates feed predominantly on detritus or other benthic invertebrates, and/or constituting prey for sub-apex predators. | Nephrops, crabs, shrimps |
| *Planktivorous Fish And Invertebrates* | Fish and invertebrates feeding predominantly on zooplankton | Anchovy, herring, horse mackerel, jellyfish |
| *Sub-Apex Pelagic Predators* | Fish and invertebrates feed pelagically on fish and other prey types | Mackerel, saithe, tunids, Loligo |

| | | |
|---|---|---|
| *Sub-Apex Demersal Predators* | Fish and invertebrates feeding demersally or on the bottom on fish and other food | Sole, hake, haddock, octopus |
| *Apex Marine Mammal Predators* | Marine mammal piscivores feeding on sub-apex predators | Killer Whale |
| *Apex Fish Predators* | Fish piscivores feed on sub-apex predators | Large tuna, large cod, large sharks |

The adoption of holistic, integrated, and quantitative approaches is therefore essential to capture ecological complexity and to identify early warning signals of anthropogenic disturbances such as eutrophication, chemical pollution, global warming, overexploitation of resources, and habitat modifications (Eriksson et al., 2011; Niiranen et al., 2013).

In this context, studying planktonic food webs and how their structure changes along environmental gradients enable scientists and policymakers to gain deeper insights into marine ecosystem functioning (D’Alelio et al., 2016; Vassallo et al., 2022), and to derive valuable guidance for its sustainable management.

### 1.1 Plankton: diversity and key role in marine ecological networks

Among the many biological components relevant to marine food web analysis, plankton plays a central role due to their pivotal position in energy and nutrient cycling. They contribute to nearly half of the global net primary production—that is, the difference between energy fixed by autotrophs and their respiration—through the activity of phytoplankton, as well as through nutrient and carbon storage and the transfer of matter and energy to higher trophic levels (e.g., metazoans) (Falkowski, 2012; Worden et al., 2015).

Plankton includes organisms that cover a wide range of sizes (Figure 2) and are classified into different size classes (Sunagawa et al., 2020): (i) Picoplankton (<2 μm) which includes unicellular prokaryotes such as bacteria, as well as microalgae; (ii) Nanoplankton (2–20 μm) which includes diatoms, dinoflagellates, and ciliates, organisms that are also present in the (iii) microplankton size class (20–200 μm), which includes representatives for the previous category and additionally other unicellular forms such as radiolarians (Mitra et al., 2023); (iv) Mesoplankton (200–2000 μm) which consists in both juvenile and adult stages of copepods (Mitra et al., 2023), that are also found in the macroplankton size class (2–20 cm), together with large planktonic crustaceans and gelatinous organisms such as salps and jellyfish.

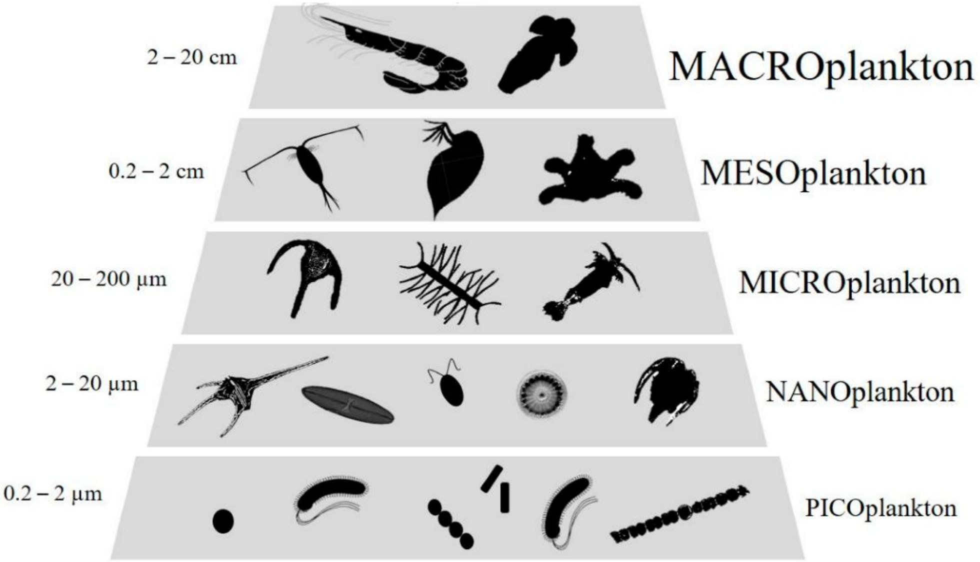


**Figure 2.** Schematic representation of planktonic size classes with their most representative organisms. Figure from Colombet et al. (2020)

In addition to the wide size diversity, plankton also shows a large trophic diversity. Planktonic communities include phytoplanktonic organisms, which can sustain themselves through photosynthesis (phototrophy) (Falkowski et al., 2004). This group, which forms the base of marine food webs, is primarily represented by diatoms and dinoflagellates (Mitra et al., 2023). All organisms that acquire nutrients by consuming and digesting their prey (phagotrophy) (Flynn et al., 2019) are classified as heterotrophic protists (or protozooplankton), mainly represented by dinoflagellates, ciliates, and radiolarians (Mitra et al., 2023; Steinberg & Landry, 2017). Among protists, there are also some protists capable of obtaining nutrients via both phototrophy and phagotrophy (Flynn et al., 2019; Stoecker et al., 2017), known as mixoplankton. Within this group, we find photoautotrophs that are also capable of phagotrophy (Jeong et al., 2010; 2021), as well as heterotrophs that acquire the ability to photosynthesize by ingesting chloroplasts from their prey or through symbiotic associations (Jeong et al., 2021). Finally, at the top of the planktonic trophic web are the zooplanktonic organisms, which show a range of feeding strategies. Zooplankton includes carnivorous species that prey on other zooplankton taxa, herbivores that feed on phytoplankton and other protists, omnivores that consume both metazoans and phytoplankton, as well as detritivores, which incorporate detrital material into their diets (Benedetti et al., 2015).

This high taxonomic, functional, and trophic diversity makes their position in ecological networks particularly complex and dynamic. Indeed, such biological complexity gives rise to intricate food webs with trophic interactions (network linkages, directed from prey to predator) between species at different trophic levels that regulate the energy and organic matter of ecosystems in streams (Legendre et al 2002; Lomartire et al 2021). Indeed, their central position in food webs means that small changes in planktonic composition or biomass can propagate rapidly along the trophic chain, affecting the stability of the entire ecosystem, such as by affecting fishery productivity (Batten et al 2019).

### 1.2 Planktonic food web models

The great taxonomic and functional diversity makes plankton a key group in assessing the health of marine ecosystems (Bedford et al 2018; Anschütz et al 2024). However, planktonic organisms are underrepresented or oversimplified in ecosystem models, following the old “phytoplankton-zooplankton” ecological dichotomy (Anschütz and Flynn, 2020). This approach misrepresents the enormous complexity within the unicellular plankton component (e.g., mixoplankton) (Jordán et al., 2018) and is a limitation that limits its use for environmental management and GES assessment purposes, significantly hampering the robustness of model results (Anschütz et al 2024; Chkili et al 2024).

Addressing the functional complexity of marine planktonic trophic networks and assessing their ecological status requires an integrated, transdisciplinary, and quantitative approach that can integrate biological, ecological, and environmental data to better understand the structure and functioning of planktonic food webs. In this context, ecological models based on food web analysis, such as Ecopath with Ecosim (EwE), are particularly effective in estimating the fluxes of matter between functional groups (i.e., groups with similar ecological functions and trophic interactions) within a target community (Figure 3) and evaluating how their structure changes as environmental gradients change (D'Alelio et al., 2016; Loschi et al., 2023).

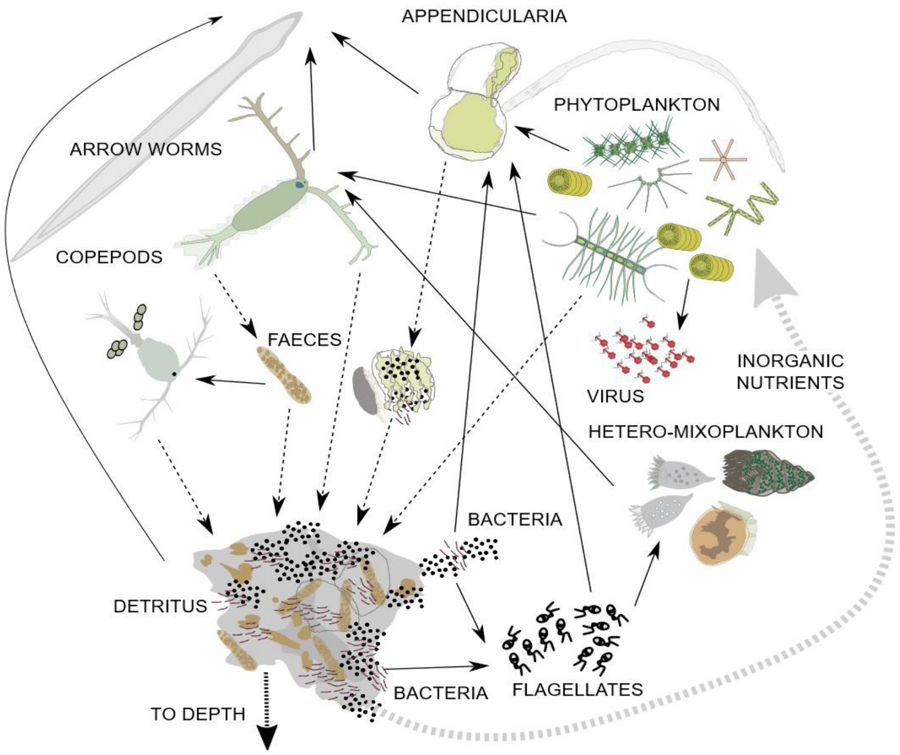


**Figure 3**. Schematic representation of a marine plankton food web. Arrows directed from prey to predators. Figure from Domenico D'Alelio "*La Microgiungla del Mare*" (2020)

Food web models, therefore, are essential tools for assessing ecosystem structure and function and are playing an increasingly important role in providing recommendations to policymakers on aquatic and marine resource management in Europe (Saint-Béat et al 2015; Piroddi et al 2015; Fath et al 2019), enabling them to gain a greater awareness of marine ecosystem functioning (D'alelio et al 2016; Vassallo et al 2021). However, because the results of these models are virtual representations of reality, their interpretation and understanding are complex for non-specialists (Baird et al 2009; Fath et al 2007; Chkili et al 2024). In this context, the application of appropriate ecological indicators can enable the effective capture and interpretation of the main forces influencing the dynamics of trophic networks, as well as facilitate more effective communication with policy makers and stakeholders (Saint-Béat et al 2015; Piroddi et al 2015; Fath et al 2019).

### 1.3 The role of plankton in the assessment of marine natural capital: an emergy-based approach

Considering the increasing availability of high-resolution biological data, the need to integrate tools capable of capturing not only the structure and dynamics of biological communities, but also their functional contribution to ecosystem processes, becomes increasingly evident. In this context, another useful perspective for enhancing the ecological role of plankton is the “emergy approach”, which allows the quantification of plankton's contribution to marine natural capital in energetic and ecosystem terms.

In recent years, natural capital assessment has gained increasing relevance as a tool to support sustainable ecosystem management. The concept of natural capital refers to the stock of natural resources-such as water, soil, biodiversity, and solar energy that fuels ecosystem functioning and generates flows of ecosystem services that are essential for human well-being and the stability of the biosphere. From this perspective, the ecological value of an ecosystem or a biotic component, such as plankton, is not solely related to its direct use by humans, but rather to the functional role it plays in bio-ecological processes, independent of its economic valuation (Odum, 1996).

One systemic way to quantify such processes is emergent accounting (“Emergy accounting”), devised by Odum (1988, 1996). This method makes it possible to assess the efficiency and sustainability of an ecological or production system based on the flows of energy and matter – both natural and anthropogenic – that are required to maintain the structure and functions of the ecosystem. All energy flows are converted into a single unit of measurement, solar emergy joules (seJ), which represent the equivalent solar energy required to sustain a given process. This makes it possible to compare very different systems and to assign energy and monetary values to natural capital, making it more understandable to policy makers and stakeholders.

The emergy approach has been widely applied to the study of terrestrial and marine ecosystems, particularly in coastal and benthic environments (Pulselli et al., 2011; Franzese et al., 2020). However, it has only recently been extended to the study of plankton, despite the crucial role this compartment plays in the functioning of marine ecosystems (Vassallo et al., 2021; Mattei et al., 2021).

Planktonic organisms are major contributors to the conversion of solar energy into biomass, feeding the upper trophic levels and facilitating the sequestration of atmospheric carbon at depth (Steinberg & Landry, 2017). However, their functional efficiency is highly sensitive to anthropogenic pressures such as pollution, climate change, eutrophication, and alien species, which can profoundly alter the productivity and resilience of coastal ecosystems. By applying emergy accounting to planktonic trophic networks, it is possible to estimate the natural capital and flows that maintain the stock, and by comparing areas with different environmental pressures or ecological status, it is possible to identify sustainable management strategies that can maximize ecological performance while preserving essential ecosystem functions.

In this sense, the emergy approach represents a complementary alternative to classical ecological methods for studying plankton, offering a systemic, energetic and economic perspective of its role in marine ecosystem processes. Integrating this methodology into marine natural capital assessment and management can provide new tools for decision support, promoting more integrated and informed governance of coastal environments.

### 1.4 Integration of environmental DNA (eDNA) in food-web studies

In recent years, the development of omics tools, such as environmental DNA metabarcoding (eDNA metaB), can detect the presence and composition of biological communities through the analysis of genetic fragments released by organisms in the environment, providing a high-resolution view of marine biodiversity (Ruppert et al., 2019). eDNA metaB involves the sequencing of specific genetic markers, allowing the simultaneous identification of multiple taxa from the total DNA present in an environmental sample (Taberlet et al., 2012) (Figures 4).

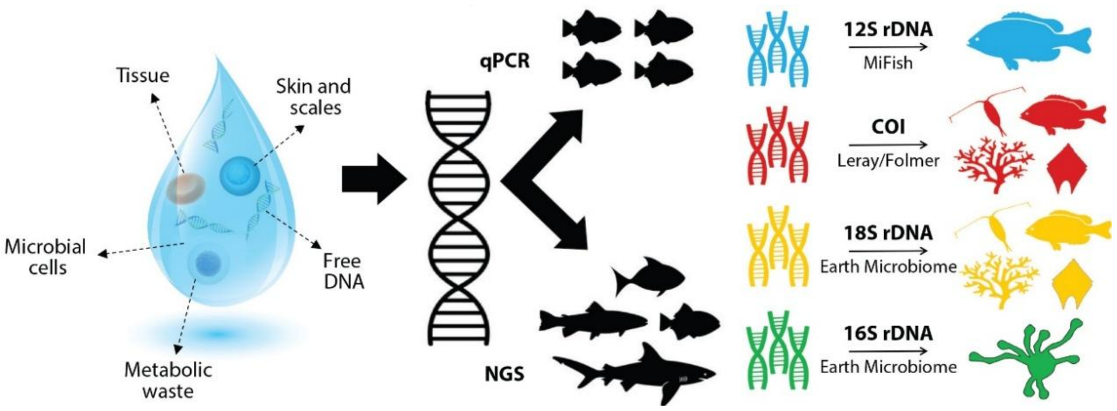


**Figure 4.** Marine eDNA suspended in seawater may contain living cells, metabolic waste, parts of organisms or dissolved material. Shown on the right are a series of gene markers used to investigate a wide range of organisms. Figure from Chavez et al. (2021).

Since trophic network models are complex and require a large amount of quantitative data that can take a long time to analyze and the need for highly specialized operators for taxonomic identification (Ruppert et al., 2019), the use of eDNA metaB make it’s possible to study the entire planktonic community relatively easily and quickly (Ruppert et al., 2019; Djurhuus et al., 2020). Several studies have used eDNA data to derive networks based on correlations between taxa abundances, which can describe qualitative biological interactions with good approximation (e.g., Djurhuus et al., 2020; Steele et al., 2011). However, the direct use of data from eDNA metaB for food web analysis still has methodological limitations, related to the qualitative nature of the information obtained, the difficulty of interpreting genetic signals in terms of biomass and actual interactions, and the lack of standardization in analytical protocols. To date, the use of eDNA to develop robust ecological network models is still limited, but one possible approach would be to employ the techniques described above (i.e., trophic network models and eDNA metaB) to study in an integrated and detailed manner possible changes in marine plankton trophic networks in relation to environmental conditions.

## 2. AIM OF THE STUDY

The aim of this PhD thesis was to develop and apply integrated and multidisciplinary approaches to understand how the structure and functioning of planktonic food webs change across different coastal marine environments, characterized by distinct ecological conditions and levels of anthropogenic pressure. Furthermore, the project also aimed to provide specific ecological indicators to support coastal environmental management tools, in line with the criteria established by the Marine Strategy Framework Directive (MSFD), particularly regarding the assessment of Good Environmental Status (GES).

The research was based on the analysis of a large dataset obtained through: (i) two sampling surveys conducted along the coasts of the Campania region (autumn 2020 and summer 2021), within the framework of the FEAMP-ISSPA project; (ii) an oceanographic cruise in the North Sea (Belgium, July 2022), carried out as part of the S4GES – JPI Oceans project. This data enabled a high-resolution characterization of planktonic communities, considering both taxonomic and functional components. This was made possible through the integration of different analytical methodologies, including traditional microscopic analysis, molecular techniques based on environmental DNA, and bio-physical assessments of natural capital. A synthesis of the research carried out in this Ph.D. project is presented in the following paragraphs.

Chapter 3 presents a comprehensive and detailed analysis of copepod functional traits and their spatial distribution in coastal Campania waters, considering local environmental conditions such as the availability of trophic resources and land-based inputs. This study provided important baseline information for the network modeling work presented in the following chapter.

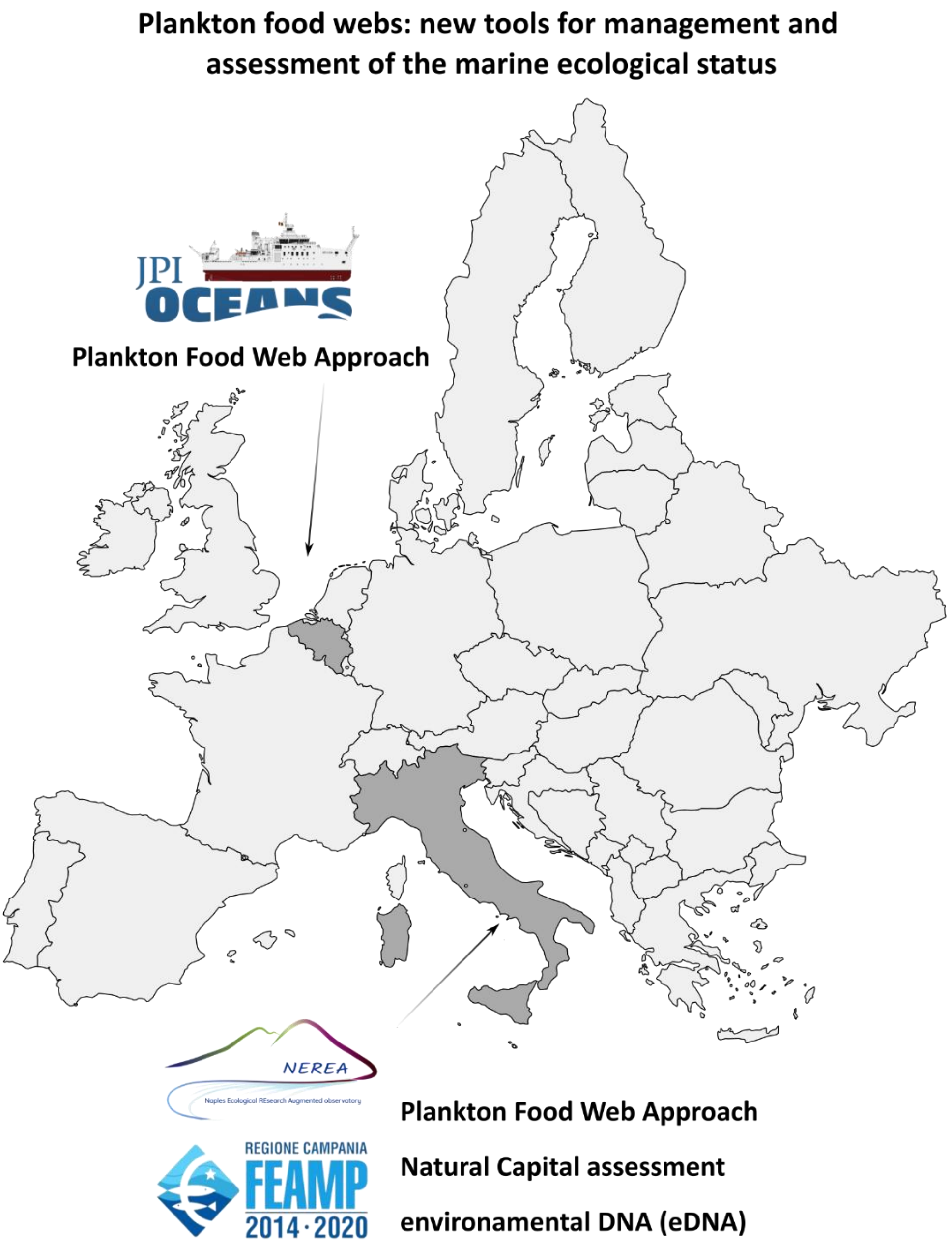
Chapter 4 focuses on the development of ecological models to analyze structural and functional changes in planktonic food webs in relation to local environmental conditions (e.g. riverine inputs). The specific aims were to: (i) investigate structural changes in the planktonic food web in response to environmental pressures (e.g., turbidity and input of organic matter); (ii) evaluate shifts in the behavior of key planktonic functional groups; (iii) test the utility of network-based trophic indicators as tools for assessing ecosystem status, supporting environmental policies and marine spatial planning.

In Chapter 5, the approach presented above was applied in the North Sea to explore how the structure and functioning of planktonic food webs vary under different tidal conditions. This also led to the identification of ecological indicators useful for environmental planning and the integrated management of coastal areas.

In Chapter 6 presents the development and application of an innovative methodological approach that integrates food-web models with emergy accounting, to quantify, in biophysical terms, the natural capital (NC) and environmental flows (ENFL) associated with plankton biodiversity during the autumn 2020 and summer 2021 surveys.

Lastly, in Chapter 7 the environmental DNA (eDNA) was used to study pelagic food webs, with the aim of evaluating the spatiotemporal variability of plankton communities and their connectivity within the Campanian coastal system, complementarily to traditional biodiversity survey and food-web modelling. By combining eDNA analysis with Lagrangian passive dispersal modeling, the study investigated how marine currents influence food web structure and biodiversity distribution, highlighting the role of spatial and temporal factors in shaping ecological interactions.

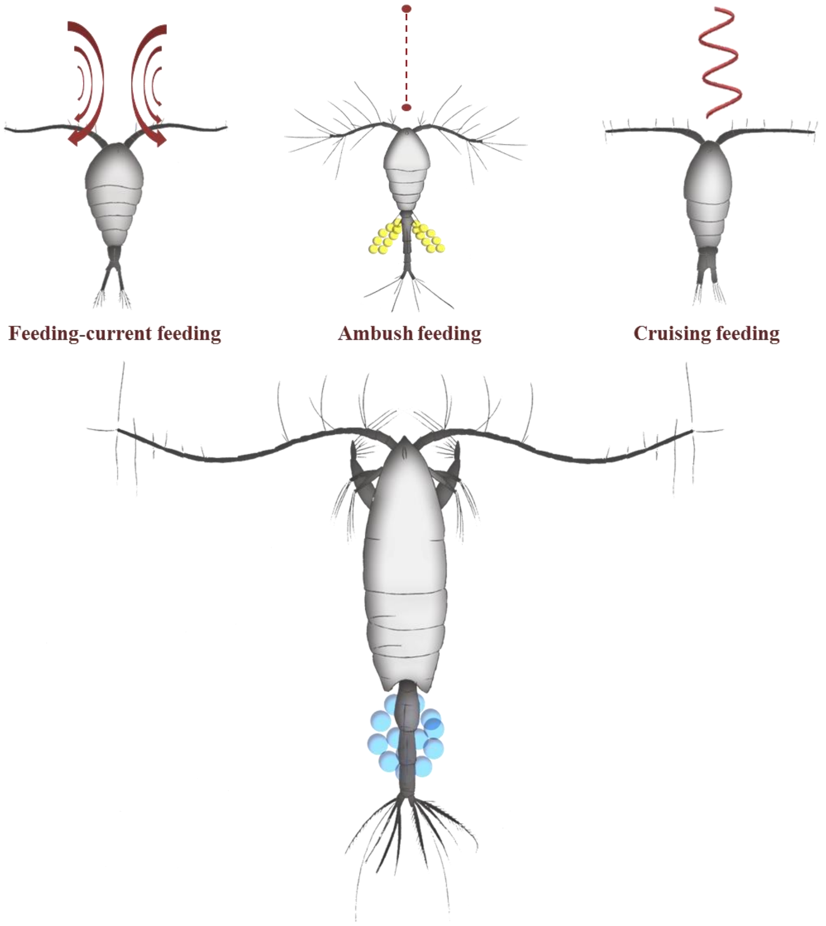
The results obtained are detailed in the following sections.



Visual concept of Ph.D. activities and study areas in which different approaches have been applied to study plankton communities.

## 3. THE SPATIAL DISTRIBUTION OF COPEPOD FUNCTIONAL TRAITS IN A HIGHLY ANTHROPIZED MEDITERRANEAN COASTAL MARINE REGION (Paper I).

This study focuses on copepods, marine zooplanktonic organisms of primary importance within marine food webs. Despite the vast literature concerning the taxonomy of these organisms, little research has focused on the analysis of their functional traits. Therefore, we conducted a thorough investigation into the functional traits of 95 copepod species along the coast of Campania, a region characterized by significant population density and marine biodiversity. We analyzed their body sizes, feeding and spawning strategies and trophic regime. Our results revealed the existence of nine distinct functional groups, each with unique functional characteristics. We observed that in the Campania marine ecosystem herbivorous groups were prevalent, accounting for 54% in autumn and 69% in summer of the total copepod abundance, characterized by a trophic current-feeding regime and spreading strategy. The predominant species in this category include *Acartia clausi*, *Centropages typicus, Temora stylifera and Paracalanus parvus.* This study, the first of its kind along the Campania coast, provides a detailed view of the functional diversity of copepods in this region. Overall, our results support an improved understanding and trophic characterization of the Mediterranean pelagic system, thus contributing to the conservation of marine resources and ecosystem services in an area of considerable ecological and economic value.



Graphical abstract of the work “The Spatial Distribution of Copepod Functional Traits in a Highly Anthropized Mediterranean Coastal Marine Region” - Figure from (Almeda et al 2017)

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# The Spatial Distribution of Copepod Functional Traits in a Highly Anthropized Mediterranean Coastal Marine Region

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**Abstract:** Copepods dominate marine zooplankton in abundance and play key roles in pelagic food webs. These small crustaceans show high taxonomic and functional diversity. Although there has been considerable research on their taxonomy, only a few studies have focused on their functional traits. In this study, we analyzed the functional traits of 95 copepod species, considering their body size, trophic regime, feeding behavior, and spawning strategy. Based on samples collected during two surveys (autumn 2020 and summer 2021) located in the coastal waters of three gulfs (Gaeta, Naples, and Salerno) in the highly populated Campania region (the central Tyrrhenian Sea, NW Mediterranean), we identified nine functional groups of copepods with different characteristics. The group that comprised herbivorous copepods with feeding currents and a broadcast strategy was the most abundant in both seasons and all gulfs. This group was dominated by *Acartia clausi*, *Centropages typicus*, *Temora stylifera*, and the *Paracalanus parvus* complex. The other functional groups showed differences in their temporal and spatial distribution. Our study reports the functional diversity of copepods along the Campania coast, thus contributing to advancing our knowledge of the planktonic trophic structure in a region of considerable importance due to its marine resources and services.

**Keywords:** zooplankton; functional diversity; Tyrrhenian Sea; feeding strategy; spawning strategy

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## 1. Introduction

Zooplanktonic communities play a vital role in marine ecosystems [1]. They are mainly represented by copepods, which are the dominant taxonomic group both in terms of their diversity and abundance of individuals [2] and account for more than 80% of the total abundance of mesozooplankton (0.2–2.0 mm) [3]. Copepods represent the key link within pelagic food webs, as they are the main pathway of energy transfer from primary producers to fish [1,4] and contribute substantially to the functioning of the biological pump [5,6]. Furthermore, copepods also show enormous diversity in functional terms: they can obtain food through ambushing, filter feeding, or cruising behavior and can feed on particles and aggregates [7]. Copepods can select their prey based on size [8,9] and motility [10], as well as mechanical and/or chemical cues [11,12]. Since the role of planktonic copepods in the marine ecosystem is influenced by their diversity and phenotypic characteristics [13], studying the functional traits of these organisms is a fundamental step toward understanding the overall ecosystem functioning [14].

Functional traits, defined as phenotypic attributes that shape the physical form of an organism, delineate species based on their physiological functions and the interactions displayed in the abiotic and biotic environment [15]. Functional traits are characteristics that, at the species or organism level, influence their fitness and have been related to survival, feeding, growth, and reproduction [14,16]. Functional traits are useful for categorizing species that show similar traits into certain functional groups (FGs) [17,18]. The categorization approach based on similarity in functional traits rather than taxonomic classification allows diversity to be summarized in distinct and parsimonious groups. These groups have the potential to improve the representation of zooplankton in ecosystem models, as FGs enrich the description of ecological functionality without introducing additional diversity and taxonomic complexity.

Few studies have been conducted on zooplankton functional diversity in marine and estuarine environments (e.g., [19,20]) and in marine plankton ecology [18,21], and most of them are focused on copepods, given their high abundance, as well as great functional and morphological complexity [22]. Several studies, both at the regional and global scales, have used the study of functional traits to group species with similar traits into FGs [17,18] to describe zooplankton diversity [23,24], with the aim of analyzing community responses to environmental disturbances [21,25] or describing marine food webs with a higher level of detail [26,27].

Although functional trait analyses have been carried out in the Mediterranean Sea [21,28], similar studies are still lacking in the Tyrrhenian Sea, which is considered the most oligotrophic region in the western Mediterranean [29]. The coastal areas of the Tyrrhenian Sea have represented globally crucial ecosystems over the last few decades [30,31], but they are constantly exposed to increasing anthropogenic pressures, which have intensified over time [32,33]. One of the areas overlooking the Tyrrhenian Sea is Campania, a region with three main gulfs, which are, from north to south, the Gulf of Gaeta, the Gulf of Naples, and the Gulf of Salerno (Figure 1). The entire coast of Campania, and particularly the metropolitan area of Naples, is severely exposed to anthropogenic pressure [34] and extensive agriculture, livestock farming, and industrial activities [35]. In addition, coastal waters may also be subject to natural forcings that act at the seasonal or multi-year scale (e.g., periods of drought/rain), as well as short-term episodic disturbances (e.g., floods, storms) [36]. Monitoring these environments is important for understanding the risks imposed by anthropogenic and natural impacts on coastal marine habitats.

In particular, the Gulf of Naples has been extensively studied thanks to the presence of the Long-Term Ecological Research site MareChiara (LTER MC), established in 1984 [37,38] to monitor the physical [39] and chemical [40] characteristics of the water column and the dynamics of plankton [41–43]. In contrast, the Gulf of Gaeta and the Gulf of Salerno have received less attention. Studies in the Gulf of Gaeta have primarily focused on assessing the effects of the Garigliano and Volturno river flows on coastal dynamics [44], while studies on both the physical [44] and biological aspects [45] of the Gulf of Salerno are scant [45].

In this study, we present a complete and detailed overview of copepods' functional traits and of their spatial distribution in the Campania region. Statistical analyses were conducted to define the copepods' FGs, based on specific biological and ecological information obtained during two oceanographic surveys (autumn 2020 and summer 2021) conducted along the Campania coast. We also discuss the spatial distribution of copepod functional traits considering local environmental conditions, such as trophic resource availability, and inputs from the land. This study aims to contribute to a better understanding and trophic characterization of the pelagic system, while providing important background information for future studies.

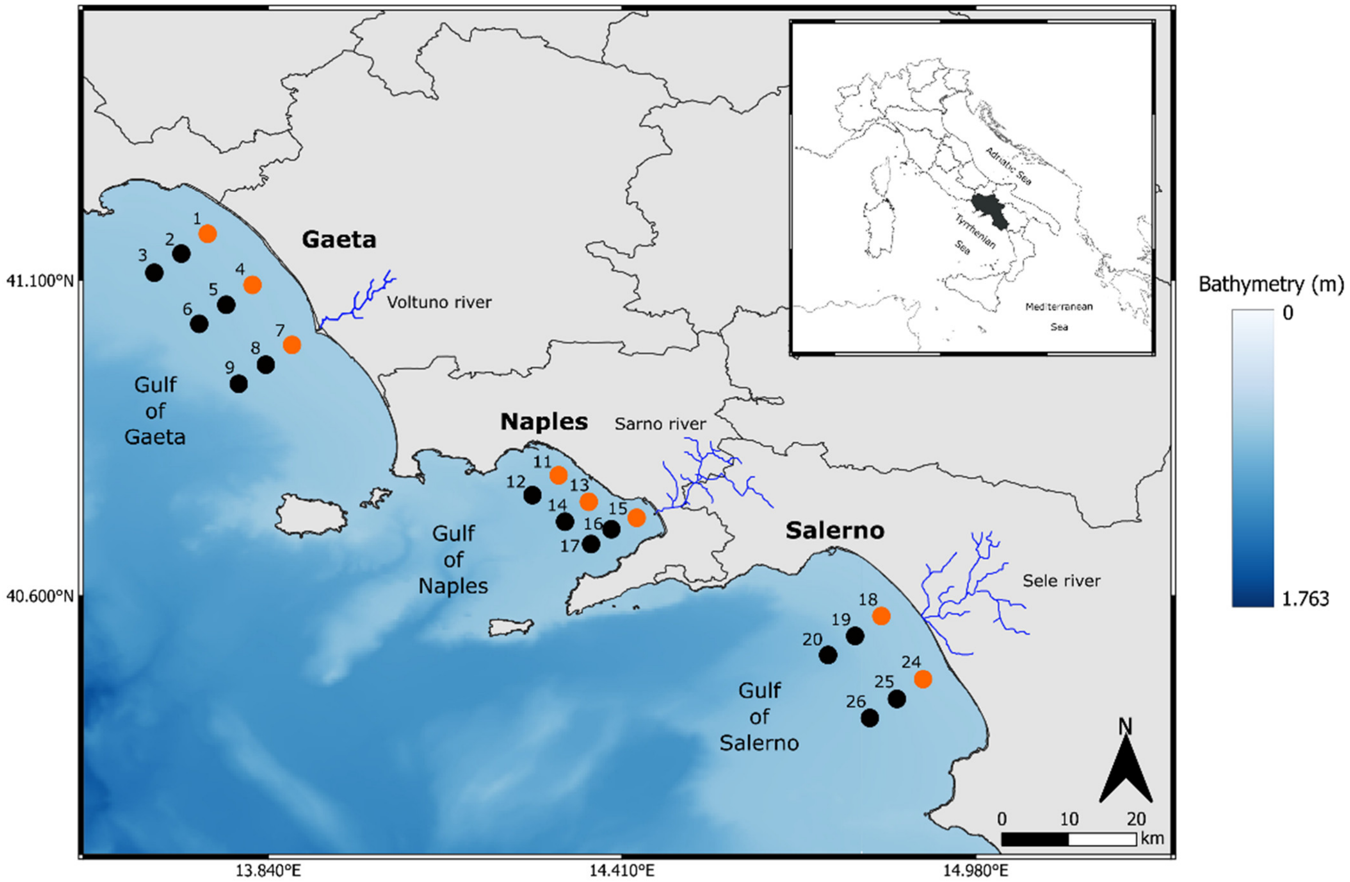

**Figure 1.** Study area (Campania region) and sampling stations. Orange circles represent inner shelf stations (<20 m depths), and black circles represent mid shelf stations (50–150 m depths).

## 2. Materials and Methods

### *2.1. Sampling Area and Environmental Data*

We carried out two surveys along the Campania coast (central Tyrrhenian Sea, NW Mediterranean Sea) on board *R/V Vettoria* in the autumn of 2020 (9 September–9 October) and summer 2021 (29 June–15 July). During each survey, we sampled a total of 22 stations among the Gulf of Gaeta (9 stations), the Gulf of Naples (7 stations), and the Gulf of Salerno (6 stations) (Figure 1). The stations were located along transects from the inner (<20 m depth) to mid (50–100 m depth) shelf waters (Table S1).

The three gulfs have different geomorphological characteristics and are influenced by different anthropogenic factors. The Gulf of Gaeta (41°06′ N 13°30′ E) is heavily urbanized, hosts fish and shellfish farms [46,47], and is placed at the mouth of one of the main rivers in southern Italy, i.e., Volturno, which is highly polluted due to discharges from local factories, sewers, and agricultural drains [48]. Volturno River has a seasonal influence on the coasts; in fact, in winter, it manifests a plume oriented more offshore, therefore without interacting with the coastal waters, while in summer, the plume is oriented both north and south of the estuary, influencing the coastal waters [49,50].

The Gulf of Naples (40°44′ N 14°16′ E) is characterized by heavy land runoff from a very densely populated area due to the Sarno River's outflow, though it is also influenced by typical Tyrrhenian oligotrophic waters due to its bottom topography and general physiography [35,51]. The Sarno River is considered the most polluted river in Europe due to heavy metal contamination from industrial activities along its course [52]. From a physical point of view, the Gulf of Naples [53,54] shows a period of stratification in July–August and a period when the water column is close to completely mixing from December to January, followed by surface re-stratification in February. Similar detailed knowledge about the water stratification processes is not available for the Gulfs of Gaeta or Salerno.

