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Seagrass-epibiont interactions in Mediterranean coastal lagoon ecosystems: biotic relationships in a changing environment

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Indice

Summary

This thesis presents a comprehensive investigation of seagrass-epibiont interactions in Mediterranean coastal ecosystems, with a particular focus on the Stagnone of Marsala lagoon (Sicily, Italy). Through a multi-faceted approach combining global analysis, long-term ecological studies, experimental research, and field observations, the work provides crucial insights into the dynamics and resilience of these important marine habitats in the face of environmental change. The research begins with a global perspective on the ecological roles of epibionts and epiphytes in seagrass ecosystems, establishing a broad context for understanding these interactions. It then narrows its focus to the Mediterranean, presenting an in-depth examination of long-term ecological and structural changes in the Stagnone of Marsala lagoon. This case study reveals valuable information about the temporal dynamics of seagrass-dominated lagoon ecosystems and the factors driving changes in biodiversity and ecosystem structure. The thesis further explores the physiological responses of key species to environmental stressors through an investigation of the thermal performance and biotic interactions of *Cymodocea nodosa*, *Paranemonia cinerea*, and its symbiont. This experimental approach provides insights into the potential impacts of warming on important Mediterranean coastal species. Finally, the research examines the spatial and temporal dynamics of seagrass-epibiont interactions within the Stagnone of Marsala, combining field observations with ecological modeling to understand the factors influencing the distribution and abundance of epibionts on seagrasses in a lagoon setting. By integrating these diverse approaches, the thesis offers a holistic understanding of seagrass-epibiont ecology in the Mediterranean context, identifies critical knowledge gaps, and provides valuable insights for the conservation and management of these vital coastal ecosystems in an era of rapid environmental change.

General introduction

Coastal ecosystems, particularly lagoons, are among the most productive and ecologically important marine habitats worldwide (Duarte et al., 2013; Nordlund et al., 2016; Orth and Heck, 2023; Pérez-Ruzafa et al., 2024). Coastal lagoons are bodies of water situated inland and separated from the ocean by a physical barrier (Rodrigues-Filho et al., 2023). One or more restricted inlets may occasionally connect them to the sea (Rodrigues-Filho et al., 2023). These ecosystems are important for many biogeochemical processes (Newton et al., 2018) and provide different ecosystem services, acting as nursery areas for fish. Indeed, during the early stages of their life cycles, opportunistic marine estuarine fishes exploit these habitats as feeding grounds and nursery places to later migrate as adults to the sea for reproduction. As a result, many lagoons sustain significant fisheries, and others continue extensive and intense aquaculture practices that provide income based on the quality and price of their natural resources (Pérez-Ruzafa et al., 2024).

These ecosystems that take in and utilize organic matter and nutrients from terrestrial systems and the coastal area include marshes, mangroves, seagrass beds, and estuaries. High productivity and additional element cycling are supported by the fact that a significant amount of the nutrients and organic matter typically stay and cycle within the system, acting as biogeochemical hotspots. Important microbiological changes and chemical interactions are facilitated by dynamic aerobic and anaerobic sediments, which influence the availability of nutrients for production and, ultimately, water quality. Vegetation and organisms can produce organic material that can either accumulate in the soil matrix as sequestered organic carbon and nutrients or mineralize into inorganic forms that are available for plant and algal uptake (Elsey-Quirk et al., 2022).

In the Mediterranean Sea, these ecosystems provide crucial services such as carbon sequestration, nutrient cycling, and habitat provision for diverse marine life (Waycott et al., 2009; Nordlund et al., 2016; Orth and Heck, 2023). However, they are increasingly threatened by climate change and other anthropogenic stressors (Orth et al., 2006; Waycott et al., 2009; Orth and Heck, 2023), being exposed to changes in sea water temperature, water salinity, sea-level rise, hydrodynamic conditions of water masses, frequency of extreme weather events and eutrophication processes (Lacoste et al., 2023).

Seagrass meadows, represent the most important habitat within the coastal lagoons, where they have a fundamental ecological role (Agostini et al., 2003), representing a biogeochemical hotspot (Elsey-Quirk et al., 2022), stabilizing sediments and regulating nutrient cycles (Garrido et al., 2013), providing a physical *refugium* from predation and acting as nursery and feeding areas for several endangered species (Franco et al., 2006, Newton et al., 2018).

Seagrass ecosystems are characterized by complex ecological interactions, particularly between the seagrasses themselves and their associated epibionts and epiphytes (Borowitzka et al., 2006). These interactions play a vital role in shaping ecosystem structure, function, and biodiversity (Jernakoff et al., 1996). In particular, seagrasses play a crucial role for their symbionts by providing refuge and offering strategic leaf positions that enhance access to food and light (Brodersen et al., 2015). Seagrass leaves are habitat to a micro and macroalgal epiphytes community of rapidly growing primary producers, which can contribute significantly to the biomass of the seagrass ecosystem (Wheeler et al., 2024).

Epiphyte community contributes significantly to primary production, provides food and habitat for different organisms, and influences nutrient cycling within seagrass meadows (Piazzi et al., 2016; Michael et al., 2008). Larger macroalgal epiphytes can increase their abundance in response to light, nutritional availability, and warm water temperatures. Their physiological and reproductive performance can be directly impacted by micro and macroalgae's ability to outcompete seagrasses for nutrients and shade the plants, lowering the amount and quality of light they obtain (Wheeler et al., 2024). Also, epiphytes can also increase oxidative stress, reduce chlorophyll-b levels (Costa et al., 2005, Wheeler et al., 2024), and alter plant metabolism (Ow et al., 2020). On the other hand, the composition of epibiont communities can serve as a predictor of environmental factors and water quality, since they are sensitive indicators of natural and long-term environmental changes, with their biomass reflecting shifts in nutrient conditions (Piazzi et al., 2016). Their study is very important because they can be useful as a marker of shifting environmental or stress conditions, especially when the temperature regime is changing (Wheeler et al., 2024).

The nature and intensity of seagrass-symbiont interactions may be altered by environmental changes, potentially affecting the resilience and functioning of these ecosystems (Martínez-Crego et al., 2010). Climate change presents a major threat to Mediterranean coastal ecosystems, with rising temperatures, sea level rise, and increased frequency of extreme events expected to have significant impacts on seagrass communities and their associated communities

(Pergent et al., 2014). Indeed, different driver of climate changes, such as marine heatwaves events can attributed their potential impact on the structure of seagrasses meadows and epifaunal communities associated with these species (Smale and Wernberg, 2013; Wernberg et al., 2013; Chen et al., 2021). Natural and human-induced disturbance can impact both seagrass healthy and the epifaunal communities they support, leading to coral bleaching, meadows fragmentation, reduced canopy size, canopy detachment and biodiversity loss (Chen et al., 2021). Marine plant populations are thought to be limited primarily by physical disturbance, light and nutrients availability and herbivory interactions (Estes and Peterson, 2000). For seagrasses, drivers of change related to nutrient supply, light availability, and/or physical factors have received most attention in the literature (Estes and Peterson, 2000; Waycott et al., 2009; Duarte, 1991), being for example the consequences of physiological stress associated with light limitation, diminished growth, increased shoot mortality, and limited depth distribution (Collier et al., 2007). Also, eutrophication, is considered to be one of the main reasons for seagrasses loss (Burkholder et al., 2007; Jephson et al., 2008). It may cause an increase in nutrient supply that enhances phytoplankton production and turbidity and also promotes the annual rapid growth of filamentous algae and epiphytic and free-floating macroalgal mats, which in turn reduce the supply of light and nutrients to the seagrass (Duarte 2000). However, literature reviews (Hughes et al., 2004 and Valentine and Duffy 2005) have shown that an increased nutrient load alone often has little negative effect on seagrass growth, as long as natural populations of meso-grazers (i.e. amphipods, isopods, and gastropods) are present to harvest the algae. Seasonal fluctuation in temperature is one of the main environmental factors influencing organismal performance and shapes their interactions (Bass and Falkenberg, 2024). Warmer months can promote higher growth rates, increases in metabolic activity and primary productivity, and changes to animal behavior; while cooler temperatures in winter typically give rise to lower rates of physiological and behavioral change (Kearney et al., 2009; Chen et al., 2021; Bass and Falkenberg, 2024). These changes in organisms due to change in environmental conditions can then influence biotic interactions, including trophic ones and their role in shaping ecosystem structure, stability and dynamics (Chen et al., 2021). Understanding how these environmental changes affect seagrass-epibiont interactions is crucial for predicting ecosystem responses and developing effective conservation strategies.

This thesis aims to increase our understanding of the complex ecological interactions within Mediterranean coastal ecosystems, with a particular focus on seagrasses and their associated epibionts and epiphytes. By integrating global analyses, case studies, and experimental approaches, we seek to elucidate how these interactions may respond to environmental change and their potential role in shaping ecosystem structure and functioning, for generating useful information able to assist conservation strategies for these vital coastal ecosystems in the face of global environmental change.

The thesis is structured into four chapters, each addressing specific aspects of seagrass-epibiont ecology and their response to environmental change: **Chapter 1** establishes a global perspective on epibiont and epiphyte roles in seagrass habitats. This chapter synthesizes current knowledge on the ecological interactions between seagrasses and their associated organisms, providing a broad context for the subsequent focused studies in the Mediterranean Sea. **Chapter 2** presents an in-depth examination of the Stagnone of Marsala lagoon, exploring long-term ecological and structural changes in this important Mediterranean coastal ecosystem. This case study provides crucial insights into the current state of these habitats and sets the foundation for assessing future changes. **Chapter 3** investigates the thermal performance and biotic interactions of key species, with a focus on *Cymodocea nodosa*, *Paranemonia cinerea*, and its symbionts. This chapter provides a mechanistic understanding of how these species cope with environmental stressors at a organismal level, offering insights into their potential resilience to climate change. **Chapter 4** explores the spatial and temporal dynamics of seagrass-epibiont interactions within the Stagnone of Marsala. This detailed study combines field observations with ecological modeling to understand the factors influencing the distribution and abundance of epibionts on seagrasses in a Mediterranean lagoon setting.

The insights gained from this research will contribute to the development of more effective management practices and policy recommendations for the conservation of Mediterranean coastal ecosystems.

This work addresses several key knowledge gaps in our understanding of Mediterranean coastal ecosystems:

1. The relative importance of different types of seagrass-epibiont interactions across various spatial and temporal scales.
2. The long-term dynamics of biodiversity and ecosystem structure in Mediterranean coastal lagoons.

3. The potential impacts of climate change on the thermal performance and biotic interactions of key coastal species, the seagrass *Cymodocea nodosa* and the sea anemone *Paranemonia cinerea*.
4. The factors driving the spatial and temporal variability of seagrass-epibiont associations in lagoon environments.

By addressing these gaps, this thesis contributes to the broader field of coastal ecology and provides valuable insights for the conservation and management of Mediterranean marine ecosystems in an era of rapid environmental change.

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Chapter 1- Epibionts and epiphytes in seagrass habitats: a global analysis of their ecological roles.

Abstract

Seagrasses ecosystem support complex biological interactions that shape marine community structure and ecosystem functioning. Thanks to their structural complexity, they support heterogeneous communities and interact with associated benthic invertebrates and fish populations, establishing complex relationships that influence the performance and fitness of the involved organisms. This study, through a systematic review, investigated the existing potential biotic interactions between seagrasses and epibionts-epiphytes on a global scale. We created a complex search string and ran it in the online databases Scopus and Web of Science, yielding a total of 62 final outcomes spanning from 1984 to 2024. Our results revealed both positives and negatives effects of different biotic interactions among these habitat formers and their associated symbionts. The review showed that the most studied interactions referred to *Posidonia oceanica* (Delile) L. and *Zostera marina* (Linnaeus, 1753), which provide refuge and habitat to different epiphytes and epibionts. The reviewed studies highlighted the importance of epiphytes, their potential role in seagrass growth, nutrient dynamics, and their implications for light absorption. Additionally, epibionts such as sea anemones, may chemically defend seagrasses from predation; however, their mucous can coat seagrass leaves and obstruct sunlight absorption. Understanding these various types of biotic interactions and studying how they can influence the performance of the species involved is vital in the current and future context of climate change.

1.1 Introduction

Biotic interactions are one of the main factors influencing the distribution and abundance of species worldwide (Jones et al., 1994; Stelling-Wood et al., 2023). Among these, the interaction between habitat-forming species and the associated biodiversity plays a fundamental role in the ecosystem functioning (Teagle et al., 2017; Stelling-Wood et al., 2023). Seagrasses are highly productive habitat-forming species worldwide creating densely populated meadows that offer a variety of important ecosystem services, including: (i) raising the pH and O_2 levels in the surrounding water column during the day, which benefits calcifying organisms like corals (Ricart et al., 2021; Brodersen and Kühl, 2022; Greve et al., 2003); (ii) protecting the coast from erosion due to wave attenuation caused by leaves (Brodersen and Kühl, 2022); (iii) providing a

variety of feeding and nursery grounds, particularly for young fish and marine invertebrates (Brodersen and Kühl, 2022); (iv) absorbing nutrients like nitrogen and phosphorus, improving the water quality (Brodersen and Kühl, 2022); and (v) efficiently sequestrating and fixing carbon into the sediment thereby mitigating climate change (Duarte et al., 2005; Fourqurean et al., 2012; Brodersen and Kühl, 2022).

Healthy and productive seagrass meadows are thus important for ensuring a good environmental state of marine waters, especially in coastal regions (Brodersen and Kühl, 2022). In shallow waters, seagrasses grow in a range of sediment types and often offer the only solid substrate that macroalgae may attach. According to Kitada et al., (2019), and Gessner et al., (1971), their presence can increase the surface area of the bottom suitable for colonization by epiphytic or epibenthic diatoms by a factor of 5 to nearly 19. The overall primary production of the seagrass ecosystem is substantially increased by offering additional photosynthetic organisms a suitable substrate (Spicer and Woods, 2022).

Different studies examining the interaction among seagrasses, epiphytes, epibionts and grazers date back to the early 1990s (Robertson and Mann 1982; Van Montfrans et al., 1984; Howard et al., 1989). At that time, these relationships were considered complex and poorly understood. Since then, our understanding has improved. Seagrasses are now recognized as providing substrate for different epibionts and epiphytes, with which they can have either positive and negative interactions.

The purpose of this systematic review was to synthesize the potential biotic interactions between seagrasses and epibionts-epiphytes at global scale. The review aimed to provide an overview of biotic interactions between habitat-forming species, such as seagrasses and their associated epibionts and epiphytes, highlighting the involved species and the advantages and disadvantages of the different types of biotic interactions existing among them. Understanding the various types of biotic interactions and studying how they can influence the performance of the species involved is crucial in the current and future context of climate change and may help to assess how these relationships influence ecosystem functioning and resilience under stressful conditions.

1.2 Materials and Methods

The systematic review was performed applying the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline proposed by (Hutton et al., 2015;

Liberati et al., 2009; Moher et al., 2009). The PRISMA method was applied to explore main research question: (1) What are the biotic interactions between epibionts (such as sea anemones)-epiphytes and seagrasses or phanerogams? And a secondary question: What are the advantages and disadvantages of different types of biotic interactions (symbiosis, mutualism, and commensalism) between these organisms? To answer these questions, a literature search was conducted using the PICO (Population, Intervention, Comparison and Outcomes) approach to define keywords. The PICO elements were as follows: Population of interest (P): biotic (species) components, Intervention (I): all different types of biotic interactions between epibionts, epiphytes, and habitat-forming organisms such as seagrasses or phanerogams, Comparator (C) different responses of communities to different types of biotic interaction, and Outcomes (O): species complex interacting, and advantages and disadvantages of the biotic interaction between epiphytes-epibionts and seagrasses. Based on these PICO elements, the following research string was developed: (biotic OR interaction OR commensalism OR mutualism OR symbiosis) AND (epiphyt* OR epibiont* OR "sea anemon*" OR anemon*) AND (seagrass* OR phanerogam*). The initial search was carried out on December 18, 2023, and repeated on June 26, 2024 on Elsevier's Scopus (www.scopus.com) and Clarivate Web of Science (www.webofknowledge.com) databases (Table 1.1). The results were reported according to the PRISMA guidelines, following four steps: (1) Identification, (2) Screening, (3) Eligibility, and (4) Inclusion. The identification step involved identifying records through database searching and removing duplicates. The screening step consisted in reviewing papers by title and abstract. The eligibility and inclusion steps were based on a set of selection criteria, involving a more detailed screening at the full-text level (Liberati et al., 2009). The inclusion criteria encompassed studies focusing on biotic interactions between seagrasses and epibionts, mobile fauna, and epiphytes; and the effects of these biotic interactions on the host. Exclusion criteria included off-topic studies, non-English language publications and incomplete book chapters. Data were extracted from the selected records using a structured matrix. This matrix included information such as publication date, habitat details (location, country, latitude and longitude), species characteristics (taxonomic order and species), specific details of biotic interactions, complex interacting species (epibionts, sea anemones, habitat-forming organisms like seagrasses or phanerogams), types of biotic interactions (mutualism, commensalism, symbiosis), advantages and disadvantages of epiphytes and epibionts, the roles of seagrass, effects of these biotic interactions, and additional comments.

1.3 Results

The initial search identified 378 records, specifically 147 records were identified in Web of Science and 231 in Scopus (Table 1.1).

Table 1.1: Complex and simple string used in this systematic review. The asterisk (*) following a search word has been used to consider and include all word variations in the search.

Table 2.1: Strings of Systematic Review

Data	Strings	Scopus	WoS	Total
2024-06-26	(biotic OR interaction OR commensalism OR mutualism OR symbiosis) AND (epiphyt* OR epibiont* OR sea anemone OR anemon*) AND (seagrass* OR phanerogam*)	231	147	378

Of these, after the removal of 145 duplicates, 233 records remained for the further screening. The first and second screening stages, which involved reviewing title and abstract, resulted in the exclusion of 143 records, leaving 90 records for further evaluation. The third screening stage, conducted at the full-text level, excluded an additional 11 records, and 79 passed to exclusion with reason. Ultimately, 62 records were included in the systematic review (Figure 1.1).

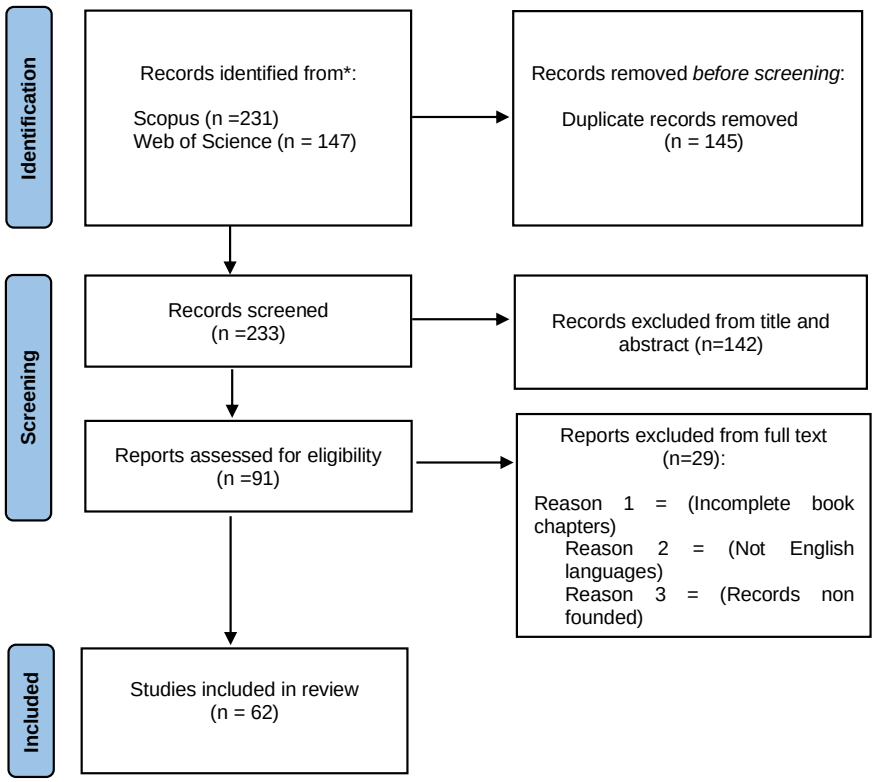

Figure 1.1: PRISMA flow diagram illustrating the systematic review process for literature selection. The diagram shows the identification, screening, eligibility, and inclusion stages of the review, detailing the number of records at each step from initial database searching to final inclusion.

The selected records spanned from 1984 to 2024, with the highest concentration of studies occurring between 2006 and 2010, averaging 4 records per year during this period (Figure 1.2).

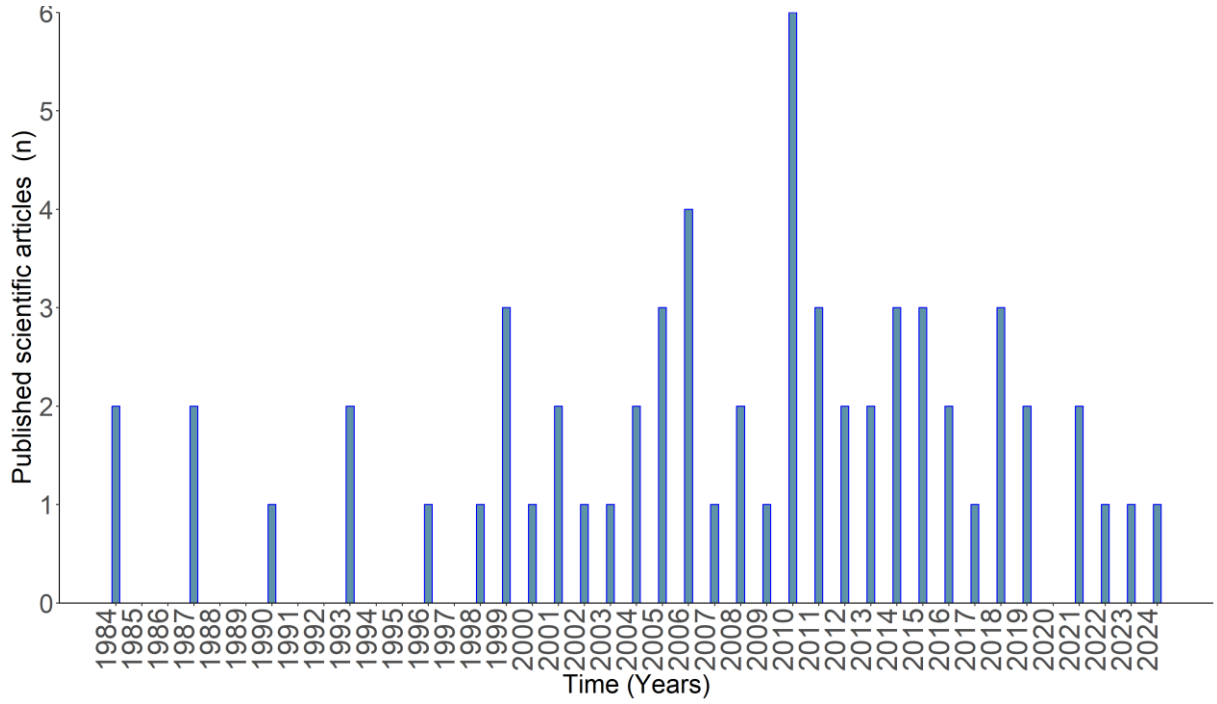

Figure 1.2: Temporal trend of published articles used in this Systematic Review

All of the selected studies focused on seagrasses or vegetal foundation species of the order Alismatales, such as *Posidonia oceanica*, *P. australis* (Hook, 1858), *P. sinuosa* (Cambridge and J.Kuo), *Cymodocea nodosa* (Ucria), *C. rotundata* (Ascherson and Schweinfurth, 1870), *Zostera marina, Z. capricorni* (Aschers, 1876), *Z. caespitosa* (Miki, 1932), species. These species cover the whole distribution worldwide, overall in the Mediterranean Sea with 55% of studies cases (Figure 1.3). Most studies examined biotic interactions between epibionts-epiphytes-seagrass or grazers-epiphytes-seagrass, focusing on the roles of epiphytes, epibionts, and grazers in their interactions with seagrasses, including both advantages and disadvantages. Regarding the impact of seagrass on epiphytes, the studies reported varied and sometimes conflicting information. Some papers indicated that seagrasses may negatively affect epiphytes by inhibiting their growth through the release of chemical compounds such as phenolics (Orth et al., 1984; Jernakoff et al., 1996). Others suggested that seagrasses are not simply inert substrates for microalgal attachment but rather influence community composition by altering competitive interactions between different microalgal groups (Jernakoff et al., 1996; Pinckney et al., 1998). Additionally, seagrasses can serve as refuges from predators.

Figure 1.3: Map representing location of the study case and relative species in this systematic review.

Seagrasses hosts

All seagrasses presented different epibionts on their leaves (Figure 1.4), with amphipods and gastropods being the most reported taxa. The most studied seagrasses were *Zostera marina* (Linnaeus, 1753), *Posidonia oceanica* and *Thalassia testudinum* (Banks ex König, 1805). Among them, studies on *Z. marina* focused on different epibionts, such as amphipods (mainly *Gammarus locusta* (Linnaeus, 1758), *Gammarus oceanicus* (Segerstråle, 1947), *Microdeutopus gryllotalpa* (Costa, 1853), *Rissoa sp., Rissoa membranacea* (j. Adams, 1800), *Littorina littorea* (Linnaeus, 1758), *Idotea balthica* (Pallas, 1766) and *Ampullaceana balthica (Linnaeus, 1758)*, decapods, nudibranchs, polychaetas, isopods (mainly *Delesseria sanguinea* (Hudson) J.V.Lamouroux 1813)), sea anemones, molluscs, turbellarians and crustaceans. Reported epibionts for *Posidonia oceanica,* whereas, included different species of gastropods (*Bittium reticulatum* (Costa, 1778), amphipods (*Apherusa chiereghinii* (Giordani Soika, 1950)), molluscs (*Jujubinus striatus* (Linnaeus, 1758)), sea anemones, arthropods (*Dexamine spiniventris* (Costa, 1853) and *Gammarus* spp.) decapods and different species of marine invertebrates. While *Thalassia testudinum,* hosted amphipods, decapods (*Tozeuma carolinense* (Kingsley, J.S. 1878) and *Pagurus maclaughlinae* (Garcìa-Gomez, 1982)), protozoan ciliates (*Lagotia expansa* (Levinson, 1893), *Lagotia depressa, Metafolliculina andrewsi* (Hadzi, 1938), *Parafolliculina tristanensis* (Dons, 1948), *Acineta tuberosa* (Ehrenberg, 1833), *Cothurnia. ceramicola* (Kahl, 1933), *Cothurnia maritima (Ehrenberg, 1838), Parafolliculina amphora* (Dons, 1914), *Vorticella campanula* (Ehrenberg, 1831), *Chaetospira muelleri* (Lachmann, 1856)), suspension feeding bivalves (*Modiolus americanus* (Leach, 1815)) and gastropods (*Turbo castanea* (Gmelin, 1971) and *Thor manningi* (Chace, 1972).