The Gulf of Salerno (40°31′ N 14°42′ E) is characterized by oligotrophic conditions and exposed to the influence of Tyrrhenian waters [45,55]. Here, the Sele River is responsible for transporting pollutants into the sea due to the presence of manufacturing companies from the textile and leather industries and agroindustry along its banks [56,57]. In general, the Gulf of Salerno has been described as a spatially homogeneous system without areas of enrichment, with its coastal influence limited to a restricted inner shelf area [45].

All three gulfs are influenced by upwelling processes, which bring nutrient-rich waters to the surface. Along the Campania coast, these processes occur due to strong winds and the presence, in the Gulf of Gaeta and Naples, of the Cuma Canyon and the Dohrn Canyon, respectively [58,59].

For each sampling site, temperature (°C), salinity (PSU), and turbidity (transmittance) profiles were acquired using a multi-parameter probe (Sea-Bird 911 Plus). In addition, the total chlorophyll *a* was analyzed at selected depths (0, 10, 25, 50, 75, 100 m) using High-Performance Liquid Chromatography analysis (HPLC—Agilent 100, Agilent Technologies, Santa Clara, CA, USA). These environmental values were integrated into the surface layer (0–10 m) and the deep layer (10 m—zooplankton sampling depth) (Table S1).

### 2.2. Mesozooplankton Sampling and Analysis

Mesozooplankton samples were collected using vertical hauls from 5 m above the bottom to the surface using a double WP2 net (57 cm Ø, 0.25 $m^2$ mouth area of each net, 200 µm mesh size) equipped with a flowmeter and towed at 0.7–1 m $s^{-1}$. One of the two samples was used to estimate the mesozooplankton abundance and taxonomic composition, and the other was used to investigate the gelatinous zooplankton taxonomy. We consider here only the first series of the samples, which were transferred from the cod-end into 500 mL plastic jars, gently concentrated on a gauze (100 µm mesh size), and then fixed in ethanol 96% in 100 mL plastic jars.

In the laboratory, each sample was resuspended in a bowl with distilled water to a final volume of 200 mL. The sample was accurately stirred with a graduated pipette, which was also used to collect two aliquots of 5 mL each that were analyzed for zooplankton identification and enumeration under a stereomicroscope (Leica MZ12.5) in a 10 mL Bogorov counting chamber. The rest of the sample was checked to account for the presence of rare species. The taxonomic identification was performed down to the species level whenever possible, following the proper literature (e.g., [60–63]). The copepods were further identified according to gender and developmental stage (adult females and males, copepodites). To allow for comparison of records collected in water columns of different depths, the abundance was expressed as the number of individuals in a square meter (ind. $m^{-2}$) (Tables S2 and S3).

### 2.3. Copepod Functional Traits

Functional traits represent various aspects of copepod ecology related to competition and habitat use. In this work, functional traits were attributed to copepod species based on their (i) mean body length (<1 mm, 1–2 mm, >2 mm), (ii) feeding strategy (FS) (ambush feeding, current feeding, cruise feeding), (iii) spawning strategy (SS) (broadcast spawners, egg sacs), and (iv) trophic regime (TR) (carnivore, omnivore, omnivore–detritivore, and omnivore–herbivore). Transitional groups were established to separate species that, while being technically omnivorous, showed a relative preference for herbivory or detritivory [23]. Information on copepod functional traits was obtained from the literature [18,63–66] and from the knowledge of experts in the field. Concerning the feeding strategy, three different categories have been considered: the ambush strategy, in which copepods encounter and intercept prey and capture them with active attacks; current feeding, in which copepods are able to generate a feeding current and convey prey to their mouth appendages; cruise feeding, in which copepods navigate through the water, catching individual prey [64].

A binary matrix of functional traits was created including all the recorded taxa, assigning 1 when a trait was present in the specific taxon or conversely 0 when a trait was absent. When it was not possible to allocate specific traits to a copepod species owing to little or no information in the literature, the category NA (Not Assigned) was used (Table S4).

### *2.4. Data Analysis*

A dissimilarity matrix (with Jaccard distance) was calculated based on the binary matrix of functional traits, and then agglomerative hierarchical clustering analysis (Ward's method) was used to identify different FGs. For this analysis, only feeding strategy, spawning strategy, and trophic regime were considered as traits. The number of FGs was determined by using the K-means values as a cut-off level, and the "Elbow method" was applied [67] to determine the optimal number of FGs.

To test the significance of seasonal and spatial differences in terms of the abundance of functional groups (data transformed by log(x + 1)) and environmental parameters (data normalized), a two-way permutational multivariate analysis (used Hellinger and Euclidian distances for biological and environmental data, respectively) of variance (PERMANOVA, $p < 0.05$), followed by a pairwise test for significant terms, was performed on three fixed factors: "Season" (two levels: autumn 2020 and summer 2021), "Gulf" (three levels: Gulf of Gaeta, Gulf of Naples, and Gulf of Salerno), and "Distance" (two levels: inner and mid shelf stations). All the analyses were performed and plots generated using the R Studio software v.4.3.2 ('*factoextra*', '*vegan*', '*Rstatix*', and '*tidyverse*' packages) [68].

## **3. Results**

### *3.1. Environmental Variables*

The environmental conditions, defined by temperature, salinity, turbidity, and chlorophyll *a*, appeared to be significantly different between the inner and mid shelf stations ($p < 0.001$) in both seasons. Only during the summer did significant differences emerge among the three gulfs, particularly between the Gulf of Naples and the Gulf of Salerno ($p < 0.01$).

The overall sea water temperature was not significantly different between the three gulfs (Figure 2). In the Gulf of Salerno, the integrated temperature was significantly higher in autumn as compared to summer, while no significant difference was recorded between seasons in the other gulfs.

The lowest values for depth-integrated temperature were recorded in summer in the Gulfs of Salerno (19.1 °C) and Gaeta (19.8 °C), and the highest values in autumn were recorded in the Gulf of Salerno (21 °C) (Figure 2). In both autumn and summer, the average sea surface temperature was higher in the upper 10 m (24.5 ± 0.98 °C and 25.7 ± 1 °C, respectively) than in deeper waters (19.1 ± 2.6 °C in both seasons) (Table S1). Salinity was consistent across regions, with the highest values recorded in the Gulf of Salerno in autumn and the lowest in the Gulf of Gaeta in summer. The seasonal distributions were not significantly different, except for in the Gulf of Gaeta, where the salinity in autumn was significantly higher than that in summer (Figure 2). Turbidity varied between 0.12 and 0.87, with the highest depth-integrated values in the Gulf of Salerno, where turbidity was significantly higher in the autumn (up to 0.48) as compared to the summer (Figure 2). In summer, the surface turbidity was higher in the Gulf of Naples (0.51) than in Gaeta and Salerno (~0.22), while in the deep layers, it was similar in all three gulfs (~0.25) (Table S1). Chlorophyll *a* peaked in summer (highest concentrations in the Gulf of Naples, 9.1 $\mu g\ L^{-1}$) and was lower in autumn (minimum in the Gulf of Gaeta, 2.9 $mg\ m^{-2}$), with significant seasonal differences in the Gulfs of Gaeta and Naples (Figure 2). The chlorophyll *a* concentration was higher in the Gulf of Naples in both autumn and summer, with surface concentrations of 3.19 $mg\ m^{-2}$ in autumn and 6.62 $mg\ m^{-2}$ in summer, than in Gaeta and Salerno (<2 $mg\ m^{-2}$). Even in the deeper layers, the concentration of chlorophyll *a* was highest in the Gulf of Naples (5.25 $mg\ m^{-2}$ in autumn and 9.07 $mg\ m^{-2}$ in summer), while in Gaeta and Salerno, it was around 4.7 $mg\ m^{-2}$ (Table S1).

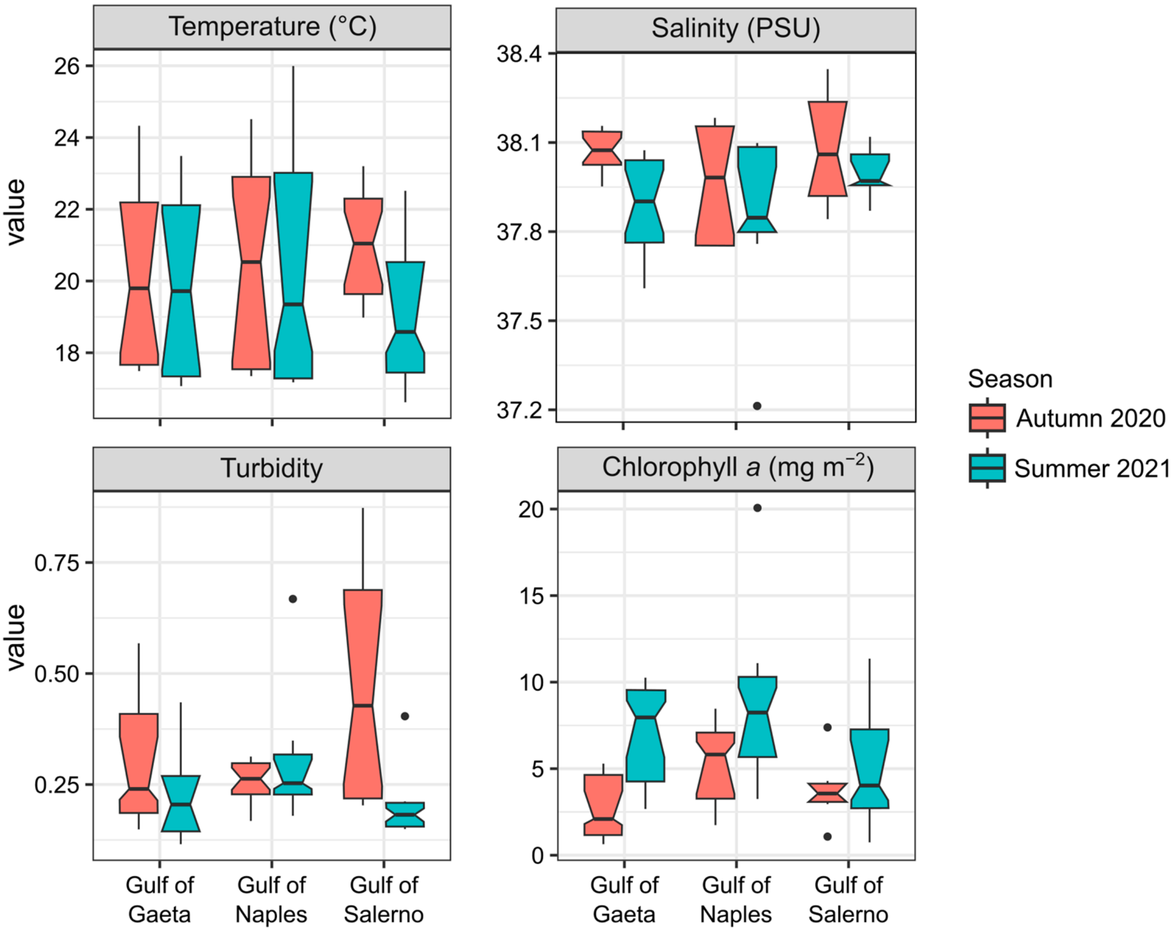

**Figure 2.** Box plots showing the depth-integrated values of environmental variables recorded during the autumn (red) and summer (blue) seasons in the three gulfs of the Campania region. The midline indicates the median value, the vertical line represents the minimum and maximum values, and the black dots indicate the outliers.

### *3.2. Copepod Functional Groups*

In autumn, the total mesozooplankton abundance was on average 3 × $10^6$ ind. $m^{-2}$, 52.6% of which was represented by copepods, followed by cladocerans, which were less represented in the Gulfs of Gaeta and Naples (7%) than in the Gulf of Salerno (19%) (Figure 3). Calanoids were overall the most abundant copepod group (on average, 81% of the total copepod abundance), with *Temora stylifera* being the dominant species, particularly in the Gulf of Naples (3% of total copepod abundance), as opposed to the Gulf of Gaeta and Salerno, where it represented 21% and 13% of the total copepods, respectively. The *Paracalanus parvus* complex, the second dominant copepod taxon, represented 13% of the total copepod abundance in all three gulfs. In summer, the total mesozooplankton abundance was on average 1.62 × $10^6$ ind. $m^{-2}$, of which 44.9% was represented by copepods, followed by cladocerans, which, also in this season, were particularly abundant in the Gulf of Salerno (14% of the total zooplankton). In summer, calanoids were the main copepod group (81%) (Figure 3) and were dominated by the *Paracalanus parvus* complex (22.6%) and *Centropages* spp. (11.2%), with both taxa present with a relatively similar abundance in all three gulfs (Tables S2 and S3).

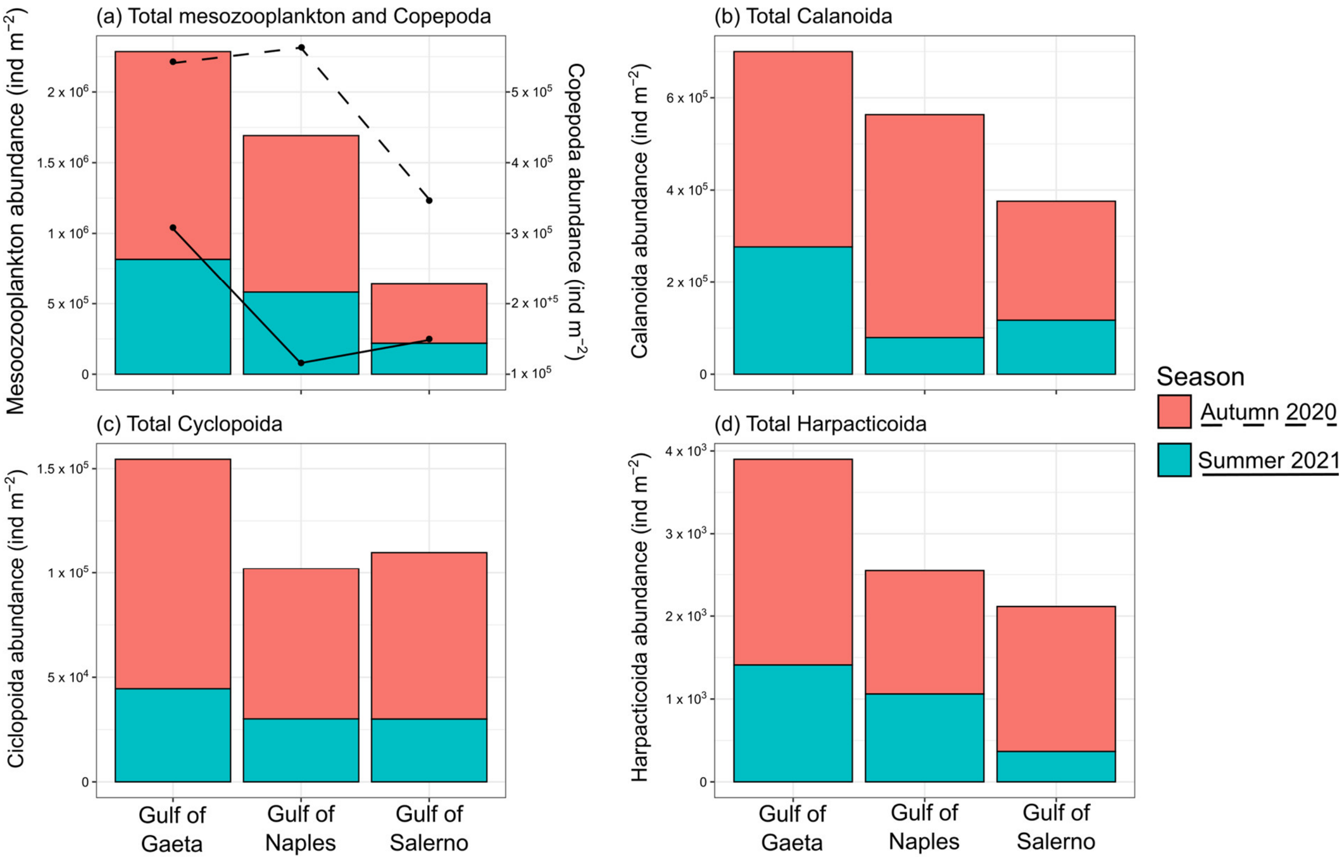

**Figure 3.** Bar plot showing the total abundance (ind. m$^{-2}$) during autumn (in red) and summer (in blue) of mesozooplankton and copepods (**a**), where the dashed lines refer to the total abundance of copepods in autumn 2020 and the solid lines to summer 2021. (**b**) Total abundance of Calanoida, (**c**) Cyclopoida, and (**d**) Harpacticoida.

We identified nine functional groups (FGs) in the overall copepod assemblage, represented by 95 species (Figure 4). FG1 encompassed the highest number of species and included broadcast, current-feeding taxa, with a tendency toward herbivory. Moreover, this assemblage included different body sizes, among them small (e.g., *Calocalanus* spp. and *Paracalanus* spp.) and medium-sized (*Acartia* spp., *Temora stylifera*) calanoids. FG2 included medium-sized, cruising, broadcasting species (*Scaphocalanus* and Scolecitrichidae). FG3 was represented by small (*Diaixis pygmaea*), medium-sized (*Isias clavipes* and Pontellidae), and large (*Labidocera wollastoni*) omnivorous species, which produce feeding currents and have a broadcasting reproductive strategy. FG4 included the cyclopoid family of Corycaeidae, small–medium-sized carnivore species, ambush feeders, and sac-spawners. FG5 grouped all the *Oithona* species, which are small, omnivorous, ambush-feeding cyclopoids and carry egg sacs. FG6 consisted mainly of large (*Haloptilus* spp., *Heterorhabdus* spp.) to medium-sized (*Candacia* spp.) cruising carnivores with a broadcast reproductive strategy. FG7 grouped small to medium-sized species of the genera *Clausocalanus* and *Macrosetella*, which feed by cruising and carry egg sacs. FG8 consisted mainly of large carnivores (e.g., the genus *Copilia*, members of the family Euchaetidae, and species of the genus *Sapphirina*), with a cruising feeding strategy and a reproductive strategy using egg sacs. FG9 included small cruising detritivores (*Oncaea* spp., *Microsetella* spp.) that use a sac-spawning strategy.

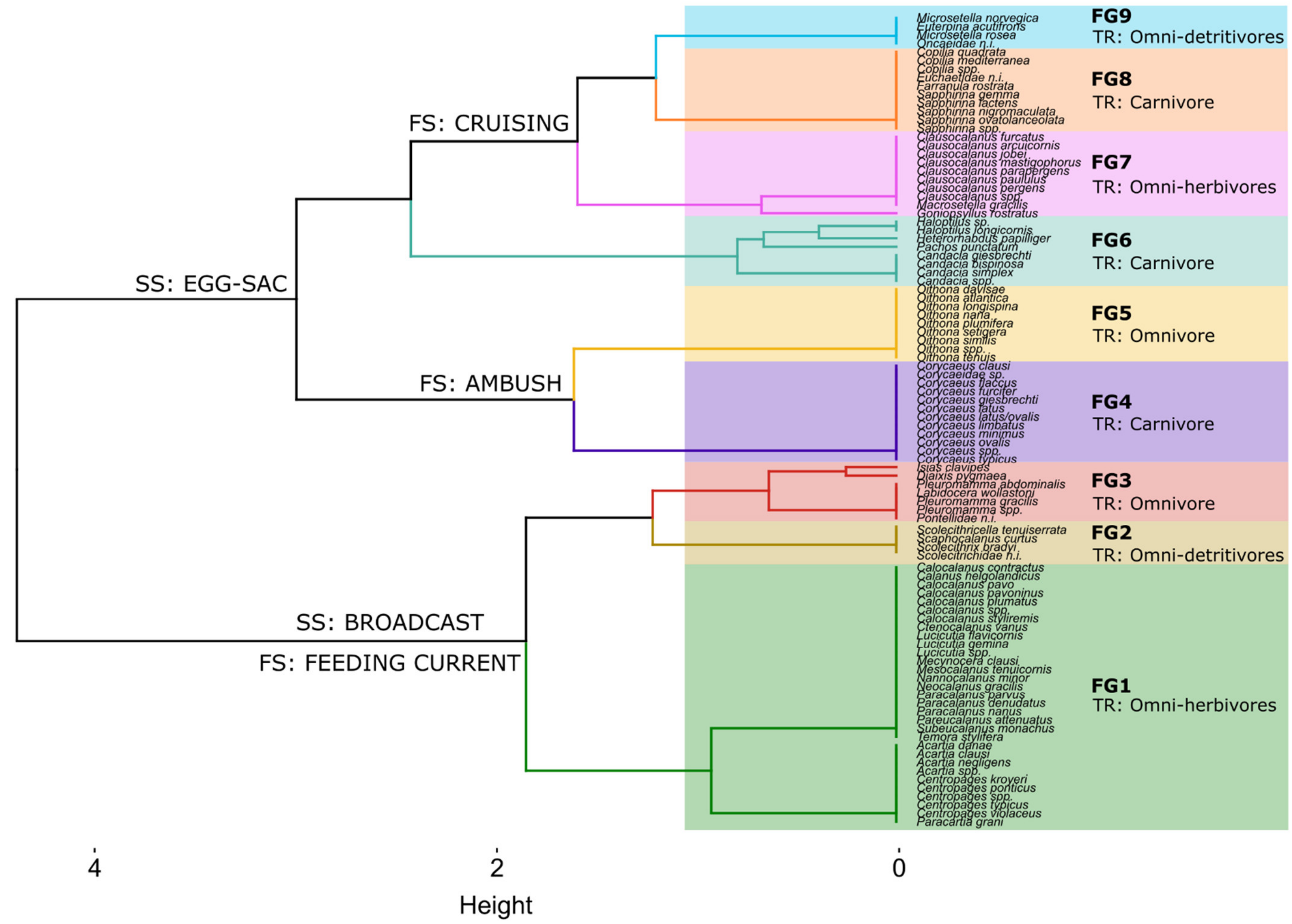

**Figure 4.** Functional dendrogram obtained from hierarchical clustering using Ward's method, showing nine functional groups (FGs) of the 95 analyzed copepod species (FS: Feeding Strategy; TR: Trophic Regime; SS: Spawning Strategy).

The occurrence of copepod FGs differed significantly ($p < 0.01$) between seasons and between the inner and mid stations. When considering the three gulfs, differences were found in autumn between Gaeta and Salerno ($p < 0.001$), whereas in summer, they were found between Gaeta and Naples ($p < 0.001$) and between Naples and Salerno ($p < 0.01$).

The prevalent group was FG1 (Figure 5a,b), more abundant in summer (69%) than in autumn (54%). In autumn, FG1 was more represented in the Gulfs of Gaeta and Naples (~65% for both) than in Salerno (34%). In summer, FG1 was more represented in the Gulf of Gaeta (83%), while it decreased in the Gulfs of Naples and Salerno (59% and 66%, respectively). In both seasons, FG1 was on average more represented at the inner shelf stations (76%) than at the mid shelf stations (62%). In autumn, the second most represented group was FG7, particularly abundant in the Gulf of Salerno (41%) as compared with Gaeta (15%) and Naples (21%), with an even distribution from the inner shelf to mid shelf stations (~23%). In summer, FG7 was poorly represented (7%). The third group in ranked order of relative abundance was FG5, which showed a similar percentage during both the summer and autumn (~12%) and higher relative abundance at the mid shelf (13%) than at the inner shelf (7%) stations. In autumn, FG5 was more represented in the Gulfs of Gaeta and Naples (16% and 12%, respectively) than in Salerno (7%), whereas in summer, it accounted for similar percentages (~13%) in the three gulfs. The other functional groups (FGs 2, 3, 4, 6, 8, and 9) were poorly represented in the three gulfs in both seasons, as they overall accounted for less than 5% of the total mesozooplankton abundance (Figure 5a,b and Table S5).

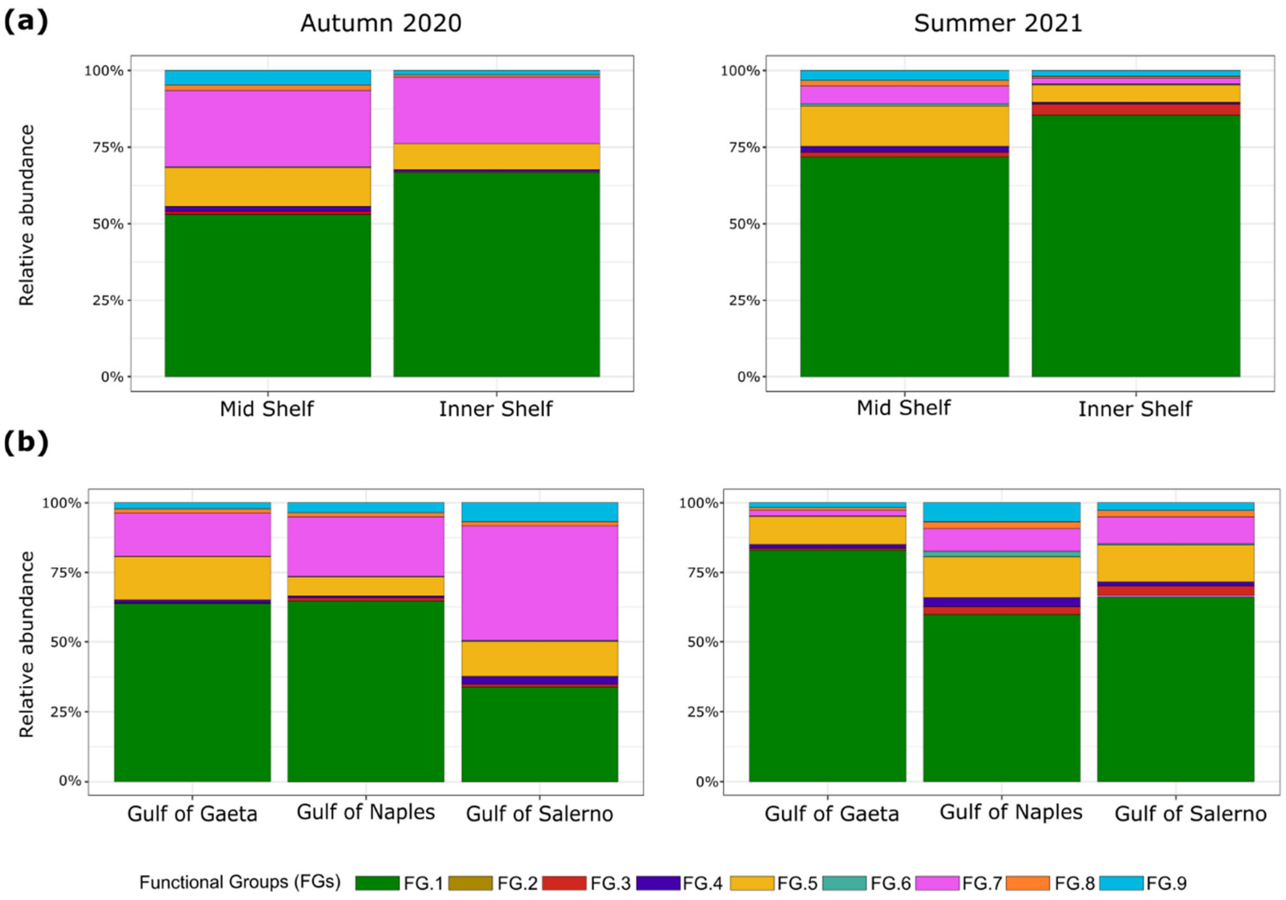

**Figure 5.** Bar plots of the relative abundance (%) of nine functional groups (FGs) found through cluster analysis, during the autumn and summer seasons at the mid and inner shelf stations (**a**) and in the three gulfs (**b**).

## 4. Discussion

This study presents the functional traits of copepods along the Campania coast, allowing for a better understanding of the patterns of the variability in and functioning of zooplankton communities in an important Mediterranean region. Our results identified nine different functional groups, which differed in their spawning and feeding strategies and trophic habits. These groups have consistently been found in other studies conducted at both the regional [18,28] and global scales [17,19,65].

Overall, we observed that the spawning strategy, represented by egg sac and broadcast spawners, was the trait distinguishing the two main copepod assemblages characterizing the Campania coast (Figure 2). Carrying egg sacs is considered an energetic strategy that reduces egg mortality, at the expense of individual fecundity and hatching rate, while egg broadcasting is a form of adaptation to protect the eggs [69]. Broadcast spawners are mostly calanoid copepods, while cyclopoids and harpacticoids mainly carry egg sacs, employing cruising and ambush feeding strategies.

Ambush feeding is frequently observed in small copepods, whereas it is less common in larger species, which tend to acquire food while cruising, probably due to differences in metabolic requirements linked to body size [70]. Ambush feeders encounter prey passively and require less energy than cruising predators, who must actively search for prey [7]; this strategy reduces metabolic costs and predation risk, although at the expense of feeding efficiency [64,71].

Our results showed that both summer and autumn were dominated by omni–herbivorous copepods, i.e., FG1. The species in this group represent the main food of many pelagic fish [72,73] and zooplanktonic predators [74,75]. Based on long-term observations, omni–herbivorous copepods, i.e., *Acartia clausi*, *Centropages typicus*, the *Paracalanus parvus* complex, and *Temora stylifera*, are the most abundant copepods in the Gulf of Naples. These species are present and consistently reproduce all year round [76] but with a succession of their peak of seasonal abundance. For the former three species, the populations begin to increase at the onset of the stratification period and reached peaks during the shallowest stratification period [43]. We observed that FG1 was more abundant under the conditions of a high chlorophyll *a* concentration, which typically characterize inner shelf stations during summer [77,78]. This seasonal preference could be possibly explained by the feeding strategy of the FG1 species, which feed through currents carrying phytoplanktonic cells and tending to reach high abundance along the inner shelf, where rivers provide a greater supply of nutrients. Considering the high concentration of contaminants and potentially toxic compounds accumulated along the Campania coast due to river runoff [34], the success of FG1 in this area could be also explained by the high capacity of some species in this group (such as *Acartia*) to tolerate eutrophicated and polluted areas [79,80].

The mid shelf stations explored in our study host a higher abundance of *Clausocalanus* species (FG7), which constitute an important numerical component of copepod communities throughout the year [81]. *Clausocalanus* in our study dominated the autumn period, when *C. furcatus* largely prevailed over the other congeners, and the autotroph biomass was much lower than in summer. In the Gulf of Naples, this species, which is reported to prefer oligotrophic conditions, where it reproduces better, is mainly found in the upper 40 m of the water column [81].

The presence of small omnivorous copepods, such as the cyclopoids *Oithona* (FG5) and Oncaeidae (FG9), was observed in both summer and autumn in all the gulfs. *Oithona* is considered one of the most abundant genera in the oceans and acts as an important link in the trophic network between the microbial loop and higher trophic levels [82]. *Oithona* spp. can adopt a predatory ambush strategy, preferring mobile prey such as flagellates, ciliates, and dinoflagellates [83,84]. This strategy reduces the risk of predation [85] and metabolic costs [86,87], while increasing tolerance to starvation due to lower energy demands [85]. These adaptations enable the genus *Oithona* to thrive in both oligotrophic and eutrophic environments [85,86], like the Gulf of Naples, which is highly intermittent in terms of the trophic resources originating from the coastline [88].

FG9 comprises detritivorous species, such as *Oncaea*, which mainly consume detritus originating from discharged appendicuralian houses and copepod nauplii [89,90] and use gelatinous zooplankton (e.g., thaliaceans and chaetognaths) as a potential substrate for survival [91], contributing to the recycling of organic matter [92]. Our analyses showed that FG9 was more represented in the Gulf of Salerno in autumn. This is probably due to a rise in turbidity caused by increased river runoff, sediment resuspension, and coastal erosion [93,94] but also by the increase in gelatinous zooplankton (such as thaliaceans and chaetognaths) observed in these area [43]. These conditions may also expose *Oncaea* to pollutants originating from urban areas. In fact, both *Oncaea* and *Oithona* species, thanks to their adaptation strategies, in particular their low metabolic rate and ability to feed on a wide range of prey [86,95], are copepods tolerant or insensitive to pollution [2,96,97].

Two-thirds of the carnivorous copepods appearing during our surveys (FG4 and FG8) were sac-spawners, and only FG6 showed a broadcast spawning strategy. Within these groups, we find species belonging to the families Corycaeidae (FG4), Candaciidae (FG6), and Sapphirinidae (FG8), which have very diversified diets, ranging from the consumption of microalgae [98,99] to small copepods [74,100], meroplankton [101,102], and gelatinous zooplankton, such as thaliaceans, appendicularians, and chaetognaths [102,103]. The low relative abundance of FG4, FG6, and FG8 in the Campania gulfs (Figure 4) is probably due to the use of a sampling method that fails to capture larger zooplanktonic organisms [104,105]. In general, our results show that only in the Gulf of Naples, during the summer, are relatively large carnivores present. This is probably due to an increase in available prey, which may include meroplankton larvae, showing a peak in the early summer [43] likely favored by the abundant presence of microalgae (indicated by the increase in chlorophyll *a*).

The presence of these available prey could also be due to the presence of nutrient-rich water from upwelling processes due to the presence of the Dohrn Canyon [59]. The appearance of typical offshore species (i.e., *Candacia*) is probably due to the surface circulation of the Tyrrhenian Sea, which arrives more regularly within the Gulf of Naples at this time of year [44,106], from oligotrophic Tyrrhenian to coastal waters [76].

## 5. Conclusions

Our results highlighted the spatial and temporal functional diversity of copepods along the Campania coast, which can be related to local environmental characteristics, thus improving our knowledge of the ecological roles of Mediterranean coastal zooplankton. Herbivorous species dominated in all the three gulfs and in both seasons, detritivorous species were observed most in autumn in the Gulf of Salerno, while carnivorous groups were most represented in summer in the Gulf of Naples. Our study represents a base for successive holistic studies to integrate the information acquired in this work with other biological, physical, and chemical data to increase our understanding of the functional complexity of plankton communities. Our study corroborates the view that functional traits in a community can be used as indicators of environmental characteristics, thus allowing for inference of ecosystem functions, such as energy transfer in the food web and nutrient cycling. Future studies should therefore focus on integrating a wider range of functional traits with multiple environmental factors and ecosystem functions to obtain a more complete and accurate view of the ecological dynamics of planktonic systems.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/environments11060113/s1. Table S1: Sampling stations and environmental data; Table S2: Abundance of mesozooplankton community (ind. $m^{-2}$) found in the sample during the autumn season (2020); Table S3: Abundance of mesozooplankton community (ind. $m^{-2}$) found in the sample during the summer season (2021); Table S4: Functional traits for each species found in the sample (the number of references is present in the main text); Table S5: Abundance of the functional groups (FGs) (ind. $m^{-2}$) found through cluster analysis.

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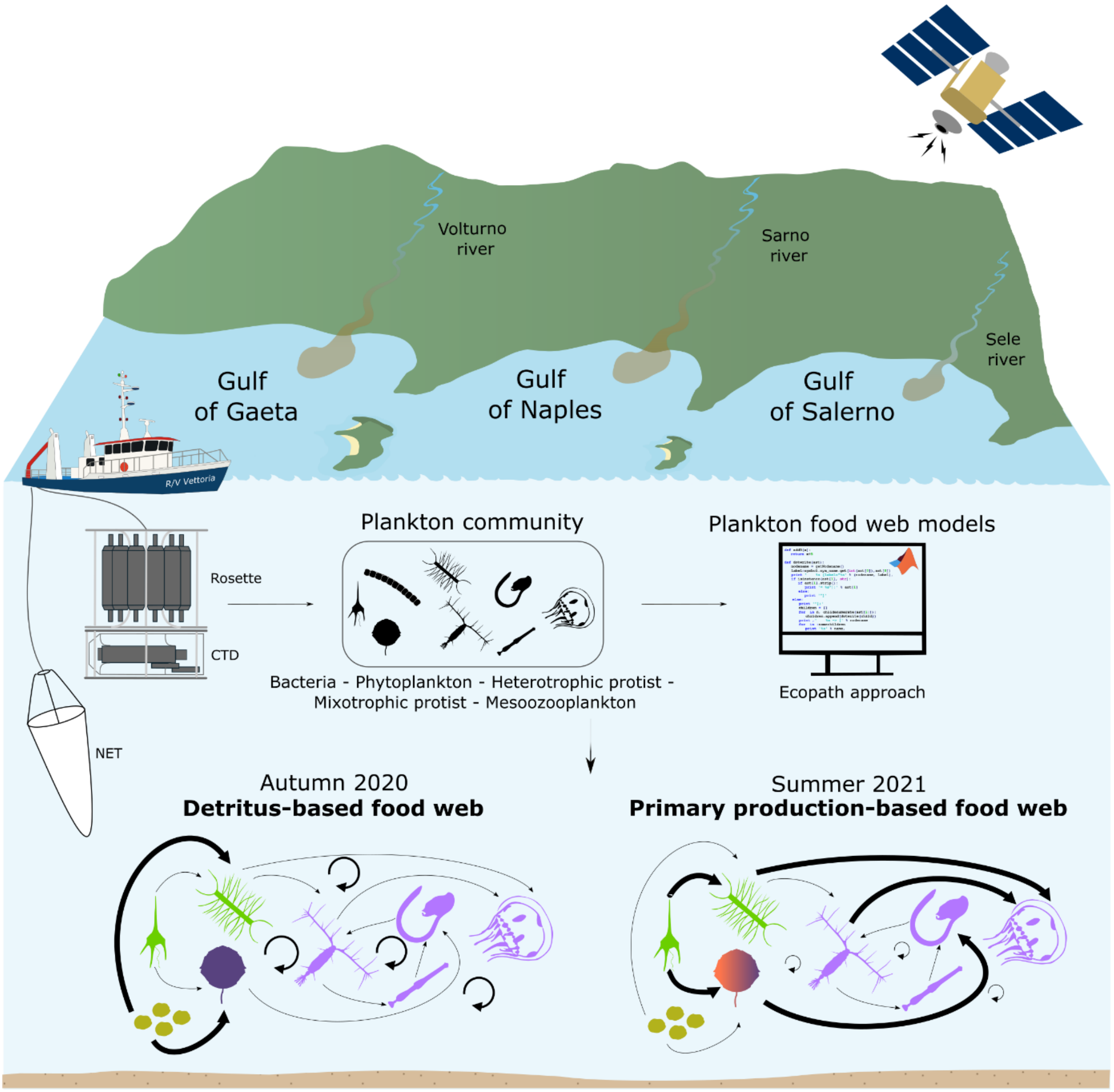
# 4. PLANKTON FOOD WEBS AS INDICATORS OF THE ECOSYSTEM STATUS TO SUPPORT THE MARINE STRATEGY FRAMEWORK DIRECTIVE: A CASE STUDY FROM CAMPANIA REGION (ITALY) (Paper II).