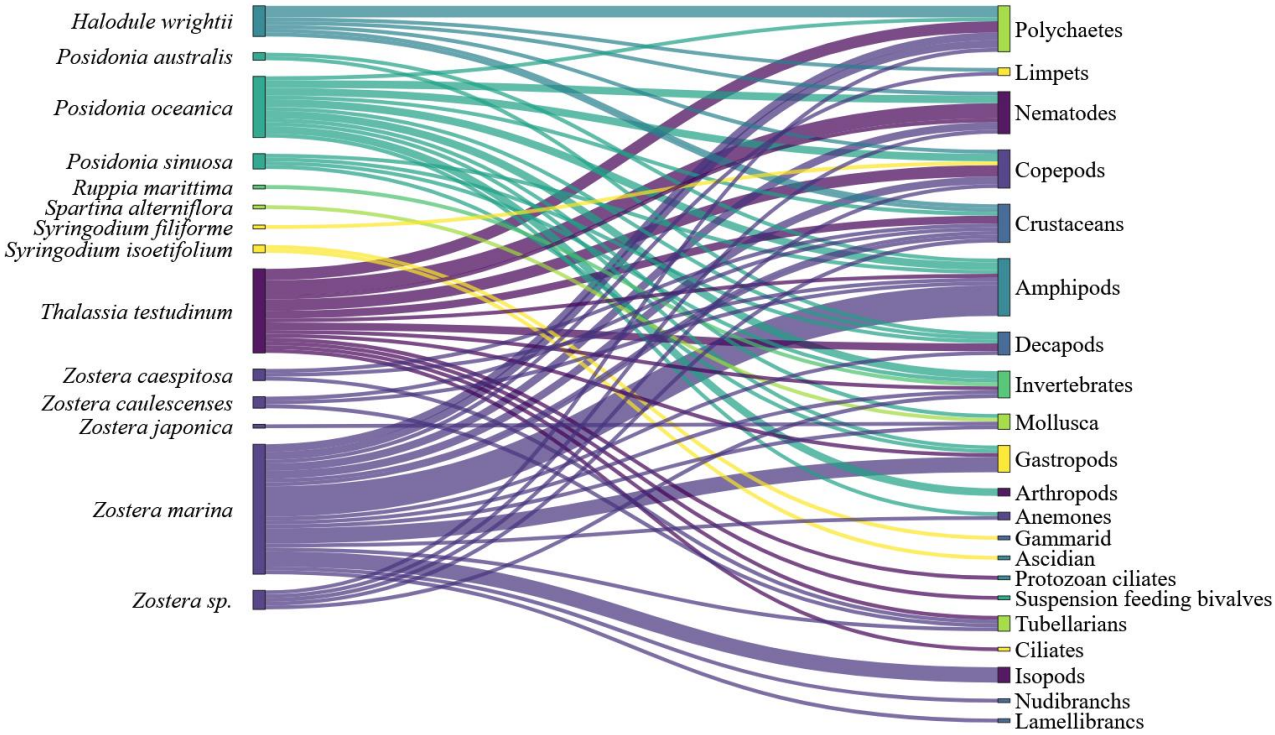

Figure 1.4: Interactions between seagrasses species and their relative epibionts reported in the analyzed case studies. The width of the bars plot fluxes is representative of the number of studies.

Regarding epiphytes on leaves of seagrasses (Figure 1.5), studies reported that *Z. marina* mainly presented green and brown algae, filamentous and encrusting diatoms (mainly *Cocconeis scutellum* (Ehrenberg 1838)) and filamentous algae (mainly *Delesseria sanguinea* (Hudson)); while *P. oceanica* hosted different species of epiphytes, epiphytic cyanobacteria, and periphyton. Finally, *T. testudinum* hosted, periphyton, epiphytes and epiphytic cyanobacteria.

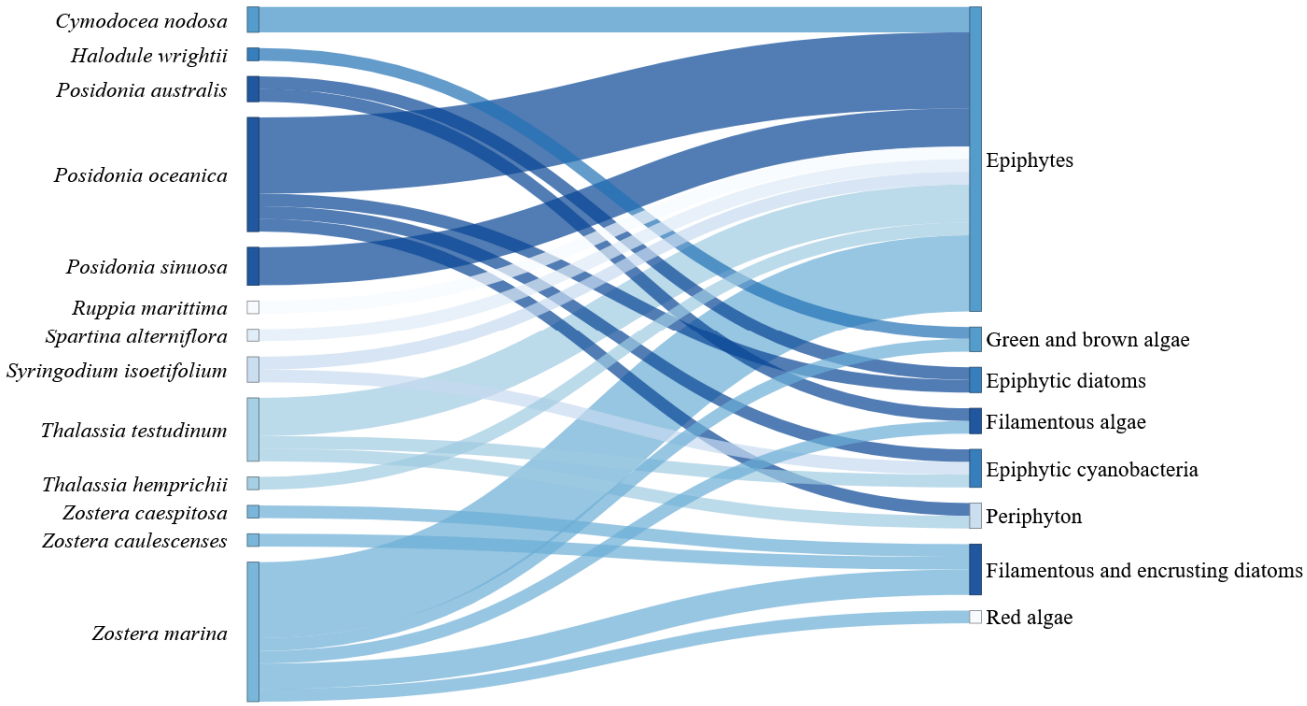

Figure 1.5: Interactions between seagrasses species and their relative epiphytes reported in the analyzed case studies. The width of the bars plot fluxes is representative of the number of studies.

Studies focused on macrofauna associated with seagrasses (Figure 1.6) showed that *Z. marina* was mainly associated with crabs (*Carcinus maenas* (Linnaeus, 1758), brittles star, fish (*Nerophis ophidion* (Linnaeus, 1758), *Syngnathus typhle, Micrometrus minimus (*Gibbons, 1854) *and Paralabrax clathratus* (Girard, 1854), and pen clam (*Atrina rigida*, (Lightfoot, 1786)). *Posidonia oceanica* leaves hosted fish (*Sarpa salpa*, (Linnaeus, 1758)), molluscs and sea urchins (*Paracentrotus lividus*, (Lamarck, 1816), while among *T. testudinum* leaves sea urchin (*Lytechinus variegatus* (Lamarck, 1816), fish (*Lagodon rhomboides* (Linnaeus, 1766)), crustaceans, turtles, waterfowl and dugongs were the main taxa reported.

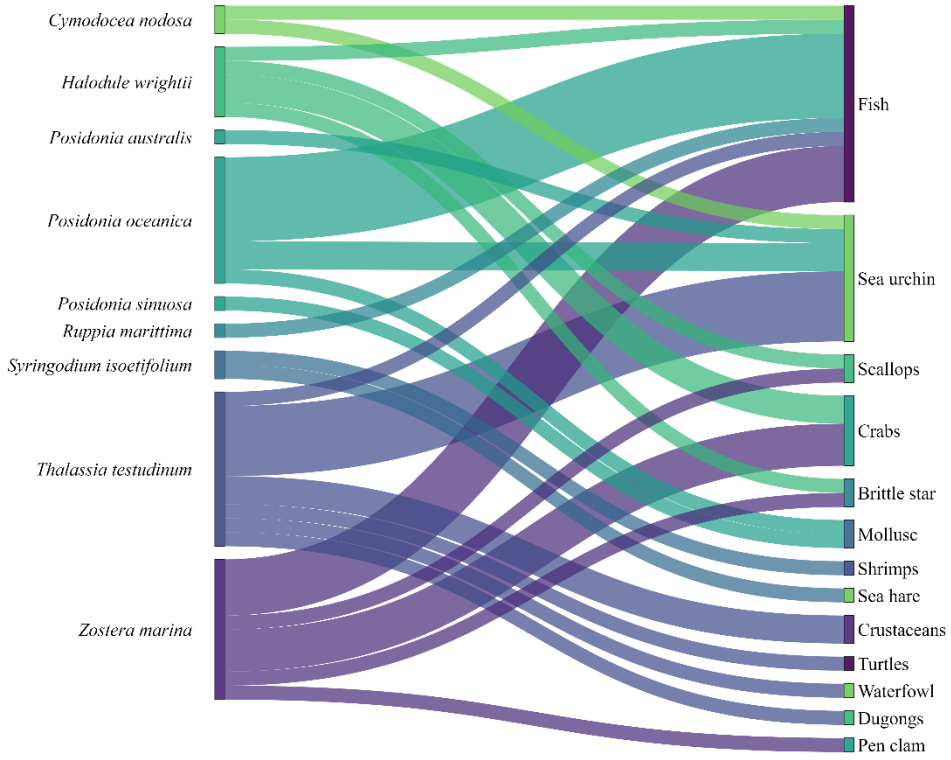

Figure 1.6: Interactions between seagrasses and macrofauna reported in the analyzed case studies. The width of the bars plot fluxes is representative of the number of studies.

Detected interaction

Studies highlighted both the benefits and drawbacks of biotic interactions between seagrasses and symbionts (Figure 1.7). For example, most of the studies focused on the positive role of epiphytes growing on seagrasses as a food source for epifauna and herbivores (Orth and Van Montfrans 1984; Horner, 1987; Klumpp et al., 1993; Martin-Smith, 1993; Mabrouk et al., 2021).

Epiphytes also improve the primary production of the system, protect seagrasses from desiccation caused by tidal and wind action, and finally improve biodiversity supporting epifauna community (Jernakoff et al., 1996; Klumpp et al., 1993; Michael Martin-Smith, 1993). Among them, diatoms and microalgae not only represent a source of food, but also improve the system's primary production and biodiversity (Jernakoff et al., 1996; Pinckney and Micheli, 1998; Michael et al., 2008); while cyanobacteria provides mainly food for epifauna (Yamamuro, 1999).

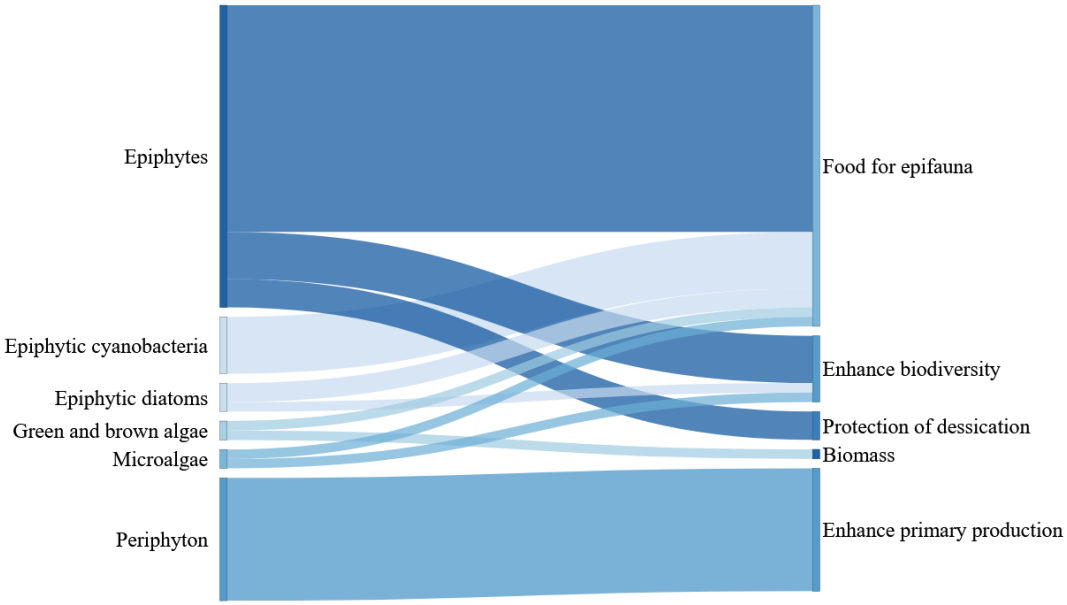

Figure 1.7: Positives roles of epiphytes interacting with seagrasses. The width of the bars plot fluxes is representative of the number of studies.

Epibionts living on seagrasses are organisms playing significant roles (Figure 1.8), increasing the physical complexity of the canopy, protecting seagrass from predation, and finally providing a very important trophic link between seagrasses and secondary consumers (Nakaoka et al., 2001).

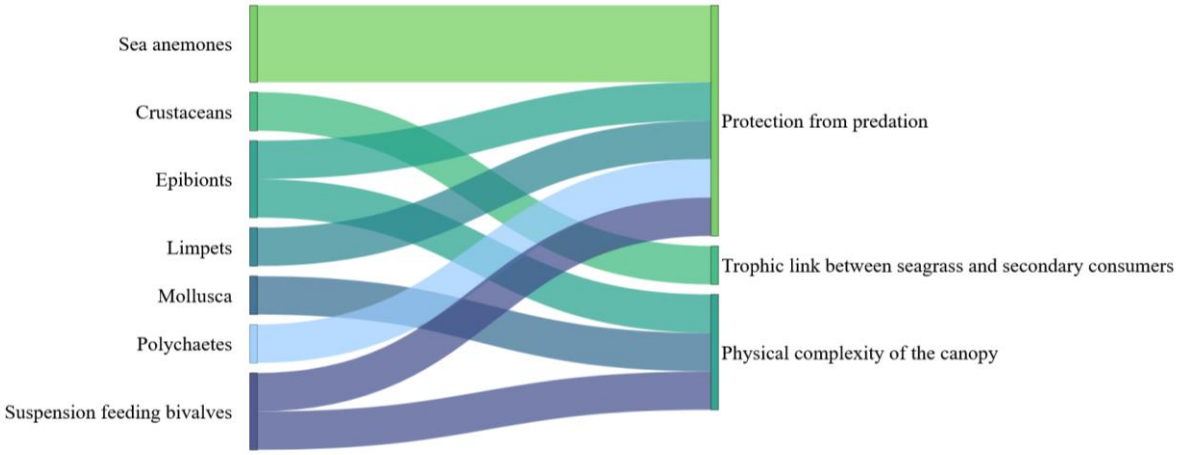

Figure 1.8: Positives roles of epibionts interacting with seagrasses. The width of the bars plot fluxes is representative of the number of studies.

Despite the many positive roles of epiphytes for their hosts and the associated macrofauna, they also can negatively affect their surrounding environment (Figure 1.9). They can limit light, significantly reducing seagrasses growth and photosynthesis, but also reducing nutrient exchanges between phanerogams and the water column (Jernakoff et al., 1996).

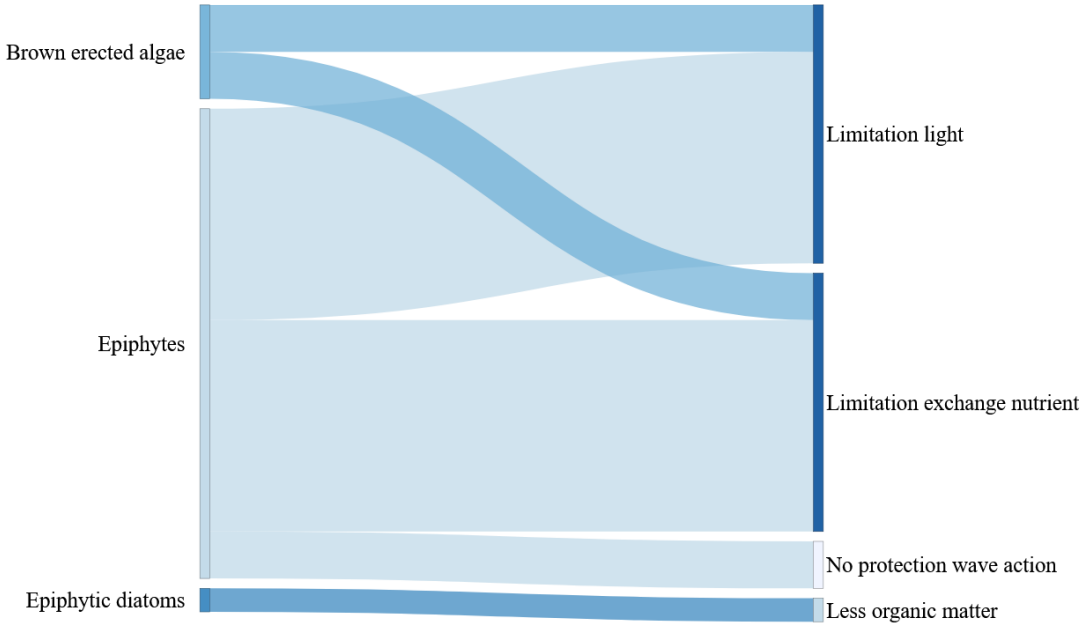

Figure 1.9: Negative role of epiphytes interacting with seagrasses. The width of the bars plot fluxes is representative of the number of studies.

1.4 Discussion

Present results revealed significant interactions between seagrasses and their symbionts (epibionts-epiphytes) emphasizing both the benefits and drawbacks for the habitat-forming species, as well as the indirect contribution of these biotic interactions to the community structure and functioning (grazers-epiphytes-seagrasses complex).

Spatial and temporal variability in epiphyte communities in hosting organisms has been widely studied (Vanderklift and Lavery, 2000; Saunders et al., 2003; Johnson et al., 2005). Seagrass leaves serve as a dynamic substratum for both sessile epifauna and epiphytes (Jernakoff et al., 1996), and their morphological traits can influence symbionts position and type of interaction. Variations in the diversity of epiphytes may be related to the seagrass species, the length of the leaves, the rate of growth or turnover of the leaves, the surrounding environment and the

availability and distribution of epiphyte propagules in terms of both space and time. Epiphyte colonization and community structure have been found to be influenced by light and temperature conditions (Robbins and Bell, 2000; Drake et al., 2003), grazing pressure, and/or nutrients (Gil et al., 2006; Lee et al., 2007; Prado et al., 2007). Moreover, water motion and the variety of algal propagules influence epiphyte growth, and colonization processes in general (Kendrick and Burt, 1997; Lavery et al., 2007).

Despite the advantages that seagrasses offer to symbionts (serving as refuge, substrate for epiphytes/epibionts strategic position in the leaf for the acquisition of food and light, etc.), seagrasses can inhibit the growth of epiphytes through the release of chemical compounds such as phenolics, that have an inhibitory effect on microalgae and bacteria (Mannino and Micheli, 2020).

The effects of epiphytes on seagrasses are among the most studied and reported in the scientific literature. One of the most positive roles of epiphytes within these interactions regards the protection of seagrass leaves from predation. Several studies have shown that epiphytes frequently make up the majority of the diets for herbivorous epifauna (Klumpp et al., 1993; Jernakoff et al., 1996; Andrade et al., 2024). Seagrass leaves and stems provide substrate where grazers-epiphytes interactions take place. Indeed, different taxa graze on epiphytes, directly or indirectly; for example, mugilids, hemiramphids and monacanthids and sparids have been reported to eat epiphytic algae in seagrass meadows and the epiphytes are more attractive than the seagrasses as food for grazing (Jernakoff et al., 1996). Also, Klumpp et al., (1993) reported that direct grazing of Australian seagrasses by fishes is probably insignificant in term of seagrasses removal, confirming that they preferred epiphytes and that the ingestion of seagrasses may be incidental. Grazing is a crucial process in seagrass ecosystems for three main reasons: it influences the productivity of the seagrass communities (Campbell et al., 2024; Jiménez-Ramos et al., 2024); it may affect the species composition of seagrass epiphytes (Andrade et al., 2024); and it provides a major trophic link for the cycling of nutrients (Klumpp et al., 1993; Jiménez-Ramos et al., 2024). There are a large diversity of grazers within seagrass meadows (Klumpp et al., 1993; Jiménez-Ramos et al., 2024), including fish, molluscs and crustaceans. In addition, filter feeding molluscs are considered important herbivores, filtering phytoplankton (Jiménez-Ramos et al., 2024). Filter-feeders transform suspended material by changing particle size distributions in the water column and by converting particulate material into dissolved constituents or biomass via metabolism. Peterson and Heck, (2001) proposed that bivalves could

create positive feedback loops where phytoplankton, consumed by suspension-feeding bivalves is quickly converted into ammonium (NH_4) for plant growth. Although their role in grazing the propagules of epiphytes is little known, it is insignificant compared to the grazing activity of gastropods (Jernakoff et al., 1996). Grazers can help control epiphytic biomass and productivity, thus reducing the potential negative effects of these on host organisms. This regulation can occur through different trophic levels, impacting seagrass ecosystems. The assemblage of small invertebrates living in seagrass beds can provide top-down control of epiphytes by directly grazing on epiphytic algae; while nutrient concentration, a bottom-up factor, can affect epiphytes and seagrasses differently (Touchette and Burkholder, 2000). The addition of nutrients can lead to either higher or lower seagrass productivity, with significant decreases often attributed to human-induced nutrient enrichment in coastal water. An abundance of nutrients can increase phytoplankton and epiphytes, restricting sunlight and harming seagrass growth (Ibarra-Obando et al., 2004).

A key element of seagrass-epiphytes interactions is related to the nutrient exchange between them (microalgae and seagrasses) (Orth and Van Montfrans, 1984). Indeed, population of bacteria on *Zostera marina*, may be supported almost entirely by carbon obtained directly from seagrass photosynthesis (Wahl et al., 2012). Seagrasses swing in response to wave action and currents, which may play a significant role in producing steep chemical diffusion gradients, eliminating potential growth-inhibiting substances, and clearing accumulated sediments (Waycott et al., 2019.). This movement improves the nutrient exchange and growth of epiphytes (Brodersen and Kühl, 2022).

Despite all these positive effects, the presence of epiphytes on seagrass leaves may entail also negative effects, such as limiting light absorption, leading to decreased growth of seagrasses (Heck et al, 2019; Jernakoff et al., 1996) and reducing photosynthetic rates (Fong et al., 2018; Hovel et al., 2016). They can compete for nutrients in the water column, and may also increase the susceptibility of seagrasses to leaf loss when subjected to physical forces such as wave-action (Prado et al., 2007). In addition, epiphytes can create a boundary layer around the seagrass leaf through which nutrients and oxygen may diffuse to be available for metabolism of the seagrasses (Burkholder et al., 2007).

Also meiofauna (epibionts) associated with seagrasses has an important role in seagrass communities. Marine epibionts are organisms that live on leaves of seagrasses, playing important roles because of their interactions with the host, their contribution to primary

production, their role as food source for heterotrophic fauna (Jernakoff et al., 1996; Ibarra-Obando et al., 2004; Crespo et al., 2014), and contributing to the canopy physical complexity (Nakaoka et al., 2001). For example, Lewis and Hollingworth (1982) suggested that meiofauna associated with seagrass might exploit epiphytic algae which foules seagrass blades. Coull and Bell (1979) referred that these epibionts may play an integral role as nutrient regenerators or food for higher trophic levels in shallow waters. Aladro-Lubel and Martínez (1999) reported that the seagrasses are one of the most important organic substrates for the establishment of epibiotic organisms diversity. The most commonly reported epibionts interacting with seagrasses included sea anemones, hydroids, bryozoans and polychaetas (Guilini et al., 2017). The composition of epibiont communities may act as a predictor of environmental conditions and water quality (Giovannetti et al., 2010). Any disruption of these species, caused by the presence and accumulation of potentially harmful compounds in the water, could impact these communities and, in turn, higher trophic levels.

Results showed that epiphytes represent overall a source of food for epifaunal organisms and play an important role in the trophodynamics of seagrass communities. They can enhance primary production thanks to periphyton, contributing significantly to the overall productivity of seagrass meadows (Fong et al., 2000; Drake et al., 2003). Moreover, they can reduce water movement and protect littoral seagrasses in the low tide from desiccation and insolation (Valdez et al., 2020), contributing to the biodiversity of these systems, and helping to determine the composition of mobile assemblages.

The total primary production of the systems is increased by photosynthetic epiphytes (Borowitzka et al., 2006), and this productivity can equal or surpass that of farmed terrestrial ecosystems (Borum, 2004; Hauxwell and O'Rourke, 2020). On the other hand, epibionts contribute to seagrasses systems trophic dynamics and enhance canopy structural complexity. Overall, epiphytes and epibionts are considered sensitive indicators of “natural” and long term environmental variation as their biomass corresponds to changes in nutrient conditions (Piazzi et al., 2016; Chen et al., 2021). They may respond to environmental changes more quickly than the seagrasses themselves (Piazzi et al., 2016). Further research on the interactions between seagrasses and their epiphytes/epibionts is crucial to understanding how changes in the strength of these interactions can affect the ecological functions of seagrass meadows, and how these effects may shift under changing environmental conditions, such as those associated with climate change. Biotic interactions, such as those between seagrasses and symbionts, can either enhance

or hinder the ability of seagrasses to adapt to changing environmental conditions, influencing ecosystem resilience and services related to carbon storage, water quality improvement and habitat provisioning. In summary, understanding these interactions may significantly guide biodiversity conservation efforts, and ensure the sustainability of crucial ecosystem services.