This study investigates the structure and functionality of planktonic food webs along the coasts of three gulfs in the Campania Region (central Tyrrhenian Sea, NW Mediterranean), providing new insights into marine ecosystem dynamics. Using an ecological-network modeling approach based on *Ecopath*, we derived specific ecological indicators to assess the trophic status of these systems during contrasting seasonal periods: autumn 2020 and summer 2021. Our findings revealed clear seasonal structural differences in planktonic food webs. In autumn, the microbial food web, particularly in the Gulf of Gaeta, demonstrated higher network organization, dominated by detritivores. In contrast, summer dynamics were driven by primary production and herbivorous processes. Integrating these ecological findings with physical analyses, such as coastal connectivity and satellite observations, highlights the critical role of planktonic food webs in supporting marine ecosystem functioning and coastal management. This work represents a significant contribution to the implementation of Descriptor 4 of the European Union’s Marine Strategy Framework Directive (MSFD), which evaluates the impact of anthropogenic pressures and climate change on marine food webs. By deepening our understanding of seasonal food web dynamics, our study has provided essential tools and insights for stakeholders in charge of monitoring and managing marine health. We believe that this work highlights the importance of applying ecological indicators for sustainable management.



Graphical abstract of the work “Plankton food webs as indicators of the ecosystem status to support the Marine Strategy Framework Directive: a case study from Campania region (Italy)”

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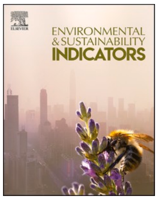

# Plankton food webs as indicators of the ecosystem status to support the marine strategy framework directive: a case study from Campania Region (Italy)

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ABSTRACT

The European Union's Marine Strategy Framework Directive (MSFD) aims to assess a "Good Environmental Status" based on the analysis of 11 Descriptors. Among them, investigating the food webs (Descriptor 4) helps to evaluate the impact of anthropogenic and climate change on the functioning of marine ecosystems. The present study analyzed, with specific ecological indicators, how environmental conditions (e.g., river inputs) can affect the structure and functionality of plankton food webs. An ecological-network modeling approach (based on *Ecopath*) was developed and applied along the coast of the Campania Region (central Tyrrhenian Sea, NW Mediterranean) in the three main gulfs (Gaeta, Naples, and Salerno), during two seasonal time-snapshots (autumn 2020 and summer 2021). The results revealed clear seasonal structural diversity of the planktonic food web, with autumn 2020 showing less network organization (Average Mutual Information, AMI: 1.18, Relative Ascendency, A/C: 23 %), especially in Gaeta, than summer 2021 (AMI: 1.30, A/C: 35 %). In autumn, Gaeta's food web was primarily dominated by filter feeders (e.g., doliolids, appendicularians and salps), whereas in summer, it was centered around primary production and grazing. Integrating the analysis from satellite data, it was possible to attribute autumn trophic condition to increased inputs of organic matter of terrestrial origin, transported by the main rivers present in the Gulf of Gaeta. The proposed approach is designed as a tool to inform marine policies and coastal planning, supporting the role of plankton food webs in highlighting those coastal marine sectors whose ecosystems are more affected by to anthropogenic and natural disturbances.

## 1. Introduction

Since the advent of the industrial era, marine ecosystems have suffered mounting pressure from anthropogenic factors (e.g., climate change, pollution) (Halpern et al., 2007). The analysis and quantification of human impacts on aquatic environments are complex due to the rapidity of abiotic condition changes and to the increasing intensity of human impact (Nõges et al., 2016). Such pressures can result in

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substantial alterations of the structure and dynamics of marine ecosystems, with direct and indirect effects on food webs (Eriksson et al., 2011; Niiranen et al., 2013). In this context, studying food webs, i.e., the networks of trophic interactions, and how their structure modifies across environmental gradients, allows scientists and policymakers to gain a deeper awareness of the functioning of the marine ecosystem (D'Alelio et al., 2016; Vassallo et al., 2022) and insights for its management.

In recent years, ecological indicators related to food webs have been frequently applied to the study of marine ecosystem dynamics, to deliver complex information to different stakeholders (Saint-Béat et al., 2015; Piroddi et al., 2015; Fath et al., 2019). In Europe, the adoption of the Water Framework Directive (2000/60/EC), the Habitats Directive (92/43/E EC), and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) highlighted the urgency of identifying ecological indicators able to assess a "Good Environmental Status" (GES). The MSFD requires EU Member States to achieve GES in marine systems by defining 11 descriptors composed of several indicators for an integrated assessment of the ecosystem state (Piroddi et al., 2015; de Jonge and Schückel, 2021). Descriptor 4 highlights the importance of studying food webs to understand the rate of energy transfer within an ecosystem, the productivity of its components, and the ecosystem structure based on the identity, size, and abundance of occurring individuals (Cardoso et al., 2010). By measuring energy flows within the food web, it is possible to map ecological changes in the productivity of specific trophic components or prey-predator relationships (2010/477/EU).

Plankton are a fundamental component of aquatic food webs as the primary conduit for energy transfer (Worden et al., 2015) and drivers of dynamics in marine ecosystems (Behrenfeld and Boss, 2014). Plankton include organisms covering a broad size range (0.2–2000 μm), and high taxonomic, functional, and trophic diversity (Mitra et al., 2023; Bellardini et al., 2024a). Such biological complexity gives rise to intricate food webs with interactions among species at different trophic levels that regulate ecosystems' energy and organic matter in flows (Legendre and Rivkin, 2002; Lomartire et al., 2021). Plankton rapidly responds to environmental changes, propagating the effects of physical-chemical perturbations from the individual to the community level and up to organisms occupying higher trophic levels (such as fish) (Batten et al., 2019; Bellardini et al., 2024b). Considerable taxonomic and functional diversity makes plankton a pivotal group in marine ecosystem health assessment (Bedford et al., 2018; Anschütz et al., 2024). Nevertheless, planktonic organisms are under-represented or oversimplified in ecosystem models, a limitation that significantly compromises the robustness of model outputs (Anschütz et al., 2024; Chkili et al., 2024). Few studies have considered the planktonic component in detail, with plankton food web models allowing to assess the environmental health (Loschi et al., 2023) and the impact of ocean warming (Russo et al., 2025).

The present study investigates plankton food webs to assess: (i) if structural change occur in response to local environmental conditions (e.g., river inputs); (ii) how these condition drive behavioral changes in plankton key functional groups; and (iii) if network-based indicators focusing on the plankton food web functioning are suitable tools to evaluate the ecosystem status in support of marine policy and spatial planning.

More specifically, we investigated the structure and functioning of plankton food webs along the entire coast of the Campania Region (Tyrrhenian Sea, Mediterranean Sea), in three gulfs located in an area exposed to significant anthropogenic pressures (Tornero and Ribera d'Alcalà, 2014). Using a numerical approach based on the *Ecopath* algorithm (https://ecopath.org), we integrated environmental and biological data from the study area, developing models of planktonic food webs with thirty-five trophically interacting Functional Groups (FGs) analyzed in two seasonal time-snapshot (autumn 2020 and summer 2021). Furthermore, considering the sensitivity of food web analysis to environmental conditions, we compared ecological indicators with physical conditions, considering the connectivity between the gulfs and satellite analysis, providing a more accurate assessment of environmental impacts, such as water mass structure and circulation, on food web structure. Finally, we discussed how this integrative approach can support sustainable ecosystem management and improve communication with policymakers and stakeholders, offering a detailed understanding of marine food webs structure and function.

## 2. Materials and methods

### 2.1. Sampling area and environmental conditions

Two sampling surveys were conducted along the coast of the Campania Region (central Tyrrhenian Sea, NW-Mediterranean Sea) aboard the *R/V Vettoria* in autumn 2020 (September 9 - October 9) and summer 2021 (June 29 - July 15). During each survey, were sampled 22 stations across the Gulf of Gaeta (9 stations), Gulf of Naples (7 stations), and Gulf of Salerno (6 stations) (Fig. 1) along transects from the inner (<20 m depth) to mid-shelf waters (50–100 m depth) (Supplementary Material 1 (SM1), Appendix 1). Details about sampling methodology and measured parameters are provided in Bellardini et al. (2024a).

The three gulfs under study have distinctive geomorphological characteristics and are subject to significant anthropogenic pressures (Tornero and Ribera d'Alcalà, 2014) and seasonal and short-term environmental variability (Paerl et al., 2002). The Gulf of Gaeta (centered in 41°06′N, 13°30′E) has a high level of urbanization, with the presence of fish and shellfish farms (Triassi et al., 2019); it is subject to the influence of the Garigliano and Volturno rivers, among the largest rivers in southern Italy, which exert a significant influence on the entire coast (Triassi et al., 2019). On the northwestern coast, the Garigliano river mainly flows from southeast to northwest following the cyclonic circulation of the Tyrrhenian Sea (Careddu et al., 2015). On the southwestern coast, the Volturno river shows a plume that, during winter, spreads offshore, far from the coast, whereas, during summer, it spreads both north and south of the mouth, influencing the coastal waters (Ferrara et al., 2017).

The Gulf of Naples (40°44′N, 14°16′E) is one of the main coastal basins in the Mediterranean. It is subject to the combined influence of the oligotrophic waters of Tyrrhenian Sea and a relevant runoff, primarily from the Sarno river (Tornero and Ribera d'Alcalà, 2014; Cianelli et al., 2012), which is a source of nutrients and chemical pollutants from intensive agricultural activities and tanning industries affecting the ecosystem state over the whole coast (Saviano et al., 2023; Gifuni et al., 2023). Runoff, combined with local climatic conditions (e.g. wind patterns) exerts a significant influence on these waters, particularly in coastal areas (Kokoszka et al., 2023).

The Gulf of Salerno (40°31′N, 14°42′E) is more oligotrophic than the other two gulfs, as it is predominantly occupied by Tyrrhenian offshore waters (Ragosta et al., 1995). The presence of the Sele River impacts the ecosystem state, contributing to the transport of marine pollutants due to the textile, leather, and agro-industrial activities along the river banks (De Rosa et al., 2022). In general, the Gulf of Salerno is a spatially homogeneous system devoid of areas of significant enrichment, with coastal influence confined to a limited area of the inland platform (Ragosta et al., 1995).

The three Campania gulfs are seldom subject to upwelling processes, whereby nutrient-rich waters are brought to the surface (de Ruggiero et al., 2018) owing to the presence of strong winds and submarine canyons, namely Cuma in the Gulf of Gaeta and Dohrn in the Gulf of Naples (Ciannelli et al., 2022).

Overall the Gulf of Naples represents the most studied area, with the *MareChiara* Long-Term Ecological Research Site (LTER MC), established in 1984 (Ribera d'Alcalà et al., 2004; Zingone et al., 2019; Russo et al., 2024) to monitor the physical-chemical and biological characteristics of the gulf (Kokoszka et al., 2022; Mazzocchi et al., 2023; Romillac et al., 2023; Saggiomo et al., 2023). In contrast, in the Gulf of Gaeta and Salerno, few studies have been carried out so far mainly from the

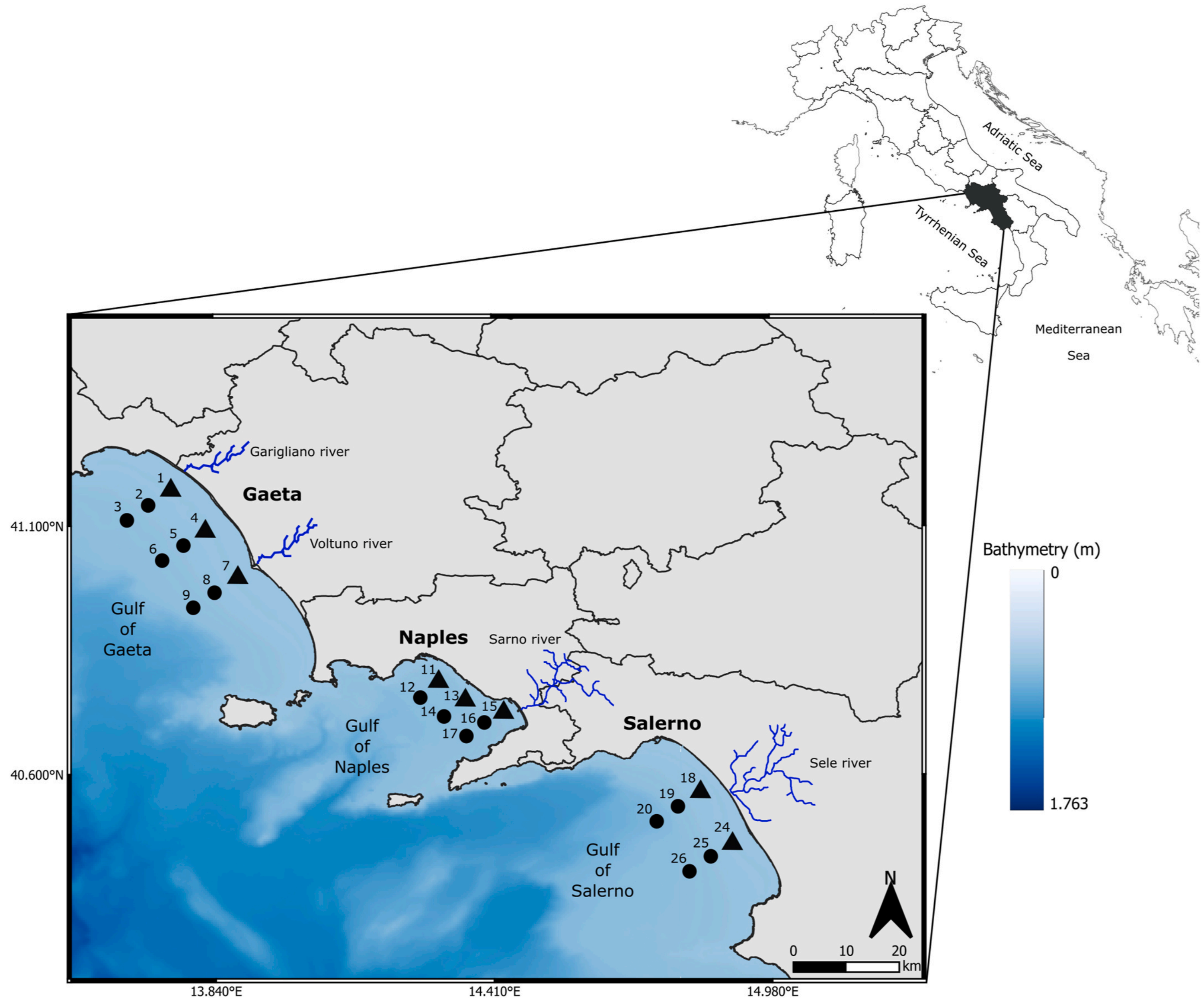

**Fig. 1.** Study area (Campania region) and sampling stations (in black). A triangular shape represents inner shelf stations (<20 m depths), and a circular shape represents mid-shelf stations (50–100 m depths).

physical (de Ruggiero et al., 2020) and biological (Bellardini et al., 2024a, 2024b; Ragosta et al., 1995) points of view.

Information regarding environmental conditions recorded during the surveys mentioned above are provided by Bellardini et al. (2024a). In summer 2021, the highest temperature was recorded in the Gulf of Gaeta and Naples (26.1 °C and 26.4 °C, respectively), while 24.3 °C was recorded in Salerno. In autumn 2020, temperature lows (22 °C) were observed in the Gulf of Salerno, while temperatures of about 25 °C were recorded in the gulfs of Gaeta and Naples recorded temperatures of about 25 °C. Salinity values (PSU) are in line with seasonal values of the study area (Romillac et al., 2023) with lower salinity at all stations near river mouths in the three gulfs, especially during autumn 2020 (Bellardini et al., 2024a). As for chlorophyll *a*, higher surface concentrations were observed in both autumn 2020 and summer 2021 in the Gulf of Naples (3.19 mg $m^{-2}$ and 6.62 mg $m^{-2}$, respectively), where a phytoplankton bloom occurred during the summer period, peaking mainly in this gulf.

### 2.2. *Assessment of coastal connectivity between the gulfs analysis*

Turbidity data over the 20-km coastal area of interest in the years 2020 and 2021 were obtained through the automatic switching algorithm described in Novoa et al. (2017), which forms the basis for the turbidity data obtained from the Sentinel-2 MSI sensor, with a spatial resolution of 100 m accessible through the Copernicus Marine Data Store (https://data.marine.copernicus.eu, accessed on August 3, 2024). The analysis was based on daily turbidity data (TUR, expressed in Formazin Nephelometric Units - FNU), selecting only images without cloud cover to assess spatial variation. Turbidity is an important indicator of water quality and can be effectively estimated using optical satellite data, providing crucial information on ecosystem health, as well as allowing us to observe the effects of the river and the behavior of its plume. For image analysis and processing, the Python programming language was used.

Coastal connectivity (CC) analysis was applied to verify whether the different gulfs were interconnected, that is, whether changes in one coastal zone affect another. CC is defined as the percentage of water particles moving from a source site (*i*) to a destination site (*j*) within a given time interval ($t$) (Mitarai et al., 2009). Connectivity between the Campania gulfs was examined using a Regional Ocean Modeling System (ROMS), implemented for the Tyrrhenian Sea and then adapted to the coastal area of Campania to obtain high-resolution simulations (Kokoszka et al., 2022). The transport module was applied to the ROMS velocity field to track the trajectories of water particles. In this methodology, virtual particles released in simulated coastal waters were used to estimate connectivity between sites. Lagrangian particles follow velocity fields and are released at a fixed depth of 1 m. CC values are calculated as the percentage of particles reaching a target location ($xj$). Given an $n \times n$ connectivity matrix for n different coastal areas, the ($i,j$) element of the matrix represents the fraction of particles originally released in area $i$ that are found in area $j$ after a certain time interval from the date of release (Bellardini et al., 2024b). Particles were released along the coast every five days for five years (2013–2017), with about 250,000 particles released annually at various time scales: 4, 8, 16, 32, 64, 96, and 120 days. In this study, the CC results at 8, 16, and 32 after the initial release time will be presented. During the numerical simulations, we assume that the Lagrangian particles are passively transported by the modeled currents, remaining constrained at the fixed release depth of 1 m.

### *2.3. Data collection and conversion to carbon biomass*

All sampling and laboratory methods to estimate the abundance of heterotrophic bacteria, hetero-mixotrophic unicellular plankton (i.e., protists), and mesozooplankton are described in Supplementary Material 2 (SM2), section A. Mesozooplankton abundance was available on the entire water column, while abundance data for hetero-mixotrophic protists were only available for two depths, 0 m and 10 m. Therefore, the water column was divided into two layers for analysis: a surface layer ($s$) from 0 to 10 m, and a deep layer ($d$) which extended from 10 m to the depth of zooplankton sampling (SM1, Appendix 1).

Then plankton abundance data (except for phytoplankton) were converted to carbon biomass data (C biomass, $\mu gC\ m^{-2}$) using specific conversion factors. The C biomass of heterotrophic bacteria was obtained by multiplying the bacterial abundances with the conversion factor of 12 $fgC\ cell^{-1}$ (Herrmann et al., 2013). Phytoplankton abundance (divided into three size fractions pico-, nano-, and micro-) was derived from diagnostic pigments obtained by HPLC analysis (Vidussi et al., 1996, 2001; Socal et al., 2010) and converted into C biomass following equations by Canuti (2023), which modified and improved the original ones proposed by Vidussi et al. (2001) (for more details see SM2, section B). The C biomass of phytoplankton size fractions was derived by multiplying the pico-, nano-, and micro-phytoplankton abundance by the conversion factor of 40 $\mu gC\ (\mu g\ Chl\ a)^{-1}$ (Fabiano et al., 1995). To determine the C biomass of hetero-mixotrophic unicellular groups, abundance data were first converted to cell biovolume calculated using cell width and length in the sample, measured with an inverted light microscope Zeiss Axiovert 200 (Carl Zeiss, Germany) at 200x magnifications, and considering cell geometry following Hillebrand et al. (1999) and Sun and Liu (2003)). Cell biovolume was then converted to C biomass as described by Menden-Deuer and Lessard (2000).

The C biomass values of heterotrophic bacteria, phytoplankton, and hetero-mixotrophic protists were integrated separately for the $s$ and $d$ layers. Since particulate organic carbon (POC) includes detritus, bacteria, phytoplankton, and all mixo- and heterotrophic protists (Andersson and Rudehäll, 1993; Ribes, 1999), the total C biomass of mixo-heterotrophic organisms in layer $d$ was derived by subtracting the total C biomass of detritus, bacteria, and phytoplankton from the total POC integrated over the layer $d$, following the approach used by Scotti et al. (2022). Then, the C biomass of each hetero-mixotrophic unicellular FGs was calculated by multiplying the total C biomass of hetero-mixotrophic protists obtained in the $d$ layer by the percentage of biomass of each hetero-mixotrophic FGs in the $s$ layer. Among hetero-mixotrophic unicellular groups, the mixotrophic ciliate *Mesodinium rubrum* was the only exception, as its C biomass values were integrated over the entire water column due to its capability for vertical migration (Lips and Lips, 2017).

Lastly, mesozooplankton C biomass was determined by multiplying the individual abundance by individual carbon content derived from dry weight (DW) values, assuming that, on average, carbon makes up 40 % of the DW of zooplanktonic individuals (Steinberg and Landry, 2017). DW data of the most representative species at the study site, i.e., *Clausocalanus* spp., *Candacia* spp., *Corycaeus* spp., and *Pleuromamma* spp. (Bellardini et al., 2024a; Mazzocchi et al., 2023), were obtained from direct measurements or the literature (SM1, Appendix 2). For juvenile copepods (copepodites from I to IV stage), C biomass was estimated by assuming that, on average, carbon represents 27 % of the individual C of adult copepods. This coefficient was derived from the average DW values (μg) of *Centropages typicus*, one of the most representative species in the Gulf of Naples (Di Capua et al., 2022; Carotenuto et al., 2023), considering both copepodite stages (CIII-CV) and adult stages (males and females) (for more details see SM2, section B).

### *2.4. Input data and modelling approach*

The plankton food web was analyzed in terms of structure and function using an ecosystem approach, based on the quantification and characterization of the C flows operating the ecosystem. The system flux analysis was carried out by developing and calibrating simulations derived through the *Ecopath* approach (Christensen and Pauly, 1992; Christensen, 2003), using an algorithm written in MatLab software v.9.13.0 (R2022b).

To reduce the complexity of the food web, species with similar ecological roles and interactions were aggregated into functional groups (FGs), i.e. groups with similar ecological functions and trophic interactions, based on expert knowledge and bibliographic research (see SM2, section B). In our model, 35 FGs were defined: one non-living FG (i.e. detritus, FG 35), 17 microbial FGs (from FG 1 to FG 17, Fig. 2) and 17 mesozooplanktonic FGs (from FG 18 to FG 34, Fig. 2). Specifically, for the identification of the main mesozooplanktonic FGs, copepods were derived from Bellardini et al. (2024a), while for the no-copepods organisms (from FG 26 to FG 34), were considered the groups showing higher biomass (for more details see SM2, section B).

To run the simulations, several ecological inputs were needed for each living FG, namely: (i) carbon biomass ($\mu gC\ m^{-2}$; see the previous methodological section), (ii) production rate per unit of biomass (μ, $d^{-1}$), (iii) consumption rate per unit of biomass (α, $d^{-1}$), (iv) unassimilated rate of biomass consumed (ε, adimensional), and (v) the proportion of phototrophy in individual metabolism (ph/het, adimensional). The latter has a value of 0 for heterotrophs, 1 for autotrophs, and intermediate values for the mixotrophs. For all metabolic rates (μ, α, and ε), the minimum and maximum values for each FGs were identified; because of the high uncertainty associated with some metabolic rates, we applied ranges of variability, defined as described by Morissette et al. (2005). Lastly, metabolic rates were calculated for each FGs, using the following equation proposed by Hansen et al. (1997), applying a specific $Q_{10}$ value:

$$\log_{10}\lambda = \log_{10}\lambda_{ref} + \log_{10}(Q_{10})\cdot(T - T_{ref})\,/\,10 \qquad [1]$$

where λ indicates the metabolic rates (μ, α, or ε), $\lambda_{ref}$ $T_{ref}$ represents, respectively, the reference value of metabolic rates and temperature value obtained from the literature. T represents the average temperature of the sampling site in this work (see SM1, Appendix 1).

All information concerning the C biomass of FGs, the derivation of metabolic rates (μ, α, and ε), and the ph/het ratio of FGs are available in SM2, section B, and SM1, Appendix 3, 4, 5, 6, 7 and 8.

In addition, the diet matrix was calculated from an adjacent matrix that represents the probable trophic interaction among FGs, following

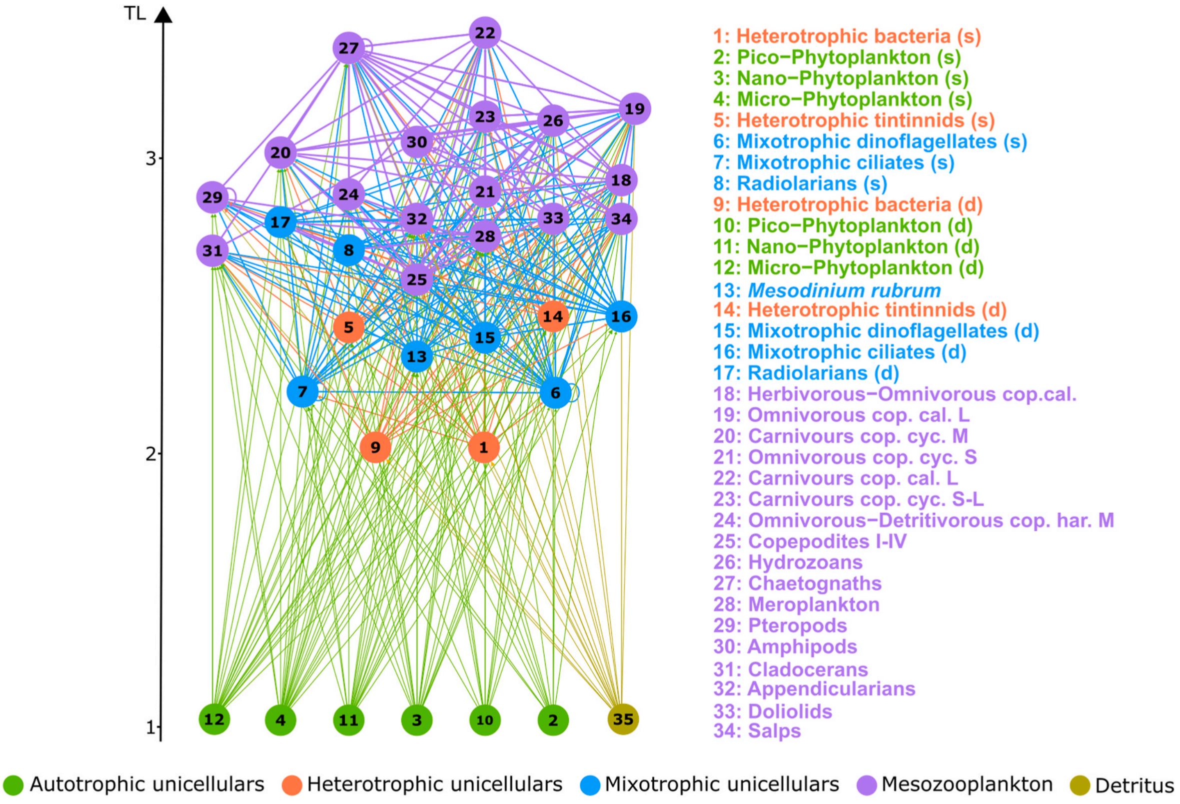

**Fig. 2.** Planktonic food-web. Arrows, directed from sources to sinks, represent trophic interactions between FGs and are colored according to the source. FGs are ordered by the trophic levels (TLs) and colored according to the trophic group, while the numbers in the node correspond to FG-IDs. Near to ID of FGs, *(s)* and *(d)* represent the surface and deep layers, respectively; while for the mesozooplankton groups, cop.: copepods; cal.: calanoids; cyc.: cyclopoids; har.: harpacticoids; L: large size; M: medium size; S: small size. The network was created using the Gephi software v.0.10.1 (www.gephi.org).

the approach proposed by Ulanowicz and Scharler (2008). This method determines predation pathways using the adjacent matrix, distributing food flows based on the possible interaction between predator demand and prey availability. The adjacent matrix was created, defining different values: 0 (no interaction), 1 (low interaction probability), 2 (medium interaction probability), and 3 (high interaction probability). This value was determined by expert knowledge and literature information (full description and reference see SM1, Appendix 9 and SM2, Fig. S1 and section B).

For each living FGs, a set of linear algebraic equations were used to balance daily consumption fluxes (input and output), respiration, unassimilated food, production, predation and natural mortality (Christensen, 2003):

$$B_i \cdot \mu_i \cdot EE_i - B_j \cdot (\alpha)_j \cdot DC_{ji} - mk = 0 \qquad [2]$$

where $B_i$ is the biomass of group *i*, μ is the biomass production ratio of group *i*, $EE_i$ is the ectotrophic efficiency of group *i*, $B_j$ is the biomass of the predator *j*, $\alpha_j$ is the biomass consumption ratio of the predator *j*, $DC_{ji}$ is the interaction between prey *i* in the diet of the predator *j* and, mk is the natural mortality of the node *k*.

Equation [2] was applied with a random sampling of metabolic input parameters (μ, α, and ε) in a specific range (minimum and maximum values). The software routine provides an error message if the simulation result is unrealistic. If inconsistencies were found (e.g. EE > 1), the diet matrix was slightly modified to obtain a successful simulation (Christensen and Walters, 2004). To this end, each time the diet matrix was modified, the predator's diet was compensated for by interpreting the decreased predation as an import (Vassallo et al., 2022). Furthermore, several ecological and thermodynamic reality constraints were adopted to respect ecological reality: (i) respiration >0, (ii) production–consumption rate between 0.1 and 0.5 for protists and 0.1–0.35 for mesozooplankton (Loschi et al., 2023). Every time the food web respected all conditions and constraints, it was accepted and a new simulation started for up to 999 simulations for each sampling site (following the rationale by Loschi et al. (2023), for a total of 21.978 food webs.

### 2.5. Ecological network analysis (ENA)

For each simulation, through a MatLab code, several ecological indicators were calculated to provide information on the structure and functionality of the plankton food web (formulas and descriptions of the network-based ecological indicators see Table 1). Furthermore, some of these indices can also be used as indicators of the level of stress, stability, development and maturity of the system (Saint-Béat et al., 2015; Ulanowicz and Scharler, 2008; Niquil et al., 2012). Stress here is understood as the altered ability to transfer organic matter or energy between trophic levels of the food web (Ulanowicz et al., 1996).

We also assessed which functional groups had the greatest impact on the food web (FGs-based ecological indicators), based on the analysis of Keystone groups (KS) and the Mixed Trophic Impact (MTI). KS, having relatively low biomass, can be limiting and thus are defined as the functional groups with a structuring role within ecosystems and food (Power et al., 1996). KS plays a fundamental role: by predating other species, they significantly influence the structure of the whole ecological community. This effect results in an outsized impact on biological diversity, with KS not only influencing the composition but also the functioning and dynamics of the community. This index was calculated based on Libralato et al. (2006), which attributes high KS values to functional groups characterized by both a low biomass proportion and a high overall effect:

$$KS_i = log\ \ log\,[\varepsilon_i(1 - p_i)] \qquad [3]$$

**Table 1**
Network-based ecological indicators used for this work. For each of these indicators, the abbreviation, the formula, and a brief description are provided.

| Acronym | Indicators | Brief description | Formula | Reference |
|---|---|---|---|---|
| TST | Total System Throughput | Defines the total amount of energy or matter circulating in a system and is an overall measure of ecosystem growth and development. | $\mathrm{TST} = \sum_{i=0}^{n} \sum_{j=1}^{n+2} \mathrm{T_{ij}}$ | Finn (1976) |
| A/C | Relative Ascendency | Defines the level of system development and organization, with values between 0 and 100 %. An increase indicates a high degree of organization, characterized by highly specialized and less redundant pathways in the transfer of organic matter between trophic levels, from the bottom to the top of the marine food web. | $\mathrm{A/C} = \dfrac{\sum_{i=0}^{n} \sum_{j=1}^{n+2} \left(\mathrm{T_{ij}} \cdot \log_2 \dfrac{\mathrm{T_{ij}\ T_{..}}}{T_{i.} T_{.j}}\right)}{-\sum_{i=0}^{n} \sum_{j=1}^{n+2} \left(\mathrm{T_{ij}} \cdot \log_2 \dfrac{\mathrm{T_{ij}}}{\mathrm{T_{..}}}\right)}$ | Ulanowicz (1986) |
| FCI | Finn's Cycling Index | Indicates the "material cycle within a given system" and measures how often flow would revisit the same node several times. It can range from 0 (no recycling) to 100 % (all organic matter/energy is recycled) | $\mathrm{FCI} = \dfrac{\sum_{i=1}^{n} \left[\left(1 - \dfrac{1}{q_{ji}}\right) \cdot T_i\right]}{\sum_{i=0}^{n} \sum_{j=1}^{n+2} T_{ij}}$ | Finn (1976) |
| D/H | Ratio of Detritivory to Herbivory | Indicates the dependence of the food web on newly produced algal material vs the detritus. Values above 1 indicate that the system maintains its activities by using mainly detritus rather than relying exclusively on primary producers. | $\mathrm{D/H} = \dfrac{\sum_{j=1}^{k} \sum_{i=k+1}^{n} \mathrm{T_{ij}}}{\sum_{j=1}^{k} \sum_{i=1}^{n} \left(\mathrm{T_{ij}} \cdot \mathrm{ph_i}\right)}$ | Ulanowicz and Kay (1991) |
| AMI | Average Mutual Information | Statistical evaluation of how much each flow acting in the considered web is forced to enter a specific compartment when released by another one. It expresses how flows within the system are connected in an ordinary and consistent way (an increase means that the system is becoming more constrained and is channeling flows along more specific paths). | $\mathrm{AMI} = K \sum_{i,j} \left(\dfrac{T_{ij}}{T_{..}}\right) \log \left(\dfrac{T_{ij} T_{..}}{T_{i.} T_{.j}}\right)$ | Finn (1976) |

$T_{ij}$ is the flow matrix with flows moving from prey *i* to predator *j*.
$Ti.$ is the sum of all outflows from compartment *i*.
$Tj$ is the sum of all inflows into compartment *j*.
T. is the sum of all flows.
*n* is the number of FGs, $n + 2$ is the number of FGs to which respiration and export are added, and $i = 0$ represents the import line.
*k* is the number of living groups, so the numbers from $k + 1$ to $n$ represent no-living groups.

where $p_i$ is the contribution of the functional group to the total biomass of the food web and is calculated from the following:

$$p_i = \frac{B_i}{\sum_{k=1}^{n} B_k} \qquad [4]$$

and $\varepsilon_i$ represents the relative overall effect and described as:

$$\varepsilon_i = \sqrt{\sum_{i \neq j} m_{ij}^2} \qquad [5]$$

where $m_{ij}$ is calculated from the MTI analysis (formula described by Ulanowicz and Puccia (1990)) as the product of all net impacts for all the possible pathways in the food web linking prey, *i*, and predators, *j*.

MTI analysis, introduced by Ulanowicz and Puccia (1990), explains the effects of any group on another group within the food web by applying hypothetical changes in biomass. MTI analysis makes it possible to assess whether the influence of a target species is positive or negative. The target species can participate in direct processes, such as predation, or indirect processes, such as cascading dynamics and competition, thus influencing the ecosystem through bottom-up or top-down mechanisms with both positive and negative interactions (Bondavalli and Ulanowicz, 1999).

### 2.6. Data analysis

To identify similarities between network-based ecological indicators the median values of the 999 simulations, log transformed to base 10, were statistically analyzed using a non-metric MultiDimensional Scaling ordination (nMDS) based on Euclidean distance. For the interpretation of the first (nMDS1) and second (nMDS2) axes, three environmental descriptors (temperature, salinity, chlorophyll *a* concentration, integrated from the surface to bottom depth) were used as supplementary variables. Only those descriptors that showed significant correlation to the nMDS1 and nMDS2 (i.e. Pearson correlation, $p < 0.05$) are presented herein. To test the significance of seasonal and spatial differences, a multivariate analysis (PERMANOVA, $p < 0.05$) was performed, using three fixed factors: "season" (two levels: autumn 2020 and summer 2021), "gulf" (three levels: Gulfs of Gaeta, Naples, and Salerno), and "distance" (two levels: inner and middle shelf stations). A one-way ANOVA (multiple comparisons) was used to test which indicators were different within the factors. The FGs-based ecological indicators (KS and MTI) were calculated using the median values of the 999 simulations. The KS values were normalized (min-max normalization) and then ranks were assigned to consider the top three FGs with the highest KS rank for each sampling site (SM1, Appendix 10). For the sake of clarity, only the top rank (#1) was considered in this paper. For the MTI analyses, the average trophic impact was calculated for each area under study, based on the total number of sampling stations within the designated study area. Analyses and plots were performed with RStudio v. 4.3.2, using the packages *vegan* (*'metaMDS'* and *'adonis2'* functions), *purr* (*'reduce'* functions) and *ggplot2* (RStudio Team, 2020).

## 3. Results

### 3.1. Satellite data and coastal connectivity analysis

Satellite images show that the highest levels of turbidity during the investigated period occurred in the autumn 2020 (SM2, Fig. S2), a season characterized by more intense river flow events. In this season high turbidity values were observed in the Gulf of Gaeta, where plumes linked to the Garigliano and Volturno rivers influenced the entire gulf, extending also offshore (Fig. 3a, left panel). Instead, during summer 2021, lower turbidity values and less pronounced plumes were recorded in association with lower precipitation and reduced river flows (Fig. 3a, right panel). CC analyses showed different patterns depending on the season. In the autumn 2020, greater connectivity between the Gulfs of Gaeta and Naples was observed compared to the Gulf of Naples and Salerno (Fig. 3b, upper figures). In contrast, during the summer of 2021, a less dynamic circulation along the coast limited the interactions between gulfs, which was observable only with particle release times longer than 16 days (Fig. 3b, lower figures).

### 3.2. Plankton community

The planktonic carbon biomass showed seasonal and spatial differences. Higher average values were found in summer 2021 ($4.55 \times 10^5$ μgC m$^{-2} \pm 9.47 \times 10^5$) than in autumn 2020 ($2.31 \times 10^5$ μgC m$^{-2} \pm 4.20 \times 10^5$), associated with an increase in the biomass of autotrophic ($5.75 \times 10^5$ μgC m$^{-2} \pm 5.38 \times 10^5$) and hetero- and mixotrophic FGs ($1.09 \times 10^6$ μgC m$^{-2} \pm 1.28 \times 10^6$ and $7.49 \times 10^5$ μgC m$^{-2} \pm 1.59 \times 10^6$,

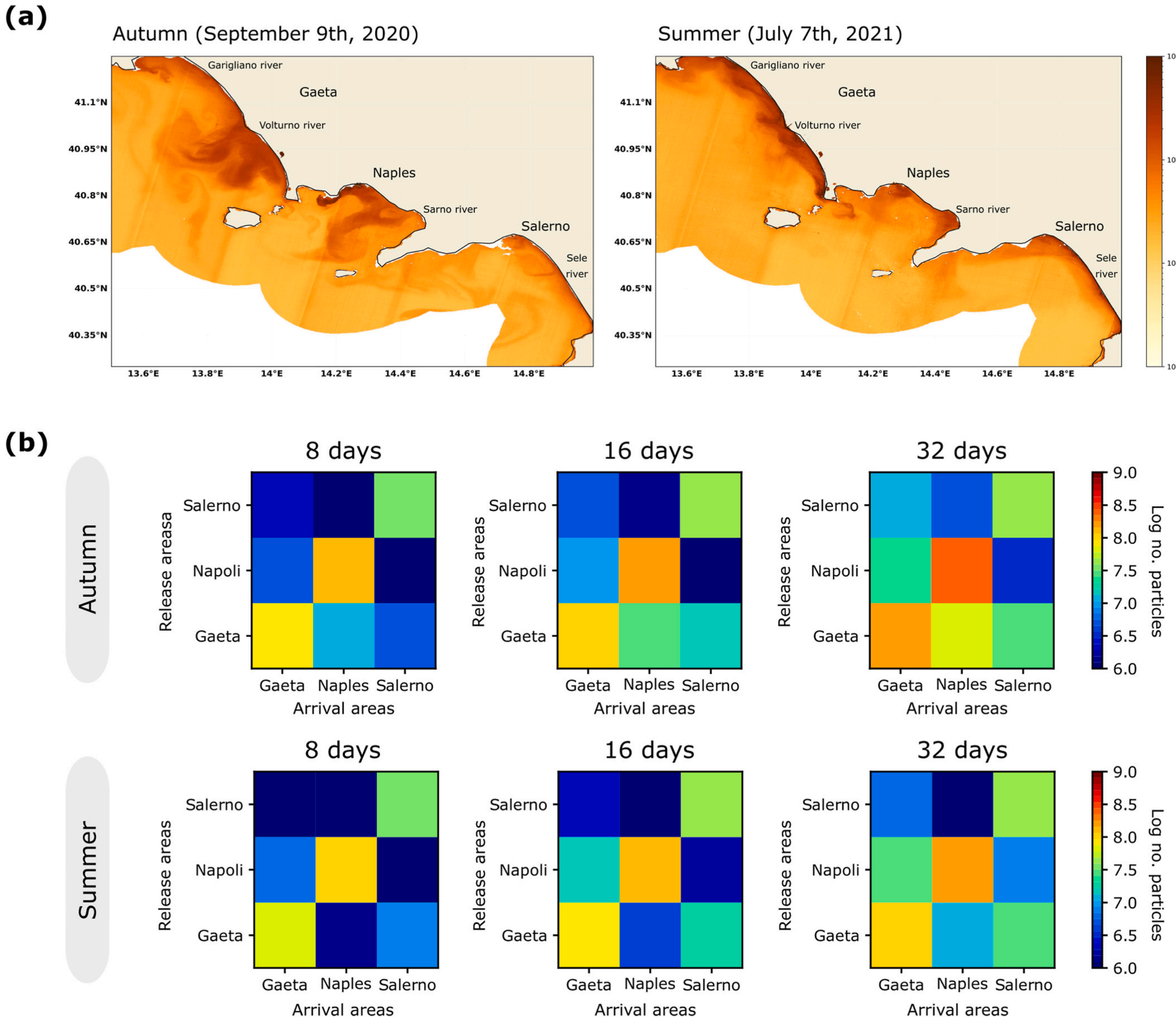

**Fig. 3.** (a) Satellite images showing turbidity (FNU) values along the coast of the Campania region. Images refer to the first sampling days for autumn (2020.09.09) and summer (2021.07.05) (other days are available in SM2, Fig. S2). **(b)** Seasonal physical connectivity matrix (average over 2013–2017) between Campania gulfs considering autumn and summer months after 8, 16, and 32 days after release (the connectivity degree increases from blue to red).

respectively). In summer 2021, in all three gulfs, the dominant FGs in terms of percentage of the total plankton community biomass were heterotrophic bacteria (FG 9, 26.3 % ± 0.06) and mixotrophic dinoflagellates (FG 15, 28.7 % ± 0.03) (Fig. 4). Overall in autumn 2020, we recorded on average a higher biomass of zooplanktonic FGs ($2.37 \times 10^5$ μgC m-$^2$ ± $5.44 \times 10^5$) compared to the summer 2021 ($1.15 \times 10^5$ μgC m-$^2$ ± $1.72 \times 10^5$), mainly due to the increase in pteropods (FG 29) and cladocerans (FG 31), which particularly in the Gulf of Gaeta contributed respectively to 34.5 % and 10 %, to the total mesozooplankton community. In contrast, in the Gulfs of Naples and Salerno, mixotrophic dinoflagellates (FG 15) and ciliates (FG 16) dominated at 12.5 % and 10.1 %, respectively (Fig. 4).

### *3.3. Ecological modelling*

The nMDS analyses of median values of network-based ecological indicators (SM1, Appendix 11) showed distinct patterns in the planktonic food webs in autumn 2020 and summer 2021 ($p < 0.05$) (Fig. 5). In autumn 2020, the Gulf of Gaeta differed significantly ($p < 0.05$) from Naples and Salerno (Fig. 5); whereas, in summer 2021, the three gulfs showed no significant differences ($p > 0.05$) (SM2, Figs. S3 and S4). Regarding the distance from the coast, the inner and mid-shelf stations showed no significant differences in both seasons ($p > 0.05$). Considering the correlation between the nMDS1 and nMDS axes with the environmental descriptors, the best and significant influence ($p < 0.05$) was shown by chlorophyll *a* during the summer 2021 and salinity during the autumn 2020 season (Fig. 5).

The autumn 2020 was characterized by lower average TST values and higher FCI values ($1.88 \times 10^6$ μgC m$^{-2}$ d$^{-1}$ ± $1.40 \times 10^6$ and 18 % respectively), in comparison to the summer 2021 ($3.74 \times 10^6$ μgC m$^{-2}$ d$^{-1}$ ± $1.96 \times 10^6$ and 5 % respectively) (Fig. 6a and b). This higher organic matter recycling was also indicated by D/H values, with an average increased contribution of detritivore organisms in autumn 2020 (8.48 ± 11.93) than in summer 2021, when in turn plankton were more herbivorous (3.59 ± 1.87) (Fig. 6c). Autumn 2020 showed lower average AMI and A/C (AMI: 1.18 ± 0.07 and AC: 23 % ± 0.02) than summer 2021 (1.30 ± 0.06 and 35 % ± 0.04) (Fig. 6d and e).

The intra-seasonal patterns highlighted greater trophic diversity

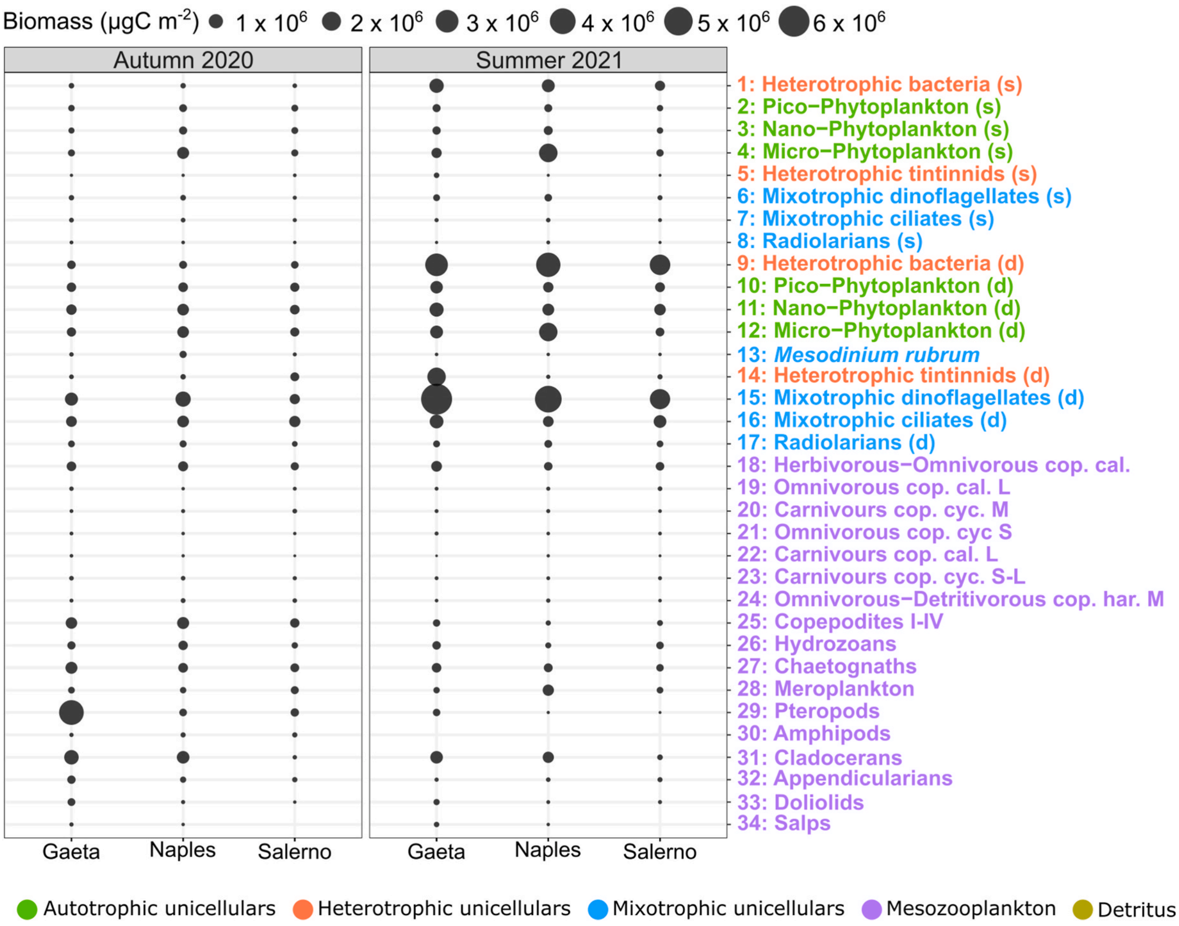

**Fig. 4.** Bubble plots show the C biomass (μgC $m^{-2}$) for each functional group (FGs) during the autumn 2020 and summer 2021 in the three gulfs (Gaeta, Naples, and Salerno). The colors distinguish the trophic group (shown in the legend). Near to ID of FGs, *(s)* and *(d)* represent the surface and deep layers, respectively; while for the mesozooplankton groups, cop.: copepods; cal.: calanoids; cyc.: cyclopoids; har.: harpacticoids; L: large size; M: medium size; S: small size.

among the gulfs in autumn 2020 than summer 2021. In autumn 2020, the Gulf of Gaeta showed greater flows and recycling of organic matter (TST: 1.92 × $10^6$ μgC $m^{-2}$ $d^{-1}$ and FCI: 28 %, respectively) compared to the Gulfs of Naples (1.27 × $10^6$ μgC $m^{-2}$ $d^{-1}$ and 11 %) and Salerno (1.18 × $10^6$ μgC $m^{-2}$ $d^{-1}$ and 11 %) (Fig. 6a and b), indicating a greater tendency towards detritivory (D/H: 17.50), than in the other two gulfs where herbivory was more important (~D\/H: 2.13) (Fig. 6c). Furthermore, Gaeta showed a higher organization of carbon flow in the food web but with lower flow efficiency (AC: 24 % and AMI: 1.10), compared to Naples and Salerno, which instead showed lower levels of organization (AC:~2 2 %) but higher flow efficiency (AMI:~1 0.21) (Fig. 6d and e).

In summer 2021, smaller differences occurred among the gulfs (SM2, Fig. S4). In the Gulf of Gaeta, the organic matter flows were higher (4.11 × $10^6$ μgC $m^{-2}$ $d^{-1}$) than in the Gulfs of Naples (2.83 × $10^6$ μgC $m^{-2}$ $d^{-1}$) and Salerno (2.28 × $10^6$ μgC $m^{-2}$ $d^{-1}$) (Fig. 6a). Considering the tendency to detritivory, the gulf of Salerno showed a higher average D/H ratio (4.96 ± 2.82), compared to Gaeta (3.40 ± 0.94) and Naples (2.64 ± 1.32) (Fig. 6c). Other ecological indicators had a similar tendency in the three gulfs (FCI:~6%, A/C:~3 5 %, AMI:~1 0.31) (Fig. 6b–d, e).

Regarding the keystone groups (KS) analysis, differences were observed between the two seasons (Fig. 7a). In autumn 2020, the top KS (rank = 1) in the three gulfs were different: in the Gulf of Gaeta, gelatinous filters feeders (i.e., doliolids, salps, appendicularians) and carnivorous organisms (i.e., chaetognaths, hydrozoans) prevailed; in the Gulf of Naples, KS was represented by copepods with a predominantly herbivorous diet and by gelatinous carnivorous (chaetognaths and hydrozoans); in the Gulf of Salerno, the top KS were chaetognaths and copepodites. In summer 2021 in all three gulfs, KS were mainly represented by gelatinous carnivorous, particularly chaetognaths. KS at lower trophic levels (i.e. meroplankton larvae) were only reported in the Gulf of Naples.

The top KS groups had different Mixed Trophic Impact (MTI) depending on their trophic position (Fig. 7b). Gelatinous filter feeders had a negative impact on unicellular FGs (autotrophic, heterotrophic and mixotrophic organisms), particularly during the autumn 2020 in the Gulf of Gaeta (Fig. 7b). Gelatinous predators exerted a higher trophic impact on the whole planktonic food web (Fig. 7b). Specifically, hydrozoans positively impacted gelatinous filters feeders (doliolids and appendicularians), specific groups of copepods (e.g., carnivorous and detritivorous copepods), and cladocerans, especially in the Gulf of Salerno during summer 2021 (Fig. 7b). Hydrozoans also had a negative impact on chaetognaths, meroplanktonic organisms and amphipods (mainly in the Gulf of Naples and Salerno, during autumn 2020). Chaetognaths represented the top predators in our models and impacted negatively on zooplankton groups they feed on (e.g., copepods and cladocerans) and positively on the food of their prey (e.g. autotrophic, heterotrophic and mixotrophic organisms). Chaetognaths also showed a negative self-impact, reflecting increased competition within the group for resources and possibly cannibalism. Lastly, herbivorous-omnivorous calanoid copepods applied negative pressure, especially during the autumn 2020, on heterotrophic (mainly tintinnids) and mixotrophic (mainly radiolarians) organisms, but showed a positive impact on the autotrophic community both in autumn 2020 and summer 2021.

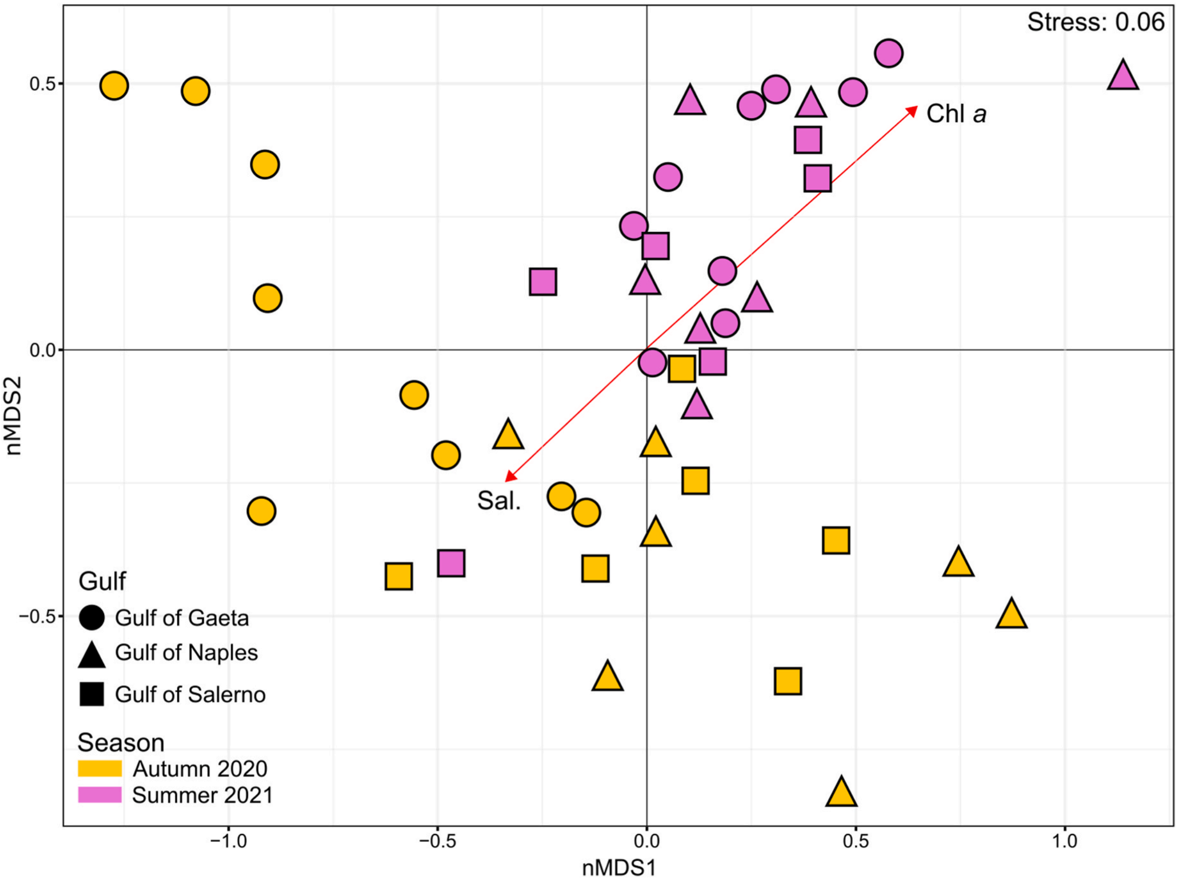

**Fig. 5.** nMDS on the ecological indicators (TST, FCI, D/H, AMI, and A/C) in the three gulfs (Gaeta as a circle, Naples as a triangle, and Salerno as a rectangle), during autumn 2020 (yellow) and summer 2021 (dark magenta). The red line shows the environmental descriptors (Sal.: Salinity; Chl *a*: Chlorophyll *a*) best correlated and statistically significant ($p < 0.05$) to the nMDS1 and nMDS2.

## 4. Discussion

Food web analysis is among the 11 Descriptors of the Marine Strategy Framework Directive (MSFD - descriptor 4) as a tool to achieve the “Good Environmental Status” of marine ecosystems. Food-web models are playing an increasingly important role in providing recommendations to policymakers on aquatic and marine-resource management in Europe (Saint-Béat et al., 2015; Piroddi et al., 2015; Fath et al., 2019). However, as the results of these models are virtual representations of reality, their interpretation and understanding are complex for non-specialists (Chkili et al., 2024; Fath et al., 2007; Baird et al., 2009). In this context, the application of appropriate ecological indicators proves crucial to delineate the structure and functioning of these ecosystems, as well as to facilitate more effective communication with policymakers and stakeholders (Saint-Béat et al., 2015; Piroddi et al., 2015; Fath et al., 2019).

In this work, the plankton food web was investigated by considering both the time factor, analyzing two seasonal time-snapshots (autumn 2020 and summer 2021), and the space factor (along the coast of the Campania region), considering thirty-five different functional groups of plankton, thus offering higher resolution and detail for understanding the ecosystem functioning through appropriate ecological indicators. Our results revealed both seasonal and spatial differences in the network organization, showing greater structural diversity among sites during autumn 2020 compared to summer 2021 (Fig. 5). These results are in line with previous works that, adopting similar methodological approaches, have analyzed changes in plankton food webs in response to different environmental conditions. For instance, planktonic food-web analysis carried out in the time-series of the Bermuda Atlantic Time Series study (BATS) in the Sargasso Sea (Russo et al., 2025) and in the Venice lagoon (Northem Adriatic Sea, Mediterranean Sea) (Loschi et al., 2023), showed that, in the presence of strong environmental forcings (i.e., decreasing net primary productivity due to global warming in open-ocean waters, sharp oligotrophication, and sediment resuspension in confined coastal environments) the structure of the planktonic food web tends to undergo drastic reorganization, with the rewiring of the trophic-interaction network and increasing of the detritivore trophic pathways. In the following sections, we discuss our results in more detail.

### 4.1. *Network-based ecological indicators highlight trophic transitions under environmental shifts*

In the models developed herein, the marine system was more productive/active (according to the TST indicator) during summer 2021 than autumn 2020, due to water column stratification, which retains nutrients from coastal runoff in the surface layer (Lavigne et al., 2013), which favors the growth of primary producers. After analyzing intra-seasonal differences, the most significant differences were found during autumn 2020, with the Gulf of Gaeta showing a more active system than Naples and Salerno. This situation was driven by the higher input of terrestrial organic matter from the Garigliano and Volturno rivers, as also highlighted by satellite data on water turbidity (Fig. 3a). This scenario is plausibly linked to the intense rainfall recorded a few days before the survey reported herein (~104 mm of rain, Meteo Data, 2020 from: https://agricolutra.regione.campania.it), which probably amplified the river discharge towards the coastal marine environment (~187 cm of hydrometric level, data from Protezione Civile Regione

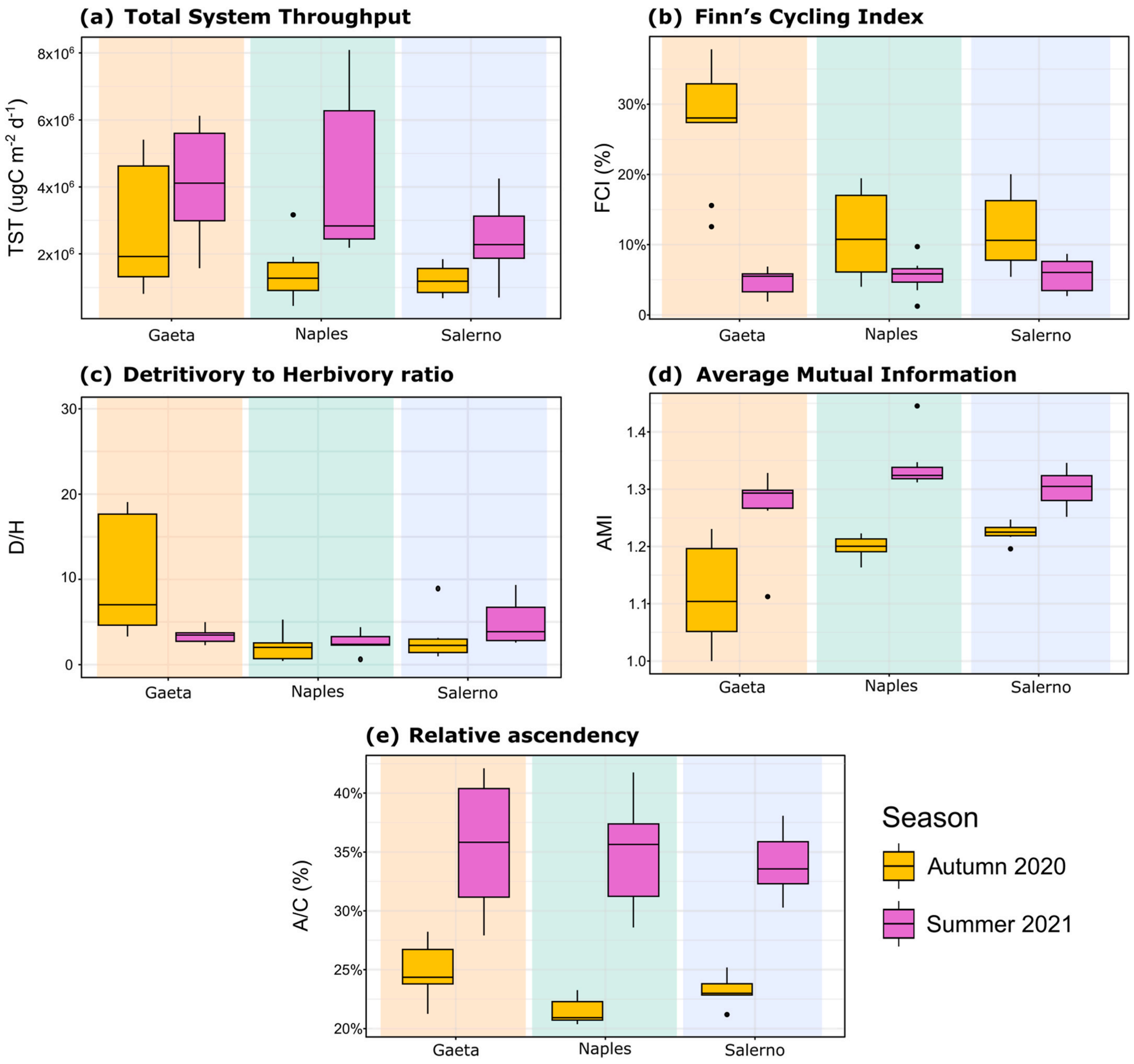

**Fig. 6.** Box plots showing network-based ecological indicators (**a** = TST, **b** = FCI, **c** = D/H, **d** = AMI, and **e** = A/C) concerning the autumn 2020 (yellow) and summer 2021 (dark magenta) seasons in the three gulfs of the Campania region (Gaeta in orange, Naples in light-green and Salerno in light blue).

Campania, https://centrofunzionale.regione.campania.it). This increase in fluvial input likely led to a significant alteration of the coastal food web, in accordance with Loschi et al. (2023). Furthermore, several studies have shown that high TST values are associated with highly productive ecosystems, such as coastal areas influenced by nutrient-rich upwelling, river inputs, and fertile continental shelves (Coll et al., 2007; Meddeb et al., 2018).

Increased turbidity in the Gulf of Gaeta during autumn 2020 impacted the ecological indicator D/H ratio, highlighting the role of planktonic detritivores in the carbon recycling process, as also shown in Loschi et al. (2023). Indeed, suspended particulate matter reduces light penetration in the water column, inhibiting phytoplanktonic primary production and favoring detritivores (Lee et al., 2002; Bonomo et al., 2014). In contrast, low D/H values were found across the three gulfs during the summer 2021 and in the Gulfs of Naples and Salerno during autumn 2020, indicating an ecosystem in which primary producers are the predominant food source for successive trophic levels (Ulanowicz and Kay, 1991; Niquil et al., 2014).

In our model output, the higher detritivory found in the Gulf of Gaeta during autumn 2020 also matches the increased organic matter recycling (FCI), compared to summer 2021 conditions characterized by low FCI values but high organic matter fluxes (high TST). This condition may indicate a less stressed system that depends on external fluxes to maintain a higher activity. FCI represents a crucial parameter for assessing system functioning changes and provides indications if a system is self-sufficient and sustained through material recycling (Ulanowicz, 1983). An increase in cycling suggests that matter persists in the system for a longer time (Fath et al., 2019). The FCI index is often considered as a possible indicator of stress, and an increase in cycling is usually interpreted as a stress response (Scharler and Baird, 2005;

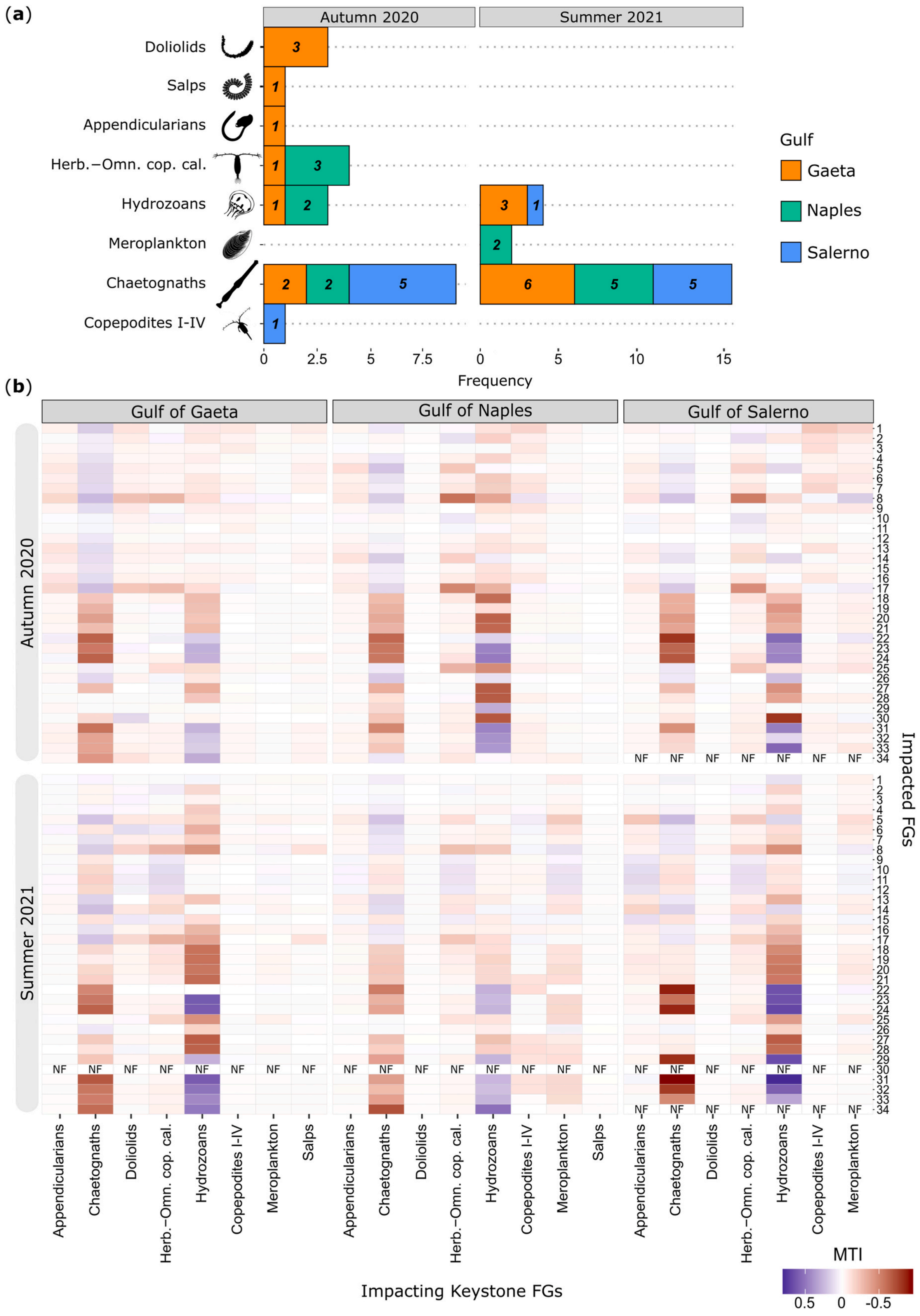

**Fig. 7.** (a) Bar Plot shows the top keystone groups (rank = 1) that were found in three gulfs (Gaeta in orange, Naples in light green, and Salerno in blue) during the autumn 2020 and summer 2021. The bold-italic numbers in the box represent the number of times (i.e. the frequency) that the FGs are at rank = 1. **(b)** Mixed Trophic Impact Analysis (MTI) for the Gulf of Gaeta, Naples, and Salerno food webs during the autumn 2020 and summer 2021 seasons. The values in the MTI matrix represent the average impacts within the investigated gulfs, calculated considering the sampling stations within the study area. When an FG was not observed in the sample, it was indicated as NF (Not Found). The number IDs of FGs (axis y) are available in Fig. 2 and 4.

Tecchio et al., 2015). Indeed, under stress conditions, cycling may act as a buffer during perturbation and increase the system's ability to resist change (Saint-Béat et al., 2015; Safi et al., 2019).

Our results suggest that the condition of increased detritus utilization in autumn 2020 and increased phytoplankton availability in summer 2021 has caused changes in the organization (A/C) and efficiency (AMI) of flows within the plankton food web. It has been observed that, for ecological networks, an A/C value of about 0.37 is optimal to represent two different trends in the development of a system, in terms of efficiency and resilience (Scotti et al., 2022; Morris et al., 2005).

When comparing the two seasonal time-snapshots, the median values of AMI and A/C show similar dynamics, with a significant increase during summer 2021. This result indicates that, in the whole region, the summer food web is characterized by greater efficiency and flow organization with more constrained flows along more specific paths, allowing energy to reach higher trophic levels (Saint-Béat et al., 2015; Baird et al., 2009). This seems to reflect a trend toward an optimal value of the A/C ratio associated with more stable and healthy ecological conditions (Morris et al., 2005; Ulanowicz, 2009).

In contrast, lower AMI and A/C values are observed during autumn 2020, when phytoplankton standing stock was relatively low, suggesting a system that is less efficient, more stressed and/or affected by perturbations (Saint-Béat et al., 2015; Baird et al., 2009) and with a less organized carbon flow in the network, in which all links transport an equal amount of material (Ulanowicz, 1980, 2009). In particular, the Gulf of Gaeta emerges as the most stressed area, characterized by a significant increase in organic matter recycling (FCI) and low AMI values. Contextually, although the A/C ratio is slightly higher in Gaeta than in Naples and Salerno, the median values are below the optimal threshold, indicating that the system may have increased its resilience at the expense of efficiency in coping with stressful conditions, as also shown in other stressed systems (e.g. Loschi et al., 2023).

### 4.2. FGs-based ecological indicators highlight structural changes under environmental shifts

The structural and functional variability in planktonic food-webs highlighted by the study of ecological indicators can be attributed to alterations in the physical and biological state of the investigated gulfs, demonstrating how food-web analyses can be sensitive to environmental conditions.

Specifically, connectivity analysis has shown that, during summer 2021, the current regime is less intense, with limited exchange between gulfs, leading to a less uniform situation and mainly local rather than regional influences, in line with previous studies (de Ruggiero et al., 2020; Cianelli et al., 2015). This situation probably allowed for a more stable and organized plankton food web, also demonstrated by the presence of hydrozoans and chaetognaths as keystone groups. Chaetognaths and hydrozoans play a significant role in shaping ecosystem structure and functioning, and this may indicate greater complexity in network organization (Choy et al., 2017; Chi et al., 2021). Gelatinous carnivorous occupy key positions in food webs due to their interactions with other functional groups (Chi et al., 2021; D'Ambra et al., 2013). This interaction led to complex food web linkages, further intensified by cannibalistic behavior, which increased resource competition and functional links (Chi et al., 2021; D'Ambra et al., 2013). Furthermore, chaetognaths and hydrozoans are also important consumers of eggs and larvae of several fish species, exerting a direct impact on the recruitment dynamics of these groups (Brodeur et al., 2008; Nakamura et al., 2024).

Conversely, more dynamic situations were observed during autumn 2020, with a greater interconnection between gulfs (Fig. 3b), particularly between Gaeta and Naples, and more characteristically local environmental conditions. Satellite images highlighted high levels of turbidity during the autumn 2020 (SM2, Fig. S5) favoring the presence of gelatinous filters feeder (i.e., doliolids, salps, and appendicularians) as the keystone groups in the planktonic food web (Spinelli et al., 2009; Höfer et al., 2015). Pelagic tunicates contribute significantly to organic matter recirculation, as they are able to feed efficiently on particles across a wide size spectrum, including viruses, pico- and micro-zooplankton, in contrast to other primary consumers that feed only on larger organisms (Condon et al., 2010). The low trophic level (in our study, see SM1, Appendix 3 and 4) reflects the role of these organisms as primary consumers and agrees with their trophic function as grazers (Madin and Purcell, 1992; Conley et al., 2018). This allows them to play a crucial role in the organization and structure of energy flows, compared to groups with higher trophic levels (Riofrío-Lazo et al., 2013; Hernandez-Padilla et al., 2017). Therefore, they facilitate the direct link between mesozooplankton and the microbial loop (Bedo et al., 1993; Sutherland and Thompson, 2022).