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Chapter 2- Unveiling ecological and structural changes in a Mediterranean coastal lagoon: current knowledge and key gaps in the face of environmental change.

Abstract

Coastal lagoons are highly productive ecosystems contributing to the overall productivity of coastal waters by supporting high levels of biodiversity and primary production, also playing a key role in local fisheries. Despite their ecological, economic, and social value these semi-enclosed ecosystems are sensitive to environmental changes such as eutrophication, pollution, biological invasions, and habitat alteration, which could influence habitat structure and biodiversity composition. The Stagnone of Marsala is a shallow semi-enclosed coastal lagoon in the western coast of Sicily that is not only relevant for its historical and archaeological value, but also for the hydrodynamic system (low depths of the northern basin with high evaporation and significant effects on surface currents due to the presence of different islands within the lagoon) and high associated biodiversity. Here, a review was performed to investigate the changes in structure and functioning of the Stagnone of Marsala in the last 30 years. To this end, we applied the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, by creating and searching a complex string in the online databases Scopus, WoS and Google Scholar, as well as different sources of grey literature, yielding a total of 51 outcomes. Overall, result covered monitoring information on a broad range of habitats, including seagrass meadows (mainly *Posidonia oceanica* and *Cymodocea nodosa*) and macroalgae (mainly *Cystoseira spp*., *Caulerpa prolifera* and *Chaetomorpha linum*), where physical variables (mainly temperature and salinity) were related to sediment and water column biochemical characteristics, and biological ones referred to species richness and communities composition, in a temporal range between 1990 and 2023.

2.1 Introduction

Coastal lagoons are recognized as transitional environments situated between continental and marine aquatic systems, covering approximately 13% of the world's coastlines (Pérez-Ruzafa et al., 2019). These lagoons exhibit a complex spatio-temporal diversity, distinguishing them from other transitional systems (Pérez-Ruzafa et al., 2019). One of the factors contributing to this diversity is the presence of various gradients of abiotic variables, including temperature,

oxygen, pH, and salinity (Martínez-Megías and Rico, 2022). These highly productive ecosystems play a crucial role in the overall productivity of coastal waters by supporting rich biodiversity, acting as hotspot of endemic species, and supporting migratory birds' populations (Pitacco et al., 2018). Moreover, they are key habitats that contribute to nutrient cycling and carbon sequestration ecosystem processes, emphasizing their significance in the regulation of key environmental functions (Martínez-Megías and Rico, 2022). They are also considered as important nursery areas for local fish populations, providing essential support for fish reproduction and recruitment (Erzini et al., 2022). The ecological and socio-economic value of coastal lagoons is substantial, as they offer fundamental ecosystem services that benefit not only biodiversity but also local fisheries and tourism sectors (Pitacco et al., 2018). These services encompass a range of ecological functions and socio-economic contributions, underscoring the importance of conserving and managing these unique coastal ecosystems.

Due to their features these semi-enclosed ecosystems are sensitive to environmental changes such as eutrophication, pollution, biological invasions, and habitat alteration, which could influence habitat structure and biodiversity composition (Martínez-Megías and Rico, 2022). The combined impacts of multiple drivers and global environmental changes pose significant threats to the sustainability of ecosystem functions and services, leading to cascading effects at various levels of the ecological hierarchy, from individuals to populations, communities, and entire ecosystems (McLean et al., 2019). Climate change has emerged as a major driver of change in coastal lagoons, significantly altering key environmental factors such as salinity, temperature, sea-level rise, sediment dynamic, and primary production processes (Pitacco et al., 2018). Other anthropogenic drivers of disturbance such as urbanization, fishing, industrial and touristic activities (i.e., salt production and aquatic-recreational activities) and the introduction of non-native species, highly contributed to habitat degradation, altering the community structure by benefitting generalist species detriment specialized ones, modifying biotic interactions and provoking biodiversity loss (Ligorini et al., 2022; Mazzola and Vizzini, 2005). The introduction of non-native species causes an alteration of the habitat, modifying the diversity, the ecosystem functioning and impacting human activities (Marchessaux and Belloni, 2021). Invasive species can generate both local impacts, such as biodiversity loss, and regional and ultimately ecosystem-wide impacts (Ligorini et al., 2022).

Thus, understanding and predicting the impact that multiple stressors may have on the structure and functioning of Mediterranean coastal lagoons is crucial to ensure their long-term

sustainability as well as to meet the quality standards set by the international regulatory frameworks that protect them, e.g., the International Ramsar Convention (Ramsar Convention Secretariat, 2010) or the Water Framework Directive for European countries (European Commission, 2000) (Martínez-Megías and Rico, 2022).

This study aims to investigate the temporal and spatial variation of biodiversity composition within the Stagnone of Marsala lagoon (Sicily, Italy) over the last 30 years, exploring potential effects of environmental changing conditions on its habitats. Specifically, our objectives are to: (1) summarize the current state of knowledge about the lagoon's ecological communities and environmental conditions; (2) identify spatial and temporal trends in biodiversity composition and habitat changes; (3) assess the available information on environmental variables and their potential influence on ecological communities; and (4) highlight key knowledge gaps and research needs for better understanding the lagoon's structure and functioning in the face of environmental change.

2.2 Materials and methods

2.2.1 The Stagnone of Marsala

The Stagnone of Marsala is a coastal lagoon located on the western side of Sicily, Italy (37.83°N, 12.45°E; Fig. 2.1).

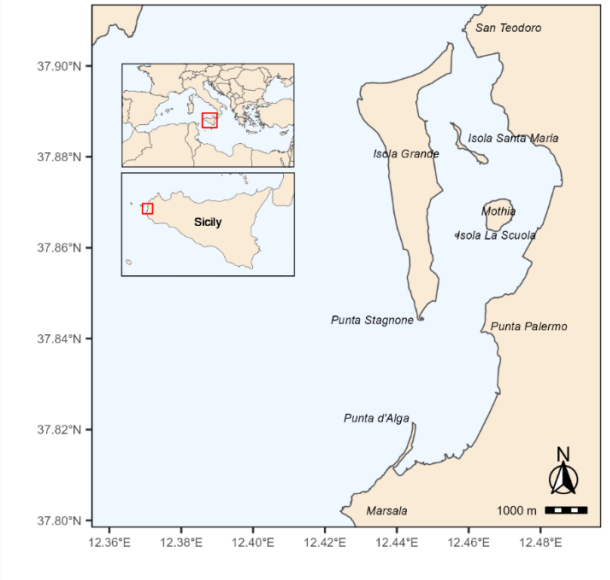

Figure 2.1. Map of Stagnone of Marsala coastal lagoon (Sicily, Italy).

Spanning an area of 21.35 km^2, it is an oligotrophic semi-enclosed brackish coastal lagoon with dimensions of approximately 1.8 km in width and 11 km in length. The lagoon features shallow waters ranging from 0.3 to 3 meters and sandy-silty bottoms, with some calcarenite outcrops (Mazzola and Vizzini, 2005; de Marchis et al., 2012). Its western boundary is formed by the narrow calcarenite tableland of Grande Island, which stretches for 7 km in a north-south direction. The lagoon is divided into two sub-basins, northern and southern, by an emergent *P. oceanica* reef barrier called Récif-barrière (Calvo and Frada` Orestano 1984), situated between Punta Palermo and Punta Stagnone. The presence of this barrier greatly decreases the wave action even in the outermost part of the lagoon (Longo et al., 2016). The northern sub-basin (14 km^2, mean depth 1 m) connects to the open sea through a shallow channel (mean depth ca. 30 cm) approximately 400 meters wide at San Teodoro, characterized by turbulent inflows of seawater (Fig. 2.1). In the past, an artificial channel was dredged parallel to the west coast of the northern mouth, measuring about 10 meters wide and 1.0 meter deep, with the purpose of enhancing local flushing capability (De Marchis et al., 2012) (Fig. 2.1). The presence of three islands (Santa Maria, Mothia and La Scuola), as well as a submerged ancient Phoenician Road, hinders water circulation within the lagoon, resulting in a renewal time of about 65 days (Bellissimo and Orestano, 2014). This sub-basin, presents a wider variability in temperature and salinity, typical of coastal lagoons, than the southern basin (Sarà et al., 1999).

The southern sub-basin, covering an area of 6 km^2 with depths ranging from 1 to 1.5 meters, is connected to the open sea through a wide mouth (approximately 2.5 km) between Punta dello Stagnone and Punta D'Alga, allowing for greater water exchange and turnover (approximately 22 $m^3\ s^{-1}$) (La Loggia et al., 2004; De Marchis et al., 2012; Bellissimo and Orestano, 2014). In this area, *P. oceanica* forms an extensive reef platform known as Plateau recifale (Calvo and Frada-Orestano, 1984). The hydrodynamic circulation within the lagoon is primarily driven by tidal and wind forces, particularly from the northwest. The impact of seasonal freshwater discharges and sporadic watershed runoff is negligible. The lagoon is characterized as oligotrophic, as evidenced by the low annual concentrations of nutrients (PO_3-: 0.03 µg L^{-1}; NH_3: 0.6 µg L^{-1}; NO_2-: 0.01 µg L^{-1}; NO_3-: 2.0 µg L^{-1}) and low annual Chlorophyll-*a* concentration (~1.0 µg L^{-1}) (Maimone et al., 1998; Sara et al., 1999; Mazzola and Vizzini, 2005). Stratification is absent in the water column due to the shallow depth and small tidal range (Bellissimo and Orestano, 2014). However, seawater temperature and salinity exhibit significant variation across seasons and different areas of the lagoon. In winter, salinity displays minimal variation among

the different parts of the lagoon (37.23 ± 0.65 psu), with the lowest values at 33 psu. Conversely, during summer, the lagoon can experience hypersaline conditions, with salinity reaching maximum values of approximately 48 psu due to limited water circulation and high evaporation rates. The north sub-basin tends to be saltier than the south sub-basin. Seawater temperatures follow a similar pattern within the lagoon, ranging between approximately 10 °C and 30 °C (Giani et al., 1996; Mazzola and Vizzini, 2005).

2.2.2 Research methodology

The systematic review was performed applying the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline proposed by Liberati et al. (2009). The PRISMA method was applied to explore the following research question: How has the composition of the ecological communities in the Stagnone of Marsala changed over time?, and a secondary question: How has environmental change influenced the potential variation in the biotic component of the lagoon?.

A literature search was conducted on electronic and printed scientific papers, as well as grey literature (which mainly consisted of PhD and master's theses carried out at the University of Palermo). The PICO (Population, Intervention, Comparison and Outcomes) approach (Liberati et al., 2009) was used for defining the keywords that were combined with various Boolean operators to finally create simple and complex search strings that were ran in Elsevier's Scopus (www.scopus.com), Clarivate Web of Science (www.webofknowledge.com), and Google Scholar (https://scholar.google.com) online databases (accessed on 8 November 2023; Table 3.1). The research question was determined incorporate the key elements, using the PICO elements: Population of interest (P): biotic (species) and abiotic (habitat) lagoon in Stagnone of Marsala ecosystem components; Intervention (I): all ecological studies conducted in this lagoon; Comparator (C): different response of communities to climatic stressor, and Outcomes (O): Measured biological responses at population and community levels. General search strings were created and run to capture all information on the Stagnone of Marsala from the scientific literature: i) "(Stagnone OR lagoon OR saltmarsh OR estuar* OR "water transition") AND (Marsala OR Trapani)", ii) "(Marsala AND lagoon)" and iii) "Marsala AND (lagoon OR Stagnone)".

Table 2.1: Search strings used in this systematic review and outputs from the online databased explored (Scopus and Web of Science – WoS). The asterix (*) following a search word has been used for considering and accept all word variation in the search.

Complex search string (search at 14/04/2022)	Scopus "Title-Abs-Key "	Wos "Title-Abs-Key "
(stagnone OR lagoon OR saltmarsh OR estuar* OR "water transition") AND (marsala OR trapani)	45	48
(Marsala AND lagoon)	34	29
Marsala AND (lagoon OR Stagnone)	43	47
Simple search string (search at 14/04/2022) on Google Scholar		
(stagnone OR lagoon OR saltmarsh OR estuar* OR "water transition") AND (marsala OR trapani)	97	

The results were reported accordingly to the PRISMA protocol, including the following steps: (1) Identification, (2) Screening, (3) Eligibility and (4) Included criteria. The first step is based on records identified through database searching (before checking for duplicates) and duplicates removal; the second step is the papers screening by title and abstract. The third and the fourth steps are based on a set of selection of exclusion and inclusion criteria, a more detailed screening, at full text level (Liberati et al. 2009). The inclusion criteria for this study were as follow: studies conducted in the lagoon of Stagnone of Marsala, analysis, and monitoring of both biotic and abiotic components within the lagoon, and relevant typologies of analysis. On the other hand, the exclusion criteria encompassed off-topic studies, such as those conducted in the lagoon of Trapani or studies specifically focused on Mozia. Additionally, incomplete chapters in books were also excluded. Subsequently, data from the selected records were extracted using a structured matrix. The matrix included various information such as the time of publication, habitat biocenosis, georeferenced location (latitude and longitude), type and time of monitoring, recorded species, or habitats measured abiotic variables, and measured ecological response variables.

2.3 Result

A total of 122 records were identified at Web of Science, 124 at Scopus, 97 at Google Scholar and 14 from grey literature, totaling 357 records. Of these, after the removal of duplicates and after the title-abstract screening only 51 records were adequate for the study, that resulted in 3895 cases studies (Figure 2.2).

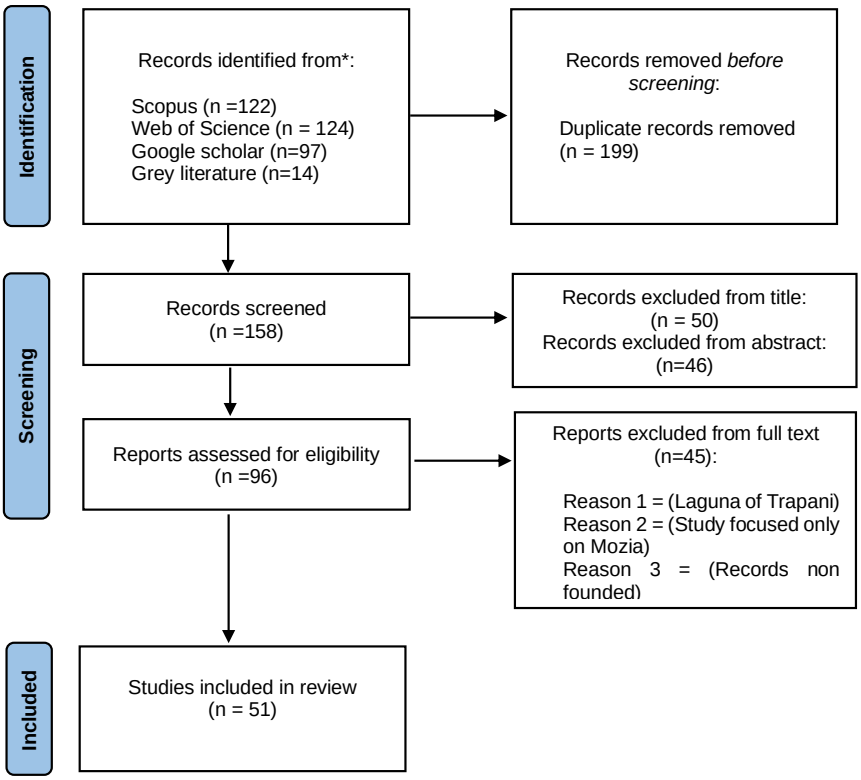

Figure 2.2: Flow of information through the different screening phases of the present review.

2.3.1 Monitoring temporal range and spatial extension

The records selected covered a temporal range of 33 years spanning from 1989 to 2023 (Figure 2.3). During the period between 1999 and 2009 there was an increased interest in the coastal lagoon, concentrating in less than 10 years more than 70% of the total monitoring case studies. Almost all analyzed records were scientific publications (84%), and just 16% of the total items consisted of grey literature, specifically bachelor's and master's theses. Based on the keywords extracted from the records, the main research topics in the lagoon were related to the characterization of sediment and water biochemistry, as well as the identification of biodiversity and the distribution of different habitat structuring species (mainly *Posidonia oceanica* (L.) Delile, and *Cymodocea nodosa* (Ucria) Ascherson, etc).

Due to the structural and hydrodynamic particularity of the Stagnone of Marsala the results of these review were reported dividing the lagoon into three sectors: north, spanning between

37.88 and 37.90 in latitude; center, between 37.86 and 37.89; and south, between 37.83 and 37.85.

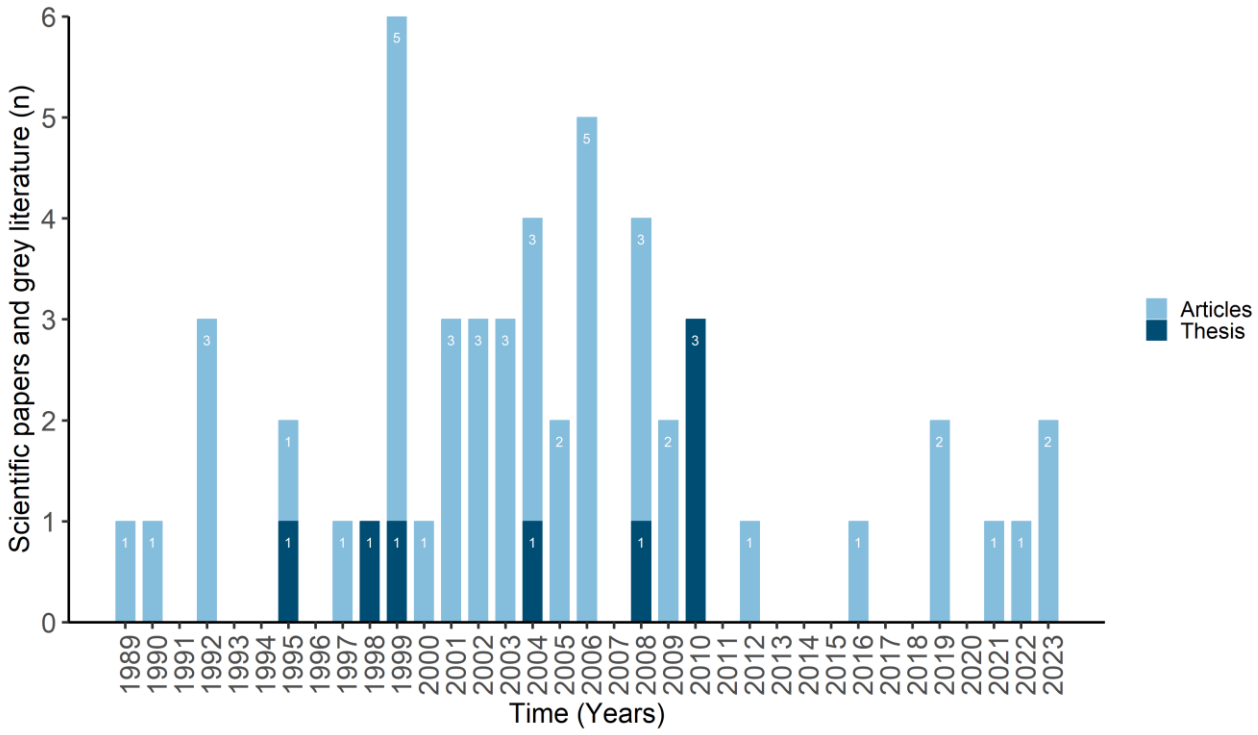

Figure 2.3: Temporal trend of published papers and thesis used in this study.

This allowed to observed that the central area presented the highest level of monitoring (Figure. 2.4), from 1984 to 2014, with a wide information collection focusing on different habitats including seagrass meadows, as well as macroalgae such as *Cystoseira* spp., *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, and *Chaetomorpha linum* (O.F.Müller) Kützing.

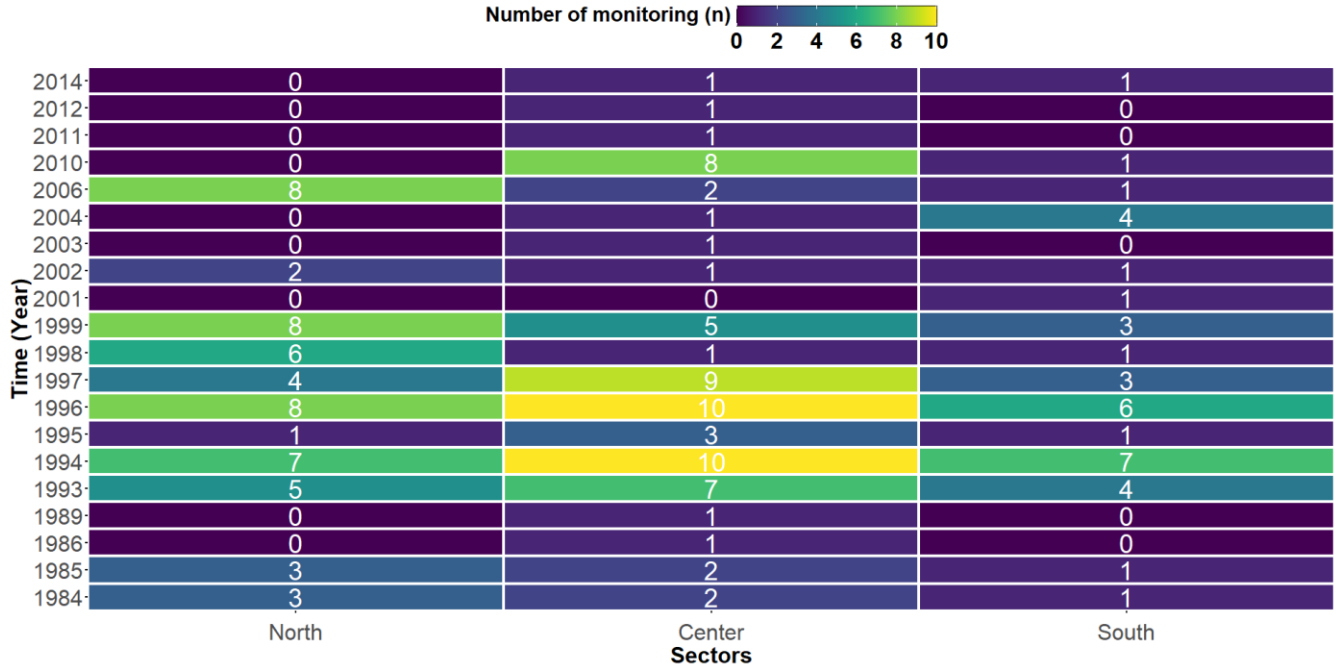

Figure 2.4: Heatmap of trend of number of monitoring in each study cases within the lagoon section area.

2.3.2 Main studied topics

Most of the included papers/reports primarily focused on habitat characterization, including response variables related to water and sediment biochemistry (32.61%), species richness (10.86%), trophic ecology (14.13%), and habitat extension (15.22%). In particular, the most used response variables for the biochemical characterization of habitats included the measurement of lipids, carbohydrates, and proteins, analysis on chl-*a* and phaeopigments, assessment of biopolymeric and bioavailable organic carbon, as well as the concentration of carbon in sediment and total organic matter (Pusceddu et al., 1997; Mazzola et al., 1999; Pusceddu, 1999; Pusceddu et al., 1999; Mazzola et al., 2000; Pusceddu et al., 2003). Case studies focused in trophic ecology included studies utilizing isotopic analysis, specifically $\delta^{13}C$ and $\delta^{15}N$, for ichthyofauna, macroalgae, and phanerogams to understand their trophic interactions within the lagoon (Vizzini et al., 2002; Vizzini and Mazzola, 2002; Vizzini et al., 2003; Vizzini and Mazzola, 2005; Vizzini et al., 2006; Vizzini et al., 2008; Andolina et al., 2022). Habitat extension records included information on abundance, density, and composition of fish, sponges and other invertebrates associated with seagrass meadows and sandy bottoms in the Stagnone (Mirto et al., 2004; Mercurio et al., 2006; Longo et al., 2016; Bellino et al., 2019; Mercurio et al., 2021).

Some studies focused on experimentally test the effect of the temperature, salinity, and food concentration on the physiological performance of *Brachidontes pharaonis*, an invasive Lessepsian bivalve, considered a foundation species within the Stagnone of Marsala (Sarà et al., 2008, 2018, 2021). Mancuso et al. (2023) focused on the adaptation to hypersaline conditions on *Posidonia oceanica* from the lagoon. La Loggia et al. (2004) investigated how local hydrodynamic conditions may influence the primary production and fate of this seagrass. The results of this study suggest that factors such as water circulation, which control temperature and salinity, could influence the metabolism and distribution of the habitat-forming species.

Just two scientific papers focused on the hydrodynamic circulation of the Stagnone of Marsala, the first use both experimental measure and numerical simulations (De Marchis et al., 2012), and the second (Campolmi et al., 2001) focused on the influence of the hydrodynamic conditions on fluctuations in zooplankton abundance and changes in population structure.

2.3.3 Spatial and temporal changes in biodiversity

Of the total studies included in the review, 16.5% focused on seagrasses and/or macroalgae. Most of the studies concerning *P. oceanica* focused on its trophic ecology, while only one study examined the epiphytic hydroid community associated with both *P. oceanica* and *C. nodosa* (Piraino and Morri, 1990). Additionally, two studies investigated the growth performance and flowering rates of *P. oceanica* (Tomasello et al., 2009; Mancuso et al., 2023).