### 4.3. Benefits of applying ecological indicators to communicate with stakeholders

Ecological models represent essential tools for assessing ecosystem structure and function, and model-derived ecological indicators can effectively capture and interpret the main forces influencing the dynamics of food webs. The ecological indicators selected and tested in this study provide meaningful and understandable information to stakeholders and policymakers analogously to previous works (Fath et al., 2019; Safi et al., 2019).

More specifically, we highlighted how environmental dynamics significantly influence the structure and functioning of the ecological systems under study. We found that, during autumn 2020, the increased flow of terrigenous matter from the Garigliano and Volturno rivers into the Gulf of Gaeta represented a significant change for the ecosystem functioning in this area (higher FCI). This condition is reflected in a less efficient and less organized structure (low A/C and AMI), with a food web dominated by detritivores (higher D/H ratio). In contrast, the summer 2021 presented more stable and favorable environmental conditions, highlighting a period of greater ecological balance (low FCI, higher A/C, and AMI), characterized by a food web oriented to herbivores and greater efficiency in transferring energy to high trophic levels (low D/H ratio) (Fig. 8). This information can help evaluate ecosystem stability, which has a cascading effect on the quality of the food provided to higher trophic levels, i.e. from plankton to fish. Indeed, in microbial food webs dominated by detritivores, primary production is sustained primarily through nutrient recycling, but a significant amount of this production is re-mineralized (Chkili et al., 2024; Meddeb et al., 2018), resulting in inefficient carbon transfer (López-Abbate et al., 2019).

In this context, the use of the D/H ratio takes on significant application value, as it is not only easily interpretable for a wide range of stakeholders but also provides policymakers and managers with insights into the relative importance of detritus as a primary source of energy for the food web, compared to primary production (Fath et al., 2019; Safi et al., 2019). In herbivore food webs, primary production is driven by nutrient inputs, with significant efficiency in transferring carbon to consumers at higher trophic level, indicating a stable and wealthy ecosystem (Chkili et al., 2024; Meddeb et al., 2018). Conversely, in food webs characterized by a predominance of recycled organic matter and a higher biomass turnover, the carbon transferred along the food web is of lower quality (Perhar and Arhonditsis, 2009; de Guzman et al., 2024), thus affecting the ability to support higher trophic levels (e.g., fish or organisms for aquaculture) with a rich and nutritious food.

Using these indicators, is possible to conduct an integrative assessment of the state of the marine ecosystem by analyzing the plankton food web functioning, and this can be a useful tool to support marine policies and spatial planning. In fact, the information synthesized by the ecological indicators tested herein can help to: (i) predict the potential quality and quantity of food deliverable to fish (*sensu lato*, being wild stocks, or aquaculture) in coastal systems; (ii) identify which coastal marine sectors are more vulnerable to anthropogenic impacts and extreme events (e.g. flooding rainfall). This approach can help policy

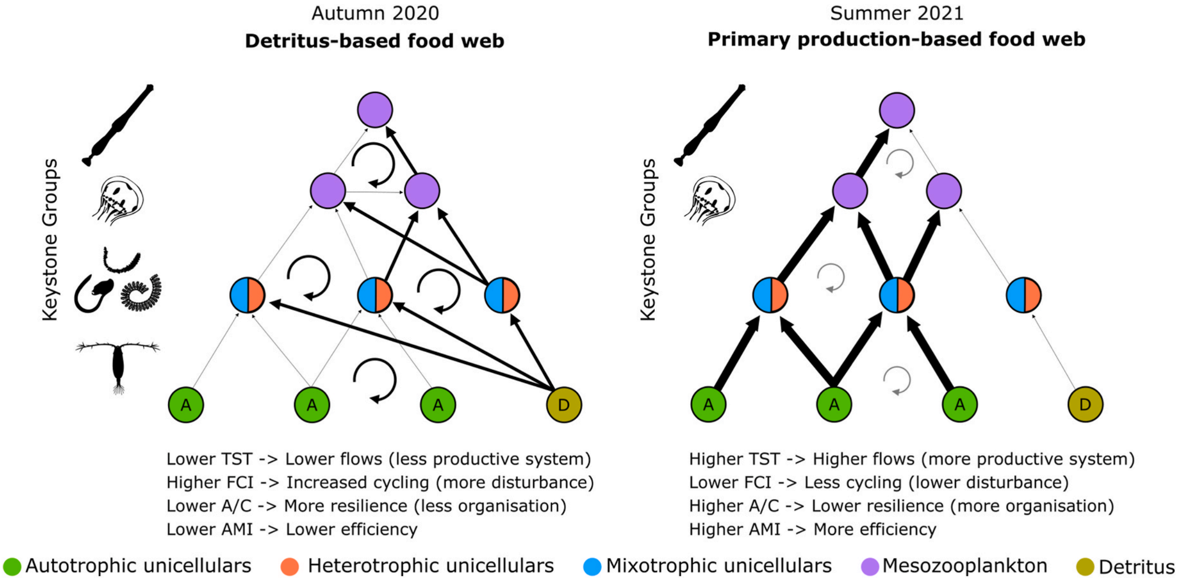

**Fig. 8.** The conceptual food web shows the main differences found through network-based ecological indicators and keystone groups between a detritus-based food web (autumn 2020, mainly in the Gulf of Gaeta) and a primary production-based food web (summer 2021).

makers to adopt more targeted, evidence-based functional management strategies, such as regulating nutrient inputs from river basins or identifying critical periods for fish stocks, to pursue the sustainable exploitation of biological resources (e.g., fisheries and aquaculture) and preserve marine ecosystems services. Integrating plankton-food-web-based ecological indicators as analytical tools within the environmental assessment required by the MSFD can add value to the continuous and adaptive monitoring of marine ecosystems, promoting the effective application of the ecosystem-based approach to coastal management.

### 4.4. *Present challenge and future perspectives*

Even though the methodology and approaches presented in this paper represent a case study limited to a specific Mediterranean coastal area, it is potentially applicable to a variety of contexts, characterized by different environmental conditions. Furthermore, although our results do not provide evidence for direct detection of the effects of warming on the plankton food web, the methodological approach adopted herein provides a potentially promising basis for future investigations to predict the impact of climate change.

One should consider that the application and development of ecological models in general remains limited for several reasons. First, food-web models are complex and require large amounts of quantitative data. For instance, the use of environmental DNA is increasing in recent years as an innovative tool – in some way, substituting the light-microscopy counts – for tracking and investigating marine biodiversity, but to date it cannot be used to develop robust ecological models because it essentially provides qualitative information. Furthermore, modelling studies (e.g. Vassallo et al., 2022; Russo et al., 2025; Loschi et al., 2023; Guida et al., 2024) are limited by the absence of a detailed taxonomic characterization of the plankton community. To this latter respect, our results show that having a more detailed taxonomic resolution, particularly for the planktonic metazoan, allows scientist to get a more accurate detection of structural changes within the trophic network and the dynamics of key functional groups that regulate its ecosystem functioning. In addition, differences in the structure and degree of aggregation of functional groups within food web models make it difficult to compare different ecosystems and related studies. Finally, there is a gap in studies on the development of reference ranges for ecological indicators, which are fundamental for better defining the 'Good Environmental Status'.

For the reasons described above, our study calls for future research to further explore these aspects, focusing both on overcoming the current methodological limitations and promoting the development of more robust and standardized comparative approaches across ecosystems, from the local to the global level. For instance, future research should be invested in the collection of quantitative data (i.e., Carbon biomass of plankton functional groups) and in the development of dynamic models to understand the effects of climate change on the structure and functioning of marine food webs. Both options can be supported by supporting long-term/time-series studies focusing on plankton ecology thus providing more effective tools for the GES assessment and management. In the context of climate change, the integration of advanced tools such as satellite remote sensing offers a strategic opportunity to expand observations to a global scale. Environmental variables retrievable from satellite data, such as the sea surface temperature or turbidity, can serve as important predictors for identifying regions most vulnerable to climate-driven impacts and consequent alterations in planktonic food web structures. Thus, high taxonomic resolution modeling approaches, combined with satellite data, can improve predictions of the effects of climate change on the structure and functioning of planktonic communities and marine ecosystems in general.

## 5. Conclusions

In recent years, the European Union has shown increasing interest in the analysis of marine ecosystem functions, adopting several holistic approaches (e.g. Water Framework Directive, the Habitats Directive, Marine Strategy Framework Directive). In this context, the use of appropriate ecological indicators is essential to communicate relevant information to policymakers and stakeholders. Our study showed how local environmental conditions (e.g. the influence of major rivers) have a significant impact on ecosystem structure and functioning, altering their stability and efficiency and modifying key functional groups in the food web. Furthermore, it was possible to provide a clearer and more accessible understanding of the trophic status of these ecosystems with ecological indicators that can help guide more informed and sustainable management choices (e.g., in the planning and installation of new aquaculture facilities). This integrated approach, based on close collaboration with the social and economic sciences, is crucial for developing ecosystem management strategies that are not only scientifically based but also effective and sustainable in the long term. Such a convergence of disciplines would allow for a comprehensive approach to complex socio-ecological dynamics, contributing to holistic and adaptive planning of environmental management.

## CRediT authorship contribution statement

**Daniele Bellardini:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luca Russo:** Visualization, Methodology, Investigation, Data curation. **Maria Abagnale:** Data curation. **Vincenzo Botte:** Methodology, Formal analysis, Data curation. **Angela Buondonno:** Data curation. **Raffaella Casotti:** Methodology, Data curation. **Gabriele Del Gaizo:** Data curation. **Iole Di Capua:** Data curation. **Marta Furia:** Data curation. **Daniele Iudicone:** Methodology, Data curation. **Florian Kokoszka:** Methodology, Formal analysis, Data curation. **Francesca Margiotta:** Data curation. **Maria Grazia Mazzocchi:** Data curation. **Maria Saggiomo:** Data curation. **Diana Sarno:** Data curation. **Simona Saviano:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Isabella Percopo:** Data curation. **Eugenia Tramontin:** Data curation. **Anna Chiara Trano:** Data curation. **Jessica Vannini:** Data curation. **Daniela Cianelli:** Methodology, Formal analysis, Data curation. **Priscilla Licandro:** Writing – original draft, Supervision, Data curation. **Paolo Vassallo:** Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Domenico D'Alelio:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization.

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## 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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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.indic.2025.100763.

## Data availability

The data are available in the Supplementary Material

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# 5. ANALYSIS OF PLANKTON FOOD WEBS IN THE NORTH SEA UNDER DIFFERENT TIDAL CONDITIONS. (Submitted paper)

The methodological approach used in paper 2 (see Chapter 4) was also applied in another geographical area to further investigate changes in planktonic food webs. Specifically, the approach was applied as part of an international project "Science for the Good Environmental Status (S4GES)," funded by JPI Ocean. The S4GES oceanographic campaign aimed to assess the environmental status of the marine environment by integrating physical, chemical and biological information from the North Sea area. During the S4GES project, an oceanographic sampling expedition focused on analyzing changes in the summer planktonic community (July 2022) in different ecosystem regimes (coastal and offshore) under different hydrographic conditions (e.g., low and high tide). Specifically, the S4GES campaign involved the measurement and sampling of several environmental variables (e.g., temperature, salinity, particulate organic carbon and dissolved organic carbon), oceanographic data (e.g., fronts, mixed layer patterns, current dynamics), contaminants (e.g., trace metals), and qualitative (metabarcoding) and quantitative (microscopic counts and flow cytometry) data on plankton biodiversity. The study (see below) investigated the functioning of the entire planktonic community along the Belgian coast by deriving trophic network models for each sampled station and assessing plankton changes in relation with different tidal conditions.

## 5.1 Material and Methods

### *5.1.1 Sampling area*

Samples were collected from July 1st1 to 15th, 2022 aboard the oceanographic ship "R/V Belgica." Two stations with different hydrographic conditions were sampled (Figure 5), one below the shore (NRT1) and the other offshore (Deep 2). Specifically, these stations were sampled during both high tide (cast A and C) and low tide (cast B and D). Continuous CTD/rosette profiles of conductivity, temperature, dissolved oxygen and salinity were recorded for each sampling site, and the entire planktonic community was sampled and analyzed, using different methodologies, detailed below.

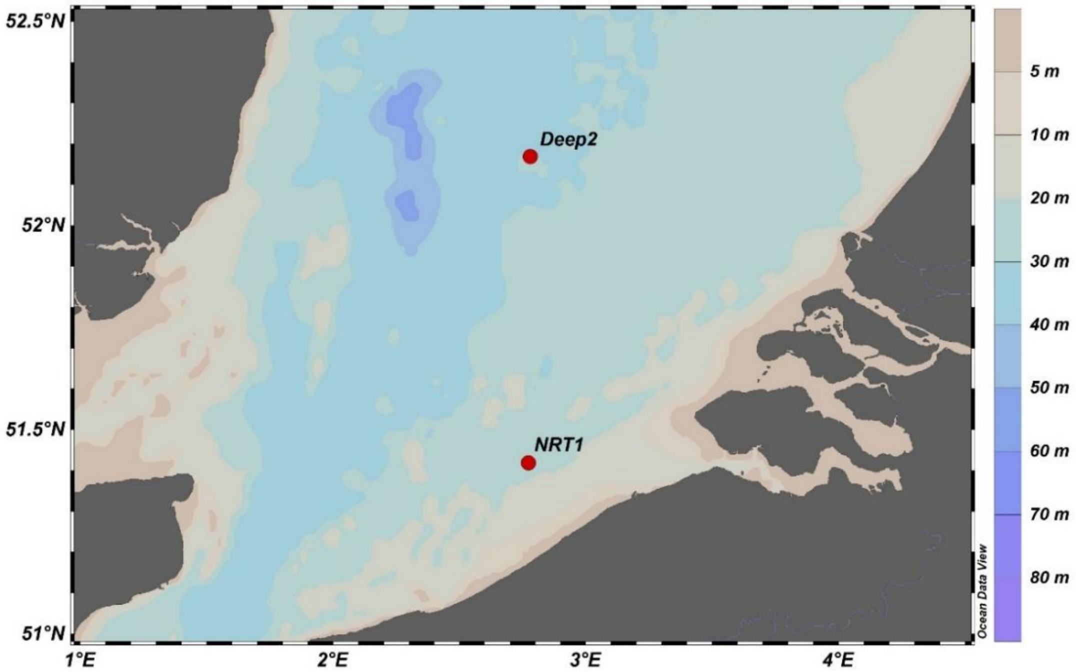


**Figure 5**. Study area along the Belgium coast. Dot point represents the offshore (Deep2, 42 m depth) and near-shore station (NRT1, 30 m depth). Legend represents the bottom depth (m).

#### 5.*1.1.1 Hetero-mixotrophic unicellular organisms*

Water samples for quantitative analyses of protozooplankton (hetero-mixotrophic unicellular organisms) were collected at the surface (0.5 m depths) from Niskin bottles. Seawater from the Niskin bottles were gently poured into 500- glass bottles, previously filled with Lugol's iodine solution (4% final concentration). Samples were set in a graduated cylinder for 48 h and concentrated to 500 mL by gently collecting the supernatant. The concentrated samples were allowed to settle in sedimentation chambers for 48 h. Taxa identification and counts were performed according to the Utermöhl method (Elder and Elbrachter 2010) with an inverted light microscope Zeiss Axiovert 200 (Carl Zeiss, Germany) at 20x (protozooplankton) magnifications. Taxonomic identification was performed at the species level whenever possible according to the most relevant and updated taxonomic literature. However, heterotrophic bacteria, were estimated with flow-cytometry and indirectly using a standard equation based on the total carbon biomass of phytoplankton (Simon et al., 1992).

#### *5.1.1.2 Mesozooplankton organisms*

Mesozooplankton samples were collected at all stations with vertically integrated tows from 5 m above the sea bottom to the surface with a double WP2 net (0.25 $m^2$ mouth area, 200 μm mesh size) equipped with a flowmeter. The samples were transferred from the cod-end to two 500 ml plastic jars and fixed in ethanol 96% and in formaldehyde (4%). Each sample in formalin was concentrated and then resuspended in a bowl with distilled water to a final volume of 200 ml; two aliquots of 5 ml each were analyzed using a stereomicroscope (Leica MZ 12.5) in a 10 ml Bogorov counting chamber. Taxonomic identification was performed at the species level whenever possible according to the most relevant and updated taxonomic literature (e.g., Castellani and Edwards 2017). The abundance was expressed as the number of individuals in a cubic meter (ind. $m^{-3}$).

### *5.1.2 Input parametrization of biological rates for food web models*

Plankton food webs were reconstructed through simulations derived with the Ecopath approach (https://ecopath.org/), using an algorithm written on the MATLAB software v.9.13.0 (R2022b). To reduce the food web complexity, species with similar ecological roles and interactions were aggregated into functional groups (FGs, see Table 4). In our model 22 FGs were defined, namely: one non-living FG (De), 11 microbial FGs (i.e.: heterotrophic bacteria [FG 5], phytoplankton [FGs 1, 2, 3, and 4], heterotrophic [FGs 7, 9, and 10], and mixotrophic unicellular organisms [FGs 6, 8, and 11]) and 10 zooplankton FGs (FGs from 12 to 21). The dinoflagellate *Noctiluca scintillans* was left as a stand-alone FG due to its peculiar characteristics, with a size and diet that are largely comparable to the zooplankton.

To run the simulations, several ecological inputs were needed for each living FG (see next paragraphs), namely:

(i) Carbon biomass (μgC $m^{-2}$);

(ii) Production rate per unit of biomass (μ, $d^{-1}$);

(iii) Consumption rate per unit of biomass (α, $d^{-1}$);

(iv) The proportion of phototrophy in individual metabolism (ph/het, adimensional);

(v) Unassimilated rate of biomass consumed (ε, adimensional),

(vi) Diets of consumers (percentage probability of interaction): 0 (no interaction), 1 (low interaction probability), 2 (medium interaction probability), and 3 (high interaction probability).

The whole metabolic rates were estimated from the literature taking as a reference the most representative taxa included in each FG and considering the effect of temperature on metabolic rates μ and α (Brown et al., 2004). Furthermore, for each FG the maximum values were calculated, while they were set to zero of the minimum values, assuming the option of zero – growth. As there is a high uncertainty on some metabolic rates, for those FGs where only average metabolic rate values were known, a range of variability (pedigree) (Morissette et al., 2014, see Table 3) was added, to calculate maximum values. Then, all metabolic rates (μ α ε, see next paragrapher) were calculated following the equation used by (Hansen et al. 1997), applying a specific $Q_{10}$ for the FGs:

$$\log_{10} \lambda = \log_{10} \lambda_{ref} + \log_{10} (Q_{10}) *(T - T_{ref})/10$$

where $\lambda_{ref}$ and $T_{ref}$ were respectively the reference λ and temperature taken from the literature, while T represents the average temperature of the sampling site.

**Table 2**. Default options for the pedigree routine. Table from Morisette et al 2014

| ***Physiological Parameters*** | ***Default CI (±%)*** |
|---|---|
| Same Group/Species, Same System | 10 |
| Same Group/Species, Similar System | 20 |
| Similar Group/Species, Same System | 30 |
| Similar Group/Species, Similar System | 40 |
| Empirical Relationship | 50 |
| From Other Model | 80 |
| Guesstimate | 90 |

### *5.1.3 Derivation of carbon biomass of planktonic microorganisms*

#### *5.1.3.1 Hetero-mixotrophic unicellular organisms*

Once cell biovolume (BV) values were calculated, the corresponding cell carbon content (pg C $cell^{-1}$) was assessed using conversion factors available in the literature (Menden-Deuer and Lessard 2000) and specific for each group of organisms:

Diatoms = $10^{-0,541+(0,811*(LOG_{10}(BV)))}$

Dinoflagellates = $10^{-0,353+(0,864*(LOG_{10}(BV)))}$

Green algae = $10^{-1,026+(1,088*(LOG_{10}(BV)))}$

Tintinnids = $10^{-0,\ 168+(0,841*(LOG_{10}(BV)))}$

Oligotrichs = $10^{-0,639+(0,984*(LOG_{10}(BV)))}$

Prasinophyta = $10^{-0,545+(0,886*(LOG_{10}(BV)))}$

Coccolitophores = $10^{-0,642+(0,899*(LOG_{10}(BV)))}$

Cryptophyta and Euglenophyta = $10^{-0,665+(0,939*(LOG_{10}(BV)))}$

Some microorganisms were not directly measured. The cells carbon content of these latter was obtained from an extensive meta-analysis on BVs and carbon contents available in the literature (Harrison et al., 2015), from Laplace-Treyture et al. (2021) in the case of freshwater cyanobacteria (i.e., *Anabaena* filaments) and from Stauffer et al. (2017) for the dinoflagellate *Noctiluca* scintillans.

Marine cyanobacteria carbon biomass was obtained by multiplying the marine cyanobacteria abundances with the conversion factor of 200 fgC cell$^{-1}$ (Caron et al., 1995). Heterotrophic bacteria (FG 5) carbon biomass was estimated indirectly by considering the carbon biomass of all autotrophic FGs derived at each sampling station by using the equation found in Meinhard et al. (1992).

Moreover, the identified microorganisms were then classified based on their trophic modes (autotrophic [A], mixotrophic [M], and heterotrophic [H]), reported in the most updated studies and reference databases available in the literature (Unrein et al., 2014; Haraguchi et al., 2018; Leles et al., 2019; Mitra et al., 2023; Schneider et al., 2020).

#### *5.1.3.2 Mesozooplankton organisms*

To determine the C biomass of zooplanktonic FGs (from 12 to 22), the species – specific conversion factors (µgC ind$^{-1}$) found in the literature were applied. Furthermore, to determine the biomass C of juvenile copepods, the individual carbon values of adult copepods were multiplied by a factor of 0.27 (see Chapter

4) (Table 3). To determine copepod FGs were used a functional traits approach (see Chapter 3).

**Table 3**. List of identified planktonic metazoans, with conversion factors and references used to derive their carbon biomass.

| ***Taxa*** | ***µgC/ind*** | ***Ref.*** | ***Taxa*** | ***µgC/ind*** | ***Ref.*** |
|---|---|---|---|---|---|
| *Acartia clausi* m | 2.53 | Nassogne, 1972 | *Euterpina acutifrons* | 0.49 | Nassogne, 1972 |
| *Acartia* spp. iuv. (CI-CIV) | 0.71 | Chapter 4 | *Evadne spinifera* | 1.70 | D'Alelio et al., 2016 |
| Amphipoda spp. (as gammaride) | 174.00 | Chapter 4 | *Fish larvae* | 24.29 | Arreola et al., 2018 |
| Anthomedusa spp. | 188.50 | Vassallo et al., 2022 | *Hydrozoa* | 188.50 | Vassallo et al., 2022 |
| Bivalvia larvae | 4.92 | Uye et al., 1982 | *Idroide* spp. | 188.50 | Vassallo et al., 2022 |
| *Calanus helgolangdicus* f | 85.63 | Chapter 4 | *Larva sipunculide* | 4.70 | Chapter 4 |
| *Calanus* spp.iuv (CI-CIV) | 23.88 | Chapter 4 | Polychaeta larvae | 4.38 | Nassogne, 1972 |
| *Centropages hamatus* m | 4.19 | D'Alelio et al., 2016 | *Limacina* spp. | 7.82 | Nassogne, 1972 |
| *Centropages* spp. Iuv (CI-CIV) | 1.17 | Chapter 4 | *Copepod nauplii* | 0.93 | Chapter 4 |
| Cirripedia larvae | 0.54 | Uye et al., 1982 | *Oikopleura* spp. | 1.40 | Uye et al., 1982 |
| *Clausocalanus furcatus* f | 3.60 | Mazzocchi and Paffenhofer, 1998 | *Oithona nana* f | 0.48 | Barth-Jensen et al., 2020 |

| | | | | | |
|---|---|---|---|---|---|
| *Clausocalanus iuv.* (CI-CIV) | 2.19 | Chapter 4 | *Ostracoda* spp. | 65.01 | Chapter 4 |
| *Clausocalanus jobei* f | 2.66 | Nassogne, 1972 | *Paracalanus parvus* | 2.04 | D'Alelio et al., 2016 |
| *Clausocalanus spp.* f | 7.87 | Nassogne, 1972 | *Paracalanus* spp. juv (CI-CIV) | 0.57 | Chapter 4 |
| *Cumacei spp.* | 27.56 | Conway, 2015 | *Podon intermedius* | 18.04 | Chapter 4 |
| *Decapod larvae* | 27.56 | Chapter 4 | *Pseudocalanus elongatus* f | 5.34 | Amin et al., 2011 |
| *Echinodem larvae* (asteroidea) | 1.54 | Chapter 4 | Pteropod larvae (Limacina) | 181.68 | Nassogne, 1972 |
| *Temora longicornis* juv | 2.39 | Chapter 4 | *Sagitta* spp. | 46.22 | Nassogne, 1972 |
| *Temora longicornis* m | 8.58 | D'Alelio et al., 2016 | | | |

The list of all the identified organisms among all the samplings can be found in Table 4.

**Table 4.** Identified planktonic organisms and the relative planktonic functional groups (FGs) from the S4GES cruise with their trophic modes (Tro): A: Autotrophs; H: Heterotrophs; M: Mixotrophs; M: Metazoans; Cyano: cyanobacteria; Green: green algae; Diat: diatom; Dino: dinoflagellate; Cil: ciliate (including both tintinnids and oligotrichs); Nano: nanoflagellate,

| Taxa | Tro | FGs | Taxa | Tro | FGs |
|---|---|---|---|---|---|
| Freshwater green microalgae | A - Green | FG 3 | *Tripos* | M - Dino | FG 8 |

| | | | | | |
|---|---|---|---|---|---|
| Freshwater cyanobacteria | A - Cyano | FG 2 | *Alexandrium* | M - Dino | FG 8 |
| *Bacteriastrum* | A - Diat | FG 4 | *Amphidinium* | M - Dino | FG 8 |
| *Cerataulina* | A - Diat | FG 4 | *Gymnodinium* | M - Dino | FG 8 |
| *Chaetoceros* | A - Diat | FG 4 | *Scrippsiella* | M - Dino | FG 8 |
| *Synedra* | A - Diat | FG 4 | *Phalachroma* | M - Dino | FG 8 |
| *Cylindrotheca* | A - Diat | FG 4 | *Akashiwo* | M - Dino | FG 8 |
| *Dactyliosolen* | A - Diat | FG 4 | *Dinophysis* | M - Dino | FG 8 |
| *Guinardia* | A - Diat | FG 4 | *Gonyaulax* | M - Dino | FG 8 |
| *Melosira* | A - Diat | FG 4 | *Gyrodinium* | M - Dino | FG 8 |
| *Navicula* | A - Diat | FG 4 | *Karenia* | M - Dino | FG 8 |
| *Odontella* | A - Diat | FG 4 | *Polykrikos* | M - Dino | FG 8 |
| *Rhizosolenia* | A - Diat | FG 4 | *Prorocentrum* | M - Dino | FG 8 |
| *Pinnularia* | A - Diat | FG 4 | *Torodinium* | M - Dino | FG 8 |
| *Thalassiosira* | A - Diat | FG 4 | *Tontonia* | M - Cil | FG 11 |
| *Pseudo-nitschia* | A - Diat | FG 4 | *Laboea* | M - Cil | FG 11 |
| *Protoperidinium* | H - Dino | FG 7 | *Mesodinium rubrum* | M - Cil | FG 11 |
| *Katodinium* | H - Dino | FG 7 | *Strombidium* | M - Cil | FG 11 |
| *Noctiluca scintillans* | H - Dino | FG 9 | Meroplankton | Met | FG 21 |
| *Pelagostrobilidium* | H - Cil | FG 10 | Oikopleura | Met | FG 20 |
| *Strobilidium* | H - Cil | FG 10 | *Acartia; Paracalanus* | Met | FG 12 |
| Prasinophyta | M - Nano | FG 6 | *Clausocalanus; Temora; Pseudocalanus* | Met | FG 13 |

| | | | | | |
|---|---|---|---|---|---|
| Coccolitophores | M - Nano | FG 6 | *Calanus; Centropages* | Met | FG 14 |
| Cryptophyta | M - Nano | FG 6 | *Oithona* spp. | Met | FG 15 |
| Euglenophyta | M - Nano | FG 6 | Copepodites I - IV | Met | FG 16 |
| Thecate dinoflagellates > 15 µm | M - Dino | FG 8 | *Evadne; Podon* | Met | FG 17 |
| Thecate dinoflagellates > 30µm | M - Dino | FG 8 | Hydrozoans | Met | FG 18 |
| Naked dinoflagellates > 15 µm | M - Dino | FG 8 | Amphipods | Met | FG 19 |
| Naked dinoflagellates > 30 µm | M - Dino | FG 8 | *Sagitta* spp. | Met | FG 22 |

### *5.1.4 Derivation of physiological parameters of planktonic microorganisms*

#### *5.1.4.1 Production rate per unit of biomass (μ, $d^{-1}$)*

The µ of heterotrophic bacteria (FG 5) was estimated using the equation found in White et al 1991:

$\log10\ \mu = -1.54 + 0.052\ (T)$

where µ is the growth rate, and T is the average of the temperature (°C).

The maximum µ of autotrophs (FGs, 1, 2, 3 and 4) and mixotrophic nanoflagellates (FG 6) were calculated using the maximum µ associated with different temperatures published in (Thomas et al., 2016). For all these groups, using R studio version 4.2.3 (R Core Team 2024), we calculated a polynomial regression between their maximum µ and temperatures reported in (Thomas et al., 2016), obtaining the following equations used to estimate the maximum µ of each autotrophic FG and mixotrophic nanoflagellates at each S4GES sampling station:

Diatoms $\mu = 0.0920647 + 0.0798232*T - 0.0018146*T^2$

Autotrophic dinoflagellates $\mu = -0.3907999 + 0.0734214*T -0.0015882*T^2$

Freshwater green microalgae $\mu = -0.0018257 + 0.0546007*T - 0.001112*T^2$

Freshwater cyanobacteria $\mu = -0.3261097 + 0.0637349*T - 0.0012807*T$

Mixotrophic nanoflagellates $\mu = -0.1942583 + 0.0982691*T - 0.0022308*T^2$

where T is the average temperature (°C) in the 0-3 m layer at each S4GES station.

The maximum μ of heterotrophic and mixotrophic dinoflagellates (FG 7 and 8, respectively) were obtained by calculating the median value of all the maximum μ at 20 °C of mixotrophic (n=10) and heterotrophic (n=14) dinoflagellates reported in (Jeong et al., 2010).

In this way, we obtained a reference μ of 0.42 and 1.105 $d^{-1}$ for mixotrophic and heterotrophic dinoflagellates, respectively. Reference μ of *Noctiluca scintillans* (FG 9) was taken from (Nakamura 1998), which reported a maximum μ of 0.27 $d^{-1}$ at 24 °C. μ of heterotrophic and mixotrophic ciliates (FG 10 and 11, respectively) were derived by calculating the median value of all the maximum μ at 15 °C of mixotrophic (n=16) and heterotrophic (n=24) ciliates reported in (Pérez et al., 1997), from which, using the equation proposed by (Hansen et al., 1997), we obtained a reference μ of 0.74 and 1.14 $d^{-1}$ for mixotrophic and heterotrophic ciliates, respectively.

For zooplanktonic groups, the reference value for μ was taken from the most representative species found within our FGs, and the values of μ are described in Table 5.

**Table 5.** Growth rate (*μ*) for zooplanktonic organisms. Ref.1 is the reference for the growth rate value; Ref.2 is the reference for the $Q_{10}$ value.

| ***Functional Groups (FGs)*** | | ***Representative species*** | $\mu_{max}$ | $\mu_{avg}$ | $Q_{10}$ | ***T(°C)*** | ***Ref.1*** | ***Ref.2*** |
|---|---|---|---|---|---|---|---|---|
| 12 | Small calanoids <1mm | *Acartia clausi; Paracalanus parvus* | 0.26 | | 2.65 | 20 | Jang et al 2013 | Escribano et al 2016 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | Medium calanoids 1-2mm | *Clausocalanus spp.; Temora stylifera; Pseudocalanus elongatus* | 0.37 | | 2.27 | 20 | Dzierzbicka-Glowacka et al 2011 | Escribano et al 2016 |
| 14 | Large calanoids >2mm | *Calanus helgolangdicu; Centropages hamatus* | | 0.14 | 2.27 | 15 | Carlotti et al 2007 | Escribano et al 2016 |
| 15 | Cyclopoids | *Cyclopina spp.; Oithona nana; Ditrichocorycaeus anglicus* | 0.34 | | 3.34 | 23 | Almeda et al 2010 | Almeda et al 2010 |
| 16 | Copepodites I-IV | | 1.20 | | 3.12 | 28 | Hopcroft et al., 1998 | Leandro et al.,2006 |
| 17 | Cladocerans | *Evadne spinifera; Podon intermedius* | 1.24 | | 2.80 | 26 | Atienza et al., 2007 | Hansen et al., 1997 |
| 18 | Hydrozoans | | 0.35 | | 2.57 | 18 | McConville et al 2017 | Larson et al 1986 |
| 19 | Amphipods | | | 0.09 | 1.59 | 20 | Xue et al 2013 | Xue et al 2013 |
| 20 | Appendicularians | *Oikopleura spp.* | 2.48 | | 1.78 | 20 | Hopcroft et al., 1995 | Broms et al., 2003 |
| 21 | Meroplankton | | 0.22 | | 2.80 | 16 | Almeda et al., 2009 | Hansen et al., 1997 |
| 22 | Chaetognats | *Sagitta spp.* | 0.25 | | 2.80 | 21 | McConville et al 2017 | Hansen et al 1997 |

Due to the high uncertainty on some production rates, we added a range of variability of 20%, 40% for FGs Large Calanoids (FG 14) and Amphipods (FG 19).

### *5.1.4.2 Consumption rate per unit of biomass (α, $d^{-1}$)*

Due to the scanty literature about specific α of heterotrophic bacteria (FG 5), we assumed that bacterial α were at least twice the respective μ.

The maximum α of mixotrophic (n=9) and heterotrophic (n=8) dinoflagellates were obtained by calculating the median value of all the maximum α at 20 °C of mixotrophic and heterotrophic dinoflagellates reported in (Jeong et al., 2005, 2010; Kim et al., 2008; Lim et al., 2019). This provided a reference α of 1.26 and

1.96 $d^{-1}$ for mixotrophic (n=8) and heterotrophic (n=7) dinoflagellates, respectively. Reference α for *Noctiluca scintillans* was derived from (Frangópulos et al., 2011), which reported a maximum α of 0.31 $d^{-1}$ at 18 °C. For the α of ciliates, we used the maximum α of 1.58 $d^{-1}$ at 15 °C and 3.68 $d^{-1}$ at 17.7 °C for mixotrophic and heterotrophic ciliates, respectively, reported in (Yoo et al., 2013; Yang et al., 2015). Concerning mixotrophic nanoflagellates, due to the lack of information available in the literature, we applied a maximum α value of twice their μ, as already done in previous investigations (D'Alelio et al., 2016).

To estimate zooplanktonic maximum α, the same $Q_{10}$ used to calculate growth rates were considered.

$\alpha_{avg}$ for FGs 12-13-14 and 16 were estimated using the equation proposed by Saiz et al 2007:

$\log(\alpha) = -1.751 + 0.355 \log(W) + 0.893 \log(C)$

where W represents Total Food Concentration (TFC - μgC $L^{-1}$) and C represents IC (μgC $L^{-1}$)

The $\alpha_{avg}$ for cladocerans (FG 17) was estimated by calculating a linear regression between ingestion rate values and total food concentration (TFC) values at 18°C, reported in (Katechakis et al 2004, Figure 4), resulting in the following equation used to estimate their $\alpha_{avg}$:

$\alpha\ (d^{-1}) = 0.0087x + 0.977$

where x represented the TFC (μgC $L^{-1}$)

The ingestion rate for the other zooplankton groups is described in Table 6.

**Table 6**. Ingestion rate (*α*) for zooplanktonic organisms. When not found *α*, the ingestion rate was estimated through the ratio between the $\mu_{max}$ and the Growth Gross Efficiency (GGE).

| ***Functional Groups (FGs)*** | | **Representative species** | **GGE** | $\alpha_{max}$ | $\alpha_{avg}$ | $Q_{10}$ | ***T(°C)*** | ***Ref.*** |
|---|---|---|---|---|---|---|---|---|
| 15 | Cyclopoids | *Cyclopina spp.; Oithona nana; Ditrichocorycaeus anglicus* | | 1.50 | | 3.34 | 23 | Almeda et al., 2010 |
| 18 | Hydrozoans | | 0.16 | | | 2.57 | 21 | Matsakis et al., 1993 |

|  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
| 19 | Amphipods |  |  | 0.37 |  | 1.59 | 18 | Berezina et al., 2007 |
| 20 | Appendicularians | *Oikopleura spp.* |  |  | 3.19 | 1.78 | 24 | Scheinberg et al., 2005 |
| 21 | Meroplankton |  | 0.23 |  |  | 2.80 | 16 | Almeda et al., 2009 |
| 22 | Chaetognats | *Sagitta spp.* |  | 0.43 |  | 2.80 | 21 | Otake et al., 2020 |

Due to the high uncertainty on some ingestion rates, we added a variability range of 20% (for FG 20), 40% (for FG 17) and 50% (for FGs 12, 13, 14, 16 and 19).

#### *5.1.4.3 Proportion of phototrophy in individual metabolism (ph/het, adimensional)*

For the mixoplankton FGs (6, 8 and 11), we also calculated the ph/het, which highlights to what extent mixotrophic protists rely on photosynthesis or grazing. The ph/het for the mixotrophic dinoflagellates was obtained by calculating the median value (n=17) of all the ph/het for mixotrophic dinoflagellates reported in (Skovgaard 2000; Smalley and Coats 2002; Jeong et al., 2005, 2021; Lee et al., 2015; D’Alelio et al., 2016; Stoecker et al., 2017). In this way, we obtained a ph/het of 0.6 for mixotrophic dinoflagellates. Mixotrophic ciliates ph/het was derived by taking a median value (n=3) of 0.14, calculated from all the H/A reported in (Stoecker et al., 1988; Johnson and Stoecker 2005; Maselli et al., 2020). Lastly, the ph/het for mixotrophic nanoflagellates was obtained by taking the median value (n=4) of all the ph/het associated with mixotrophic nanoflagellates found in (Unrein et al., 2014; Leles et al., 2021). This resulted in a ph/het for the mixotrophic nanoflagellates of 0.82.

#### *5.1.4.4 Unassimilated rate of biomass consumed (ε, adimensional)*

The unassimilated food parameter is the complement to 1 of the assimilation efficiency (ε=AE-1), which is the amount of food that is used for respiration and growth. The unassimilated food released by all the organisms was directed at the detritus.

Egestion of heterotrophic bacteria and cyanobacteria (FG 5 and 1, respectively) was set to 0.05 as in (Heymans and Baird, 1995; Vassallo et al., 2006). It was observed that the assimilation efficiency of nanoflagellates was 0.84 (Chase and Price, 1997), thus we assumed a value of egestion of 0.16 for the mixotrophic nanoflagellates (FG 6). Egestion of mixotrophic and heterotrophic dinoflagellates, *Noctiluca scintillans* and mixotrophic and heterotrophic ciliates were set to 0.13 following (Buitenhuis et al., 2010).

The assimilation efficiency for the FGs 11-12-13 and 17, were estimated following a linear regression (Katechakis et al., 2004) at 25.5°C:

$AE_{Ac}\%= 55.01 – 0.12 * TFC$, used to FGs 11, 12 and 13 (Calanoids).

$AE_{Pen}\%= 47.67 – 0.02 * TFC$, used to FG 17 (Cladoceran).

where TFC corresponds to the total food concentrations (µgC $L^{-1}$) of predators

The assimilation efficiency (AE) for the other zooplankton groups is described in Table 7.

**Table 7.** Assimilation Efficiency (AE) for zooplanktonic organisms, used to calculate egestion rate (ε=AE-1).

| ***Functional Groups (FGs)*** | | **Representative *species*** | ***$AE_{max}$*** | ***$AE_{avg}$*** | ***$Q_{10}$*** | ***T(°C)*** | ***Ref.*** |
|---|---|---|---|---|---|---|---|
| 15 | Cyclopoids | *Cyclopina spp.;*<br>*Oithona nana;*<br>*Ditrichocorycaeus anglicus* | 0.86 | | 3.34 | 20 | Almeda et al., 2011 |
| 16 | Copepodites I-IV | | | 0.73 | 3.12 | 20 | Almeda et al., 2011 |
| 18 | Hydrozoans | | 0.38 | | 2.57 | 25 | Marshalonis et al., 2008 |
| 19 | Amphipods | | | 0.73 | 1.59 | 22 | Pederson 1984 |
| 20 | Appendicularians | *Oikopleura spp.* | 0.05 | | 1.78 | 25 | D'Alelio et al., 2016 |
| 21 | Meroplankton | | | 0.70 | 2.80 | 15 | Steinberg and Landry 2017 |
| 22 | Chaetognats | *Sagitta spp.* | | 0.25 | 2.80 | 25 | Ikeda et al., 2021 |

Due to high uncertainty on some unassimilated rates, a range of variability of 20% (for the FGs 12, 13, 14 and 17), 30% (for the FGs 16 and 21), 40% (for the FGs 18 and 22).

### *5.1.4.5 Diets of consumers*

The food sources for the heterotrophic bacteria (FG 5) were the detrital matter (Dafner et al 2002; del Giorgio et al 2002). Diets of consumers were taken from the most representative taxa for each FG (Figure 6). Diets of mixotrophic nanoflagellates (FG 6) are mainly based on pico-planktonic organisms like heterotrophic bacteria and cyanobacteria (Mitra et al., 2023). Mixotrophic and heterotrophic dinoflagellates (FGs 8 and 7, respectively) presented similar diets, being able to feed on pico-plankton, nano-plankton and micro-planktonic organisms like ciliates and dinoflagellates (Jeong et al., 2010; Sailley and Buitenhuis 2014). However, the mixotrophic dinoflagellates presented a narrower size range of edible prey compared with the heterotrophic dinoflagellates that can feed on prey showing comparable sizes with predators (Jeong et al., 2010). *Noctiluca scintillans* (FG 9) has a generalist diet being able to feed both small organisms like heterotrophic bacteria, cyanobacteria and nanoflagellates, but also on micro-plankton like diatoms, ciliates and dinoflagellates (Kirchner et al., 1996; Zhang et al., 2016; Nishitani et al., 2020). Among micro-plankton, *Noctiluca scintillans* showed a large preference for diatoms and a very low preference for ciliates (Zhang et al., 2016). Moreover, *Noctiluca scintillans* are also able to feed on metazoans like meroplankton and the larval stages of copepods (Nishitani et al., 2020). Lastly, mixotrophic and heterotrophic ciliates (FGs 11 and 10, respectively) showed similar diets being able to feed on all the unicellular organisms from pico-plankton to micro-plankton with the exclusion of diatoms, which seem not predated by ciliates (Sailley and Buitenhuis 2014).

Regarding copepods groups (FGs 12 ,13 and 14), they show a diet composed mainly of nano-micro plankton organisms (both autotrophs and heterotrophs) and detritus (Katechakis et al 2004; Fileman et al 2010; Isari et al 2011; Camatti et al 2019). This group, mainly represented by *Temora stylifera, Acartia clausi* and *Centropages ponticus*, also shows a feeding on juvenile copepods (Calbet et al 2007; Saage et al 2006; Vincent et al 2000; D’Alelio et al 2016).

The Cyclopoids groups mainly represented by the family Oithonidae (FGs 15), show a food source characterized by microzooplankton, juvenile copepods and detritus (Paffenhöfer et al 1993, 2002; Turner 1986; Sun et al 2022; Gonzalez et al 1994). Furthermore, *Oithona* spp. show a dietary preference mainly for ciliates, which constitute more than 80% of their diet (Castellani et al 2005, 2008). The food source of copepodites (FGs 16) consists of bacteria, nano-autotrophs, and heterotrophs (Finlay et al 2004; Roff et al 1995), with a large preference for dinoflagellates and ciliates (Calbet et al 2007; Paffenhöfer et al 1996). Furthermore, it has been assumed that these groups may also feed on particulate detritus.

Regarding non-copepod groups, the main carnivores are represented by hydrozoans (FG 18), chaetognaths (FG 22) and amphipods (FG 19). Hydrozoans feed on pico-nano autotrophs, heterotrophic unicellular, and small-sized copepods (Purcell et al 1982; Boero et al 2007), but the major food preferences are represented by juvenile copepods and meroplanktonic larvae (Licandro et al 2017a,b). Chaetognaths represented the top predator in this food web. These show a food source consisting mainly of copepods and meroplanktonic larvae (Tönnesson et al 2005; Durò et al 2000), but also microzooplankton, especially tintinnids (Pearre et al 1981), detritus (Grigor et al 2020) and cannibalism is also reported for these organisms (Kehayias et al 1996; Durò et al 2000). The food sources of amphipods are represented by the consumption of salps, ctenophores and detritus, which together constitute more than 95% of their diet (Zelickman et al 1995; Berezina et al 2007). They can also prey on chaetognaths and micro-dinoflagellates (Haro-Garay et al 2003). The diet of meroplanktonic larvae (FGs 22) and is mainly composed of nano – flagellates' organisms (both autotrophs and heterotrophs), ciliates, pico-plankton (Almeda et al 2011b; Harms et al 1990; Martin et al 1996) but in some cases they may prey on juvenile copepods and particulate detritus (Lindeque et al 2015). The group of cladoceran (FG 17) also feed on a wide range of particulate food ranging from 2.5 to 100 µm, including cyanobacteria, pico- and nanoflagellates, ciliates, diatoms, and detritus (Atienza et al 2006; Wong et al 2006; Miyashita et al 2010), but have a feeding preference for micro-dinoflagellates organisms (Atienza et al 2007).

The filter-feeding groups, consisting of appendicularians (FG 20) feed mainly on all particulate material, from bacteria to microzooplankton (Gorsky and Fenaux 1998; Nakamura et al 1997). Appendicularians feed particularly on pico-nanoplanktonic organisms (Scheinberg et al 2005), while doliolids and salps may also feed on small detrital aggregates (Ahmad Ishak et al 2017; Frischer et al 2021).

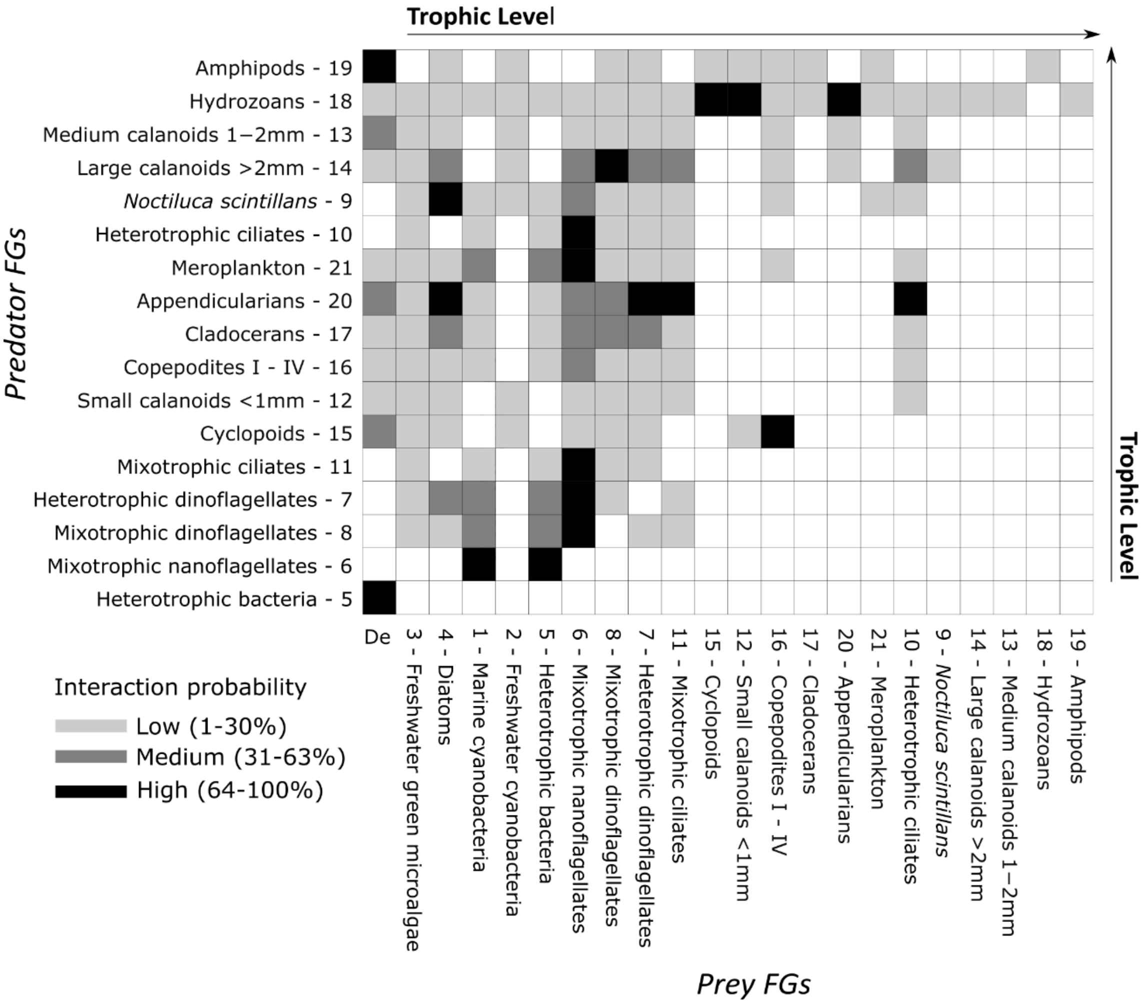


**Figure 6.** Adjacent matrix used for the analysis where the FGs were ordered respect to their no-weight trophic level (TL was calculated with RStudio using '*igraph'* package ("*TrophInd*" function). De indicate "Detritus" group. The different color represents the probability of interaction (no interaction in white; low in light gray; medium in gray and high in black).

### *5.1.5 Food web models and ecological indicators*

For each living FG, linear algebraic equations were used to balance the daily consumption flows (input and output), respiration, unassimilated food, production, predation, and natural mortality, based on the following equation:

$$Bi \times \mu\ i \times EEi - Bj \times (\alpha)j \times DCji - mk=0 \qquad [1]$$

where Bi is the biomass of group i, μ is the biomass production ratio of group i, EEi is the ecotrophic efficiency of group i, Bj is the biomass of the predator j, αj is the biomass consumption ratio of the predator j, DCij is the interaction between prey i in the diet of the predator j and, mk is the natural mortality of the node k.

Equation [1] was applied with random sampling of metabolic input parameters in a specific range. The software routine provides an error message if the simulation result is unrealistic. If inconsistencies were found (e.g. EE > 1), the diet matrix was slightly modified to obtain a successful simulation (Christensen et al., 2004). Each time the diet matrix was modified, the predator's diet was compensated by interpreting the decreased predation as an import (Vassallo et al., 2022). Several ecological and thermodynamic constraints were adopted to respect ecological reality: (i) respiration > 0, (ii) production–consumption rate between 0.1-0.5 for protists and 0.1-0.35 for mesozooplankton (Loschi et al., 2023). Every time the food web respected all conditions and constraints, it was accepted and a new simulation up to 999 simulations for each sampling site. Then, for each simulation, different ecological indicators were calculated (Table 8) to investigate if different tidal conditions affected the planktonic functional groups and how the structure and functionality of the food web changed.

**Table 8.** Ecological indicators used in this study.

| *Acronym* | *Indicators* | *Definition* | *Ref.* |
|---|---|---|---|
| **TST** | Total System Throughput | Indicates the sum of all flows in the model and is considered an overall measure of the 'ecological dimension' of the system | Finn et al., 1976 |
| **A/C** | Relative Ascendency | Relative ascendency and show the organization of the food web | Ulanowicz et al., 1986 |
| **FCI** | Finn’s Cycling Index | Indicates the "material cycle within a given system" and measures how often flow would revisit the same node several times | Finn et al., 1976 |
| **D/H** | Ratio of Detritivory to Herbivory | Indicates the dependence of the trophic network on newly produced algal material or the detritus compartment | Ulanowicz et al., 1991 |
| **-** | Modularity | Indicates group of nodes that are more connected with each other than with nodes belonging to other modules | Blondel et al., 2008 |

To explore the effect of the tide, a non-metric MultiDimensional Scaling ordination (n-MDS) was carried out on the calculated ecological indicators using R version

4.3.2 (RStudio Team). This nMDS was developed considering the 999 food web simulations per each tidal phase.

## 5.2 Results

Based on the carbon biomass dynamics at the Deep2 station, the FG's underwent minimal demographic oscillations during the tidal cycle phases, which were more evident in diatoms (FG 4), crustaceans (e.g., small-medium (FG13) and large calanoids (FG14)), amphipods (FG 14 and 19, respectively), and hydrozoans (FG 18). However, in the NRT1 station minimal difference in biomass budget were found between the tidal cycle phases, such as higher biomass of autotrophs organisms, in particular marine cyanobacteria (FG1) and diatoms (FG4) and large calanoids (FG14) during the high tide phases (Figure 7).

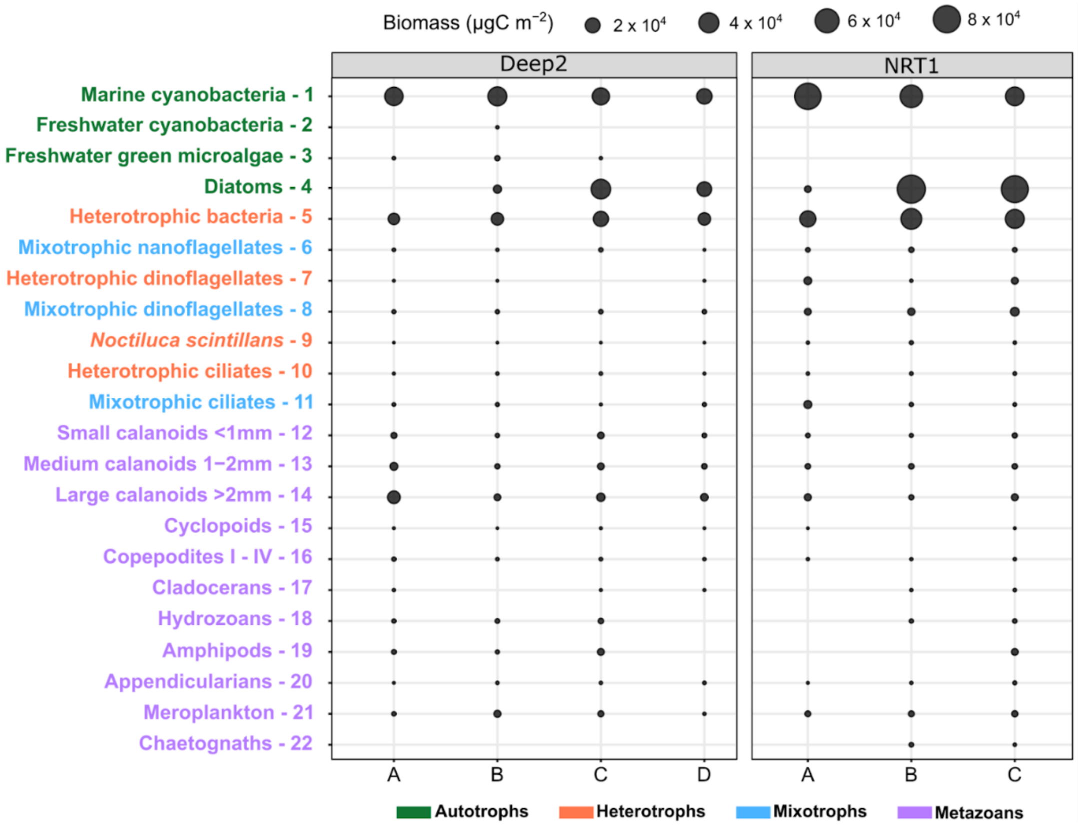


**Figure 7**. Bubble plots show the carbon biomass (µgC $m^{-2}$) for each living functional group (FGs) at the Deep2 and NRT1 stations during the tidal phases (max tide corresponds to phases A-C, low tide corresponds to phases B-D). FGs are colored based on the trophic group shown in the legend.

The tidal cycle mostly affected the plankton food-web at the offshore station Deep2 (Figure 8 and 9a), whereas, although a small change in the abundance of some FGs was observed, this did not lead to a change in the food web at the near-shore station NRT1 (Figure 8 and 9b). In particular, the Deep2 station highlighted a different functioning of the plankton food web across tidal phases (Figure 8): *Deep2_A* and *Deep2_C* showed similar patterns, and were distinct from those of *Deep2_B* and *Deep2_D*.

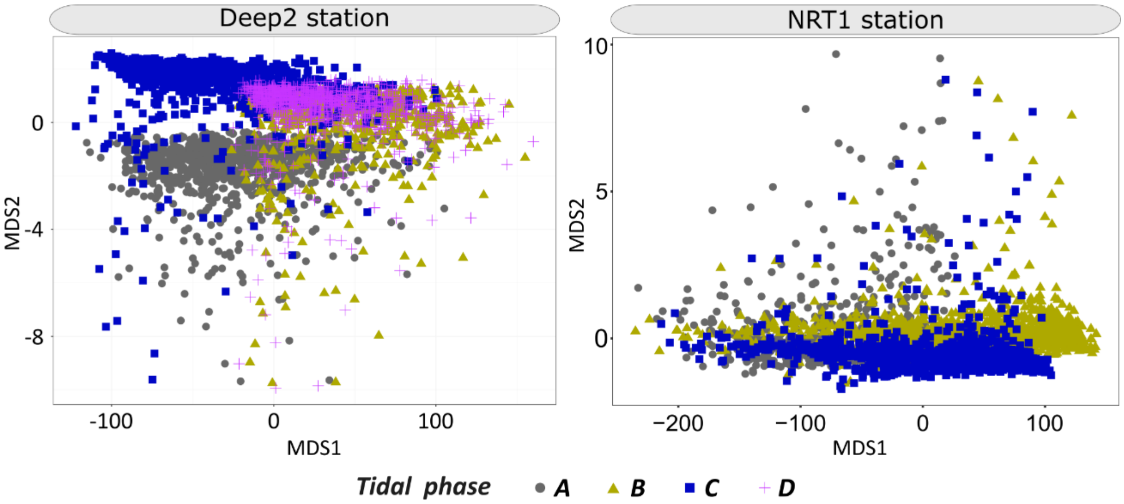


**Figure 8.** Plankton food-web analysis at the Deep 1 and NRT1 stations in the North Sea across the diel tidal cycle (max tide: phases A-C, low tide: phases B-D). Results from a nMDS analysis conducted on ecological network indicators estimated at each tidal phase using 999 replicated models per phase – each data point is a model, whose position in the plot is determined by values of ecological-network indicators.

The shift of the food-web structure and functioning, could be highlighted in the network structure of the plankton food web (Figure 9), phases *Deep2 A* and *Deep2 C* showed two sub-networks, or modules, one smaller and driven by detritus (FG De) and a second one including all other trophic interactions; phases *Deep2 B* and *Deep2 D* showed a third module involving trophic interactions being driven by mixotrophic organisms (FGs 6, 8, and 11).

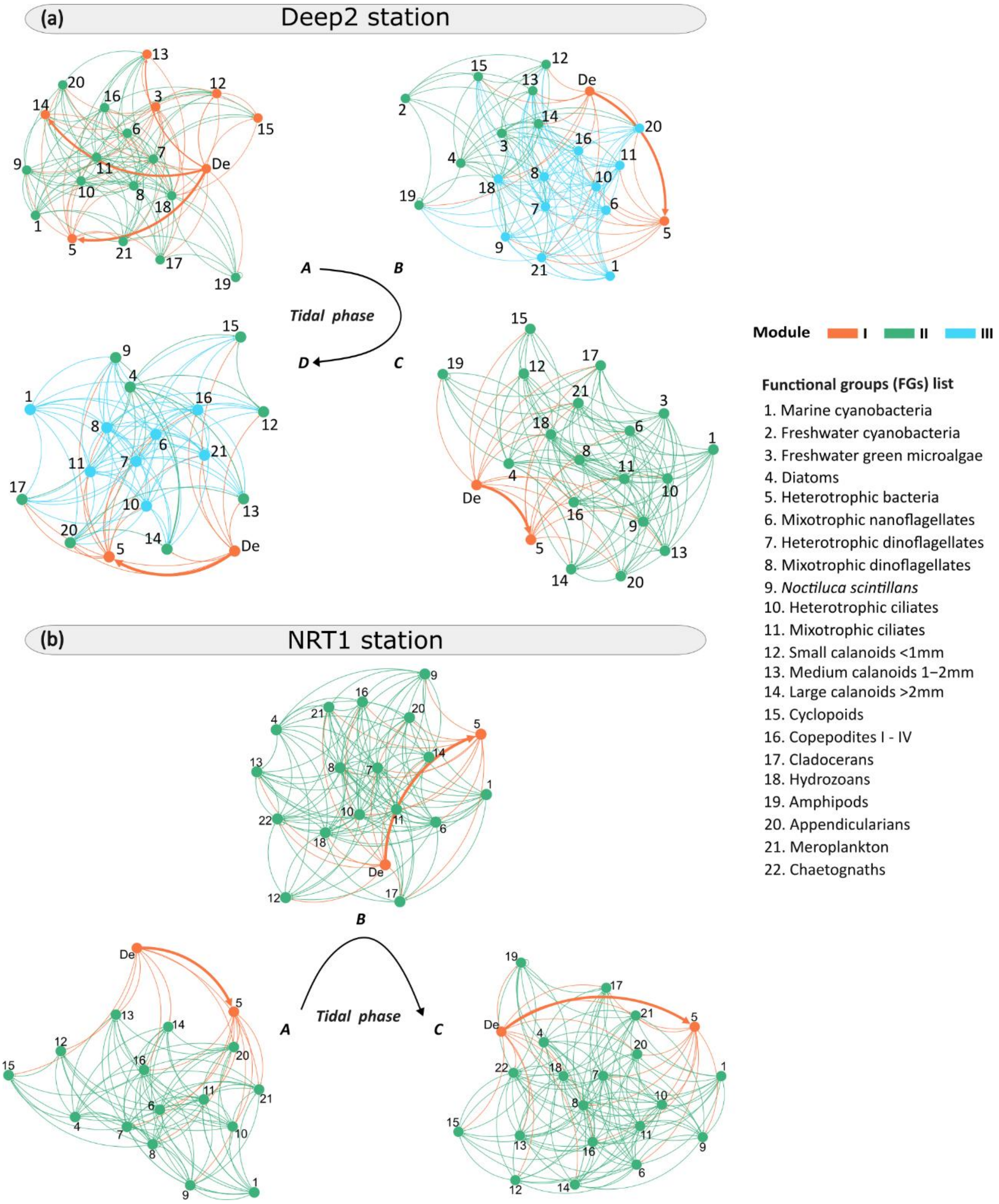


**Figure 9.** Plankton food-web changes at the (**a**) Deep2 and (**b**) NRT1 stations in the North Sea across the diel tidal cycle (max tide: phases A-C, low tide: phases B-D). Structural changes in the plankton food web at different tidal phases; network nodes are FGs, network edges are carbon-biomass flows. Different colors indicate distinct groups of FGs constituting three sub-networks, or modules (I-III). De indicate “Detritus” group.

## 5.3 Discussion

A detailed representation of the lower food web, i.e., planktonic organisms, supported by biomass data (Hassoun et al., 2024, McQuatters-Gollop et al., 2024), revealed that planktonic food webs vary during the tidal cycle, resulting in a plastic and somewhat ‘elastic’ shifting of the planktonic food web between two different network structures, operating on hourly time scales. This finding is extremely relevant, as similar observations have so far been limited to weekly time scales (D'Alelio et al., 2016), and our new results provide much more detail than previous findings on the North Sea pelagic system (Llope et al., 2012; Pecuchet et al., 2018). The observed model did not apply to the coastal station, where limited differences were found in the structure of plankton food webs under different tidal conditions (Figure 8).

Using this approach based on ecological network models and indicators sensitive to changing trophic states (Fath et al., 2019), our results highlight the importance of the synergy between biodiversity and the marine food web as a monitoring tool for assessing ocean health (Rombouts et al., 2013; Hassoun et al., 2024, McQuatters-Gollop et al., 2024). Our results support the hypothesis that the marine food web can capture both the state of the ecosystem and the processes underlying its dynamics (Loschi et al., 2023) by integrating biodiversity, structure, and functioning at the ecological-community level (Thompson et al., 2012). Indeed, we observed that the plankton food-web has altered the magnitude and directionality of its biomass fluxes, leading to changes in network modularity, as previously observed under environmental shift (D'Alelio et al., 2019). Modularity is a characteristic of the food web that changes due to human impact and climate change (Bartley et al., 2019) and modifies marine food web functioning (e.g., Eskuche-Keith et al., 2023), stability (e.g., Grilli et al., 2016), and persistence in polluted environments (e.g., Garay-Narváez et al., 2013).

The modularity alterations in the plankton food web analyzed in this study originated mainly from the modulation of trophic pathways induced by mixotrophic protists, unicellular plankton taxa capable of switching from autotrophy to heterotrophy depending on resource availability (Millette et al., 2023). Although a growing number of studies reveal that so-called “mixoplankton” can radically influence ecosystem functioning, several monitoring programs for assessing marine environmental status, e.g., the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC), still do not consider these organisms as indicators (Anschütz et al., 2024). For example, Descriptor 4 “Food webs” of the MSFD only identifies phytoplankton, i.e., strictly autotrophic unicellular metazoans, and plankton, which adds further value to our study.

# 6. EVALUATING COASTAL PLANKTONIC NATURAL CAPITAL THROUGH EMERGY ANALYSIS: CONCEPTS, METRICS, AND APPLICATIONS. (Submitted paper)

## Abstract

Understanding the biophysical foundations of coastal systems is essential for sustainable coastal management and rests on the principle of preserving natural capital in at least an intact state. This study applied emergy analysis to assess the natural capital (NC) and environmental support flows (ENFL) associated with planktonic communities within the three main gulfs along the coast of the Campania Region (NW Mediterranean, Italy) during two seasons (autumn 2020 and summer 2021). Emergy allows all the resources supporting a system to be expressed in a single unit: the solar emergy joule (sej). For the first time, the entire planktonic community was comprehensively evaluated as a key contributor to ecological functioning. Seasonal differences revealed distinct patterns in system organization and resource dependency. Higher NC stocks ($4.67 \times 10^{10}$ sej $m^{-2}$) and a more complex food web structure were observed in summer 2021, with increased self-organization and decreased reliance on external input. In contrast, autumn 2020 exhibited a greater demand for environmental support (ENFL: $7.61 \times 10^{10}$ sej $m^{-2}$ $day^{-1}$), driven by increased rainfall and geothermal heat, which emerged as key drivers of ecosystem dynamics. Solar emergy joules were also translated into monetary equivalents, facilitating interpretation in socio-economic contexts without altering the ecological meaning of the analysis. The results highlight the potential of emergy analysis as a decision-support tool for policymakers, providing critical insights into the function of ecosystems, improving the allocation of conservation efforts, and strengthening the integration of natural capital into regional and national policy frameworks.

## 6.1 Introduction

Scientific research on natural capital assessment has grown significantly in recent years, driven by several international initiatives, such as the Millennium Ecosystem Assessment (MA, www.millenniumassessment.org) and The Economics of Ecosystems and Biodiversity (TEEB, www.teebweb.org). From an anthropocentric perspective, the general concept of capital is that it must be productive, capable of producing goods and services useful to people (Dickie et al. 2014). Hence, the term value is related to the human use of resources. From a more ecological perspective, natural capital can be defined as the stock of natural resources that generate energy and matter flows and ecosystem services. In this context, the "ecological value" of natural resources is not necessarily related to their use by humans, but to the role they play in the functioning of the biosphere at different scales and in sustaining different species (Odum 1996). To explain the role of matter and energy flows in maintaining natural ecosystems, Odum (1996) introduced emergy analysis, an environmental accounting method designed to assess environmental performance and sustainability on a global scale of the biosphere (Odum 1988, 1996).

Emergy accounting methods have been widely employed to investigate terrestrial and marine ecosystems (Pulselli et al. 2011; Franzese et al. 2020). Recent research has assessed the biophysical value of natural capital and ecosystem services in the marine ecosystem using the emergy accounting method. Vassallo et al. (2017) developed a biophysical and trophodynamic model based on emergy accounting to assess the value of natural capital in marine protected areas (MPAs). The model described in Vassallo et al. (2017) has been applied in several studies (Franzese et al. 2017; Picone et al. 2017; Paoli et al. 2018; De La Fuente et al. 2019; Rigo et al. 2021, 2024; Vassallo et al. 2022; Bordoni et al. 2023) to assess the biophysical value of natural capital in selected Mediterranean MPAs. Several authors have used emergy accounting to assess natural capital and ecosystem services; for instance, Berrios et al. (2017, 2018) used it to assess the ecosystem health of three benthic marine networks in northern Chile, while also exploring their contributions to the welfare of the regional economy. Yang et al. (2019) implemented an emergy-based assessment for coastal and marine ecosystem services in China. Buonocore et al. (2020) used the emergy method to assess the value of marine natural capital stock in southern Italy. Most of the work using emergy analysis has been applied to explore benthic marine ecosystems, while few studies have focused on planktonic communities (e.g., Mattei et al. 2021; Vassallo et al. 2022).

Planktonic organisms are taxonomically and physiologically diverse and include unicellular and multicellular organisms, autotrophs, heterotrophs, mixotrophs (de Vargas et al. 2015; Chakraborty et al. 2020), and a myriad of feeding strategies (Benedetti et al. 2016). Hence, plankton plays a key role in several ecological and economic processes. From an ecological point of view, plankton forms the basis of food webs and plays important roles at many levels of the marine food web, regulating the biogeochemical cycles of carbon and other nutrients in the aquatic environment (Litchman et al. 2015; Archibald et al. 2019; Richon and Tagliabue 2021), and contributing to the transfer of matter and energy to higher trophic levels (e.g., fish) (Worden et al. 2015; Lomartire et al. 2021). Planktonic organisms are also important from an economic point of view, as they are essential for sustaining fisheries and other marine life by altering the concentration of predator populations (to be consumed), resulting in an effect on fish biomass (Vanni et al. 2002).

In this work, the Natural Capital (NC), the stock of natural resources (Natural Capital Committee, 2017), and environmental flows (ENFL), those flows essential to the annual maintenance of natural capital itself (Paoli et al. 2018) associated to the plankton community was compared considering two seasons (autumn 2020 and summer 2021) and three gulfs (Gaeta, Naples and Salerno) along the coasts of the Campania Region (North-Western Mediterranean Sea, Italy). The development and application of this approach for the biophysical assessment of planktonic diversity, realized by combining results derived from food web modelling and environmental accounting models based on emergy analysis, represents a novel contribution. This work offers a point of reflection for the development of environmental management tools and practices, considering the ecological, social, and economic spheres.

## 6.2 Materials and methods

### *6.2.1 Sampling area, laboratory, and data analysis*

Biological and environmental data were collected through two marine surveys conducted in the Tyrrhenian Sea along the coast of the Campania Region (central Tyrrhenian Sea, NW-Mediterranean Sea, Italy), on board *'R/V Vettoria'* during autumn 2020 (from 9 September to 9 October) and summer 2021 (from 29 June to 15 July). Sampling was conducted at a total of 22 stations across three gulfs: the Gulf of Gaeta (n= 9), the Gulf of Naples (n=7), and the Gulf of Salerno (n=6) (Figure 10). The Gulf of Gaeta (41°06′N, 13°30′E) is a highly urbanized area and hosts aquaculture activities, including fish and shellfish farming (Triassi et al. 2019). The coastal environment is strongly influenced by the input of the

Garigliano and Volturno rivers, which have a major impact on the entire gulf (Triassi et al. 2019). The Gulf of Naples (40°44′N, 14°16′E) is affected both by the oligotrophic waters of the Tyrrhenian Sea and by substantial continental runoff, mainly from the Sarno river (Tornero and Ribera d'Alcalà 2014). This river delivers nutrients and chemical pollutants originating from intensive agricultural practices and tanning industries (Saviano et al. 2023). The Gulf of Salerno (40°31′N, 14°42′E) show more pronounced oligotrophic conditions compared to the other two gulfs, as it is largely dominated by offshore Tyrrhenian waters (Ragosta et al. 1995). However, the Sele River contributes to pollutant transport, affecting the ecological status of the area (De Rosa et al. 2022). Furthermore, the three Campania gulfs are occasionally subject to upwelling events, driven by strong winds (Ciannelli et al. 2022) and the presence of submarine canyons (i.e., Cuma and Dohrn canyons) that facilitate the rise of nutrient-rich deep waters to the surface (de Ruggiero et al. 2018).

Sampling and laboratory methods used to estimate plankton abundance are described in Bosso et al. (2025). In this work, 34 planktonic functional groups (FGs) (i.e., groups showing similar ecological functions and trophic interaction) were investigated: 6 autotrophs groups, 4 heterotrophs, 7 mixotrophs, and 17 metazoans groups (see Chapter 4).

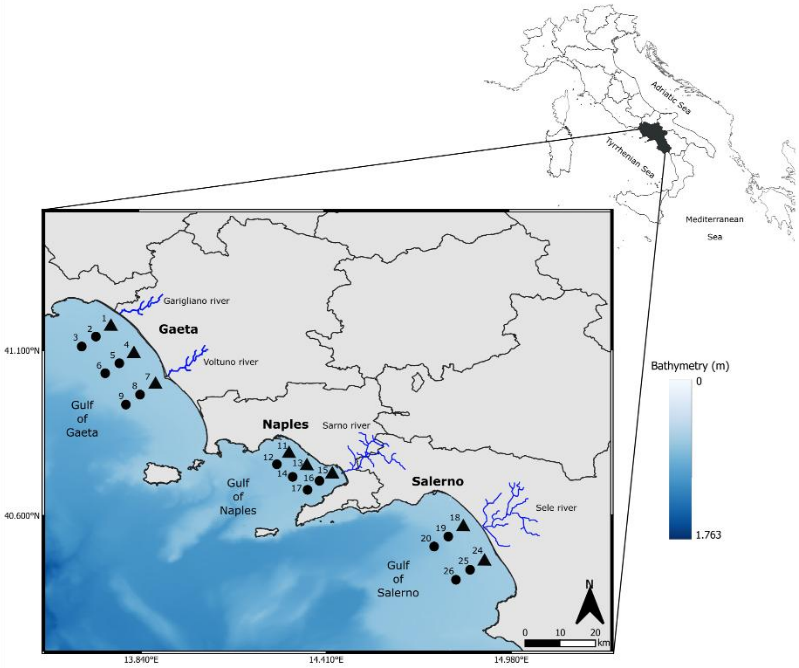


**Figure 10**. Study area and sampling stations. Triangular shape represents inner shelf stations (<20 m depth), and circular shape represent mid shelf stations (50 – 100 m depth)

To calculate Natural Capital (NC) and Environmental Flows (ENFL) several input data are required: (i) biomass data in carbon units (μgC $m^{-2}$), (ii) productivity (P), (iii) weighted trophic levels (TLs), (iv) phototrophs/heterotrophs ratio (ph/het) and (v) consumption matrices. All inputs used for the analyses in this work were obtained from the results of planktonic trophic network models (see Chapter 4). Analyses and graphical representations were performed using the software RStudio v.4.3.2, with the packages '*ggplot*' and '*tidyverse*' (RStudio Team 2020).