Studies focusing on biodiversity within the lagoon identified over 200 different species. Of the total case studies, 6.6% focused on Porifera, 24.2% on ichthyofauna, and 8.8% on the zooplankton community (Figures 2.5, 2.6). Overall, the ecological community composition changed over time, with some species apparently disappearing, while others remained or were replaced. For example, as reported in La Loggia et al. (2004), meadows of *P. oceanica* within the Stagnone of Marsala were regressing with the presence of different dead mattes (Calvo and Orestano, 1984). Indeed, it was observed that *Cymodocea nodosa* and *Caulerpa prolifera* meadows largely replaced the highly structuring seagrass meadows with changes in the hydrodynamic conditions and sediment composition within the lagoon (Calvo et al., 1982; Bellissimo and Orestano 2014: Pergent et al., 2014). Different species of Porifera, as reported by Longo et al. (2016), exhibited marked differences in taxonomic composition, as well as spatial and temporal distributions in the Stagnone of Marsala. A total of 47 sponges species were recorded, following different spatio-temporal dynamic over the study's monitoring period (1990,

2001, 2010). Species richness changed slightly over time, with 41 porifera species in 1990, 47 species in 2001 and 45 in the last monitoring time in 2010. Stable population of some species were observed over time such as *Clathrina coriacea* (Montagu, 1814), *Clathrina rubra* (Sarà, 1958), *Aplysina aerophoba* (Nardo, 1833). Some other species were initially present (*Phorbas topsenti* (Vacelet and perez, 2008), *Halichondria semitubulosa* (Lieberkuhn, 1859)), but disappeared with time; while other appeared lately, being recorded just during the last monitoring period in 2010 (*Tethya meloni*, (Corriero, Gadaleta and Bavestrello, 2015)). Specifically, Mercurio et al. (2021) studied the occurrence and distribution over time of *Geodia cydonium*, a sponge species often associated with rhizomes of *Posidonia oceanica.* They observed a regression in the species distribution from 1997 to 2021 along the coasts of the Stagnone, potentially due to changes in the hydrodynamism of the lagoon.

Ichthyofauna composition was widely reported in the analyzed scientific and grey literature (e.g. Cavallaro et al., 1977; Sarà et al. 1996; Scilipoti, 1998). Overall, the fish stock within the lagoon consisted of resident species: *Atherina boyeri* (Risso, 1810), *Aphanius fasciatus* (Valenciennes, 1821), *Syngnathus abaster* (Risso, 1827) *and Syngnathus typhle* (Linnaeus,1758), *Pomatoschistus tortonesei* (Miller, 1869), *Gobius niger* (Linnaeus, 1758), etc.; and migratory species (n = 30) that use the lagoon occasionally or as a nursery area: *Chelon auratus* (Risso, 1810), *Sarpa salpa* (Linnaeus, 1758), *Chelon ramada* (Risso, 1810), *Chelon saliens* (Risso, 1810), *Diplodus puntazzo* (Walbaum, 1792), etc. Spatial and temporal distribution of ichthyofauna was studied (Figure 2.5c), observing that the spring months presented the highest levels of biological diversity in the fish stock due to the significant input of migrant species. Conversely, the summer months show a significant reduction in diversity values for specific richness and equitability (Sarà et al. 1996). Few studies focused on distribution of fish populations in relation to some environmental characteristics, underlining that *Atherina boyeri* (Risso, 1810), is the most abundant species present within the Stagnone, followed by different *Syngnathus* species, whose distribution was positively correlated with density of *C. nodosa* meadows.

Recently, Bardelli et al. (2023) focused on the potential establishment of a population of blue crab *Callinectes sapidus* in the Stagnone of Marsala, recorded in the southern sector of the Stagnone. A total of 22 specimens of crabs (five males and 19 females, witch three were ovigerous) were sampled in five sites within the Stagnone of Marsala (in the northern and southern area, but not in the center of the lagoon).

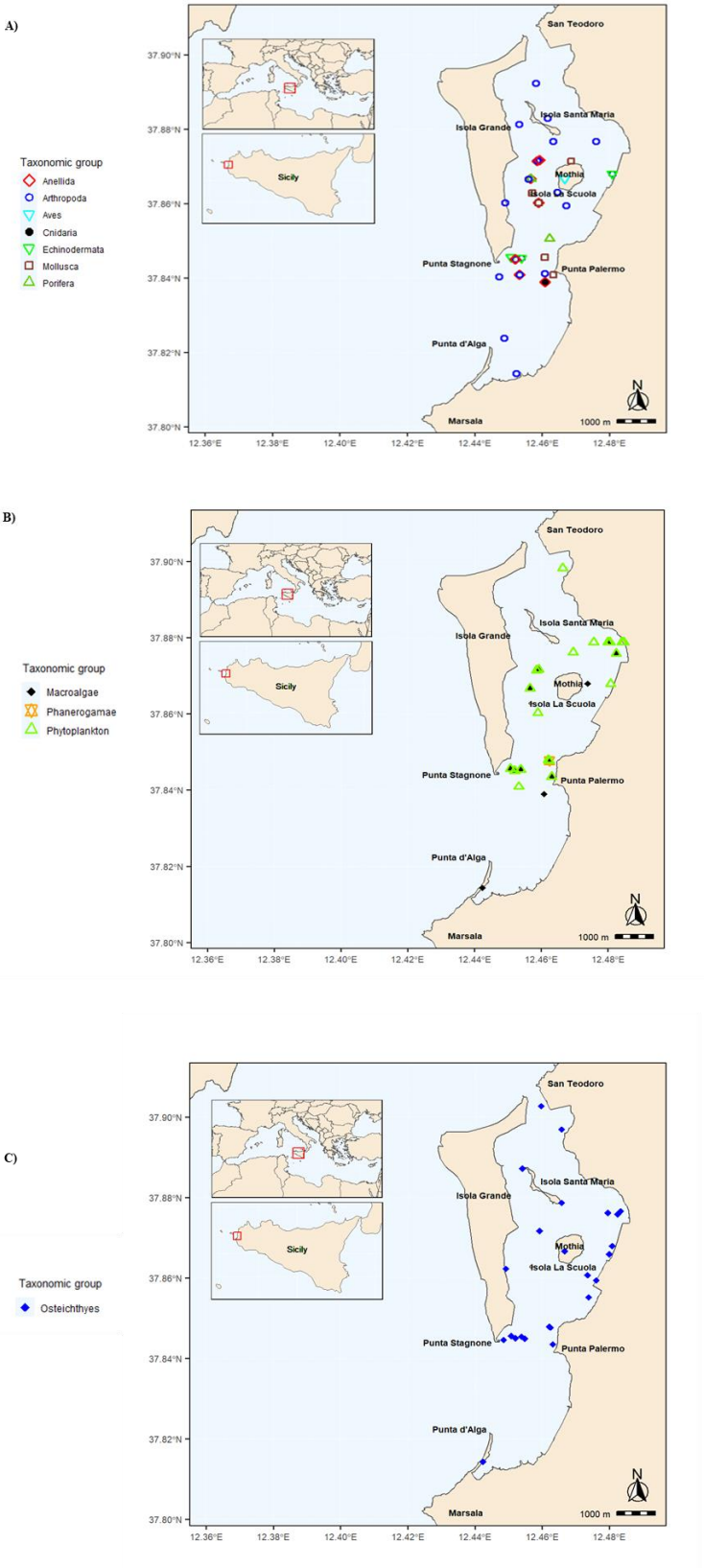

Figure 2.5: Spatial distribution of taxonomic groups (A, B, C) within the Stagnone of Marsala lagoon.

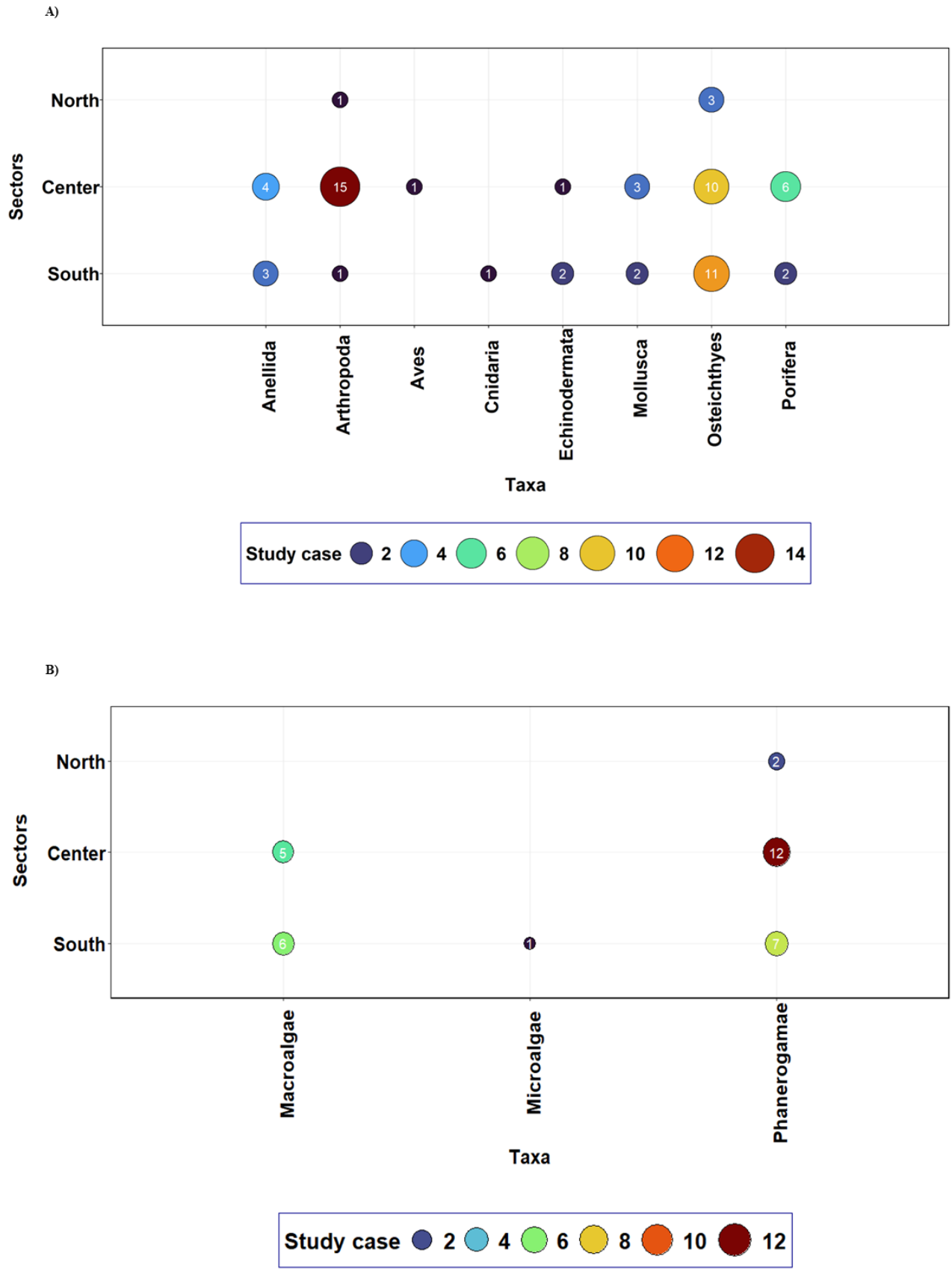

Figure 2.6: Bubble charts reporting the number of case studies for each taxonomic groups (A, B).

2.3.4 Trophic structure

Spatial and temporal variability in trophic structure within the Stagnone of Marsala has been extensively studied. Different studies focused on stable carbon and nitrogen isotopes on primary producers (*Posidonia oceanica, Cymodocea nodosa*, *Enteromorpha* sp., *Chaetomorpha linum* (Kützing, 1845)) (Vizzini et al., 2002, 2003, 2005; Costa et al., 2018), and consumers, particularly fish (*Atherina boyeri, Mullus surmuletus* (Linneo, 1758), *Sarpa salpa, Diplodus puntazzo, Syngnathus abaster* and *Syngnathus typhle*) (Mazzola et al., 1999; Lopiano et al., 2001; Vizzini and Mazzola, 2001, 2004, 2005; Andolina et al., 2022). High values of $^{13}\delta C$ of fish from the Stagnone suggest that they rely ultimately on seagrass and epiphytic algal material, and not on the main available source (macroalgae), a component of primary importance in the other lagoons. Moreover, seagrasses' broad distribution and quantity indicated that they were a significant final food source for fish in the Stagnone of Marsala (Vizzini et al., 2002; Vizzini and Mazzola, 2006). Fish use of organic material from seagrass meadows is consistent with findings from earlier research on the role that seagrass and epiphytes play in providing food for consumers in seagrass meadows from coastal lagoons (Vizzini et al., 2002; Vizzini and Mazzola, 2006).

2.3.5 Environmental and biochemical variables.

Different studies have demonstrated the oligotrophic status of the Stagnone di Marsala (Pusceddu et al., 1997; Sarà et al. 1999; Pusceddu et al., 2003). Due to the oligotrophic nature of the adjacent marine system, nutrient input primarily comes from internal recycling, driven by a high density and biomass of heterotrophic bacteria (Mirto et al., 2004). These nutrients are absorbed in the low-turnover seagrass beds, mainly present in the southern sector of the lagoon. Regarding the composition of sediment organic matter, the uncoupling between large amounts and relatively low nutritional value of sedimentary organic matter suggested that the Stagnone di Marsala may act as a detrital ‘trap’ (Pusceddu et al. 1999). These information allows a fuller understanding of the nutrient dynamics in the Stagnone, where low nutrient availability in the water column coexists with internal recycling and the accumulation of detrital organic matter in sediments.

Regarding environmental conditions, it is well known that environmental conditions (especially temperature) play a fundamental role in defining species' ecological niches and the structure and composition of ecological communities. Despite the great effort to characterize the ecological communities of the Stagnone and its trophic status, there is still a significant lack of

knowledge regarding the potential cause-effect between environmental conditions and ecosystem functioning. Data refer to temperature, salinity and chlorophyll are limited to point data, often consisting in single measurements representing the entire lagoon with low temporal resolution. For this reason, it has not been possible to conduct a correlation analysis between changes in environmental variables and community composition over time.

2.4 Discussion

The review was able to summarize the current status of knowledge about the ecological studies carried out in the Stagnone di Marsala, between the 1989 and 2023. Outputs from this review have provided a general overview of the research carried out in one of most important Mediterranean coastal lagoon and the level of ecological knowledge on the lagoon structure and functioning. Also showed that the Stagnone of Marsala is an ecologically significant coastal lagoon, as evidenced by the extensive scientific research conducted over a span of more than 30 years, with the first scientific work published in 1989. Indeed, in general after the apparent decrease in the interest of the scientific community in the study of coastal lagoons, and the disappearance of specific Committees such as this one of the CIESM, the number of publications appearing in high impact journals has increased significantly in the last two decades, and now covers ecosystems throughout the world (Pèrez et al., 2011).

Even if the lagoon is a target site for monitoring and ecological studies since 1989 (date of the first scientific publication focused on the Stagnone), there is a partial understanding of its dynamics, communities structures and composition. Outputs from the review showed that there is a lack of homogeneity in studied areas within the lagoon, being the central sector the most investigated and better characterized. The scant of information in the northern and southern sectors, which have different geological and hydrological features with respect to the central area, is a critical point, preventing a complete understanding of the lagoon structure and functioning. Particularly, north and south lagoon mouths represent the entry point for non-native species, such as *Brachidontes pharaonis* (Fischer, 1870), *Caulerpa taxifolia* (M.Vahl) C.Agardh and *Callinectes sapidus* (Rathbun, 1896). Indeed, coastal lagoons are considered as key points for the establishment of invasive species populations in the Mediterranean sea (Lacoste et al., 2023), being exposed to high risk of biodiversity loss and community structure changes, with a cascading effect on the main ecosystem services they provide related to fisheries and tourism (Lacoste et al., 2023).

Soft-benthic communities play a key role in the functioning of theses shallow ecosystems, especially in the regulation of nutrient and oxygen fluxes, and provision of high levels of biodiversity (Lacoste et al., 2023). Benthic macrofauna, such as polychaetas, convert the detrital material into biomass, that becomes available for other organisms from other trophic levels (Rodrigues-Filho et al., 2023). Ichthyofauna can help to recycling nutrients, mineralizing nitrogen and phosphorus through excretion and defecation, and making these nutrients available for primary production (Rodrigues-Filho et al., 2023; Naiman et al., 2002; Oliveira et al., 2014). Moreover, microbial communities are important elements of food webs, consuming and decomposing organic matter in the nutrient cycle; and zooplankton communities are essential in aquatic biogeochemical cycles, such as those of carbon, phosphorus and nitrogen, due to respiration process and conversion into inorganic matter, supporting primary producers (Rodrigues-Filho et al., 2023). Our study showed also that 14.3% of scientific papers were related to trophic ecology and sediment composition characterization, both fundamental approaches and information providing a more comprehensive understanding of nutrient dynamics in the Stagnone, highlighting the coexistence of low nutrient availability in the water column, internal recycling processes, and the accumulation of detrital organic matter in the sediments.

Even if these vulnerable habitats are threatened by climate change, there are few studies that focused on effect of different anthropogenic pressures on the soft-bottom benthic compartments in Mediterranean coastal lagoons (Lacoste et al., 2023). The major five human pressures influencing these habitats are i) habitat destruction, such as creation of ports, marinas or urbanizations, ii) pollution and eutrophication, iii) impact of economic activities such as aquaculture on different physical processes (erosion, sedimentation and composition of sediments), iv) the introduction of invasive species, and v) the climate change with the increasing of warmer temperatures, the alterations in salinity and nutrient levels, and the successive anoxia processes (Lacoste et al., 2023).

The effect of climate change on the lagoon structure and functioning has not been addresses in literature. There is an absence of long-term spatial and temporal data series of environmental variables in published literature, except for sea surface temperature within the saltmarshes present in the central basin of the lagoon (Sarà et al., 2021). Even if thermal anomalies or increasing temperatures over time has not been reported, Sarà et al. (2021) highlighted the presence of a marine heatwave during 2017 exceeding 32.5 ºC and responsible of *Brachidontes pharaonis* population mass mortality. Some experimental species-specific studies have been

performed to assess the effects of different environmental factors on the metabolism of key structuring species. These studies highlight the importance of the highly variable conditions in these habitats on the performance of habitat-forming species and the structure of associated communities (La Loggia et al., 2004, Sarà et al., 2008, Tomasello et al., 2009).

The potential effect of anthropogenic non-climate related stressors has been studied for seagrasses and sponges structuring habitats. Tomasello et al. (2009) reported the presence of different stressor that led to regression, lower growth rate and the lack of flowering of *P. oceanica* within the lagoon; while Mercurio et al. (2021) attributed the regression of the distribution of porifera populations to the closure of the southern opening month of the Stagnone di Marsala, reducing water circulation and turnover, and causing the burial of the bottom depression, previously characterized by rich vegetal and non-sessile spherical sponges (Calvo et al., 1982). Significant silting also damaged the meadows of the seagrass *Posidonia oceanica*, whose rhizomes serve as an optional substrate for sponges, provoking a regression of the species distribution within the lagoon (Telesca et al., 2015; Calvo et al., 2021).

This review highlighted significant knowledge gaps in a site considered a natural laboratory for over 30 years. While considerable foundational work has been done to understand the trophic status, nutrient cycle and the structure and composition of the ecological communities within the Stagnone, critical information is still lacking, preventing the connection between collected data and changing environmental conditions. Although environmental data for marine environments are more readily available through satellites-fed online databases, such data for coastal and lagoon systems are either scarce or unavailable due to technological limitations (Newton et al., 2014). Therefore, long-term local data series are essential. Furthermore, although the Stagnone of Marsala is a Natura 2000 site, none conservation monitoring actions aim capturing long-term changes in the lagoon over time. Understanding the complex connection between biological traits, ecological processes, environmental variables and ecosystem functions and services, are important to conduct the efficient management actions of this vulnerable ecosystem (Gordillo et al., 2020). There is an urgent need for a continuous monitoring plan to assess effects of various local and global stressors on the lagoon's structure and functioning. Such action is fundamental to maximizing the value of sampling and analysis efforts for defining the lagoon's ecological status and functioning under current and future climate change scenarios.

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Chapter 3 - Thermal performance of *Cymodocea nodosa* and its epibionts: assessing climate resilience in Mediterranean lagoons.

Abstract

Biotic interactions drive key ecological and evolutionary processes and mediate ecosystem responses to climate change. This study aimed to understand the interaction between seagrass and its associated epibionts. Specifically, we measured the thermal performances of two Mediterranean lagoon interacting species complex: the structuring seagrass *Cymodocea nodosa* and its epibiont, the sea anemone *Paranemonia cinerea,* which is in mutualistic association with the zooxanthellae *Symbiodinium* sp. Respiration rate, oxygen production, and photosynthetic performance traits were used as proxies for metabolism and were measured at 15 different temperatures (from 8°C to 36°C). The Thermal Performance Curve (TPC) of *Cymodocea nodosa* showed a wide thermal window with an optimum temperature of 27.5°C for oxygen production and an upper critical limit (CT_{max}) over 40°C, while respiration rate did not show an optimum temperature, highlighting the species' potential ability to acclimate and adapt to increasing temperature scenarios. The TPC of *P. cinerea* showed a left-skewed trend (typical of a warm-tolerant species), with a thermal optimum identified around 31.8°C and a CT_{max} at 37.6°C. Along with the changes in metabolic traits, changes in morphological traits (bleaching and mucus production) was observed around 32°C indicating a stress response when surpassing optimal temperature. *Symbiodinium* sp. instead, had a lower optimum and CT_{max} (28.6 °C and 31.1°C respectively) with a decrease of photosynthetic performance at higher temperatures (i.e. an impairment of symbiotic photosynthesis function) which was coupled with the observed bleaching phenomenon. Understanding the complex interplay between climate and biotic interactions is crucial to predict how ecosystems will respond to the rapid rates of current warming and how biotic interactions may change over time.

3.1 Introduction

Biotic interactions play a key role in ecological and evolutionary processes, mediating ecosystem responses to climate change (Blois et al., 2013). These interactions can vary across time and space, and may shift due to the impacts of environmental change, which can alter abiotic conditions. Such changes can affect the performance and functioning of interacting species, potentially influencing the strength and dynamics of their interactions (Brooker and Callaghan,

1998; Choler et al., 2001; Callaway et al., 2002; Brooker et al., 2007; Gilman et al., 2010; Blois et al., 2013).

The combination of different drivers and global environmental changes are threatening the sustainability of ecosystem functions and services, impacting the whole ecological hierarchy through cascading events at the individual, population, community, and ecosystem levels (McLean et al., 2019). Temperature is one of the most important stressors conditioning the functional traits of organisms, particularly ectotherms (Abram et al., 2017; Litchman and Thomas, 2023). Increased temperatures influence the physiological and behavioral mechanisms of organisms, primarily by accelerating metabolic processes (Angilletta et al., 2010; Abram et al., 2017). In this context, predicting organismal responses becomes essential, and a trait-based approach offers a valuable framework for assessing organisms performance - across different thermal scenarios (Litchman and Thomas, 2023). Among the most important seagrasses in the Mediterranean Sea, *Cymodocea nodosa* (Ucria) Ascherson is widely distributed throughout the basin (Borum, 2004), being the most common species in shallow waters such as coastal lagoons (Marín-Guirao et al., 2016; Deguette et al., 2022). This species provides various ecosystem services, such as increasing habitat diversity and biodiversity, stabilizing sediment, sequestering carbon, contributing to primary production, and decreasing irradiance, thereby creating an array of microhabitats not found in unvegetated bottoms (Borum, 2004). Additionally, *C. nodosa* leaves can host a diverse community of epiphytes, which are a very important component of the ecosystem (Borowitzka et al., 2006; Prado, 2018).

Although epiphytes occupy portions of the leaves, potentially reducing the photosynthetic capacity of seagrasses, they can protect the seagrasses from high irradiance. Furthermore epibionts can contribute to removing large amounts of nutrients from the water column (Cornelisen and Thomas, 2002; Prado, 2018). Understanding the complex interplay between climate and biotic interactions is crucial to predicting how ecosystems will respond to the rapid rates of current warming and how these interactions may change over time (Blois et al., 2013).

In this study, we investigated the interaction between *C. nodosa* and its epibiont, the sea anemone *Paranemonia cinerea* (Contarini, 1844), which is in mutualistic association with the zooxanthellae *Symbiodinium* sp., for which data is scant throughout the current scientific literature. While some studies explore the photosynthetic performance and physiological stress in symbiotic marine invertebrates and seagrass, providing information on how stressors such as temperatures fluctuations and light intensity affect the photosynthetic apparatus (Bhagooli et al.,

2021; Figueroa et al., 2019), this particular complex biotic interaction remains largely unexplored. As first step toward understanding of the interaction between *C. nodosa*, *P. cinerea* and *Symbiodinium* sp., we investigated their thermal tolerance range and identified their thermal limits. In addition, we assessed the photosynthetic performance of *C. nodosa* and *Symbiodinium* sp. at different temperatures. This approach helps us evaluate how these organisms respond to increasing temperatures, which is essential for predicting their resilience to climate change. By identifying their thermal thresholds, we can better understand the conditions under which this mutualistic relationship can thrive or break down. Furthermore, this knowledge will contribute to our broader understanding of how temperature fluctuations might affect the health and stability of seagrass ecosystems, which play a critical role in coastal marine environments by providing habitat, stabilizing sediments, and cycling nutrients. Ultimately, this research will enhance our ability to develop conservation strategies aimed at preserving these key ecological interactions in the face of global warming.

3.2 Materials and Methods

3.2.1 Sampling and respirometry measurements.

A total of 180 shoots of *C. nodosa*, and 180 individuals of *P. cinerea* (Figure 4.1c), in mutualistic association with the zooxanthellae *Symbiodinium* sp., were sampled in February 2022 at the site of Punta Palermo (37°50’29.867”N, 12°27’48.25”E) (Figure 3.1), within the Stagnone of Marsala lagoon (Sicily, Italy).