### *6.2.2 Biophysical and monetary assessment of natural capital and environmental flows*

#### *6.2.2.1 Emergy analysis*

The emergy analysis is a methodology developed by Odum (1983, 1996), based on a thermodynamic approach. Odum (1996) defines emergy as the total equivalent solar energy used, both directly and indirectly, to produce goods and services and then its unit of measurement is the solar emergy Joule (sej) (Odum 1996). The greater the energy stored for the generation of a product, the greater its value (Odum 1988, 1996), as the greater the cost incurred by nature to maintain them in terms of resources employed to obtain it. For emergy analysis, the first step is drawing a system diagram, using a standardized emergy system language proposed by Odum (1996) to organize the relationships between the system's compartments. Diagram allows us to list in detail all input resources that support, directly or indirectly, the analyzed process or a product and that converge through a transformation link of energy and materials, both in time and in space (Ulgiati and Brown 2009). All these inputs powering the system are then converted from traditional units of measure (U.M.) into solar emergy Joules using conversion factors defined as Unit Emergy Values (UEVs). The latter defines the equivalent solar energy required to obtain a single unit of product (sej/U.M. e.g. sej $J^{-1}$ or sej $g^{-1}$). The greater the number of transformations required to obtain a product, the greater the UEV as well as the energy quality of the product (Brown and Ulgiati 1997). Furthermore, biophysical emergy results can be converted into a monetary equivalent using a specific conversion factor: emergy to money ratio (EMR). The EMR represents the ratio between a nation's emergy and its Gross Domestic Product (GDP) (Odum 1996). The monetary value of a product is then given by the ratio between its total emergy and EMR. This conversion translates the values of the biophysical biosphere's resources investment into equivalents of a specific currency. Expressing the physical value of natural capital in monetary terms does not change the biophysical foundation of the methods. Still, it helps convey its importance to local managers and policy-makers responsible for decision-making, and it supports more effective communication in socio-economic contexts (Turcato et al. 2015; Vassallo et al. 2022).

The protocol for emergy analysis has been partially adapted from the protocol originally developed for the assessment of NC and EFNL in MPAs described by Vassallo et al. (2017) and Paoli et al. (2018), so that it could be applied to the planktonic community, consisting non only of autotrophic and heterotrophic organisms but also of mixotrophic organisms. NC and EFNL are evaluated adopting a donor-side approach which considers the environmental costs (as solar emergy Joules) required to generate the stocks of biomass living in a specific habitat (NC) and maintain the system's operativity (ENFL). This methodology includes: trophodynamic analysis, biophysical accounting and monetary conversion.

*6.2.2.2 Trophodynamic analysis*

Biomass stocks (μgC) of planktonic organisms were converted to the primary biomass (Bp) required for its generation using the following equation, considering a transfer efficiency of 10% between trophic levels (Pauly and Christensen 1995):

$$B_p = \sum B_i \cdot 10^{(TL_i - 1)} \text{i= 1,2,3…n} \qquad [2]$$

where $B_i$ is the biomass and $TL_i$ represents the trophic level of the *i-th* group, obtained from the trophic network simulation (see Chapter 4).

Then, the time required to create the primary biomass used to generate and store biomass stocks within the system was calculated (stock formation time) as the ratio of Bp to an average primary productivity (PP) rate typical of the investigated marine environment (Romillac et al. 2023).

Further, environmental flows (ENFL) that support the maintenance of natural capital were assessed. To determine the primary production (Pa), the production values (P) of the autotrophic groups obtained from the trophic network simulations (see Chapter 4) were added together, and for the mixo-planktonic groups the production values were obtained by multiplying the P values by the proportion of phototrophy in the individual metabolism:

$$P_a = (\sum P) \qquad [3]$$

Similarly, the daily consumption for heterotrophs groups ($Pe_i$) was calculated using the consumption matrix derived from the trophic network model analyses (see Chapter 4) at the sampling sites, converting it into the required primary biomass according to the following equation derived from Eq. [2]:

$$P_{e_i} = C_i \cdot 10^{(TL_i-2)} \quad \text{i= 1,2,3…n} \qquad [4]$$

where $Pe_i$ is the primary production supporting the consumption of the *i-th* heterotrophic taxonomic group, $C_i$ is the consumption and $TL_i$ is the trophic level of the *i-th* taxonomic group.

The total primary production ($Pe_{tot}$) supporting consumption in each habitat was calculated as follows:

$$P_{e_{tot}} = (\sum P_{ei}) \qquad \text{i= 1,2,3…n} \qquad [5]$$

The sum of the internal primary production (Pa) and the primary production supporting the consumption (Pe) equals the total productivity required to maintain the biomass stock (supporting production – $_{supp}P$). The ratio between internal production (Pa) and required production (Pe) defines whether the system is self-sufficient (Vassallo et al. 2017) and, in turn, provides information about the supporting area required to maintain the system. Two different scenarios are possible: (i) If Pa ≥ Pe, the system can support its internal consumption and export the surplus of primary production to external habitats. The system's support area is therefore less than or equal to its physical surface area; (ii) If Pa < Pe, the system cannot sustain its internal consumption and needs additional primary production, imported from outside the system, to sustain itself. In this case, the supporting area of the system is considered greater than its physical area.

### *6.2.2.3 Biophysical accounting and monetary conversion*

All natural resources, used for the assessment of the NC and ENFL, which are essential for the maintenance of processes within the habitats, were estimated using the calculations described in the previous paragraphs.

In particular, the following were estimated:

- The nutrient content of the organic matter (i.e. carbon, nitrogen and phosphorus): carbon is obtained from the total primary biomass (Bp) for the calculation of NC and from the supporting production ($_{supp}P$) for the calculation of ENFL, while nitrogen and phosphorus were evaluated by applying the ratio of Redfield et al. (1963), with respect to carbon content.
- The natural resources that support biomass production (i.e., solar radiation, rain, wind, geothermal heat, currents, tides, and runoff) are expressed in energy terms (Joules - J). These were calculated using specific formulas shown in Table 9. Time scaling factors were applied, using the Bp/PP ratio for the NC calculation, while the Pe/Pa ratio was used for ENFLs.

**Table 9.** Natural resources for the calculation of NC and ENFL (in the square brackets, the reference values for calculating ENFL).

| ***Natural resources*** | ***Formulas*** | ***Unit*** | ***Ref.*** |
|---|---|---|---|
| Carbon | Bp or [$_{supp}P$] | μg | This work |
| Nitrogen | 7/41 · Bp or [$_{supp}P$] | μg | This work |
| Phosphorus | 1/41 · Bp or [$_{supp}P$] | μg | This work |
| Solar radiation | Solar radiation per unit area · (1-albedo) · (Bp/PP) or [Pe/Pa] | J | Odum 1996 |
| Rain | Daily rainfall · water density · Gibbs free energy · (Bp/PP) or [Pe/Pa] | J | Odum 1996 |
| Wind | (Geostrophic coeff. · wind velocity)$^3$ · air density · drag coeff. · second per days · (Bp/PP) or [Pe/Pa] | J | Campbell et al. 2005 |
| Geothermal heat | Geothermal flux · (Bp/PP) or [Pe/Pa] | J | Odum et al. 2000 |
| Currents | Evaporation water height · sea water density · 0.5 · (current velocity)$^2$ · (Bp/PP) or [Pe/Pa] | J | Brown and Bardi 2001 |

| | | | |
|---|---|---|---|
| Tide | (Number of tides per year/2) · (height tides)$^2$ · gravity · seawater density · (Bp/PP) or [Pe/Pa] | J | Odum 1996 |
| Runoff | (River flow · Water density · Gibbs free energy)/Area extension | J | Brown and Bardi 2001 |

All meteo-oceanographic input data, used to calculate natural resources (see equation in Table 9), were  obtained from several regional sources, including different monitoring stations, instruments, or reference datasets, and refer to daily values corresponding to the days on which the sampling was carried out in different locations of the study area; subsequently the natural resources data was averaged for the three gulfs investigated in this work. Furthermore, in this work for both the NC and ENFL calculations, the following were calculated daily (day$^{-1}$). Once the resources needed to maintain the system were obtained, they were converted into emergy units (sej) using specific UEVs (Table 10), for carbon, nitrogen, and phosphorus the UEVs were converted into µg.

**Table 10.** Values of the Unit Emergy Values (UEVs) used in this work

| ***Natural resources*** | ***UEVs (sej/unit)*** | ***Ref.*** |
|---|---|---|
| Carbon | 1.02 x$10^2$ | Odum 1996 |
| Nitrogen | 7.40 x$10^3$ | Odum 1996 |
| Phosphorus | 2.86 x$10^4$ | Odum 1996 |
| Solar radiation | 1.00 | Odum 1996 |
| Rain | 2.93 x$10^4$ | Odum 1996 |
| Wind | 2.41 x$10^3$ | Odum 1996 |
| Geothermal heat | 5.53 x$10^4$ | Odum 1996 |
| Currents | 1.77 x$10^7$ | Brown and Bardi 2001 |
| Tide | 2.71 x$10^4$ | Odum 1996 |
| Runoff | 6.61 x$10^4$ | Odum 1996 |

Lastly, the NC and ENFL values were obtained from the following sum:

$NC_{tot}$ *or* $ENFL_{tot}$ = *max* (C, N, P) + *max* (solar radi., wind, rain, currents) + tide + runoff + geothermal heat [6]

The formula [6] considers the *max* value between carbon, nitrogen, and phosphorus and between solar radiation, rain, and wind (Odum 1996) because they are co-products of the same processes occurring in the biosphere. Lastly, the NC and ENFL biophysical values were converted into a monetary equivalent value, expressed as emergy euro (em€), as previously described in Par 6.2.2.1. The EMR used in this work is 2.81 x$10^{12}$ sej €$^{-1}$ (derived from https://www.emergy-nead.com/country/data).

## 6.3 Results

### *6.3.1 Emergy analysis*

Emergy analysis gives, as the first output, the emergy diagram as shown in Figure 11. This diagram shows the natural resources associated with the system needed to (i) sustain the planktonic community and (ii) to calculate the value of natural capital (NC) and environmental fluxes (ENFL). In this case, mixoplankton communities, being organisms capable of both autotrophy and heterotrophy, were grouped within a rectangular box.

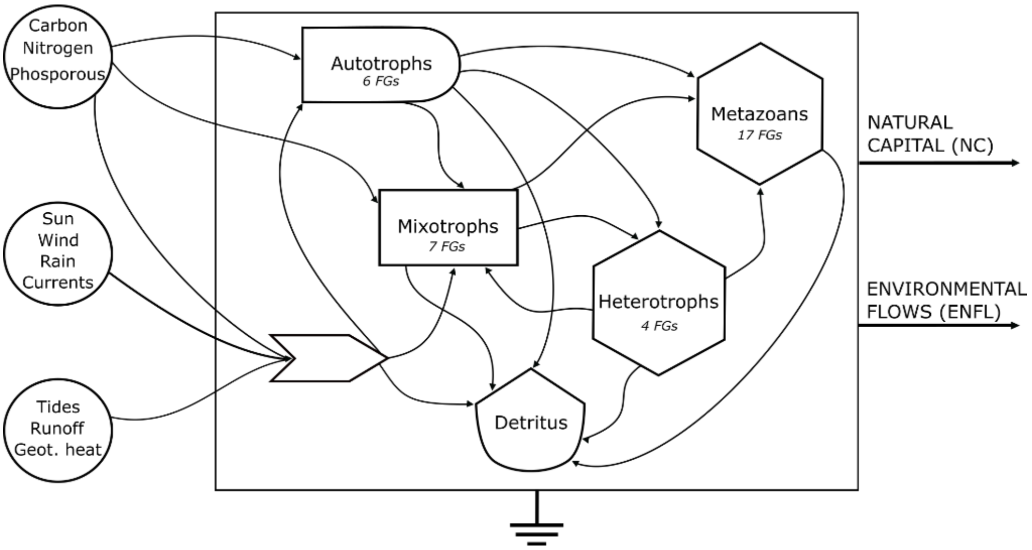


**Figure 11**. Emergy diagram of the Campania coastal zone. In *italics,* the number of functional groups (FGs) representing each trophic group. According to Odum (1996) the system boundaries are represented by the main box; compartments are represented within the main box where producers are indicated as bullets, consumers as hexagons,

while compartments with mixed behavior are represented as rectangular boxes; inputs are depicted as circles arranged around the main box, outputs are represented on the right and finally energy loose on the bottom of the main box.

### *6.3.2 Trophodynamic analysis*

Analyzing the total biomass of the FGs (see Chapter 4), grouped into the main trophic groups (autotrophs, heterotrophs, mixotrophs and metazoans groups), it was observed that the summer 2021 showed a higher biomass than the autumn 2020 (4.46 x$10^7$ µgC $m^{-2}$ and 2.34 x$10^7$ µgC $m^{-2}$, respectively) (Figure 12). The Gulf of Gaeta showed a higher biomass in both autumn 2020 and summer 2021 (1.05 x$10^7$ µgC $m^{-2}$ and 1.90 x$10^7$ µgC $m^{-2}$, respectively). During autumn 2020, the biomass was mainly influenced by a higher presence of metazoans than in summer 2021 (1.19 x$10^7$ µgC $m^{-2}$ and 5.40 x$10^6$ µgC $m^{-2}$, respectively). In contrast, in summer 2021, the biomass was mainly dominated by autotrophic, heterotrophic and mixotrophic groups (averaging 3.83 x$10^6$ µgC $m^{-2}$ in autumn 2020 and 1.29 x$10^7$ µgC $m^{-2}$ in summer 2021). The greatest contribution was made by the mixoplankton component, which showed a biomass of 1.57 x$10^7$ µgC $m^{-2}$ in summer 2021 compared to 4.85 x$10^6$ µgC $m^{-2}$ in autumn 2020. Furthermore, during the summer 2021, a slightly higher average trophic level (2.06) was found than in autumn 2020 (1.77) (Figure 12).

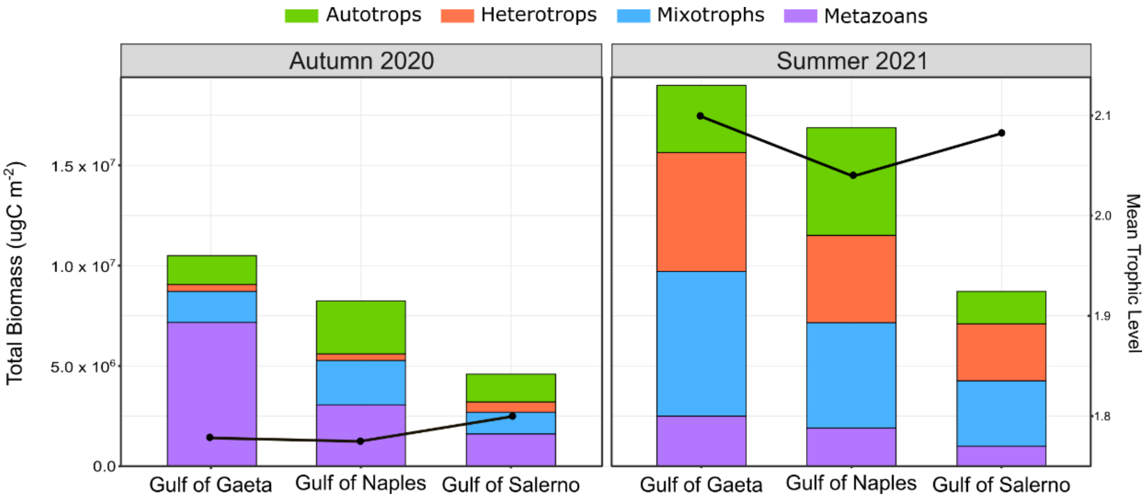


**Figure 12**. Bar plots show the total biomass (µgC $m^{-2}$) in the three gulfs during the autumn 2020 and summer 2021 (left y-axis). The colors represent the planktonic trophic groups (illustrated in the legend). The black solid lines refer to the mean trophic levels in the three gulfs (right y-axis).

### 6.3.3 *Biophysical and monetary assessment of natural capital and environmental flows*

The average contributions of various natural resources to the production of NC across the three examined gulfs reveal that nutrients represent the predominant component in both seasons. Among nutrients co-products (see formula [6]), nitrogen is always the most influential element ($1.08 \times 10^5 \pm 2.48 \times 10^4$ in autumn 2020 and $1.73 \times 10^5 \pm 4.45 \times 10^4$ in summer 2021) (Figure 13a), contributing 59% to NC formation during autumn 2020 and 78% during the summer 2021 (Figure 13b). Furthermore, in the autumn 2020, rain and runoff contributed to the NC across the three gulfs with an average of 18% and 13% respectively (with rain always representing the largest element among the co-products, $5.98 \times 10^4 \pm 1.26 \times 10^4$). The remaining resources contributed less than 10% (Figure 13b). Conversely, during the summer of 2021, geothermal flows played a relatively more significant role, with an average contribution of 11%, whereas the impact of the other resources remained marginal (<5%).

Regarding ENFL, rain and geothermal heat represent the most significant contribution for the maintenance of NC during the autumn 2020 (26% and 56%, respectively on average), with the rain being the most representative of the co-products (see formula [6], $4.69 \times 10^5 \pm 4.06 \times 10^5$) (Figure 13a). Rain has the greatest impact in the Gulf of Gaeta and Salerno (~61%), compared to Naples (46%) (Figure 13b). Geothermal heat shows a main contribution in the Gulfs of Gaeta and Salerno (52% and 57%, respectively) compared to Naples (33%). In the summer of 2021, geothermal heat and nitrogen emerged as the main contributors to ENFL (47% and 40% on average). Geothermal heat accounted for ~54% in Gaeta and Salerno and ~33% in Naples. Nitrogen also contributes significantly, with a share equal to ~32% in Gaeta and Salerno, and 56% in Naples (Figure 13b).

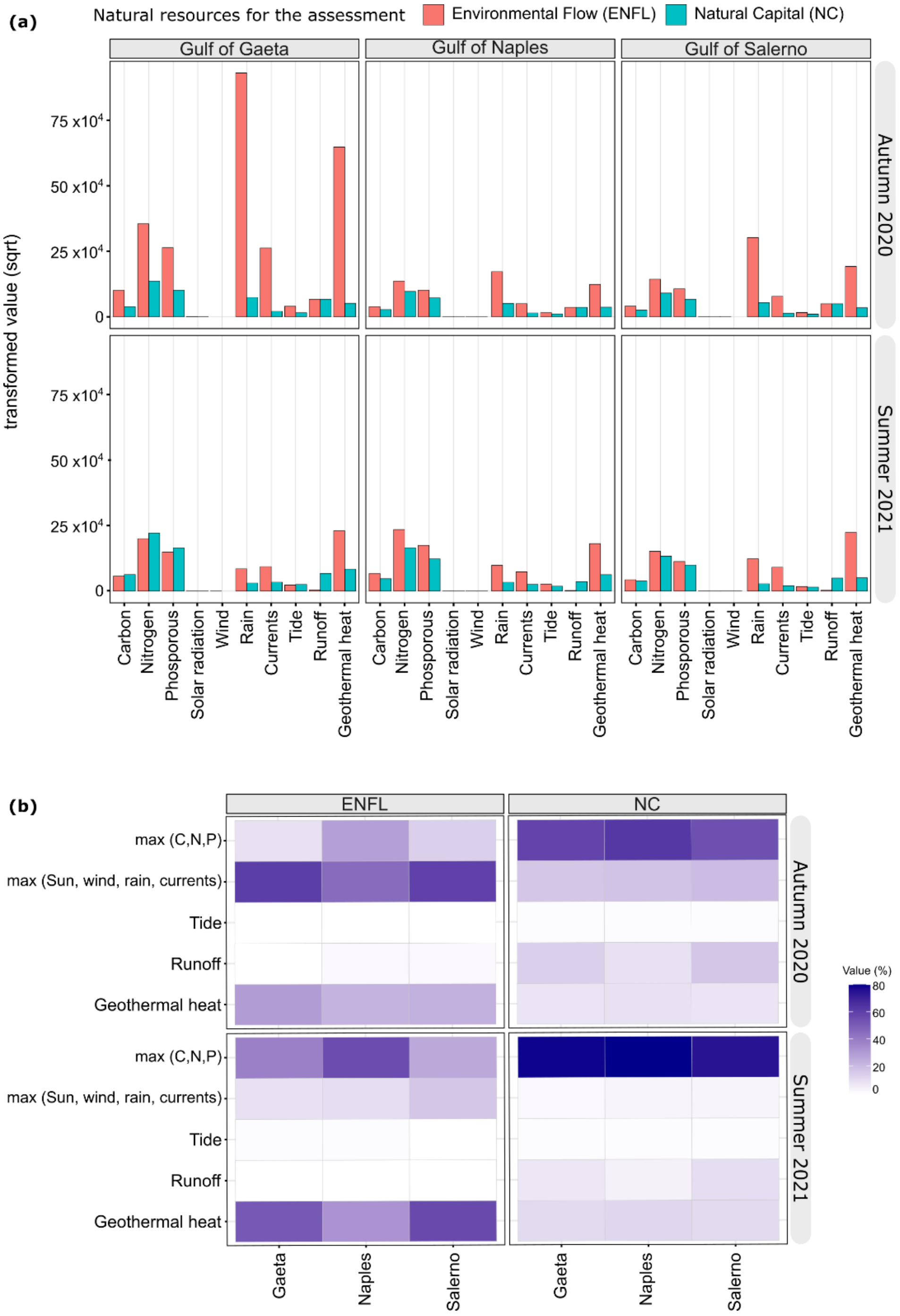


**Figure 13**. (**a**) Bar plots show all natural resources (square root transformed values), used for the assessment of the Natural Capital (NC, in blue) and Environmental Flow (ENFL, in red) during the autumn of 2020 and summer of 2021 in the three gulfs of the Campania Region (Gaeta, Naples, and Salerno). Natural resources on the x-axis are inserted following equation [6]. (**b**) Heatmaps show the percentage contribution of considered inputs to the total value of Natural Capital (NC) e Environmental flow (ENFL).

Furthermore, for the entire study area, summer 2021 shows higher NC average values (6.26 x10$^{10}$ sej m$^{-2}$ ± 4.13 x10$^{10}$) than autumn 2020 (2.39 x10$^{10}$ sej m$^{-2}$ ± 2 x10$^{10}$), indeed the ENFL average values are higher in autumn 2020 (3.88 x10$^{10}$

sej m$^{-2}$ day$^{-1}$ ± 7.61 x10$^{-3}$) than in summer 2021 (1.04 x10$^{10}$ sej m$^{-2}$ day$^{-1}$ ± 7.82 x10$^{9}$) (Figure 14a,b). Observing the difference between the three gulfs across the seasons, during the autumn 2020, the Gulf of Gaeta shows higher NC and ENFL median values (1.89 x10$^{10}$ sej m$^{-2}$ and 3.90 x10$^{10}$ sej m$^{-2}$ day$^{-1}$, respectively), compared to Naples (1.51 x10$^{10}$ sej m$^{-2}$ and 2.99 x10$^{9}$ sej m$^{-2}$ day$^{-1}$, respectively) and Salerno (1.35 x10$^{10}$ sej m$^{-2}$ and 8.29 x10$^{9}$ sej m$^{-2}$ day$^{-1}$, respectively). In the summer of 2021, the Gulf of Gaeta showed a higher NC median value (8.19 x10$^{10}$ sej m$^{-2}$) than Naples (3.53 x10$^{10}$ sej m$^{-2}$) and Salerno (3.36 x10$^{10}$ sej m$^{-2}$) (Figure 14a), but lower ENFL median values (6.06 x10$^{9}$ sej m$^{-2}$ day$^{-1}$), compared to Naples and Salerno (6.17 x10$^{9}$ sej m$^{-2}$ day$^{-1}$ and 9.74 x10$^{9}$ sej m$^{-2}$ day$^{-1}$, respectively) (Figure 14b).

Regarding the balance per unit area within each Gulf, a high ratio required (Pe) to generate (Pa) productivity was found in Gaeta during the autumn of 2020 (median value: 22.2), compared to Naples (1.44) and Salerno (4.68). In summer 2021, similar median values were found in the Gulf of Gaeta and Naples (3.44 and 3.04, respectively), while in Salerno the ratio increased (5.42) (Figure 14c).

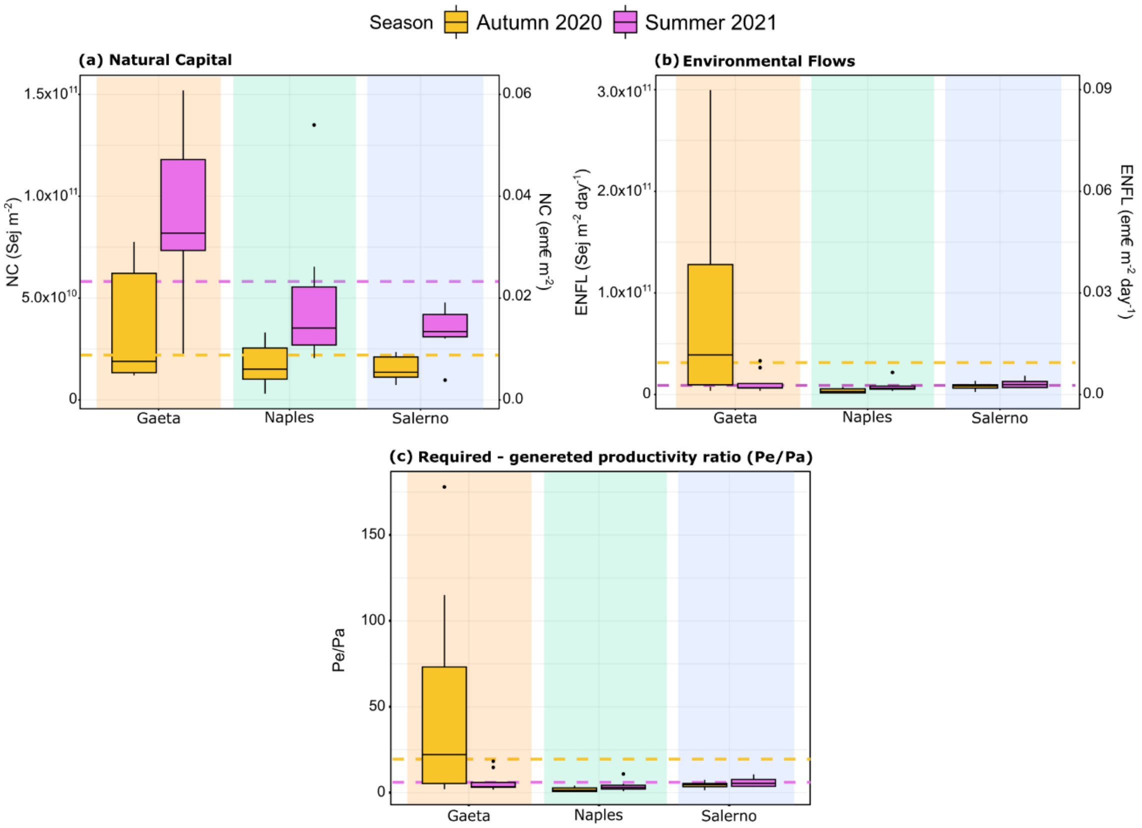


**Figure 14**. Box plots showing (**a**) NC (in sej m$^{-2}$ and em€ m$^{-2}$), (**b**) ENFL (in sej m$^{-2}$ day$^{-1}$ and em€ m$^{-2}$ day$^{-1}$) and (**c**) Pe/Pa values, during the autumn 2020 (dark yellow) and summer 2021 (dark magenta) seasons in the three gulfs of the Campania Region (Gaeta

in orange, Naples in light-green and Salerno in blue). The broken lines (dark yellow for autumn 2020 and dark magenta for summer 2021) indicate the average of the three gulfs.

## 6.4 Discussion

In this study, emergy analysis was applied to evaluate the biophysical effort required to generate and maintain stocks (ENFL) of natural capital (NC) along the coastal areas of the Campania Region (southern Italy) during autumn 2020 and summer 2021. For the first time, the analysis comprehensively included the entire planktonic community, which is known for its high responsiveness to environmental fluctuations (Russo et al. 2025b). As widely reported in the literature, plankton communities deliver crucial ecosystem services (Steinberg and Landry 2017; Russo et al. 2025a), such as nutrients and organic matter cycling and storage (Worden et al. 2015; Steinberg and Landry 2017) and serve as a fundamental food source for both small and large marine organisms (Lomartire et al. 2021). However, despite their central role in ecosystem functioning, plankton are often underrepresented in marine spatial planning and are typically only considered in broad functional groups, such as phytoplankton and zooplankton. The approach presented here introduces a novel application of emergy analysis that can provide valuable insights and support decision-makers in designing more effective and sustainable coastal management plans.

Among the main biotic drivers that contribute to generating NC, the nutrients (particularly nitrogen) were found to be most important in both seasons and each gulf considered. This result reinforces previous research highlighting the influence of nutrient inputs from upwelling events or riverine discharges in coastal areas (Corrales et al. 2015; Meddeb et al. 2018). In particular, the greater contribution of nutrients, the higher NC, demonstrating how they affect the entire system beyond just primary productivity. Emergy, which quantifies the solar energy required to build biomass at each trophic level (Jørgensen 1994), reflects this increase in ecological complexity, consistent with Odum’s hierarchical framework (Odum 1996). The main contribution of nutrients is observed during the summer of 2021, probably due to the water column stratification promoting nutrient retention in the surface layer (Staehr and Sand-Jensen 2006).

Seasonal differences were observed in the system's ability to store natural capital. During summer 2021, the system exhibited higher NC values, particularly in the Gulf of Gaeta, associated with greater planktonic biomass and higher mean trophic levels. This complexity appears to be driven in part by the increased biomass of hetero-mixotrophic planktonic groups. A key strength of this study is the explicit inclusion of mixotroph groups, those capable of both photoautotrophy and phagotrophy, as a distinct functional group (Flynn et al. 2019; Anschütz et al. 2024). These organisms are often overlooked in current monitoring programs and legislative frameworks (e.g., the Marine Strategy Framework Directive, MSFD), despite evidence of their substantial influence on trophic dynamics (Lehtinen et al. 2016; Anschütz et al. 2024), including enhancing food quality, facilitating nutrient transfer to higher trophic levels (Traboni et al. 2021; Balzer et al. 2023), and potentially generating adverse ecological and socioeconomic effects (Anschütz et al. 2024).

Concerning the maintenance of natural capital stocks (ENFL), an important contribution, in autumn 2020, is associated with rainfall. Indeed, intense precipitation events were recorded a few days before sampling, and rainfall events not only affected physical parameters such as temperature and salinity but also increased river discharge, delivering additional organic matter and nutrients to the coastal system (Gifuni et al. 2022; Kokoszka et al. 2023; Bosso et al. 2025). These inputs can have dual effects: stimulating primary production or, conversely, increasing turbidity and reducing light penetration, thereby inhibiting autotrophs and favoring detritivores (Lee et al. 2002; Bonomo et al. 2014).

In addition to nutrients and rainfall, geothermal flows emerged as significant natural drivers supporting the maintenance of natural capital stocks (ENFL), particularly in the Gulf of Gaeta and Salerno, during autumn 2020. Although often considered negligible in emergy analyses, they assume an important role in this study because of the area's pronounced volcanic activity, influenced by the presence of Vesuvius (in the Gulf of Naples) and the Campi Flegrei caldera (which influences the Gulf of Gaeta), which contribute to geothermal emissions in terrestrial and marine environments (Mangoni et al. 2016; Caliro et al. 2025). These geothermal flows can alter local hydrodynamics and nutrient transport (Scott et al. 2001; Mashayek et al. 2013), and may release trace metals (e.g., iron, manganese, zinc) and gases (e.g., hydrogen sulfide, methane), thereby modifying water chemistry. While iron enrichment can stimulate primary production, excessive mineral concentrations may lead to ecological imbalances, including harmful algal blooms (Lan et al. 2024).

During summer 2021, the planktonic system demonstrated a high degree of self-organization and self-sufficiency, as indicated by low Pe/Pa ratios, consistent with previous findings (Merquiol et al. 2023). This suggests efficient energy flows and a stable trophic configuration, driven by the flourishing primary production. In contrast, the autumn 2020 system showed a reduced level of self-sufficiency, with higher Pe/Pa values suggesting increased dependency on external inputs to sustain natural capital levels, in accordance with Vassallo et al. (2022). Although storage levels were maintained, especially in the Gaeta Gulf, this occurred at a higher biophysical cost, requiring continuous regeneration of natural capital supported by external and additional resource inputs. This condition reflects a less efficient and potentially less resilient configuration, with faster turnover and higher metabolic demand. In the Gulf of Gaeta, this dynamic was likely exacerbated by elevated turbidity resulting from increased river discharge (especially from the Volturno and Garigliano Rivers), which delivered organic matter and nutrients to the coastal zone (Bosso et al. 2025). The resulting trophic shift favored recycling-based food webs and an increase in gelatinous filter feeders (e.g., salps, chaetognaths) (as shown in Chapter 4), which play a key role in detritus remineralization and nutrient recirculation (Condon et al. 2010; Riofrío-Lazo et al. 2013).

These results are also in line with other studies (Buonocore et al. 2021; Rigo et al. 2024), conducted in different environmental conditions, which observed how the presence of external flows (e.g., nutrient inputs and geothermal sources) significantly affects emergy values. It was observed that in oligotrophic environments, the capacity for natural capital accumulation is generally lower but also more stable, with Pe/Pa closer to self-sufficiency. Conversely, contexts that are eutrophic or subject to strong anthropogenic and natural pressures (e.g., coastal urbanization, intense weather events) show accelerated ecological cycles and a greater need for external inputs to maintain functional balance. Similar patterns have been reported in benthic systems within Marine Protected Areas of Liguria (Vassallo et al. 2017; Paoli et al. 2018), where natural capital values were more temporally stable but generally lower due to slower regeneration rates and reduced trophic complexity compared to planktonic systems (Vassallo et al. 2022). Furthermore, it has been observed that the benthic system, similarly to the planktonic system, also tends to increase its metabolic effort in the presence of environmental pressures to cope with external disturbances, storing natural capital in response to more intense environmental fluxes (Vassallo et al. 2013, 2022).

Lastly, to integrate biophysical assessment with economic assessment, emergy values were converted into monetary units. This conversion is not intended to monetize nature, but rather to make the results more readable and comparable within decision-making contexts where economic language is predominant (Vassallo et al. 2017; Paoli et al. 2018). Both in summer 2021 and autumn 2020, the Gulf of Gaeta showed the highest total monetary values of natural capital stock and environmental flows. This indicates a significant capacity for the local ecosystem to generate ecological services and benefits that, when expressed in monetary terms, can be more easily communicated. However, it is important to highlight that this conversion to currency does not change the biophysical foundations of the results or compromise the integrity of the environmental accounting model used. Instead, it enhances its communicative and strategic reach, allowing for more effective interaction with economic stakeholders, public administrators, and policymakers, who often need concise and comparable indicators to guide their choices. In this context, monetary values may serve, for instance, to monitor changes in the system over time, by tracking variations in NC and ENFL in response to the modification of environmental conditions or the occurrence of extreme and adverse events. In this way, ecological information takes on a more central role in decision-making processes, contributing to more informed management (Vassallo et al. 2017; Paoli et al. 2018; Bordoni et al. 2023).

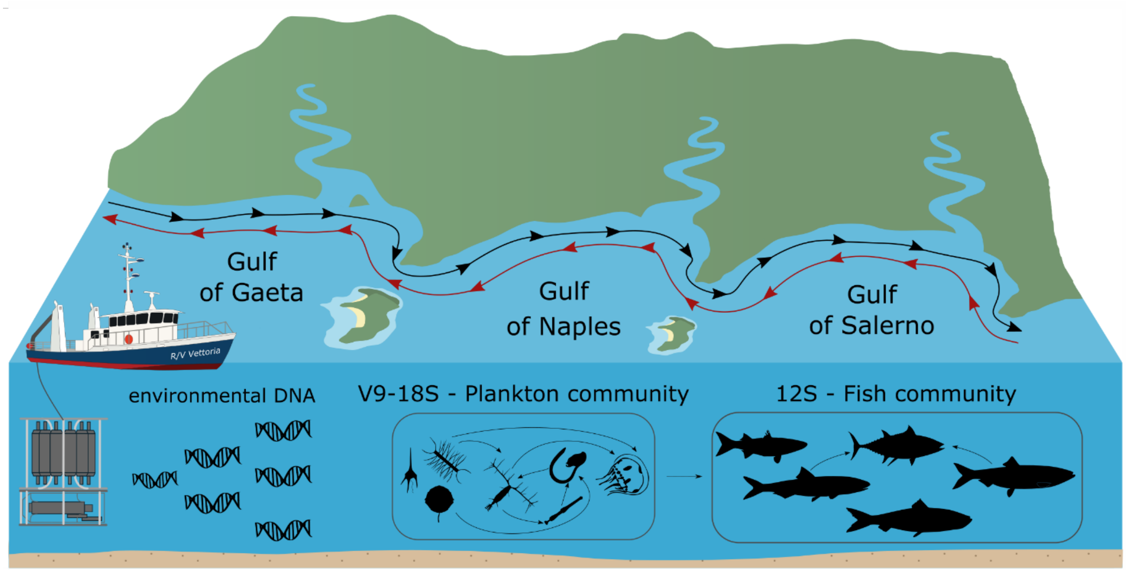
## 6.5 Conclusion

This study demonstrates that emergy analysis offers a robust framework for quantifying the biophysical effort underlying natural capital generation and maintenance in dynamic coastal systems. Moreover, in line with observations from other studies, the sensitivity of the emergy approach to local environmental conditions is evident, making it particularly useful for comparing ecological systems subjected to different natural and anthropogenic pressures. By including the entire planktonic community, the analysis captured trophic complexity often neglected in traditional assessments. Future applications could expand this approach to other coastal and marine ecosystems under varying anthropogenic pressures (e.g., eutrophication, climate change), enabling comparative evaluations of ecological efficiency and vulnerability. The integration of biophysical and monetary valuation also enhances the relevance of emergy-based metrics for environmental policy and marine spatial planning. This method can serve as a valuable tool for stakeholders and decision-makers in designing adaptive management strategies that align ecological sustainability with socio-economic priorities across diverse socio-ecological systems.

# 7. SPATIOTEMPORAL CHANGES OF PELAGIC FOOD WEBS INVESTIGATED BY ENVIRONMENTAL DNA METABARCODING AND CONNECTIVITY ANALYSIS (Paper III).

This study investigated the seasonal (autumn 2020 and summer 2021) and spatial (Gulf of Gaeta, Naples, and Salerno) variability of planktonic communities and small pelagic fish along the coast of the Campania region (Italy), by integrating environmental DNA metabarcoding (eDNA metaB) with Lagrangian particle connectivity modelling. This multidisciplinary approach enabled us to explore the role of physical connectivity in shaping the pelagic food web across different environmental gradients and in different seasons, offering new insights into how biotic interactions and hydrodynamic processes influence biodiversity patterns and pelagic food web organization.

This work provides a significant contribution by demonstrating how the combination of molecular techniques and oceanographic modelling can enhance our understanding of marine food web dynamics in environments characterized by high spatiotemporal variability. The results have important implications for ecological monitoring, sustainable fisheries management, and marine spatial planning, particularly in the Mediterranean Sea and other coastal ecosystems affected by anthropogenic pressures and climate change.



Graphical abstract of the work “Spatiotemporal changes of pelagic food webs investigated by environmental DNA metabarcoding and connectivity analysis”

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# Spatiotemporal changes of pelagic food webs investigated by environmental DNA metabarcoding and connectivity analysis

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Environmental DNA metabarcoding (eDNA metaB) is fundamental for monitoring marine biodiversity and its spread in coastal ecosystems. We applied eDNA metaB to seawater samples to investigate the spatiotemporal variability of plankton and small pelagic fish, comparing sites with different environmental conditions across a coast-to-offshore gradient at river mouths along the Campania coast (Italy) over 2 years (2020–2021). We found a marked seasonality in the planktonic community at the regional scale, likely owing to the hydrodynamic connection among sampling sites, which was derived from numerical simulations. Nonetheless, spatial variability among plankton communities was detected during summer. Overall, slight changes in plankton and fish composition resulted in the potential reorganization of the pelagic food web at the local scale. This work supports the utility of eDNA metaB in combination with hydrodynamic modelling to study marine biodiversity in the water column of coastal systems.

This article is part of the theme issue 'Connected interactions: enriching food web research by spatial and social interactions'.

## 1. Introduction

Unlike on land, most primary and secondary producers are organisms smaller than a few millimetres in the ocean water column [1,2]. These floating communities belong to the plankton and tightly drive aquatic food webs [1]. Plankton have huge functional diversity, with autotrophs, heterotrophs, mixotrophs, herbivores, carnivores and detritivores coexisting at the microscale (up to 100 m) [3,4]. Such diversity displays unexpectedly long trophic pathways in a few cubic metres of seawater and feeds important categories of fishes [5], like those playing as 'keystones' in marine food webs [6].

By definition, plankton drift with currents [7], and their communities show high spatiotemporal variability owing to the tight interplay between environmental factors and water transport [8,9]. On the one side, local physicochemical conditions select for plankton organisms with different physiology, biological cycles and feeding behaviours [9,10]; on the other side, ocean currents can reciprocally segregate or mix different water masses with distinct physicochemical properties which plankton can benefit from, or not [8,9,11]. This variability quickly scales up to fish communities [10,12], whose distribution in space and time is shaped by both evolutionary and ecological factors, from spawning timing and migratory abilities to salinity tolerance and food preferences [13–15].

Pelagic food webs, spanning from plankton to fish, can be, therefore, highly dynamic in space and time, especially at the regional scale (1–100 km) and close to the coast [16], where riverine inputs and intensified water flows can profoundly modify environmental conditions and the ecological state of the water column [17,18], with cascading effects on fish populations and pelagic food webs [12,19,20]. In this context, an important question is whether the variability of plankton–fish consortia, whose predator–prey relationships are a fundamental factor for fish recruitment, is higher in space or time in coastal systems, posing fundamental implications in the marine ecological study and management of ecosystem goods and services, and economic activities like fisheries and fish aquaculture [5,21].

This article is a proof-of-concept for the study of spatiotemporal changes in pelagic food webs in a coastal system at the regional scale (Campania region, Mediterranean Sea) and across different seasons (2020–2021). The study integrated the plankton–fish biodiversity inventory with the environmental DNA technique [22] and coastal connectivity (CC) analyses carried out with Lagrangian modelling to assess the probability that ocean currents transport plankton from one site to another over a given time interval [23]. Such an integrated approach allowed us to evaluate the degree of ecological and physical connectivity among communities and geographical sites and to assess the relative contribution of space and time in pelagic food web variability.

## 2. Material and methods

### (a) Physical connectivity among coastal sites

To track estimated connectivity among nearshore sites we first performed numerical simulations using a regional ocean modelling system (ROMS) developed for the Tyrrhenian Sea (2 km resolution); then, the results of this first simulation were used as initial and boundary conditions for a finer grid model called Gulf of Naples Advanced Model (GNAM), covering the Campania coast with a 500 m resolution to obtain high-resolution output. GNAM is a free-surface, terrain-following, primitive equations ocean model widely used for a broad range of applications [24] and recently validated for the Gulf of Naples (GoN) area using a multiannual comparison with coastal high-frequency radar data and hydrological measurements [25]. We then used the ROMS velocity fields to run a Lagrangian transport package of virtual passive particles released along coastal areas, following velocity fields and constrained to fixed release depths (1 m).

CC is defined as the percentage of numerical particles, representing small water volumes and the plankton therein, leaving a source site ($i$) and arriving at a destination site ($j$) over a time interval $t$. Given $n$ different coastal areas, an $n \times n$ connectivity matrix was evaluated for each given time scale, where the ($i,j$) element was the fraction of the particles from source area ($i$) to destination area ($j$), in the released time ($t$). In this study, we released particles along the Campania region coast (figure 1*a*) every 5 days for 5 years (2013–2017, around 250,000 particles per year), and tracked them for 96 days (for IDs, the number of release areas and the seasonal connectivity, see electronic supplementary material, table S1 and figure S1). Finally, a connectivity network was produced by summing the particle fraction of the areas of the sampling sites. Connectivity networks were visualized with Gephi v. 0.10 [26].

### (b) Seawater sampling

We sampled environmental DNA (eDNA) onboard the *R/V Vettoria* between January 2020 and September 2021 in different sites along the coast of the Campania region (Southern Tyrrhenian Sea, Italy; figure 1*a*). Sampling in the GoN occurred at an approximately monthly scale; sampling at the plumes of three rivers in the GoN, Gulf of Gaeta (GoG) and Gulf of Salerno (GoS) occurred during summer (see electronic supplementary material, table S2). In the GoN, we sampled the long-term ecological research site MareChiara (DEIMS iD: https://deims.org/0b87459a-da3c-45af-a3e1-cb1508519411) (40°48′ N, 14°15′ E) [27], the Sarno River mouth (40°43′ N, 14°27′ E), and an offshore site localized above a canyon, i.e. the Dohrn Canyon (40°36′ N, 14°08′ E). Other stations were at the Volturno River (40°58′ N, 13°50′ E) and Sele River (40°28′ N, 14°55′ E) mouths, in the GoG and GoS, respectively.

At each sampling site, we collected surface seawater using Niskin bottles, we filtered 0.5–2 L of seawater on nitrocellulose filters (porosity 0.45 µm, diameter 47 mm, GVS North America, two replicates per each sample) that were flash-frozen in liquid nitrogen and then preserved at −80°C until further analyses. A SeaBird 911 Plus multi-parametric probe provided temperature, salinity, density, conductivity, dissolved oxygen, fluorescence and turbidity data.

### (c) Metabarcoding analyses

We extracted DNA using the E.Z.N.A. Mollusc DNA kit (Omega Bio-Tech) following the manufacturer's instructions. Metabarcoding libraries were prepared using the primers Euk1391F and EukBr [28] for the V9-18S region and 12S MiFish_U forward

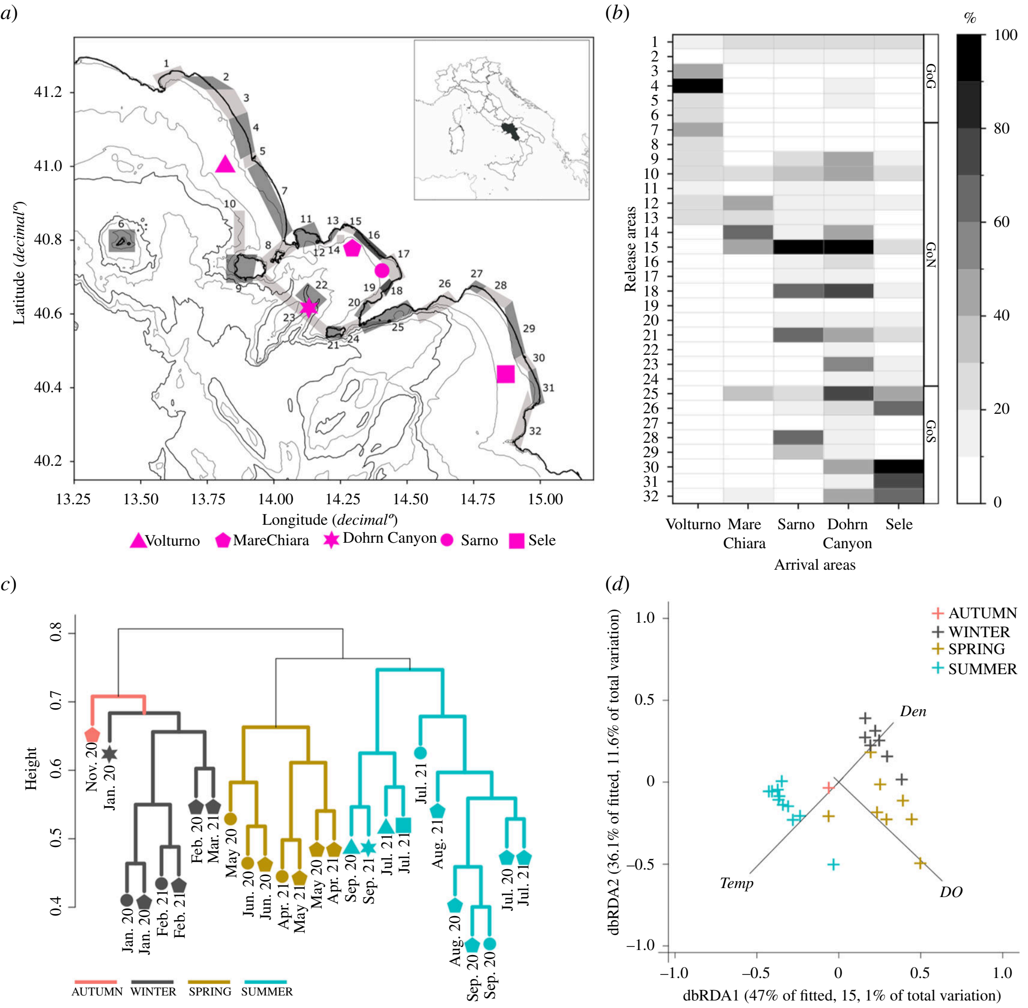

**Figure 1.** (*a*) Sampling map where numbers correspond to particle release areas (defined by the contiguous rectangles) along the Campanian coast; geometric shapes represent sampling stations. (*b*) Annual physical connectivity matrix showing particle migration rates, from release to arrival areas, as percentages (%). Cluster dendrogram (*c*) and dbRDA plot (*d*) based on Hellinger transformation of the reads count for V9-18S eDNA. (*d*) dbRDA plot fitted to significant predictor variables determined by BEST selection distLM (Temp, Temperature; DO, Dissolved Oxygen; Den, Density).

(fw) and reverse (rv) [29], respectively, and sequenced by GenomiX4Life (Illumina MiSeq; https://www.genomix4life.com/it/) following published protocols [30,31]. The V9-18S region was chosen for its capability to detect most of the planktonic taxa, as already done in the GoN [32] and in other marine systems [33,34]. On the other hand, the 12S region was used only to selectively detect fish presence, since the number of reads referring to each amplicon sequence variant (ASV) did not allow for an estimate of the relative abundance of fish taxa.

Illumina paired-end V9-18S raw reads (FASTQ format) were pre-processed to generate ASVs in RStudio [35] using the dada2 pipeline [36]. Primer sequences were removed, and fw and rv reads were trimmed based on the quality score (the first 150 bases of each fw and rv reads were kept; the maximum number of 'expected errors' allowed in a read = 2; max number of ambiguities = 0). Filtered reads were used to train the error model from the data using a machine-learning approach. Fw and rv reads were then denoised to generate ASVs by applying the trained error model and using the option 'trimOverhang = TRUE' to account for the fact that the sequenced amplicon was smaller than the read size. Finally, fw and rv reads were merged and checked for chimeras. 12S ASVs were also generated with the dada2 R library; adapter trimming and preliminary filtering were instead performed using cutadapt [37] with the 'linked adapter' option in paired-end mode, allowing 20% mismatch, truncating 3′ bases when quality was <15 and discarding untrimmed reads. All reads with ambiguities were then removed in dada2 before read error estimation and denoising; denoised reads were then merged into contigs, allowing a maximum of nine mismatches, and finally checked for chimeras.

To account for differences in the number of V9-18S region ASVs across samples, data were normalized at the median value of reads across samples ($n$ = 91,446) using the function 'rrarefy' of the vegan R package [38]. Taxonomy was assigned to ASVs using a consensus taxonomy approach through the Python script 'taxonomy_assignment_BLAST.py' (https://github.com/Joseph7e/Assign-Taxonomy-with-BLAST/blob/master/taxonomy_assignment_BLAST.py) from five BLAST hits, using a minimum coverage of 70% and assigning taxa to species if the percentage of identity was ≥99%, to genus if < 99% and ≥ 95%, or to any other taxonomic categories if higher than such thresholds The script was run twice, the first time against the SSU eukaryotic rRNA database of NCBI (https://ftp.ncbi.nlm.nih.gov/blast/db/SSU_eukaryote_rRNA.tar.gz, last modified 7 December 2022) and the second time against the PR2 database v4.14.0 (https://github.com/pr2database/pr2database/releases).

### (d) Ecological data analysis

We performed statistical analyses using data without replicates and, where present, relative abundance was averaged, as done in similar studies (e.g. [33]), to a total of 26 eDNA samples. Environmental and biological (V9-18S eDNA) data were analysed separately (see electronic supplementary material, tables S2 and S3); the similarity matrices for environmental data were based on Euclidean distances of normalized data, while for biological data, we employed Hellinger transformation to reduce the impact of highly abundant taxa [39]. As the first exploratory analysis of beta-diversity, we conducted cluster analysis on V9-18S Hellinger-transformer data in the *vegan* package ('*decostand*', '*vegdist*' and '*hclust*' functions) [40]. To test for the presence of seasonal differences between samples, we performed a two-way permutational multivariate analysis of variance (PERMANOVA, $p < 0.05$) with the fixed factor 'season' (three levels: winter, spring, summer), followed by a PAIRWISE test for significant terms.

We examined relationships between biological and environmental variables using the distance-based linear models (DistLM) routine that models linear relationships between dissimilarity matrices of biological data and predictor environmental variable(s) [41]. This routine allows fitting one or more environmental predictors to one or more biological variables. Among the model-building options, we selected the 'Best' procedure for the variables selection and 'An Information Criterion' ('AIC'; [42]) criterion for model comparisons. The criterion comes from the likelihood theory and smaller AIC values indicate a better model. Before the DistLM analysis, we used the Draftsman plot to reduce the effect of redundant variables and examine the correlation among environmental parameters before the analyses [43]. Conductivity, the only redundant variable showing >90% correlation, was excluded from the analyses. Statistical significance (PERMANOVA, $p < 0.05$) of the DistLM routine was assessed by permutation tests where each set of samples was randomly permuted 9999 times [44]. Analyses and plots were performed using the software PRIMER v.6.1.11 [44] and RStudio v.4.3.2 [35].

To investigate pelagic food webs, we identified potential trophic relationships between plankton and small pelagic fish detected, respectively, by V9-18S data and 12S on each site in different seasons. Therefore, plankton ASVs were summed and aggregated into specific functional groups (FGs) based on their taxonomic, dimensional [45,46] and physiological similarities [3]. The use of FGs is important as it increases the representation in food web models of ecological roles played by planktonic organisms within marine ecosystems, reducing complexity and functional redundancy [19,45]. Based on this rationale, we identified in our dataset seven FGs: autotrophic protists, heterotrophic/mixotrophic protists, crustaceans, gelatinous filter feeders, jellyfish, arrow worms and terrestrial organic matter (see electronic supplementary material, tables S3 and S4).

Planktonic FGs and small pelagic fish taxa were represented in conceptual food webs considering, respectively, their relative abundance (based on reads count) and presence–absence. Putative trophic interactions among FGs and fish were obtained from the literature and GloBi [47] (see electronic supplementary material, table S5). We represented conceptual pelagic food webs using the software Gephi, v. 0.10 [26].

## 3. Results

Lagrangian particle simulations showed a higher annual average connectivity among sampling stations in the GoN. Within these latter stations, the MareChiara site was largely connected to the Sarno River mouth, while the Volturno and Sele River mouths were less connected (figure 1*b*). However, model results showed that all sites were connected to different extents, thus allowing us to intercompare biological samples and their taxonomic composition studied with eDNA metabarcoding (eDNA metaB) and interpret spatial differences in light of connectivity among sites.

We annotated 4,344 planktonic and 13 fish ASV/taxa (seven pelagic and six demersal fish ASV) using the V9-18S and 12S regions, respectively (for more details about annotation results, see electronic supplementary material, tables S3 and S6). V9-18S samples showed seasonal partitioning, with three groups of samples, the first including all winter samples and one autumn, and the second and third groups, which differed more from the first group, including the spring and summer samples, respectively (figure 1*c*).

The PERMANOVA test found significant differences in biological data among seasons ($p < 0.05$). DistLM analysis showed that environmental parameters drove biological data partitioning, with temperature, density and dissolved oxygen explaining 26.7% of the total variance (figure 1*d*). The summer group was the largest one and included two subgroups, one including samples from the inner GoN (MareChiara and Sarno stations), and one including samples from the outer GoN (Dohrn Canyon) and other gulfs' sites. The latter observation indicates that spatial partitioning occurred, though to a lower extent than the seasonal one.

To map compositional differences in time and space, we combined plankton FGs and small pelagic fish presence and derived conceptual pelagic food webs (figure 2). MareChiara and Sarno stations had the best spatiotemporal coverage and strong connectivity all over the year (figure 1*b*), allowing seasonal comparisons (figure 2). At both sites, heterotrophic/mixotrophic protists were predominant and more abundant in winter and spring than in summer. Autotrophic protists were higher in summer at both sites. Crustaceans, which predate the previous groups, were third in rank and found at MareChiara during summer and winter but virtually absent during spring; however, during the latter season, crustaceans were detectable at Sarno. Other FGs were weakly detected overall.

Concerning small pelagic fish communities, we also observed signals of spatiotemporal differentiation at MareChiara and Sarno. The small pelagic *S. aurita* occurred in all the seasons analysed at the MareChiara and Sarno sites, while *E. encrasicolus* was always present but not at Sarno in spring. Winter was the richest season at the MareChiara and Sarno sites, which differed for the presence of *S. pilchardus* and *C. auratus* (present only at the MareChiara site).

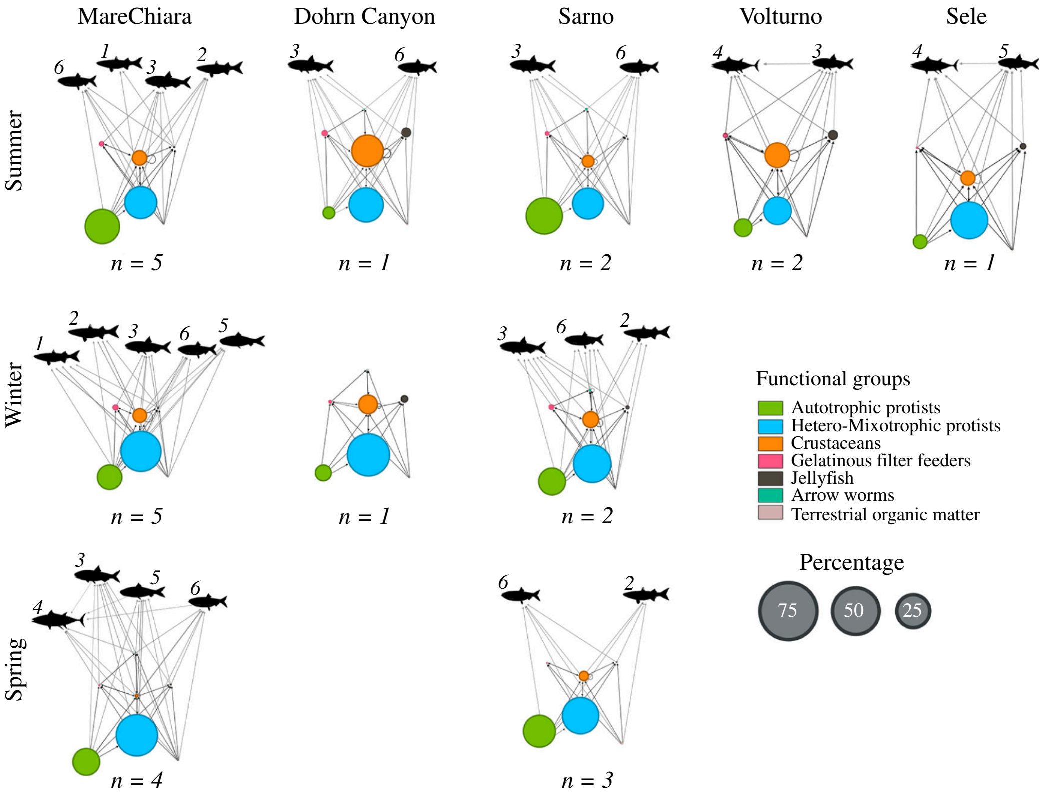

**Figure 2.** Conceptual pelagic food webs at the sampling sites in the summer, winter and spring seasons (samples collected during autumn, i.e., Nov_20 at the MareChiara station, were grouped into the winter samples). Edges represent putative trophic interactions between plankton FGs and small pelagic fish. Node size is proportional to the relative percentage of reads for V9-18S eDNA, while the information obtained from 12S (fish) was used to indicate the presence of taxa. Fish IDs: 1, *Chelon auratus*; 2, *C. labrosus*; 3, *Engraulis encrasicolus*; 4, *Euthynnus alletteratus*; 5, *Sardina pilchardus*; 6, *Sardinella aurita*. *n* is the number of samples used to calculate the percentage of total V9-18S reads.

Dohrn Canyon samples allowed us to describe only winter and summer communities, which were markedly different from those found in the inner GoN. About plankton, autotrophic protists were scanty if compared with MareChiara and Sarno, while heterotrophic/mixotrophic protists showed similar values. Crustaceans were in higher percentage during summer and showed almost double the relative abundance of the inner GoN. The other FGs were less represented, except for jellyfish, which showed a higher relative abundance than in the inner GoN. Finally, the Dohrn Canyon and Sarno sites showed the same summer small pelagic fish community.

We also explored the spatial differences among summer communities from the GoN and the other gulfs, i.e. studied at the Volturno and Sele stations. The Volturno plankton community was similar to that present at the Dohrn Canyon but showed a higher relative number of autotrophic protists. At Sele site, heterotrophic/mixotrophic protists were the most relevant, reaching the highest relative abundance among all summer communities. Still, autotrophic protists showed a lower amount than in other coastal sites during summer. Concerning planktonic animals, Volturno was similar to the Dohrn Canyon (higher crustaceans and jellyfish than in other stations), while Sele was more similar to inner GoN stations (lower crustaceans and jellyfish). Concerning small pelagic fish, *E. alletteratus* and *E. encrasicolus* were present at both Volturno and Sele, but this taxa combination was not found in any other site during summer.

## 4. Discussion

The advance in the eDNA metaB analysis in marine ecology has profoundly increased the amount of information available on marine biodiversity. The use of eDNA presents many advantages: for instance, it is less invasive and more cost-effective than traditional surveys [48,49]. So far the eDNA technique has been mainly applied to temporal or spatial studies with only a few investigations comparing the spatiotemporal changes of marine communities at the regional scale (e.g. [50,51]).

Our study shows the importance of integrating eDNA data and connectivity analysis to understand the dynamics of coastal planktonic communities, which are carried by physical dynamics and may be moved to different sites, influencing the higher trophic levels. Our results confirmed the plankton seasonality previously described at the MareChiara station (e.g. [32,52]), but extended this observation at the whole regional scale, with the planktonic communities in the Campania coast that differed more by seasonal than by spatial dynamics. This observation is in line with a recent scientific reference about plankton we can invoke as a comparison for the study area, i.e. the observation that the genetic fingerprint of populations of the diatom species *Pseudo-nitzschia multistriata* collected in the different Campania gulfs was spatially very similar, but far different over time [53].

Environmental factors determined these seasonal differences. Summer and winter communities detected with V9-18S eDNA metaB were mainly influenced by temperature and density, respectively, which remark the water column stratification cycles affecting nutrient availability in the photic zone influencing the metabolism, growth, reproduction, development, distribution

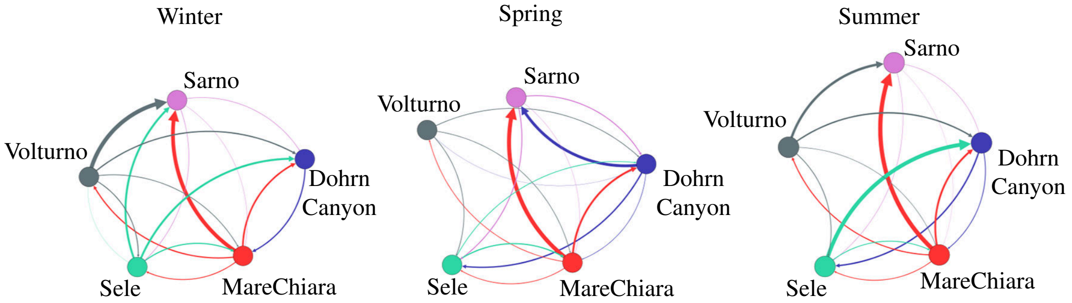

**Figure 3.** Connectivity network. Network edges represent connections between sites. Edges directed from release to the arrival site represent the particle release and are colour-coded by the release site. Edge width is proportional to the sum of the values of particle migration rate, grouped by the sampling site's area of interest.

and food availability of marine organisms [54,55]. The higher presence of phototrophs in summer and of heterotrophic/mixotrophic protists in winter we observed in the GoN matched that from a longer metabarcoding time series from the MareChiara site [32]. Spring communities are also influenced by dissolved oxygen (DO), which is regulated by both biotic (e.g. production and consumption), and abiotic factors (e.g. pH, temperature, salinity and hydrodynamic processes) [56,57]. DO concentrations can lead to changes in planktonic community structure and trophic transfer [58,59], with crustacean zooplankton peaking in hypoxic waters where they seek refuge from predation by fish, which instead exhibit lower abundance at lower oxygen levels [60,61].

Most of the spatial homogeneity that we detected could be the result of high connectivity at the regional scale (figure 3). For instance, the strong connection between the Volturno and GoN areas matches previous modelling studies' results [62,63].

However, spatial differentiation can also emerge from the multiple stimuli that local environmental conditions may exert on the pelagic communities, which are highly dynamic entities. The tangled action of species immigration and local selection can affect spatial differentiation among communities and their food webs' functioning during the same season, like summer.

As a general pattern, phototroph-driven food webs and higher fish diversity occurred at eutrophic stations closer to the coast undergoing stronger inputs from land runoff and showing higher retention times, like in the inner GoN (MareChiara and Sarno), suggesting a more direct flux of matter from primary producers to higher consumers. Conversely, food webs at offshore stations (Dohrn Canyon), or those more exposed to the action of open sea currents (Volturno and Sele), showed a lower dependence on strictly phototrophic plankton, higher prevalence of heterotrophic/mixotrophic protists adapted to oligotrophic conditions, and less diverse small pelagic fish communities, suggesting a more dissipative microbial loop-based food web with longer trophic chains [64]. Oligotrophic sites also included more jellyfish, which compete with planktivorous fish [65], probably owing to upwelling transporting specimens from deeper waters [66].

Overall, we observed that slightly different plankton assemblages co-occurred with the different small pelagic fishes (figure 2) showing mainly a planktivorous diet composed of copepods, and to a lesser extent other crustaceans, molluscs, pelagic tunicates and other fishes [67,68]. The assembly of small pelagic fishes can be influenced by several interplaying drivers, such as food quality [13,14], biological (e.g. spawning timing) and environmental (e.g. salinity tolerance) factors [15,69].

Concerning biological factors, small pelagic fishes show different spawning times, which may have affected the occurrence of fish DNA in our study. Sardine (*S. pilchardus*, #5 in figure 2) spawns during autumn–winter in a mixed water column with salinities around 37–38 psu [68,69], which corresponds with the environmental conditions that we retrieved. Other species have their optimum reproduction and spawning during spring and summer when the water column is stratified and warmer, as in the case of skipjack tuna (*E. alletteratus*; #4) (23–27.5°C) and anchovy (*E. encrasicolus*; #3) (13–25.5°C) [70], which can spawn in a wide range of salinity (36.7–37.9 and 29.1–38.2 psu, respectively) [70,71]. Sardinella (*S. aurita*; #10) also spawns at temperatures above 23°C, from July to October in agreement with its tropical origin [69].

Regarding environmental factors, salinity tolerance can also drive fish assemblages. For instance, we found *C. auratus* (#1) only at MareChiara station, and *C. labrosus* (#2) also at the River Sarno mouth. This is in agreement with observations of *C. auratus* having mainly a pelagic behaviour, while *C. labrosus* is euryhaline and frequently found in estuaries [72]. The genus *Chelon* includes generalist planktivorous–detritivorous fish [73,74] often found around sea bream and sea bass farms [72,75] and polluted environments [76].

However, we must consider that the small pelagic fish species found in our survey may not reflect the entire diversity present in the study area. Indeed, fishes are characterized by a scattered spatial distribution [70]; water sampling can fail to catch all the taxa retrieved from traditional approaches, such as visual census and fishing nets [49,50]; eDNA shows operational limits like primer specificity [50], and it cannot provide information on the true abundances of organisms [47,48].

## 5. Conclusion

The application of eDNA metabarcoding analysis in marine ecology has expanded our understanding of the spatiotemporal dynamics of marine biodiversity at the regional scale. By applying an integrative approach, this study highlighted the importance of combining eDNA data with connectivity analyses to reveal the complex dynamics of coastal planktonic communities, which are influenced by physical processes and can spread between different sites affecting, in this way, the higher trophic levels, like fishes. In this respect, our study highlights the need to further investigate the intricate interactions that regulate coastal marine communities and their underlying ecological processes, by intensifying the fish eDNA sampling effort at the spatial level and integrating these observations with traditional approaches like fishery surveys, which could provide more precise information on the distribution of fish in the water column. Such knowledge is critical for the effective conservation and management of marine ecosystems in the face of environmental change.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material [77]. Raw V9-18S and 12S metabarcoding data are available in the Sequence Read Archive (SRA) under BioProject PRJNA1023387.

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. D.B.: data curation, formal analysis, investigation, software, visualization, writing—original draft, writing—review and editing; L.R.: data curation, formal analysis, investigation, software, visualization, writing—original draft, writing—review and editing; V.D.T.: investigation; D.D.L.: data curation, investigation, methodology, visualization, writing—review and editing; G.D.G.: investigation; G.Z.: data curation, investigation, methodology, software; F.K.: data curation, investigation, methodology, visualization; V.B.: data curation, investigation, methodology; F.C.: investigation, writing—review and editing; F.C.: data curation, funding acquisition, resources; P.D.L.: methodology, resources; D.I.: funding acquisition, methodology, resources; F.M.: investigation, methodology, resources; S.S.: data curation, investigation, methodology, visualization; P.V.: funding acquisition, supervision; D.C.: conceptualization, methodology, supervision, writing—review and editing; D.D'A.: conceptualization, funding acquisition, methodology, supervision, writing—review and editing, project administration.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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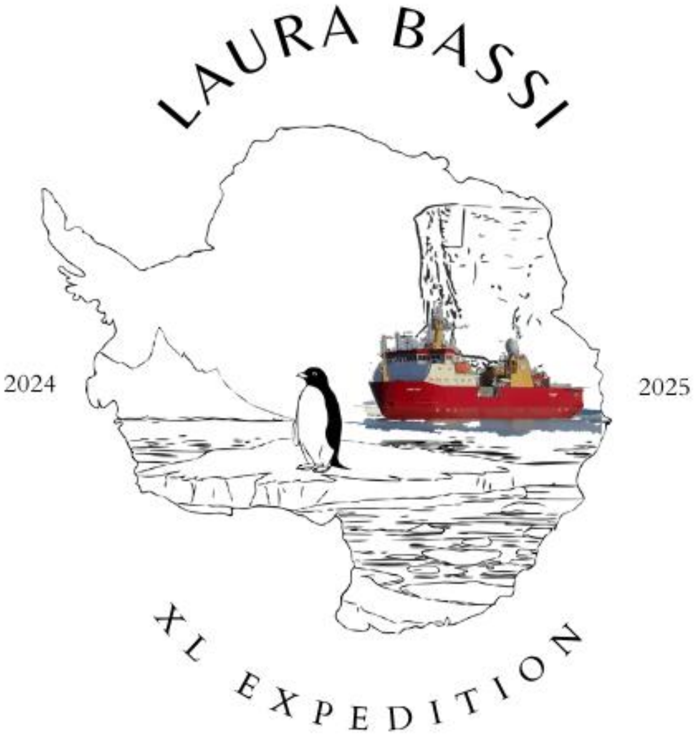
# 8. CONCLUSIONS AND FUTURE PROSPECTIVES

This Ph.D. thesis explored the study of food webs in different coastal environments subjected to distinct anthropogenic pressures in a multidisciplinary way, with the aim of providing scientifically based tools to support the sustainable management of marine ecosystems and the assessment of good environmental status according to Marine Strategy Framework Directive (MSFD) criteria. Through the analysis of data from two different oceanographic sampling campaigns (FEAMP-ISSPA Project in the Tyrrhenian Sea and S4GES Project in the North Sea), it was possible to obtain a high-resolution characterization of planktonic biodiversity, considering both taxonomic and functional components. The integration of traditional approaches (microscopic analysis) and innovative methodologies (eDNA) allowed us to explore the complexity of trophic networks and their response to different environmental gradients.

The results presented in this thesis offer a holistic and multidisciplinary perspective on the role of plankton in the functioning of marine ecosystems. Specifically: (i) it was shown that the functional distribution of copepods is influenced by local environmental conditions (Chapter 3), laying the groundwork for a more detailed analysis of trophic networks; (ii) it was demonstrated that planktonic food webs respond both structurally and functionally to natural pressures such as riverine inputs (Chapter 4) and different tidal regimes (Chapter 5), highlighting the usefulness of network indicators as tools for ecological assessment; (iii) the use of innovative technologies, such as environmental DNA, has improved our understanding of how plankton communities vary across different environmental contexts. Furthermore, through Lagrangian modeling, we observed how physical and biological processes interact to shape the connectivity and composition of plankton communities (Chapter 7); (iv) Furthermore, while omics and modeling technologies allow for a detailed description of plankton biodiversity and community structure, it is also essential to assess their ecological efficiency and contribution to natural capital. To this end, a new methodology based on emergy accounting (Chapter 6) was proposed, capable of quantifying in biophysical terms the natural capital associated with plankton biodiversity, thus bridging quantitative ecology with systemic evaluation of ecosystem services.

Taken together, these results highlight the need to move beyond sectoral approaches and embrace an integrated, holistic vision of marine ecosystems. In this framework, plankton – too often underrepresented in food-web models – emerges as a key component in trophic dynamics and the maintenance of ecosystem functions. The implications of this work are manifold. First, the methods developed contribute to improving the assessment of Descriptor 4 of the Marine Strategy Framework Directive (MSFD), by providing more robust indicators for the analysis of food webs. Second, the integration of innovative tools such as environmental DNA and emergy accounting opens new perspectives for studying marine biodiversity, quantifying natural capital, and assessing ecological sustainability in biophysical terms—ultimately fostering a more effective dialogue between ecological science and environmental governance. Finally, this thesis proposes a methodological framework that is both replicable and adaptable to other coastal systems, offering a valuable tool to address the challenges posed by climate change, pollution, and increasing human pressure on marine environments.

In continuity with the work done, an important development perspective concerns the application of the analytical and methodological approaches described in this thesis to remote ecosystems characterized by extreme environmental conditions, such as Antarctica. From January to March 2025, I took part in Italy's 40th scientific expedition (PNRA – ENEA) to Antarctica aboard the oceanographic icebreaker ship "*Laura Bassi*", during which I collected biological and environmental samples with the goal of analyzing the planktonic trophic networks of a system still little affected by anthropogenic pressures. Indeed, Antarctica represents an invaluable natural laboratory for understanding the functioning of ecological networks in nearly pristine conditions and comparing them with more impacted ecosystems. The analyses of the Antarctic samples were not completed in time to be included in this thesis but will form the basis for future scientific publications. These studies will provide a better understanding, and at larger geographic scales, of the vulnerability of marine ecosystems to global climate change.



Visual concept of 40th Antarctica Expedition (2025) aboard the oceanographic icebreaker ship “*Laura Bassi*”

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