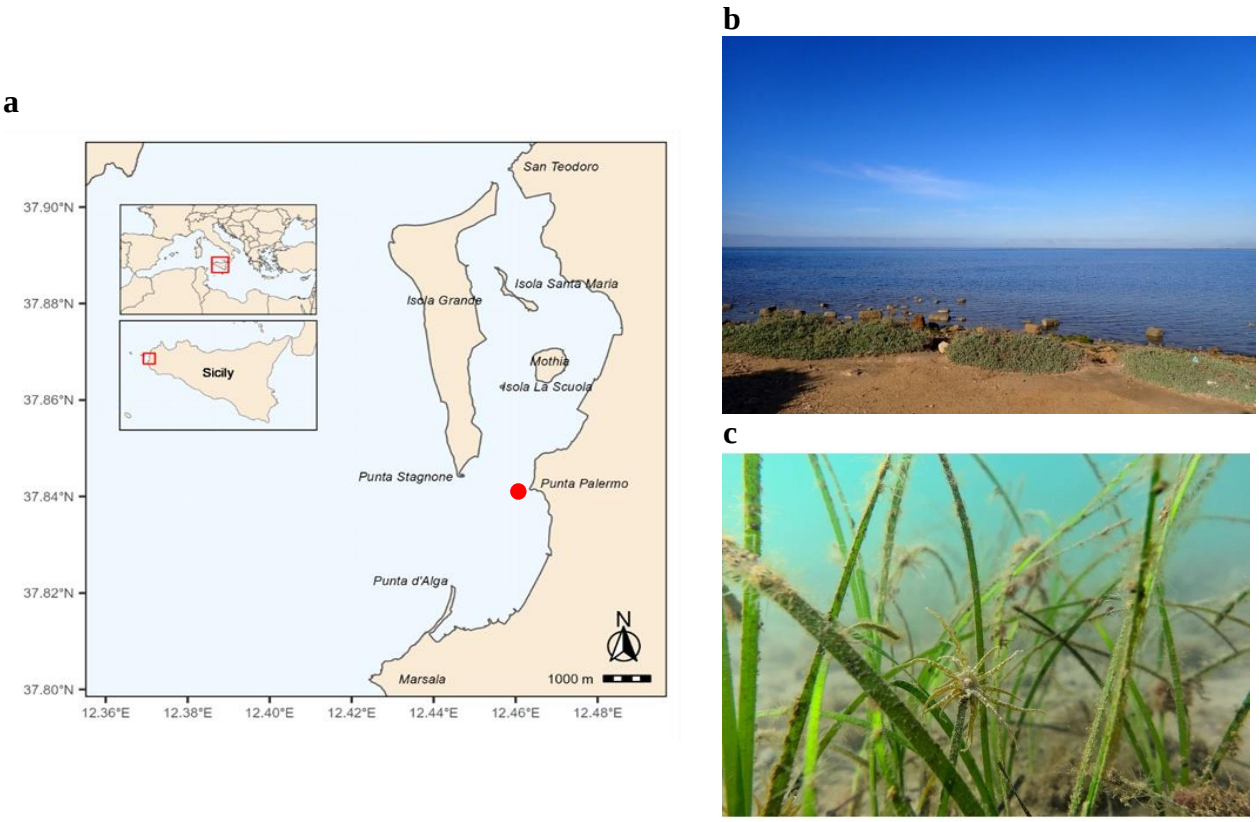

Figure 3.1: Map of sampling site, Punta Palermo (a, b), and a picture showing *Paranemonia cinerea* of *Cymodocea nodosa* leaves (c).

Organisms were carried out in the laboratory in coolers filled with aerated seawater, and were acclimated for 5 days following the environmental conditions of the sampling site (temperature: 18°C, salinity: 35 psu, photoperiod: 12 hours dark and 12 hours light) (Figure 3.2).

Figure 3.2*:* Individuals of *Paranemonia cinerea* in aquarium at dark (A) and light (B) conditions.

Respiration rate, oxygen production and photosynthetic performance traits were used as proxies of species metabolism (Angilletta et al., 2010; Sokolova et al., 2012) and were measured under 15 different temperatures: from 8 to 36 °C with step increase of 2°C. The temperature range was based on the extremes measured at the sampling site. Starting from the water acclimation temperature, each experimental temperature was reached by increasing or decreasing temperature with a rate of 1 °C per hour using a thermal bath (Grant Optima TX150 and TECO 150 chiller systems) (Prusina et al., 2014; Montalto et al., 2017; Bosch-Belmar et al., 2021).

Oxygen consumption (under dark conditions) and production (under light conditions) were measured using 4 optical oxygen meters (Pyro Science Firesting O_2), each equipped with four probs (3 probs for respirometric chambers containing organisms and one without an organism, used as a control). Twelve individuals per treatment level of each species were individually introduced into respirometric chambers (130 ml volume for *P. cinerea*, 100 ml for *C. nodosa*), containing filtered (Whatman GF/C 0.45 µm) saturated-air, and 35 psu seawater. To ensure the constant mixing of the water, each chamber was stirred with a magnet bar and an individual stirring device (Bosch-Belmar et al., 2021; Marchessaux et al., 2022). Chambers were placed inside thermostatically controlled baths (Grant Optima TX150 and TECO 150 chiller systems) to guarantee a stable temperature during the measurements that lasted 3 h (1.5 h dark : 1.5 h light conditions) with measurements taken every second.

Oxygen consumption (*RR*) and production (*PR*) rates were measured under dark and light conditions, respectively, and were calculated using the following equations (Rosewarne et al., 2016; Svendsen et al., 2016):

$$RR_{(mg\ O2\ h^{-1}\ g^{-1}\ DW)} = \frac{|\beta_{[O2]} \times 3600 \times Vol|}{DW}$$

where $\beta_{[O2]}$ is the slope of the regression line between oxygen concentrations and incubation time, standardized per time unit (3600, to convert values in hours) and per the volume of the respirometric chamber (Vol, L), and calculated in relation to the organism body mass as dry weight (DW, 48 h at 60 °C).

3.2.2 Chlorophyll-*a* fluorescence

At the end of the incubation measurements, the photosynthetic activity of each individual of *C. nodosa* and *P. cinerea* was assessed by measuring in vivo chlorophyll-*a* fluorescence of

photosystem II (PSII) with a portable pulse amplitude modulation fluorometer (Junior-PAM, Waltz), (Figure 4.3). For each sample, a Rapid Light Curve (RLC) was performed to evaluate differences in the photosynthetic capacity of *C. nodosa* and *P. cinerea*'s symbiont at different irradiance levels. To ensure reliable measurements, RLC was performed immediately after individuals were adapted to light conditions to prevent individuals from dark-adapting (Beer et al., 2014) RLCs were obtained by calculating the electron transport rate (ETR) through PSII for nine incremental quantum flux density of Photosynthetically Active Radiation (PAR) (0, 66, 90, 125, 190, 285, 420, 625, 845 µmol photons m^{-2} s^{-1}) with 20 s intervals. The ETR was calculated as:

$$ETR_{(\mu mol\ electrons\ m^{-2}s^{-1})} = \Phi PSII \times PAR \times AF \times 0.5$$

were ΦPSII is the effective quantum yield of PSII calculated according to (Genty et al., 1989) as ΦPSII = (Fm'-Ft)/Fm, obtained by applying a saturating light pulse at each irradiance level; PAR is the incident irradiance of PAR; AF is the absorption factor, which is the fraction of PAR absorbed by the leaves. It was calculated by covering a quantum sensor with the leaves of *C. nodosa* and calculating the fraction of PAR absorbed by the leaves as AF = (incident PAR – transmitted PAR)/incident PAR (Beer et al., 2014). Finally, the factor 0.5 represents the distribution of photons between photosystems II and I, assuming that 4 of the 8 electrons required to assimilate 1 CO_2 molecule are supplied by PSII (Beer et al., 2014). Maximum electron transport rate (ETR_{max}), an estimator of photosynthetic production, was obtained from the tangential function reported by Eilers and Peeters (Eilers and Peeters, 1988).

Maximum quantum yield (F_v/F_m) was determined on 30-minute dark-adapted samples (Page et al., 2021) in order to oxidise the reaction centres of photosystem II. These values indicate the maximum capacity of the photosynthetic apparatus (Rasmusson et al., 2021). Then, the minimum (basal) fluorescence (F_o), and the maximum fluorescence (F_m) were measured after a saturation pulse (9000 mmol photon m^{-2} s^{-1}, 800 ms) of actinic light and estimated the maximum quantum yield as $F_v/F_m = (F_m - F_o)/F_m$ (Murchie and Lawson, 2013). F_v/F_m ratio represents the maximum potential yield and it is usually used as measure of stress that affects components of photosystem II, such as the D1 protein (Beer et al., 2015). The maximum quantum yield of PSII (F_v/F_m), obtained using PAM fluorometry, can serve as an indicator of plant stress (Maxwell1 and Johnson, 2000; Beer Sven, 2015; Bhagooli et al., 2021; Rasmusson et al., 2021).

3.2.3 Thermal Performance Curves

By using the "rTPC" R package (Padfield et al., 2021), a total of 23 thermal performance models were performed and compared. The choice of the best model was based on the lowest AIC (the Akaike Information Criterion). Main information and parameters provide by the model included the thermal breath, the optimum temperature (T_{opt} at which the species presents maximized performance) and the lower and upper critical thresholds (CT_{min} and CT_{max}). Sharpe-Schoolfield model is a non-linear regression model describing the thermal performance curves (TPC) of an organism based on enzymatic deactivation at high temperatures (Schoolfield et al., 1981). The equation of this model is:

$$Rate = \frac{r_{\_tref} * exp^{-\frac{e}{k}\left(\frac{1}{temp+273.15}-\frac{1}{t_{ref+273.15}}\right)}}{1 + exp^{\frac{eh}{h}\left(\frac{1}{th}-\frac{1}{temp+273.15}\right)}}$$

Where: **k** is Boltzmann's constant with a value of 8.62e-05, $r_{\_tref}$ is the rate at the standardized temperature, **e** is activation energy, **eh** is high temperature de-activation energy, **th** is the temperature (ºC) at which enzyme is 1/2 active and 1/2 suppressed due to high temperatures and **tref** is standardization temperature in degrees centigrade. Temperature at which rates are not inactivated by high temperatures.

Flinn model is another non-linear regression model describing the thermal performance curves (TPC) of an organism. The equation of this model is:

$$Rate = \frac{1}{1 + a + b * temp + c * temp^2}$$

where: **temp** is the temperature in degrees centigrade, **a** is the parameter that controls the height of the curve, **b** is the parameter that controls the slope of the initial increase of the curve and **c** is the parameter that controls the position and steepness of the decline of the curve.

3.3 Result

3.2.1 Responses and thermal performance curves of *Cymodocea nodosa, Paranemonia cinerea* and *Symbiodinium* sp.

The thermal performance curve of *C. nodosa* was based on species oxygen production at the different temperature treatments. The oxygen production ranged between 0.83 ± 0.35 mg h^{-1} g^{-1} DW (estimated experimentally at 8°C) and 3.2 ± 0.56 mg h^{-1} g^{-1} DW (estimated experimentally at 28°C). At high temperature, between 30°C and 36°C, the production of oxygen decreased, with values between 2.92 ± 0.49 mg h–1 g–1 DW at 30°C and 1.73 ± 0.40 mg h^{-1} g^{-1} DW at 36°C. While, respiration rate increased with temperature with values ranging from 0.34 ± 0.21 mg h^{-1} g^{-1} DW (estimated experimentally at 8°C), to 1.44 ± 0.71 mg h^{-1} g^{-1} DW (estimated experimentally at 36°C).

From the complete set of thermal models fitted with the rTPC package, the Flinn model resulted the best fitting data distribution (presenting the lowest AIC) (Figure 3.3). The canopy optimum temperature was identified at 27.5 ºC and the CT_{max} was estimated over 40 ºC.

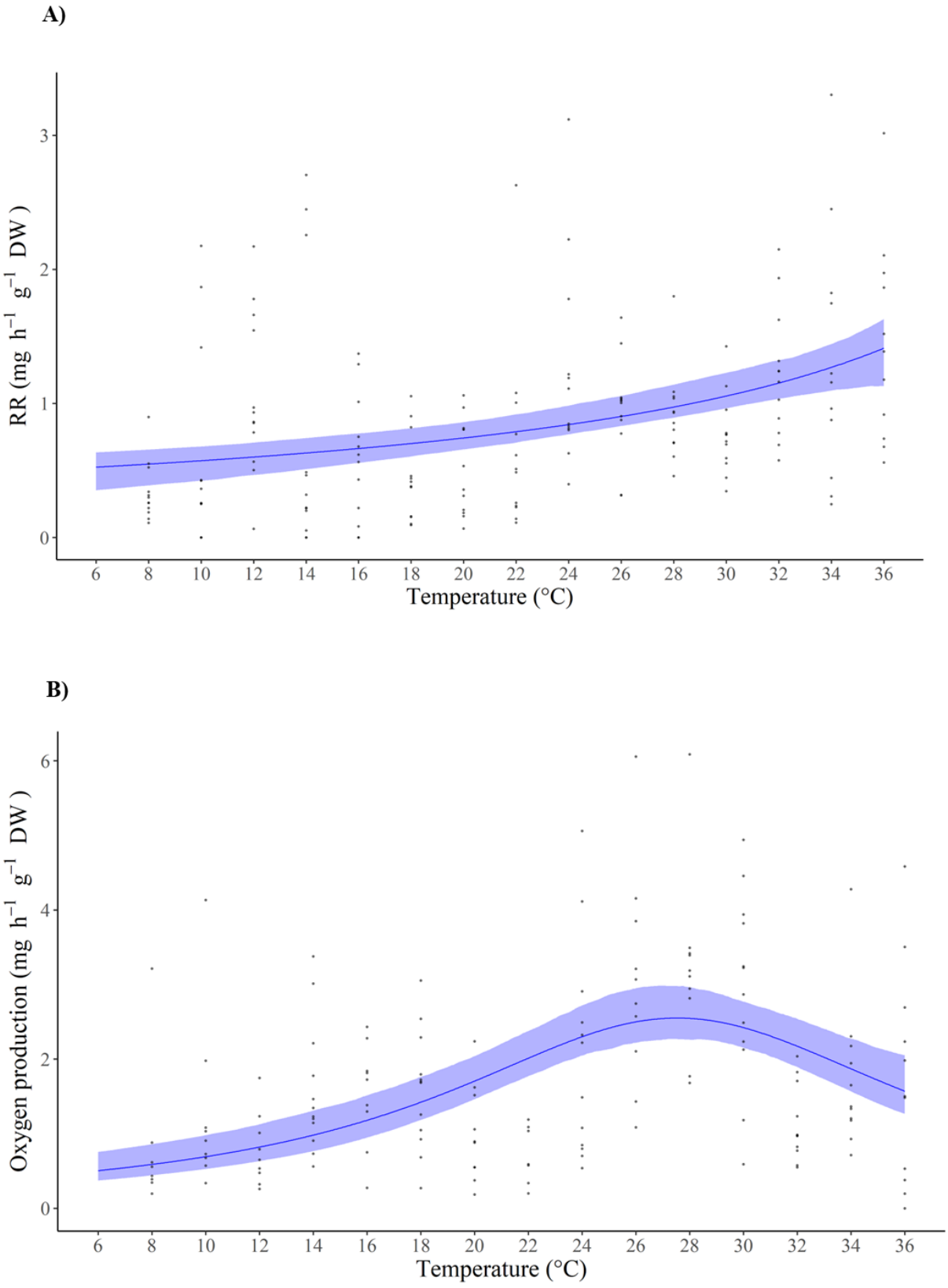

Figure 3.3 Thermal performance curve of *Cymodocea nodosa* based on respiration rate, RR (a) and oxygen production (b) according to the selected Flinn model. Shaded area corresponds to the confidence intervals. Black dots represent overlapped individual respiration rate values.

The thermal performance curve of *Paranemonia cinerea*, obtained from the Sharpe-Schoolfield high model, showed a left skewness shape, typical of species that prefer warm water (Figure 3.4). The species presented an optimum temperature of 31.8°C, and a critical thermal maximum limit (CT_{max}) of 37.6 °C. The consumption of oxygen ranged between 0.31 ± 0.16 mg h^{-1} g^{-1} DW at 8 °C and 2.49 ± 0.47 mg h^{-1} g^{-1} DW at 32 °C. At high temperatures, there was a drastic decrease in oxygen consumption, which dropped from 1.62 ± 0.36 mg h^{-1} g^{-1} DW at 34 °C to 0.51 ± 0.32 mg h^{-1} g^{-1} DW at 36 °C.

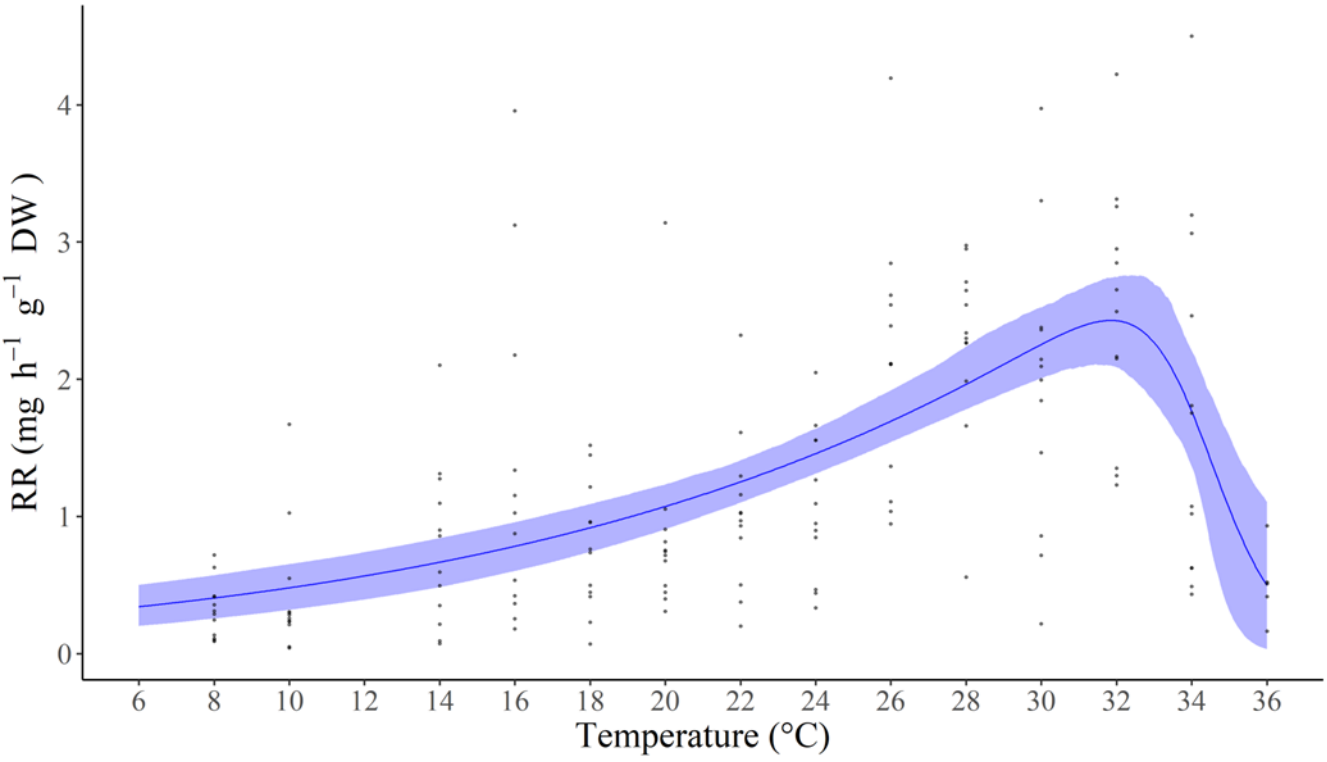

Figure 3.4: Thermal performance curve of *Paranemonia cinerea* based on respiration, according to the selected Sharpe-Schoolfield high model. Shaded area corresponds to the confidence intervals. Black dots represent overlapped individual respiration rate values.

Moreover, along with the metabolic traits, we also observed changes in the morphological traits. Specifically, at 32°C, the expulsion of zooxanthellae from the anemone occurred, followed by bleaching and mucus production as a response to thermal stress (Figure 3.5).

Figure 3.5: Changes in morphological traits in *Paranemonia cinerea* (expulsion of zooxanthellae) between 30 °C and 32 °C.

The oxygen production curve of *Symbiodinium* sp. (Figure 3.6), was obtained fitting the Sharpe-Schoolfield high model. It showed an optimum temperature of 28.6 °C, and estimated CT_{max} at 31.3°C. The production of oxygen ranged between 0.16 ± 0.13 mg h^{-1} g^{-1} DW at 8°C and 0.85 ± 0.53 mg h^{-1} g^{-1} DW at 28°C, then underwent a metabolic collapse at high temperatures from 32°C to 36°C.

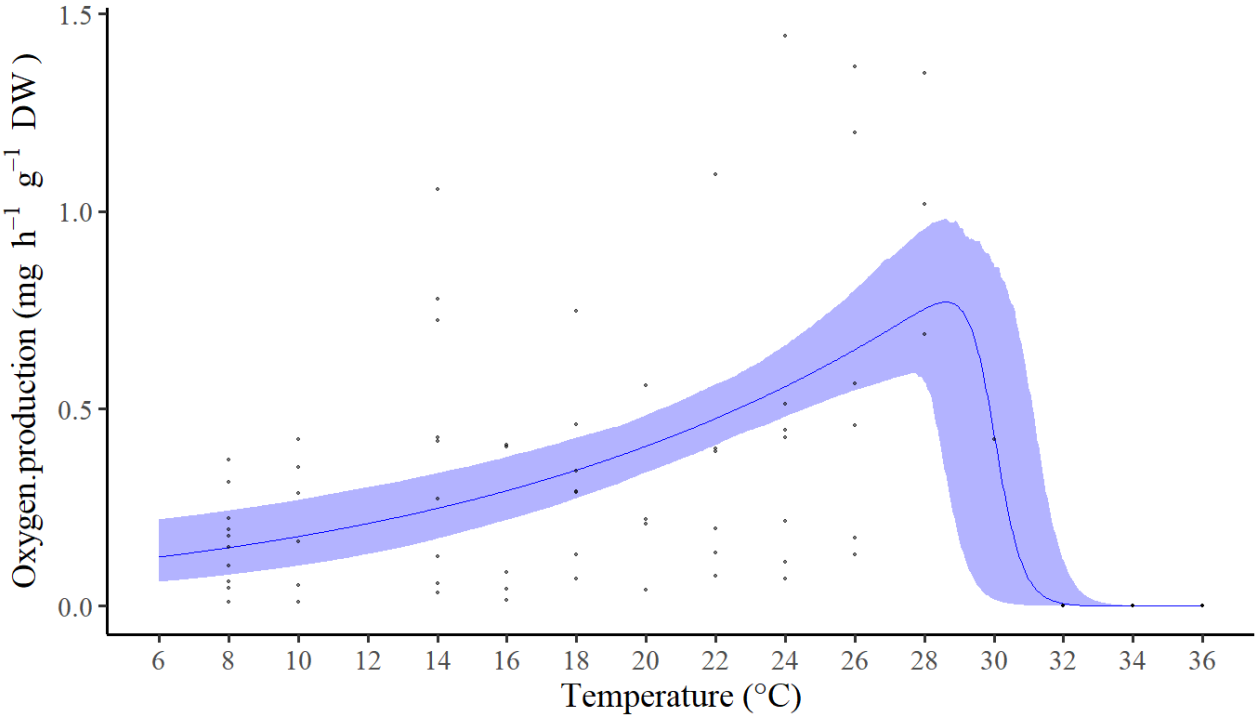

Figure 3.6: Thermal performance curve of *Symbiodinium* sp., based on oxygen production according to the selected Sharpe-Schoolfield high model. Shaded area corresponds to the confidence intervals. Black dots represent overlapped individual respiration rate values.

3.3.2. Photosynthetic performance curve of *Cymodocea nodosa* and *Symbiodinium* sp.

The maximum quantum yield of *C. nodosa* ranged between 0.72 ± 0.04 at 8°C and 0.76 ± 0.03 at 36°C, and tended to increase with increasing temperatures (Figure 3.7).

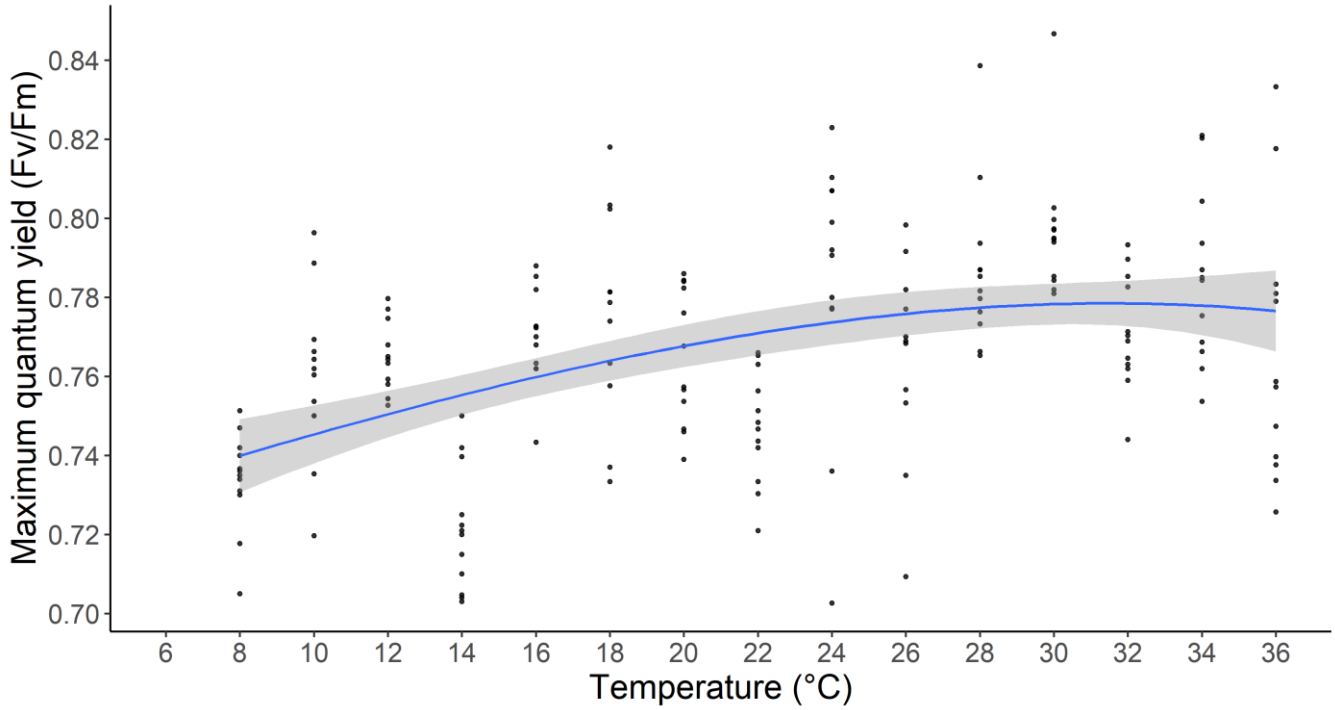

Figure 3.7: Maximum quantum yield (F_v/F_m) of *Cymodocea nodosa* at different temperatures (°C).

Maximum electron transport rate (ETR_{max}) of *C. nodosa* (Figure 3.8) showed a similar trend of F_v/F_m, at higher temperatures, values increased; indeed, at 8 °C the value was 8.6 ± 1.09 µmol e^- m^{-2} s^{-1} but at 36°C it was 27.8 ± 1.94 µmol e^- m^{-2} s^{-1}.

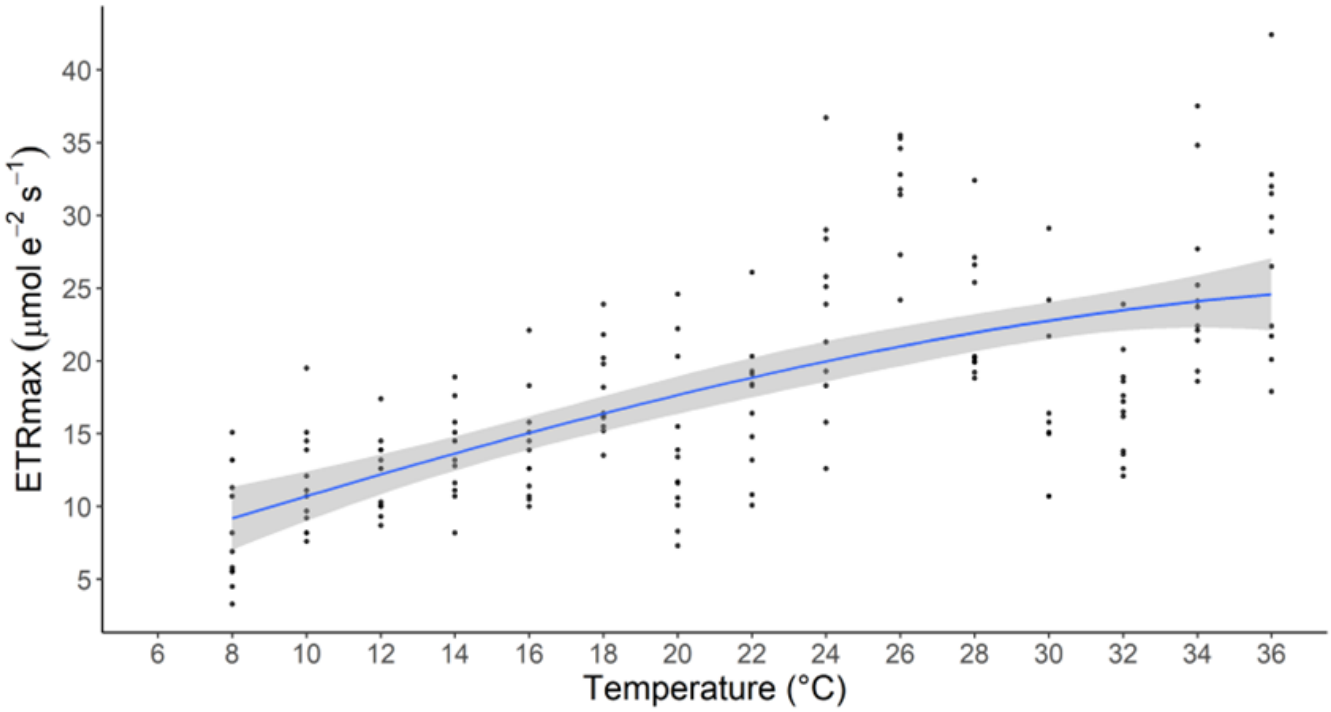

Figure 3.8: Electron transport rate (ETR $_{max}$) of *Cymodocea nodosa* over temperature (°C).

Conversely, the maximal quantum yield of *Symbiodinium* sp. tended to decrease with increasing temperatures (Figure 3.9). F_v/F_m values tended to be constant between 8 °C (0.64 ± 0.03) and 28°C (0.65 ± 0.06), after which they decreased, reaching their minimum at 36°C (0.09 ± 0.12).

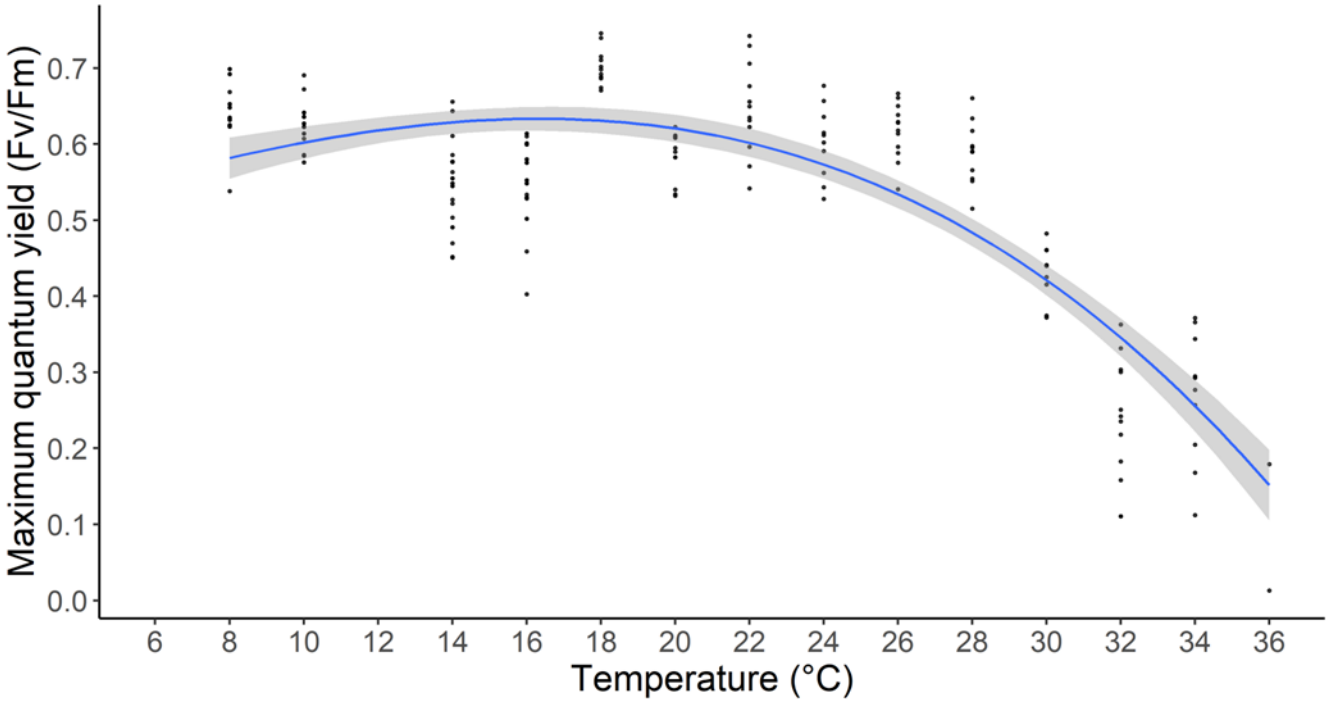

Figure 3.9: Relationship between Maximum quantum yield (F_v/F_m) of *Symbiodinium* sp. and temperatures (°C).

Maximum electron transport rate (ETR_{max}) of *Symbiodinium* sp. showed a similar trend to F_v/F_m with an optimum temperature of 27.14°C (Spain model) (Figure 3.10).

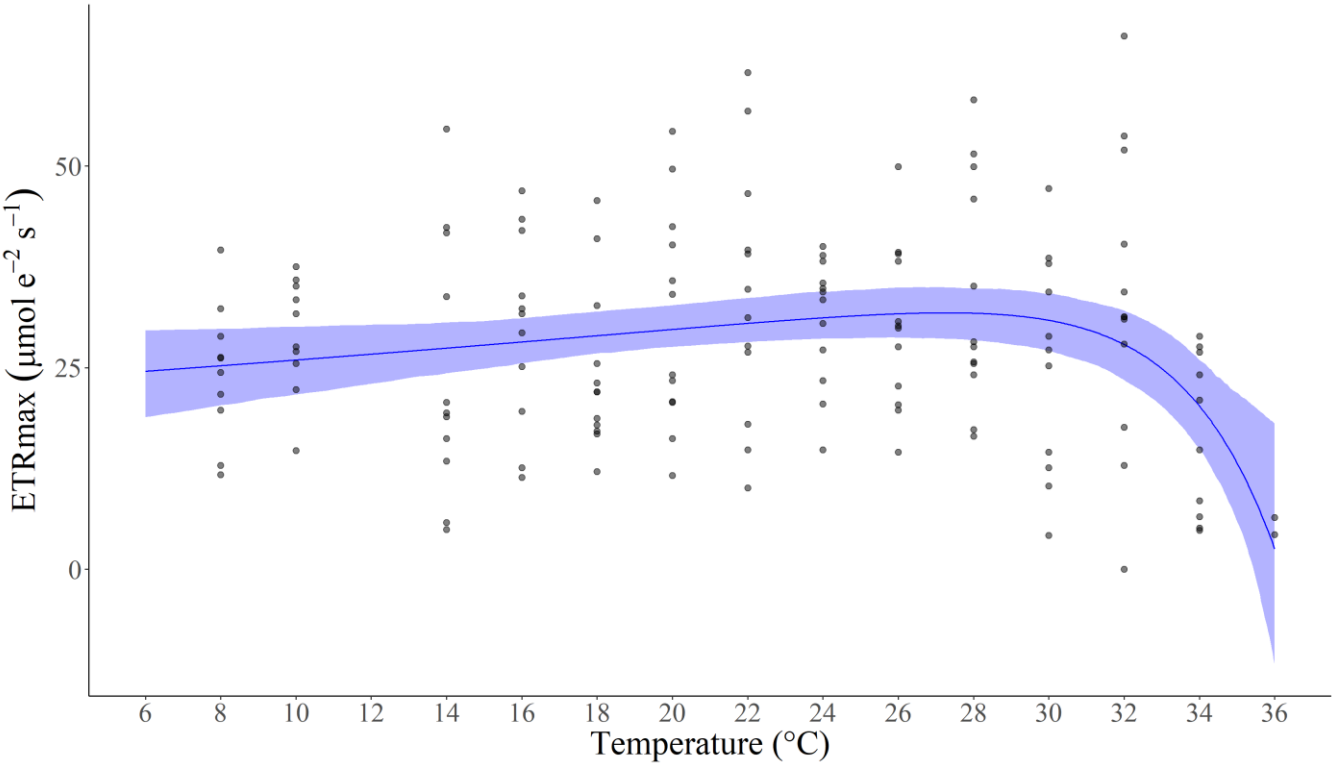

Figure 3.10: Relationship between Electron Transport Rate maximum (ETR_{max}) of *Symbiodinium* sp. and temperature (°C).

3.3 Discussions

In this context of climate change, organisms have to adapt to both increasing temperatures and more frequent and intense extreme events. One way they do this is through phenotypic plasticity, which allows them to maintain physiological processes by adjusting to their changing environment (Kellermann et al., 2019). Understanding how plasticity influences organism performance is crucial for predicting species responses to climate change. In this study, we adopted a mechanistic trait-based approach to investigate the thermal tolerance breadth and limits of interacting species, as well as their implications for interaction strength under climate change scenario.

Cymodocea nodosa.

The thermal performance curve of *C. nodosa* presented a wide thermal tolerance range with a large breath and parameters typical of a temperature warm-water affinity species. While it presented an optimum temperature at 27.5ºC based on oxygen production, respiration rate increased with temperature up to the warmest experimental temperature (36ºC). Mismatching

between the species response in both processes can be explained through several biological and physiological aspects of seagrasses. Indeed, respiration and photosynthesis (which produces oxygen) are distinct metabolic processes that can have different responses to temperature. Respiration is a catabolic process that is generally more efficient at higher temperatures, while photosynthesis is influenced by factors such as light and temperature in a more complex way. These temperatures reflect how this seagrass species responds to varying environmental conditions. High temperatures, especially during heatwaves, can lead to an increase in respiration rates and alterations in photosynthetic activity, as seen in several experiments (Silva et al., 2013; Olivè et al., 2013; Olsen and Duarte, 2015). For instance, at temperatures around 40°C, *C. nodosa* shows increased dark respiration, while photosynthesis efficiency tends to decline, confirming the detrimental effects of extreme heat on oxygen production. These findings align with broader studies on marine seagrasses, where elevated temperatures generally promote respiration but hinder photosynthesis (Olsen et al., 2012).

This is consistent with previous studies that characterize *C. nodosa* as a warm-water species with wide plasticity, capable of growing and surviving stressful environments, including high temperatures and varying nutrient availability conditions (Borum, 2004). The maximum quantum yield (F_v/F_m) of *C. nodosa,* showed an increase with rising temperatures, suggesting that the species is not significantly stressed at high thermal conditions (Marín-Guirao et al., 2016; Deguette et al., 2022). Similarly, the ETR_{max} of *C. nodosa* increases with increasing temperature until 36°C; at this temperature, *C. nodosa* reaches a plateau, suggesting that the photosynthetic system is able to maintain efficiency up to that temperature. This indicates that the plant's photosynthetic capabilities can adapt to extreme heat conditions up to that limit. This wide thermal plasticity can be attributed to the tropical origin of its genus and its geographical distribution (Pèrez and Romero, 1992; Borum, 2004). As a pioneer seagrasses with eurobiotic characteristics, *C. nodosa* has shown no evidence of negative effects on its populations in the context of climate change (Marín-Guirao et al., 2016).

Paranemonia cinerea **and** ***Symbiodinium*** **sp.**

Paranemonia cinerea, a symbiotic anthozoan that lives in mutualistic association with *Symbiodinium* sp. (Casado-Amezúa et al., 2014), displayed a thermal performance curve skewed towards higher temperatures, typical of warm-water preferring species. The optimum temperature of *P. cinerea* for respiration rate was 31.8°C, with an estimated critical thermal

maximum (CT_{max}) of 37.5°C. Moreover, the oxygen production curve of *Symbiodinium* sp. showed an optimum temperature of 28.6°C, with a critical thermal maximum CT_{max} of 31.1°C. Our results showed different optimum temperatures and critical thermal limits for *P. cinerea* and *Symbiodinium* sp. Specifically, the optimum temperature of *P. cinerea* is higher than that of its symbiont; this can be attributed to physiological and ecological differences between the two organisms. In particular, Davy et al., (2012) showed that hosts have more robust defense mechanisms against heat stress than symbiotic algae, whose photosynthetic function is rapidly impaired by heat. *Symbiodinium* sp. strains can start showing signs of heat stress and photobleaching when temperatures exceed around 30-31°C, with some studies reporting optimal photosynthetic efficiency around 28-30°C (Iglesias-Prieto et al., 1992; Goulet et al., 2017). Beyond this threshold, photosynthetic processes and associated mechanisms, such as chlorophyll production, appear to be impaired, leading to a decrease in symbiotic efficiency and possible bleaching.

In contrast, *Paranemonia cinerea*, like other cnidarians, can tolerate slightly higher temperatures. This mismatch/shift in thermal tolerance, with optimal temperatures of 31.8°C for the anemone and 28.6°C for the symbiont, illustrates the delicate balance of their symbiosis, as the host can withstand warmer waters better than its symbiotic algae, which are more vulnerable to heat-induced stress (Iglesias-Prieto et al., 1992; Warner et al., 1999; Goulet et al., 2017).
Outputs regarding to the thermal tolerance and optimum temperature of *Symbiodinium* sp. align with findings in the scientific literature for different clades of *Symbiodinium* (A-I). This agreement reflects their ability to inhabit multiple environments and the specificity of their tolerance to environmental changes (Sampayo et al., 2009; Brading et al., 2011; Oakley et al., 2014; Pochon et al., 2014; Casado-Amezúa et al., 2016). Indeed, for example, the “temperate A” clade is regarded to exhibit greater physiological resistance to short-term increases in temperature compared to conspecific types present in other areas (Rodolfo-Metalpa et al., 2006).

However, our findings also indicate that high temperatures (32°C) can result in stress in *P. cinerea*, leading to the expulsion of symbionts (i.e. bleaching process) (Dani et al., 2016) and excessive mucus production. Consequences for the anemone's fitness can be detrimental, as the host often cannot survive if the symbiont is not re-incorporated (Doering et al., 2023). This observation is consistent with studies showing that high irradiances and temperatures can cause an overproduction of toxic reactive oxygen species (ROS) from symbionts, damaging the symbiont’s PSII apparatus and compromising photosynthesis (Doering et al., 2023). The

decrease in F_v/F_m of *Symbiodinium* temperate A clade at higher temperatures further supports this finding. This decrease can be attributed to the increase in ROS due to photoinhibition, which can be harmful to both the host and symbiont (Lesser and Farrell, 2004; Casareto et al., 2016). The reduction in F_v/F_m and ETR_{max} and the decreased photosynthetic efficiency are primarily due to thermal stress, which affects the algal photosynthetic apparatus (Iglesias-Prieto et al., 1992; Warner et al., 1999; Goulet et al., 2017). Outputs emphasized the vulnerability of these symbiotic algae to thermal stress, and highlight the delicate balance of mutualistic interactions under changing environmental conditions, potentially having critical implications for the health of coastal marine ecosystems.

Implications for biotic interactions and conservation.

Understanding the thermal tolerances and responses of these interacting species is crucial for predicting ecosystem responses to climate change, especially in a vulnerable habitat such a coastal lagoon. While *C. nodosa* appears to have a high thermal tolerance, the more sensitive response of *P. cinerea* and its symbiont to increasing temperatures could lead to changes in their interactions and potentially affect the broader ecosystem. If thermal stress disrupts the interaction between *P. cinerea* and its symbiont, it may lead to changes in the abundance and distribution of the anemone, thereby negatively affecting the relationship between the seagrass and its epibiont. The main benefits of this interaction include the protection from herbivory that the epibiont provides to the plant (Vergès et al., 2011), and the availability of substrate along the dynamic water column that the seagrass offers to the anemone, which can benefit from feeding since it is a suspension feeder (Orth et al., 2006). The loosening or loss of this interaction would result in increased vulnerability of the canopy to predation, which could negatively impact the species' performance and distribution.

The differential responses of these species to temperature increase highlight the complexity of predicting climate change impacts on marine ecosystems. This raises the importance of considering not just individual species responses, but also the dynamics of their interactions when developing conservation strategies. As studies on the effects of climate change on biotic interactions continue to increase (Jaeschke et al., 2012), there remains a need for better integration of these interactions into species distribution models (Elith and Leathwick, 2009; Kearney and Porter, 2009). Our findings contribute to this growing body of knowledge and

emphasize the importance of considering species interactions in climate change research and conservation planning.

In conclusion, this study provides valuable insights into the thermal tolerances and potential responses of *C. nodosa*, *P. cinerea*, and *Symbiodinium* sp. to climate change. These findings can inform future research and contribute to the development of more effective conservation strategies for Mediterranean coastal ecosystems in the face of global warming (Olsen et al., 2012).

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Chapter 4 - Seagrass-sea anemone interactions: spatial and temporal dynamics in a Mediterranean coastal lagoon.

Abstract

Seagrass ecosystems are vital components of coastal marine environments, providing numerous ecological services and supporting diverse communities. However, the complex interactions between seagrasses and their associated fauna, particularly in dynamic environments like coastal lagoons, remain understudied. This research focuses on the relationship between the seagrass *Cymodocea nodosa* and the sea anemone *Paranemonia cinerea*, two species that play important roles in Mediterranean coastal ecosystems. This study investigates the spatial and temporal dynamics of the interaction between both interacting species in the Stagnone of Marsala, a coastal lagoon in Sicily, Italy. We conducted seasonal surveys at two sites (Punta Palermo and Salina Mozia) over a one-year period, measuring *C. nodosa* characteristics (density, cover, leaf morphology), *P. cinerea* abundance and distribution, as well as environmental variables. Our results reveal complex patterns in the seagrass-sea anemone relationship, influenced by water depth, site-specific conditions, and temporal changes. *Cymodocea nodosa* density and cover showed negative correlations with water depth, while *P. cinerea* abundance was positively associated with *C. nodosa* density, particularly at Punta Palermo. Biochemical analyses of water and sediment samples suggest that the presence of *P. cinerea* may influence local nutrient dynamics. This study highlights the importance of species interactions in shaping seagrass ecosystems and provides insights for conservation efforts in coastal lagoons.

4.1 Introduction

Seagrasses ecosystems are critical components of coastal marine environments, providing essential ecosystem services and supporting diverse biological communities (Orth et al., 2006; Duarte et al., 2008, 2013; Nordlund et al., 2016; Jongen et al., 2024). Biological interactions play a crucial role in shaping the distribution and abundance of species worldwide (Wisz et al., 2013). In particular, the interactions between habitat-forming organisms and their associated species play a fundamental role in structuring these ecosystems (Nordlund et al., 2016; Teagle et al., 2017; Stelling-Wood et al., 2023). Seagrasses, as foundation species, create complex habitats that support diverse populations and fosters interactions with various organisms, including benthic invertebrates (e.g., sea anemones, isopods, gastropods) and fish (Nordlund et al., 2016).

These interactions forms complex relationships that directly influence the performance and fitness of both the seagrasses and their associated species (Pringle, 2016). Among these interactions, those between seagrasses and suspension-feeding organisms, such as sea anemones, are of particular interest, especially in confined environments like coastal lagoons (Rinkevich et al., 1983). These interactions not only contribute to the overall ecological balance but also enhance the resilience of these ecosystems in the face of environmental stressors (Duarte et al., 2008).

Cymodocea nodosa, a warm-water seagrass species widely distributed in the Mediterranean and eastern Atlantic, plays a significant role in these ecosystems (Borum, 2004). However, the specific interactions between *C. nodosa* and most of its epibionts remain understudied.

Epibionts, such as sea anemones found on seagrass leaves, are significant contributors to primary production in these ecosystems (Jernakoff et al., 1996; Crespo et al., 2014). Furthermore, the structure of epibiont communities can serve as an indicator of environmental conditions and water quality (Giovannetti et al., 2010; Piazzi et al., 2016). Disturbance to these epibiont communities, whether due to pollution or environmental changes, can have cascading effects on the broader ecosystem (Crespo et al., 2014).

Sea anemones are typically sessile organisms that attach to seagrass leaves, where they feed both autotrophically (via symbiotic zooxanthellae) and heterotrophically (by capturing organic matter) (Brooker et al., 2019). Their presence can affect seagrass health both positively, by potentially defending against herbivores, and negatively, by obstructing light absorption (Ugarelli et al., 2017). The expected increase in global temperatures due to climate change may significantly impact seagrass-epibiont interactions (Orth and Heck, 2023). Rising temperatures could affect seagrass metabolism, growth, and reproduction, potentially leading to shifts in species distribution, altering the composition of associated epibiont communities and the strength of their interactions. Despite the ecological importance of seagrass-epibiont interactions, there is a lack of quantitative data on the relationship between seagrasses, particularly *C. nodosa*, and their associated sea anemones. This study aims to address this knowledge gap by investigating the spatial and temporal dynamics of the interaction between *C. nodosa* and the sea anemone *Paranemonia cinerea* in the Stagnone of Marsala, a coastal lagoon in Sicily, Italy. Specifically, our research objectives are to: (1) quantify the distribution and abundance of *P. cinerea* on *C. nodosa* leaves across different environmental conditions; (2) assess the relationship between *C.*

nodosa characteristics (density, cover, leaf morphology) and *P. cinerea* presence; (3) investigate how environmental factors, particularly depth and water quality, influence this seagrass-sea anemone interaction; and (4) examine temporal variations in this interaction over a one-year period. By addressing these objectives, we aim to contribute to a better understanding of the complex interactions within seagrass ecosystems and provide insights that can inform conservation and management strategies in the face of environmental change.

4.2 Material and Methods

4.2.1. Study area

Monitoring activities were conducted in the Stagnone of Marsala lagoon, a Mediterranean shallow semi-enclosed coastal lagoon on the western coast of Sicily, Italy (37.83°N, 12.45°E). Two sites were selected for monitoring: Salina Mozia (37.86°N, 12.47°E) and Punta Palermo (37.84° N, 12.46°E) (Figure 4.1). Salina Mozia is characterized by shallow waters (average depth < 1 meter), dominated by *Cymodocea nodosa* meadows. Punta Palermo, closer to the sea, has a greater water depth (average depth 1.5 meters) and stronger currents. Vegetation here includes *C. nodosa* and various macroalgae species (Sará et al., 1999).

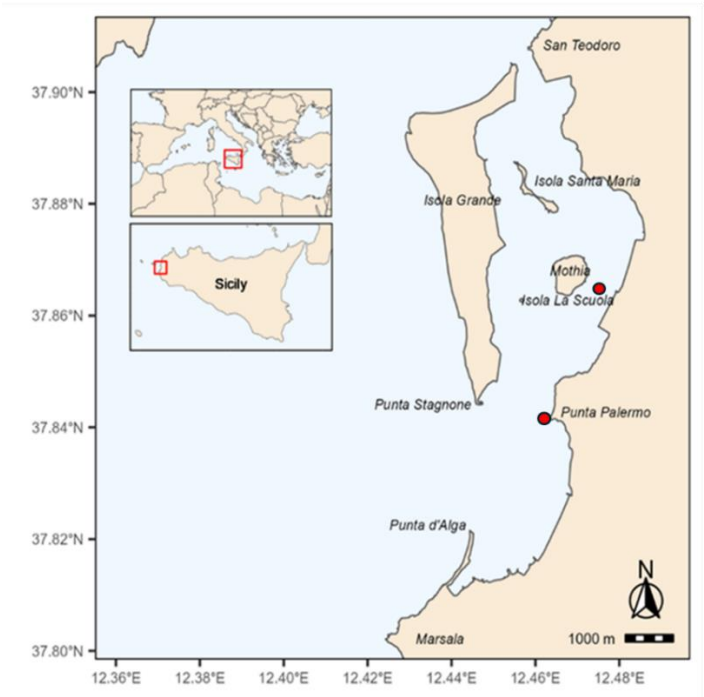

Figure 4.1: Study sites (Salina Mozia and Punta Palermo) within the Stagnone of Marsala lagoon.

4.2.2 Sampling

Sampling monitoring was performed in four periods: January 2023, November 2023, January 2024, and May 2024. At each site, three 50-meter-long transects were established parallel to the shoreline. Along each transect, five quadrats (30 x 30 cm) were placed at 10-meter intervals, resulting in a total of 15 quadrats per site during each sampling period. In each quadrat, the following parameters were recorded: (i) *C. nodosa* cover percentage, canopy height, and number of shoots; (ii) presence and abundance of *P. cinerea*, as well as its position on *C. nodosa* leaves (apical, middle, proximal); (iii) environmental variables: depth, temperature, and salinity. In detail, depth of each quadrats were measured with a meter, but temperature and conductivity with the multi-parameter probe. Both transects and the quadrats were randomly deployed. Samples of *C. nodosa* (six shoots per quadrat) were also collected for phenological and biomass analyses. Moreover, sediment cores (20 cm in length, 3.5 cm in diameter) were taken in three replicates per condition (i.e., *C. nodosa* alone, *C. nodosa* with *P. cinerea*, and sediment only) using plexiglass tubes. Water samples were collected using a 10L Niskin bottle. After collection, *C. nodosa*, *P. cinerea and sediments* samples were stored at -20°C, water samples were filtered using Whatman GF/F and GF/C filters before freezing. Water sample, were filtered with a filtering ramp. For analyses chl-a and TSM concentrations we filtered 2 L of water in Whatman GF/F 47 mm (TSM) and GF/C 47 mm (chl-a) filters, but for lipids, carbohydrates and proteins, 0.5 L in Whatman GF/F 24 mm filters were used. All samples were transported to the Ecology Laboratory at the University of Palermo for subsequent analyses.

4.2.3 Functional descriptors of *Cymodocea nodosa*

Functional descriptors of *C. nodosa* were estimated according to (Cancemi et al., 2002), which categorizes leaves into differentiated (leaf blade and base) and non-differentiated (leaf blade only) types. This classification allows for the assessment of functional plant descriptors, such as leaf length, width, and biomass, as well as the biomass of rhizomes and roots. In the laboratory, *C. nodosa* samples were rinsed in fresh water for phenological and biomass analyses. Then, the length and width (cm) of *C. nodosa* leaves were measured, along with presence of leaves predated, and wet weight. Epiphytes were then separated from the *C. nodosa* leaf by the use of a scalpel and weighted separately.

4.2.4 Biochemical analysis in the water column and in the sediment

Fluorometric analysis was employed to quantify the amounts of chlorophyll-*a* and phaeopigment (Lorenzen and Jeffrey, 1980). Samples were left overnight at 4 °C in the dark in 90 % acetone. Phaeopigments were quantified by acidifying the mixture with 0.1 N HCl. Following phenol interference adjustment, protein analyses were performed using spectrophotometry (Hartree, 1972; Rice, 1982). Carbohydrates were measured and expressed as glucose equivalents (Gerchakov and Hatcher, 1972). Lipids were extracted using a two-phase separation method with a chloroform:methanol:water ratio (Bligh and Dyer, 1959), and their concentrations were determined spectrophotometrically (Marsh and Wenstein, 1966) using standard tripalmitin solutions. All fluorometric analysis were performed following a different protocols, and after we used the spectrophotometer for lecture of absorbances. The procedure was similar for both water and sediment samples, but for the water samples, pre-treatedfilters were used for different analyses. To determine total suspended matter (TSM) and photosynthetic pigments, subsamples were filtered onto pre-washed, precombusted (450 °C, 4 h), and pre-weighed (±1µg) Whatman GF/ F filters. TSM determined gravimetrically after desiccation (60 °C, 24 h). The organic fraction of seston (OSM) was determined by loss on ignition (450 °C 4 h); the material remaining after combustion was the inorganic fraction (ISM) of TSM (Strickland and Parsons, 1972).

4.2.5 Data analysis

Data analysis was performed using three-way ANOVA to determine the effects of site (2 levels fixed; Punta Palermo, Salina Mozia), transect (A, B, C), and time (4 levels random; January 2023, November 2023, January 2024, May 2024) on *C. nodosa* functional descriptors (density, cover, leaf length, width, biomass) and the relationship with *P. cinerea*. Post-hoc Tukey HSD tests were conducted to identify significant differences between factor levels. Biochemical data were analyzed for differences between sites, times, and conditions (i.e., *C. nodosa* with and without *P. cinerea*). To test how the three variables (depth, cover and time) interact influencing density, we calculated a linear model with interactions.

Statistical analyses were carried out in R open access statistical software version 4.1.2 (R Core Team (2021).

4.3 Results

Since ANOVA mostly showed no significant differences between transects within sites and over time, the results are presented with transect values pooled by site.

4.3.1 Habitat characterization

Environmental variables, such as temperature and salinity, showed distinct seasonal patterns at the two sites. In Salina Mozia, temperatures ranged from 10.5°C ± 0.02 in January 2023 to 23°C ± 0.03 in May 2024, while at Punta Palermo, temperatures varied between 13.5°C ± 0.03 and 26.4°C ± 0.03. Salinity remained stable, with minor fluctuations between the sites. Water depths varied from 11-100 cm in Punta Palermo and 25-100 cm in Salina Mozia.

Water column analysis revealed no significant differences in chlorophyll-*a*, proteins, carbohydrates, or lipids between sites or conditions (Table 1, Supplementary materials (S)). However, total suspended matter (Figure 5, S) (TSM) exhibited significant temporal variation ($p = 0.0235$). Chlorophyll-*a* concentrations (Figure 1, S) were higher in Punta Palermo (0.70 ± 0.05 µg/L) compared to Salina Mozia (0.49 ± 0.3 µg/L) (Table 1), though the difference was not statistically significant. Protein concentrations (Figure 2, S), were higher in Punta Palermo (0.32 ± 0.07 mg/L) compared to Salina Mozia (0.23 ± 0.06 mg/L), though not statistically significant (Table 2); whereas, carbohydrate concentrations (Figure 3, S) were higher in Punta Palermo (0.86 ± 0.04 mg/L) compared to Salina Mozia (0.09 ± 0.01 mg/L), though not statistically significant (Table 1, S).

In sediment samples (Table 2), chlorophyll-*a* concentrations (Figure 6, S) differed significantly between different conditions ($p < 0.01$) with higher values in Punta Palermo. Protein (Figure 7, S), carbohydrates (Figure 8, S) and lipids concentrations (Figure 9, S) in sediments showed temporal variations but no significant differences between sites or conditions (Table 2, S).

Table 1: Average values (± SE) of the results from the biochemical analysis in the water column among conditions and time.

Table of biochemical analysis in the water column

Time	Conditions	Sites	Chl-a [µg/L]	se	Carbohydrates [mg/L]	se	Lipids [mg/L]	se	Proteins [mg/L]	se	TSM [mg/L]	se
November_2023	Sediment	Punta Palermo	0.563	0.020	0.105	0.042	0.160	0.094	0.245	0.043	60.030	2.41
November_2023	*Cymodocea nodosa*	Punta Palermo	0.787	0.012	0.216	0.098	0.090	0.009	0.455	0.075	38.219	1.53
November_2023	*Cymodocea nodosa*	Salina Mozia	0.396	0.009	0.073	0.041	0.093	0.030	0.157	0.030	35.588	2.17
November_2023	*Cymodocea nodosa + Paranemonia cinerea*	Salina Mozia	0.617	0.051	0.118	0.073	0.053	0.014	0.224	0.031	54.542	0.57
November_2023	Sediment	Salina Mozia	0.553	0.020	0.090	0.022	0.047	0.005	0.196	0.034	77.830	3.98
January_2024	*Cymodocea nodosa*	Salina Mozia	0.620	0.155	0.090	0.023	0.055	0.017	0.241	0.100	42.577	2.08
January_2024	Sediment	Salina Mozia	0.585	0.106	0.246	0.112	0.172	0.133	0.362	0.055	45.525	0.44
January_2024	*Cymodocea nodosa + Paranemonia cinerea*	Punta Palermo	0.510	0.005	0.035	0.014	0.034	0.008	0.235	0.018	56.039	3.21
January_2024	*Cymodocea nodosa*	Punta Palermo	0.293	0.014	0.037	0.000	0.058	0.007	0.091	0.002	39.283	2.71
May_2024	*Cymodocea nodosa*	Punta Palermo	0.403	0.009	0.110	0.042	0.045	0.004	0.208	0.063	86.400	8.54
May_2024	*Cymodocea nodosa + Paranemonia cinerea*	Punta Palermo	1.682	0.268	0.355	0.153	0.052	0.032	0.700	0.294	69.020	4.06
May_2024	*Cymodocea nodosa*	Salina Mozia	0.415	0.010	0.035	0.009	0.034	0.007	0.189	0.032	74.970	8.70
May_2024	Sediment	Salina Mozia	0.307	0.090	0.037	0.016	0.058	0.035	0.238	0.031	80.470	6.67

Table 2: Average values (± SE) of the results from the biochemical analysis in the sediment among conditions and time.

Table of biochemical analysis in the sediment

Time	Conditions	Sites	Chl-a [µg/g]	se	Carbohydrates [mg/g]	se	Lipids [mg/g]	se	Proteins [mg/g]	se
November_2023	Sediment	Punta Palermo	3.359	0.354	2.012	0.228	0.392	0.026	1.165	0.036
November_2023	*Cymodocea nodosa*	Punta Palermo	1.643	0.159	0.991	0.055	0.146	0.006	1.383	0.037
November_2023	*Cymodocea nodosa*	Salina Mozia	1.394	0.131	1.394	0.111	0.173	0.018	1.297	0.084
November_2023	*Cymodocea nodosa + Paranemonia cinerea*	Salina Mozia	2.907	0.106	3.284	0.156	0.253	0.018	1.163	0.054
November_2023	Sediment	Salina Mozia	2.725	0.095	3.686	0.232	0.353	0.020	1.570	0.046
January_2024	*Cymodocea nodosa*	Salina Mozia	0.909	0.044	1.894	0.090	0.219	0.010	1.546	0.060
January_2024	Sediment	Salina Mozia	1.135	0.056	1.556	0.120	0.266	0.080	1.728	0.133
January_2024	*Cymodocea nodosa + Paranemonia cinerea*	Punta Palermo	2.549	0.065	2.126	0.090	0.520	0.060	1.575	0.070
January_2024	*Cymodocea nodosa*	Punta Palermo	1.902	0.108	4.821	0.817	0.444	0.040	1.019	0.050
May_2024	*Cymodocea nodosa*	Punta Palermo	1.713	0.040	1.375	0.117	0.443	0.030	2.375	0.320
May_2024	*Cymodocea nodosa + Paranemonia cinerea*	Punta Palermo	3.924	0.060	1.399	0.070	0.301	0.010	1.645	0.050
May_2024	*Cymodocea nodosa*	Salina Mozia	1.479	0.192	0.791	0.060	0.205	0.010	1.019	0.060
May_2024	Sediment	Salina Mozia	1.797	0.129	1.697	0.128	0.465	0.060	1.704	0.210

4.3.2 Functional descriptors of *Cymodocea nodosa*.

Cymodocea nodosa density exhibited significant temporal variation (ANOVA, $p < 0$) (Table 3, S). In January 2023, densities were highest (Punta Palermo: 154 ± 15 shoots/m²; Salina Mozia: 224 ± 24 shoots/m²), decreasing in subsequent samplings. By May 2024, density in Salina Mozia decreased to 184 ± 33 shoots/m², while Punta Palermo increased at 169 ± 19 shoots/m² (Figure 4.2).

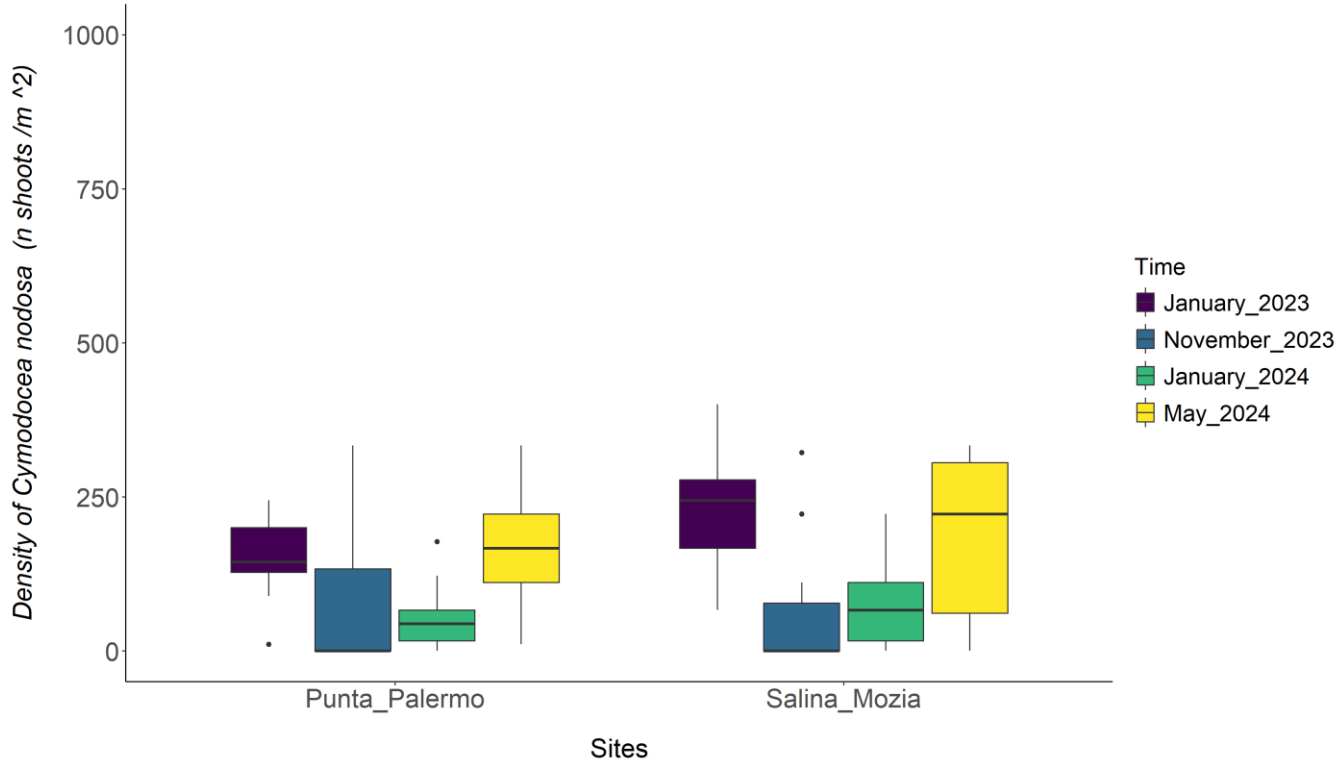

Figure 4.2: Variation of *Cymodocea nodosa* density (n. shoots / m^2) among sites and sampling periods.

Cover percentage of *C. nodosa* followed a similar trend, with the highest values observed in January 2023 (18% at Salina Mozia and 11% at Punta Palermo). There was a significant increase in cover in May 2024 at both sites, particularly at Salina Mozia (30%). Significant differences in cover were detected between sites ($p < 0.05$) and times ($p < 0.05$), with interaction effects between site and time ($p < 0.05$) (Figure 4.3) (Table 3, S).

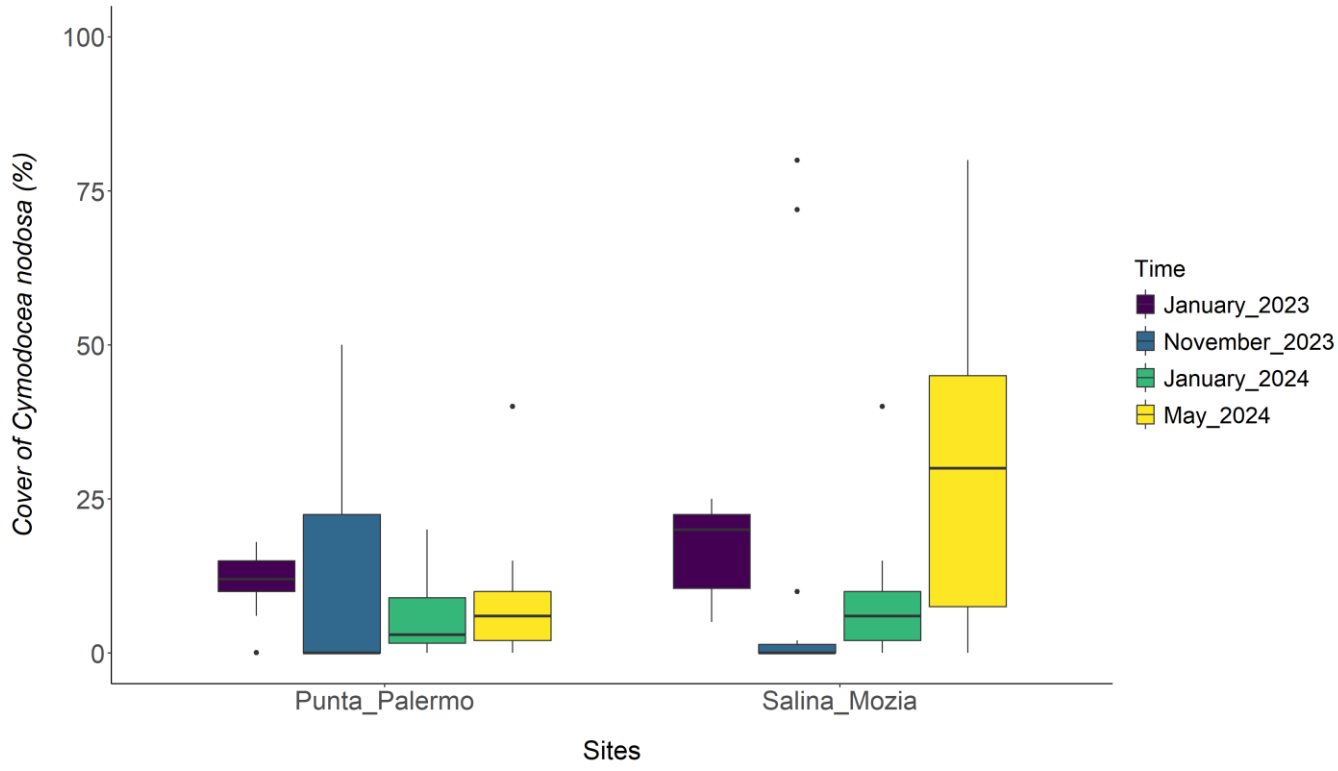

Figure 4.3: Variation of cover percentage of *Cymodocea nodosa* (%) among sites and different monitoring times.

Leaf length differed significantly between sites and sampling periods ($p < 0.001$) (Table 3, S), with Salina Mozia displaying longer leaves (Figure 4.4). Leaf width differed significantly between sites ($p < 0$) with interaction effects between site and time ($p < 0$), may 2024 seems to be particularly relevant, showing significant differences both between the two sites (Salina Mozia and Punta Palermo) and with respect to other time periods (Figure 4.5). Leaf biomass showed no significant differences between sites ($p > 0.05$) or sampling periods ($p > 0.05$) (Figure 4.6) (Table 4, S).

Epiphyte biomass on *C. nodosa* leaves did not differ significantly between sites ($p > 0.05$) or sampling periods ($p > 0.05$), averaging 0.003 ± 0.007 g in Punta Palermo and 0.006 ± 0.003 g in Salina Mozia (Figure 4.7) (Table 4, S).

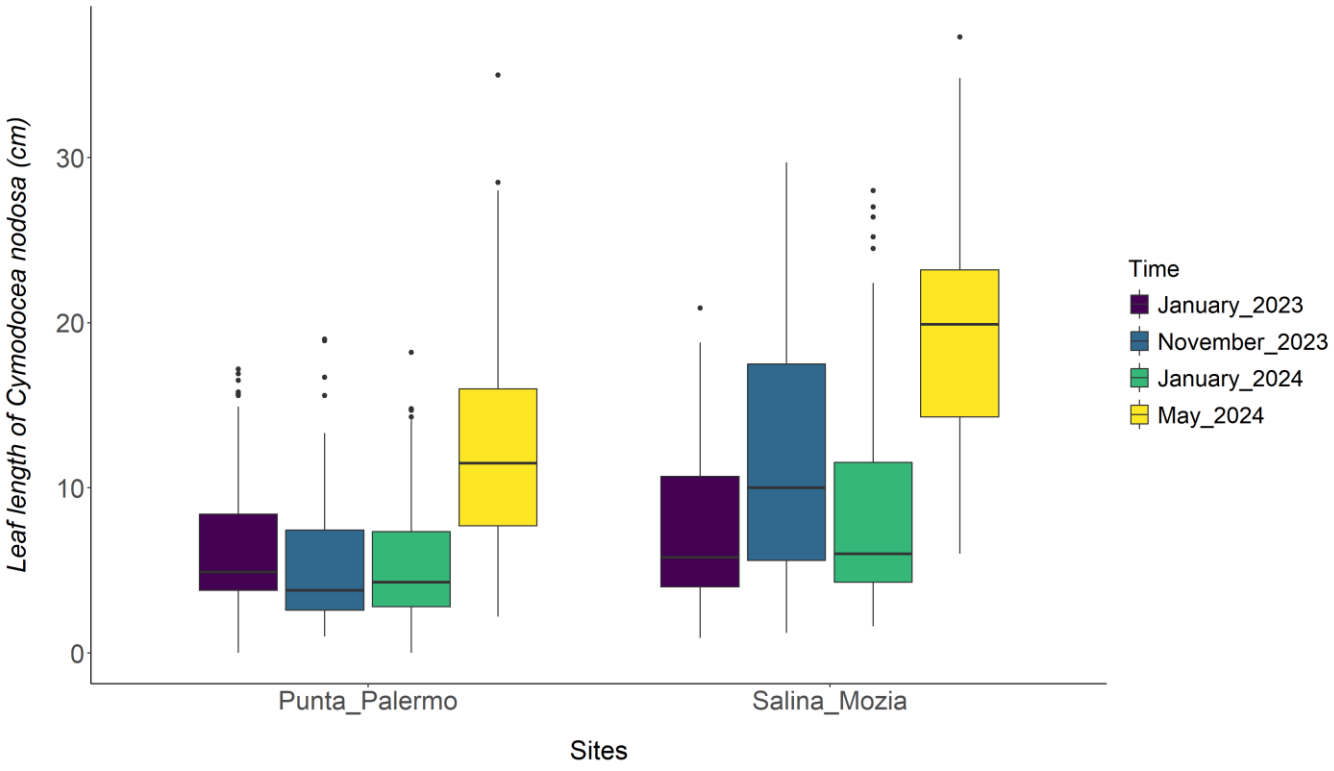

Figure 4.4: Variation of *Cymodocea nodosa* leaf length (cm) among sites and sampling times.

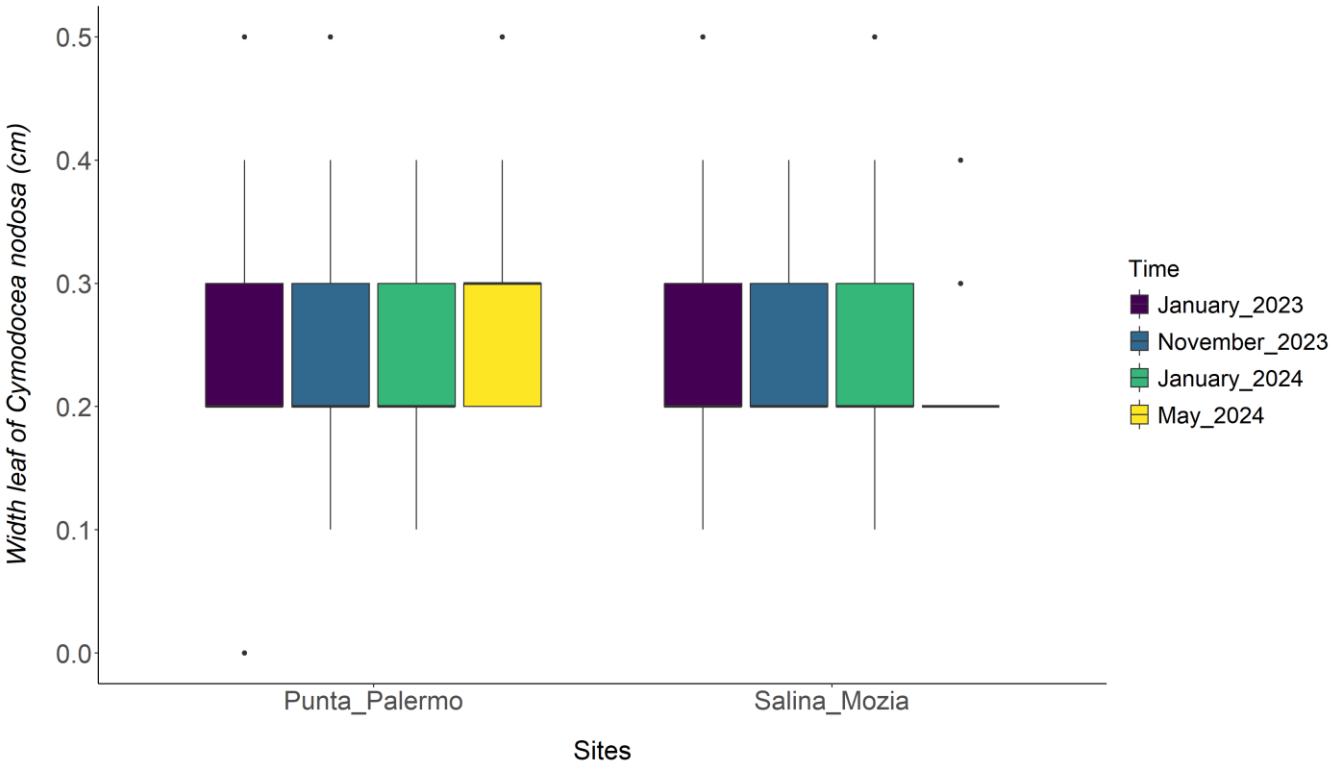

Figure 4.5: Variation of *Cymodocea nodosa* leaf width (cm) among sites and sampling time.

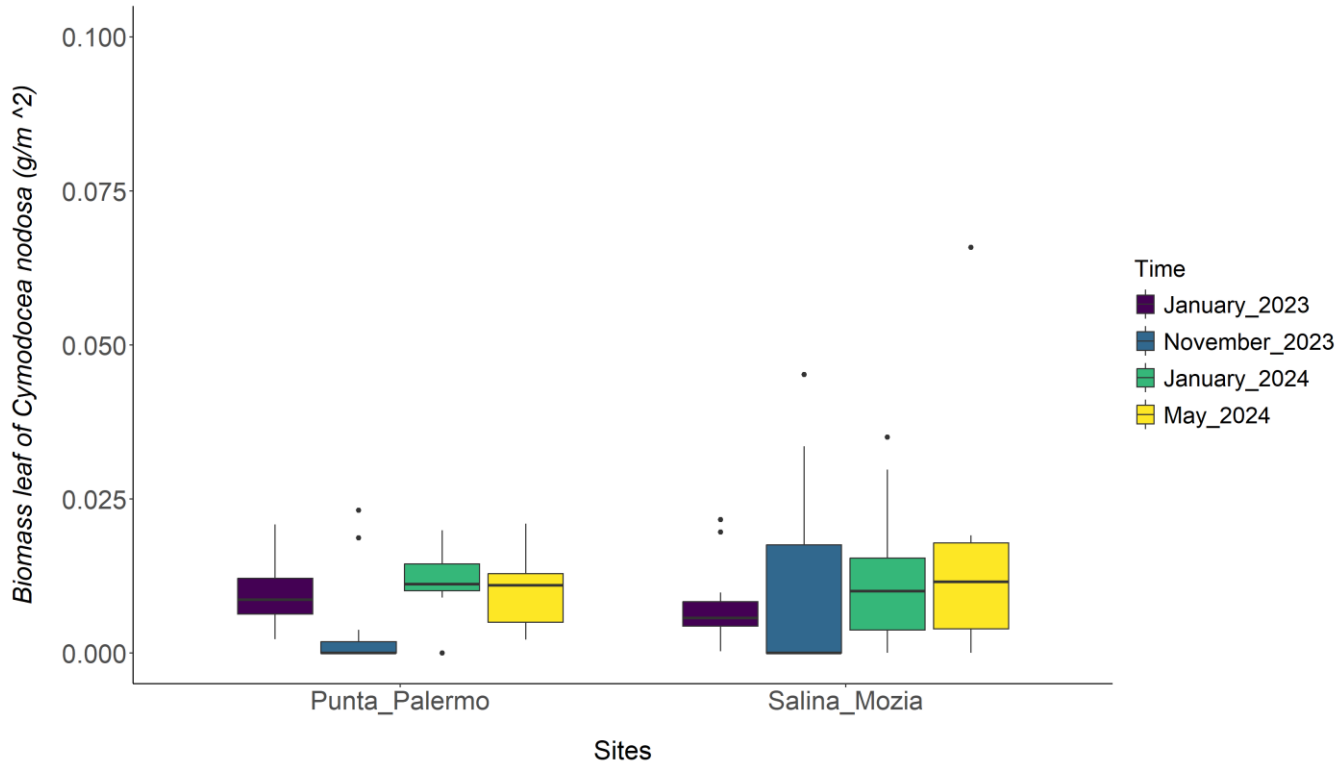

Figure 4.6: Variation of *Cymodocea nodosa* biomass (g/m^2) among sites and sampling time.

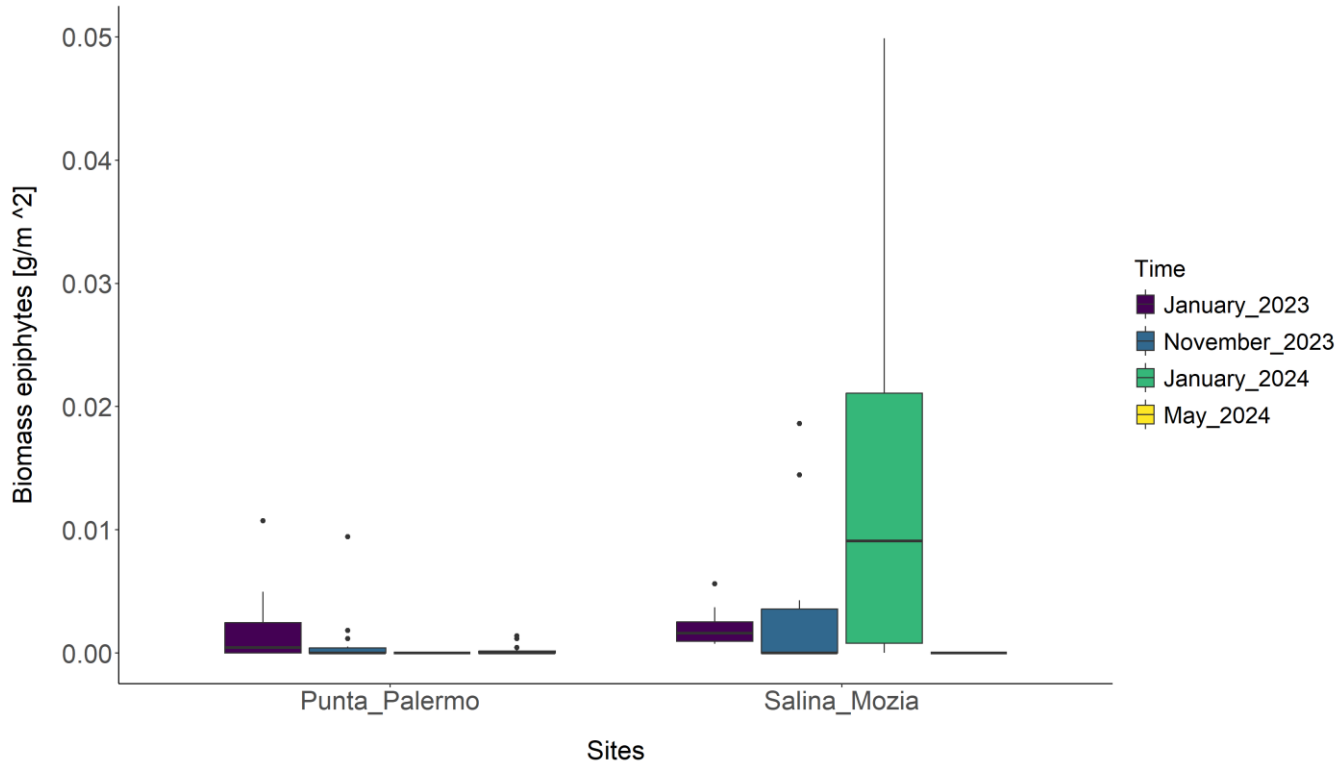

Figure 4.7: Variation of *Cymodocea nodosa* biomass epiphytes (g/m^2) among sites and sampling time.

The percentage of predated leaves varied temporally and spatially. In Punta Palermo, it ranged from 1.8% (January 2023) to 10.3% (November 2023), while in Salina Mozia, it varied from 0% (January 2023) to 12.8% (May 2024).

4.3.3 *Paranemonia cinerea* distribution

Paranemonia cinerea was more abundant in Punta Palermo (96 individuals) than in Salina Mozia (13 individuals) (Table 3), with significant differences between sites ($p < 0.01$) and sampling periods ($p < 0.01$). In Punta Palermo, abundance peaked in January 2023 (73 individuals) and January 2024 (21 individuals), with only one individual found in November 2023 and May 2024. In Salina Mozia, *P. cinerea* was only observed in January 2023 (6 individuals) and November 2023 (7 individuals) (Table 5, S).

Table 3: Temporal and spatial evolution of sea anemone observed in field during each time of monitoring.

Temporal and spatial evolution of sea anemone *Paranemonia cinerea*

Observations in field

Sites	Data	Number_sea_anemones
Punta Palermo	January 2023	73
Punta Palermo	November 2023	1
Punta Palermo	January 2024	21
Punta Palermo	May 2024	1
Salina Mozia	January 2023	6
Salina Mozia	November 2023	7
Salina Mozia	January 2024	0
Salina Mozia	May 2024	0

Paranemonia cinerea was found in both apical and intermediate positions on *C. nodosa* leaves. In Punta Palermo, multiple anemones were observed on single leaves, with positions ranging from 2.0 to 9.8 cm along the leaf length (Table 4).

Table 4: Position of sea anemone *Paranemonia cinerea* in each leaves of *Cymodocea nodosa* in Punta Palermo and Salina Mozia, in different time of monitoring.

Position of *Paranemonia cinerea* in each leaves of *Cymodocea nodosa*

Data elaborated in laboratory

Sites	Data	Height_leaves[1]	Height_sea_anemone[1]
Punta Palermo	January 2023	10.3	9.8
Punta Palermo	January 2023	9.2	5.0
Punta Palermo	January 2023	9.2	2.0
Punta Palermo	January 2023	9.2	3.0
Salina Mozia	November 2023	24.2	15.5
Salina Mozia	November 2023	29.7	17.5
Salina Mozia	November 2023	17.6	13.0
Salina Mozia	November 2023	24.0	14.5
Salina Mozia	November 2023	17.6	15.0
Punta Palermo	January 2024	18.2	16.0
Punta Palermo	January 2024	5.8	3.0
Punta Palermo	January 2024	8.4	7.0
Punta Palermo	January 2024	14.7	12.0
Punta Palermo	January 2024	12.5	7.0
Punta Palermo	January 2024	2.8	1.8
Punta Palermo	January 2024	12.0	11.3
Punta Palermo	January 2024	2.8	1.9
Punta Palermo	January 2024	4.8	4.8
Punta Palermo	January 2024	3.5	2.0
Punta Palermo	May 2024	4.8	4.2

[1] Measured in **cm**

4.3.4 Relationship between water depth and functional descriptors of *Cymodocea nodosa*

Cymodocea nodosa density and cover showed a negative correlation with water depth (R^2= 0.74, $p < 2.2e^{-}16$) (Figure 10, S). *Paranemonia cinerea* abundance was significantly related to water depth ($p < 0.01$) and *C. nodosa* density ($p = 0.053$), with the effect varying between sites (depth-site interaction: $p < 0.01$; density-site interaction: $p < 0.01$). The interaction between *C. nodosa* and *P. cinerea* showed clear spatial and temporal patterns. In January 2023, both species were present at both sites. By May 2024, the interaction was only observed in Punta Palermo, while Salina Mozia had only *C. nodosa* and bare sediment.

4.3.5 Relationship between number of *Paranemonia cinerea* and depth of water column.

Number of sea anemones *P. cinerea* (Figure 4.8) ranged with depth and depth-site interaction, but does not depend directly on the site. Depth of water column is very important factor ($p < 0.001$), only site factor has no significant effect ($p = 0.636$), but if we considered both interaction between depth and site is significant ($p < 0.001$), and depth and time is significant ($p < 0.001$), suggesting that the effect of depth on the number of anemones changes by site (Table 6, S).

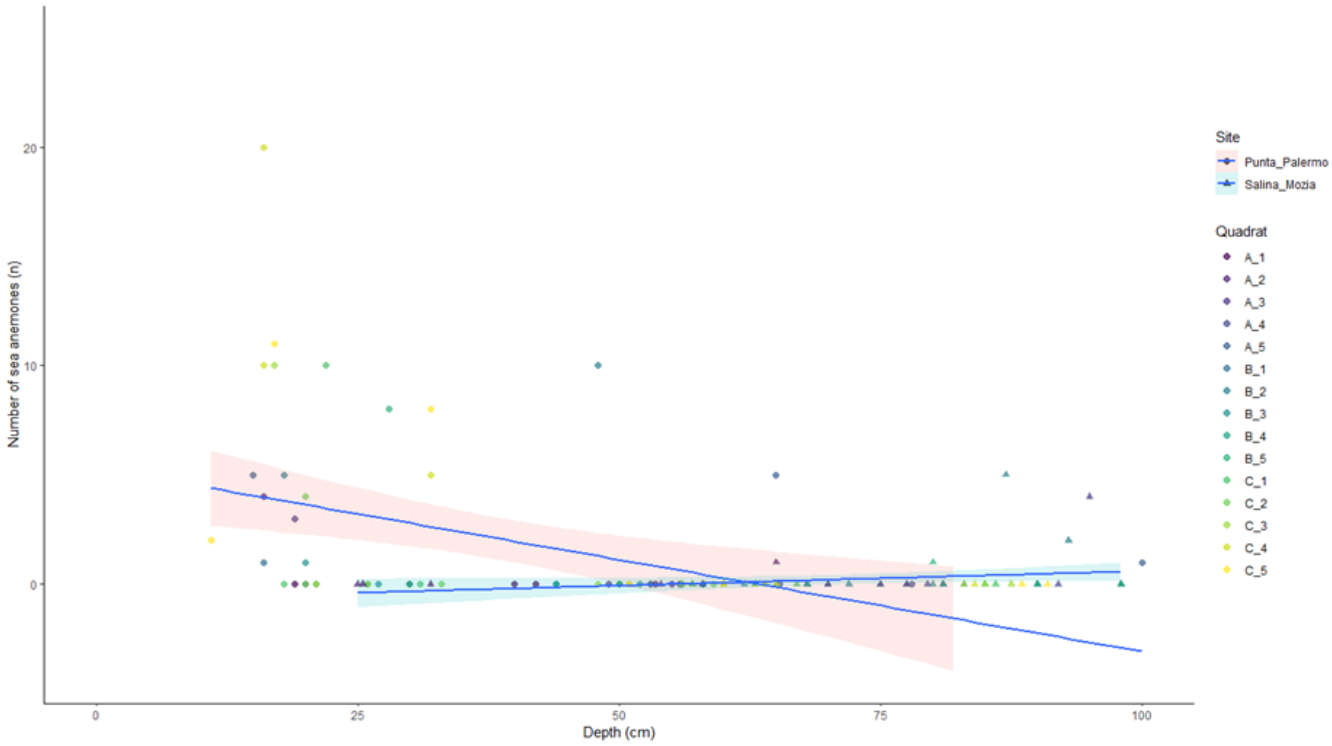

Figure 4.8 : Changes of number of sea anemone (n) across depth (cm), regarding the two studied sites (Punta Palermo and Salina Mozia).

4.3.6 Relationship between number of *Paranemonia cinerea* and density of *Cymodocea nodosa*.

The density of *C. nodosa* (Figure 4.9) has a marginally significant effect ($p = 0.053$) on the number of anemones. Site has a highly significant effect on the number of anemones ($p < 0.001$), confirming that site is a very important factor. The interaction between density of *C. nodosa* and site is highly significant ($p < 0.001$), indicating that the effect of density varies by site (Table 7, S).

The interaction between *C. nodosa* and *P. cinerea* was significantly different between sites, and dependent on the density of the habitat-forming species. In particular, in Punta Palermo, higher density of *C. nodosa* was associated with increasing abundance of *P. cinerea*. This relationship was not observed in Salina Mozia.

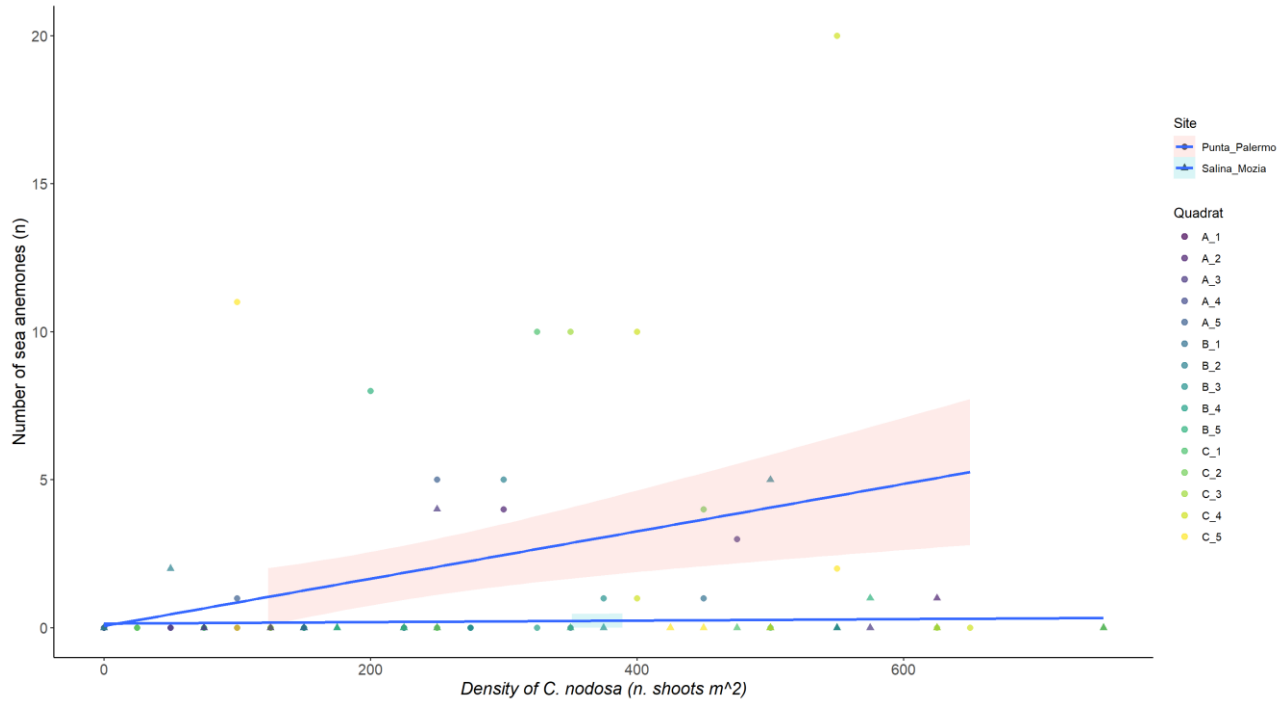

Figure 4.9: Variation of number of sea anemones (n) in base of *Cymodocea nodosa* density (n. shoots m^{-2}) among sites.

5.4 Discussions

Our study reveals complex spatial and temporal dynamics in the interaction between *Cymodocea nodosa* and *Paranemonia cinerea* within the Stagnone of Marsala. The key findings include variations in *C. nodosa* density and cover, the distribution of *P. cinerea*, and the biochemical characteristics of the water column and sediment.

The density and cover of *C. nodosa* exhibited distinct patterns at the two study sites. At Punta Palermo, we observe a decrease over time, while in Salina Mozia showed a more variable pattern with a notable increase in May 2024. These site-specific differences highlight the importance of local environmental conditions in shaping seagrass populations. Observations on *C. nodosa* decreased density and cover with increasing depth align with previous scientific research, suggesting that water column depth is crucial for the growth of seagrasses (Estes and Peterson, 2000; Ibarra-Obando et al., 2004). Ibarra-Obando et al. (2004) reported that marine

plant populations are limited primarily by physical disturbance, herbivory, light, and nutrients. Furthermore, light availability influences seagrass productivity, depth distribution, and abundance (Waycott et al., 2009).

An intriguing aspect of our results is the relationship between *C. nodosa* and *P. cinerea*. In January 2023, particularly in Punta Palermo *C. nodosa* density and cover were coinciding with a higher number of *P. cinerea* individuals. This positive association suggests a potential mutualistic relationship, which aligns with previous studies on epibiont-seagrass interactions. For instance, resident filter feeders can clear the overlying water column daily and control the density of suspended organic matter within seagrass meadows (Kikuchi, 1980; Lemmens et al., 1996; Heck and Valentine, 2006). The presence of epiphytes and epifauna, including sea anemones, play a crucial role in seagrass ecosystem health. Sea anemones, are often used as bioindicators to monitor the health of coastal and lagoon ecosystems. Because they are sensitive to environmental changes, such as pollution and chemical alterations, their presence or absence can indicate the health status of a lagoon (Rinkevich et al., 1983).

Our biochemical analyses provide further insights into the *C. nodosa* - *P. cinerea* interaction. The concentration of Chlorophyll-*a* in the water column was higher in areas where *P. cinerea* was present, particularly in May 2024. This could be attributed to several factors: 1) presence of zooxanthellae in *P. cinerea* which can increase primary productivity, 2) released chl-*a* from zooxanthellae due thermic stress, 3) excess symbionts being expelled, or 4) symbiont division and release (Coffroth et al., 2006). These released symbionts may temporarily persist in the water column or sediment, potentially affecting local nutrient dynamic (Coffroth et al., 2006; Littman et al., 2008; Fransolet et al., 2012; Fujise et al., 2014; Pochon et al., 2014). Also, *C. nodosa* is an important source of habitat and food for many marine species, including algae and phytoplankton. Their presence could promote the growth of algae at the surface, further increasing the concentration of chlorophyll (Borum, 2004).

The observed differences in biochemical parameters between sites and in the presence of *P. cinerea* suggest complex ecological interactions. Higher protein concentrations near *C. nodosa* and *P. cinerea* in Punta Palermo could be related to the sea anemones' biological activities. These results, align with the findings of Loret et al. (2020), who reported that sea anemones have different molecules that help them to capture prey or defend against predations. Specifically, their venoms are complex mixtures of peptides and small proteins. Similarly, seaweeds also show a complex composition, consisting primarily of polysaccharides, proteins, lipids and minerals.

Notably, they contain a high protein content, accounting for up to 30-40% of their dry weight (Loret et al., 2020). Presence of higher lipids concentrations near *C. nodosa* and *P. cinerea*, can be due to sea anemone can accumulate lipids as an energy reserve (Casado-Amezúa et al., 2016). Also, these increases of values was due to a high nutritional values of lipids, carbohydrates and proteins in *C. nodosa* (Nihal et al., 2013).

The observed variations in Total Suspended Matter (TSM) concentrations provide further evidence of the ecological role of *P. cinerea*. The higher TSM concentrations in areas with both *C. nodosa* and *P. cinerea*, particularly in Punta Palermo, suggest that sea anemones can take advantage of these environments rich in organic matter. In that the abundance of suspended particles helps to increase food availability (as it attracts prey). Also, mats made of *Cymodocea nodosa* have the ability to naturally filter out suspended particles from the water. Their leaves' structure slows the flow of water, which makes it easier for suspended organic particles, like dead phytoplankton or detritus, to settle more easily to the bottom instead of remaining in suspension (Yamuza-Magdaleno et al., 2024). Furthermore, seagrasses, such as *C. nodosa*, have the ability to aid in the sedimentation process, which helps retain organic matter and makes the water clearer by removing suspended particles. This suggests that the synergy between *C. nodosa* and *P. cinerea* may contribute to higher suspension of solids in the water, probably due to ecological interactions between these organisms, which may generate turbulence in the water or change sedimentation (Borum, 2004; Duarte et al., 2013).

In conclusion, our study provides valuable insights into the spatial and temporal dynamics of the *C. nodosa* - *P. cinerea* interaction in the Stagnone of Marsala. The complex relationships observed underscore the importance of considering species interactions in seagrass ecology and conservation efforts.

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Final considerations

This work focused on the important role of biotic interactions between seagrasses, as habitat formers, and their associated epiphytes and epibionts in coastal lagoon ecosystems, with a particular emphasis on how environmental change may influence the strength of their interactions.

The global review on seagrass-epibiont interactions highlighted the complexity and importance of these relationships. We found that seagrasses such as *Posidonia oceanica, Thalassia testudinum,* and *Zostera marina* play crucial roles supporting biodiversity in coastal ecosystems worldwide, providing refuge from predation (Prescott, 1990), enhancing abundance of mobile epifauna (Michael Martin-Smith, 1993b), and supporting invertebrate and fish nurseries (Michael et al., 2008). Epiphytes and epibionts emerged as sensitive indicators of environmental change, often responding more quickly than seagrasses themselves to shifts in nutrient conditions and other environmental factors (Jauralde et al., 2016; Piazzi et al., 2016).
One of the most important Italian coastal lagoons, the Stagnone of Marsala (Sicily), was chosen as study case for investigating seagrass biotic interactions. In-depth examination on the ecological status of the lagoon provided valuable insights into long-term ecological changes in this important Mediterranean coastal ecosystem. The study revealed distribution shifts in seagrass species composition over time, with *Cymodocea nodosa* meadows and beds of the algae *Caulerpa prolifera* largely replacing *Posidonia oceanica* in many areas. These changes were associated with alterations in hydrodynamic conditions and sediment composition within the lagoon (Bellissimo, 2014; Pergent et al., 2014) and were accompanied by changes in the ecological associated communities over time.

Currently, *Cymodocea nodosa* is the main structuring species within the Stagnone. Recently and unexplored interaction between the seagrass and the sea anemone *Paranemonia cinerea* was addressed through manipulative experiments in the laboratory and correlative experiments in the field. The experimental investigation of thermal performance and biotic interactions of *Cymodocea nodosa*, *Paranemonia cinerea*, and its symbiont *Symbiodinium* provided crucial insights into their potential resilience to climate change. *Cymodocea nodosa* demonstrated a wide thermal tolerance range with warm-water affinity, consistent with its characterization as a eurytopic species (Borum, 2004). This suggests that *C. nodosa* may be relatively resilient to warming temperatures in the Mediterranean. *Paranemonia cinerea* and its

symbiont showed different thermal optimum, with the anemone displaying higher thermal tolerance than *Symbiodinium*. This differential response highlights the potential for climate change to disrupt this mutualistic relationship, possibly leading to bleaching events at elevated temperatures (Dani et al., 2016; Doering et al., 2023), compromising the anemone survival and population structure. Correlative field study on the spatial and temporal dynamics of *C. nodosa* and *P. cinerea* interactions in the Stagnone of Marsala revealed that water depth, temperature, and *C. nodosa* density were key factors influencing the distribution and abundance of *P. cinerea*. The sea anemone showed a preference for shallower areas with denser seagrass coverage, suggesting that changes in seagrass meadow structure due to environmental shifts could significantly impact *P. cinerea* populations. This disruption could also have cascading effects throughout the entire ecosystem. The weakening or disappearance of the interaction between the anemone and the seagrass would likely alter the composition and dynamics of the benthic-pelagic coupling, affecting the ecosystem functioning.

In conclusion, this thesis provides a comprehensive understanding of seagrass-epibiont interactions in Mediterranean coastal lagoons, from global patterns to local adaptations. The findings underscore the complex nature of these relationships and their sensitivity to environmental change. As climate change continues to impact coastal ecosystems, understanding these interactions will be crucial for predicting ecosystem responses and developing effective conservation strategies. Future research should focus on long-term monitoring of these interactions across different Mediterranean sites, coupled with manipulative experiments to further elucidate the mechanisms underlying observed patterns.

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Supplementary materials

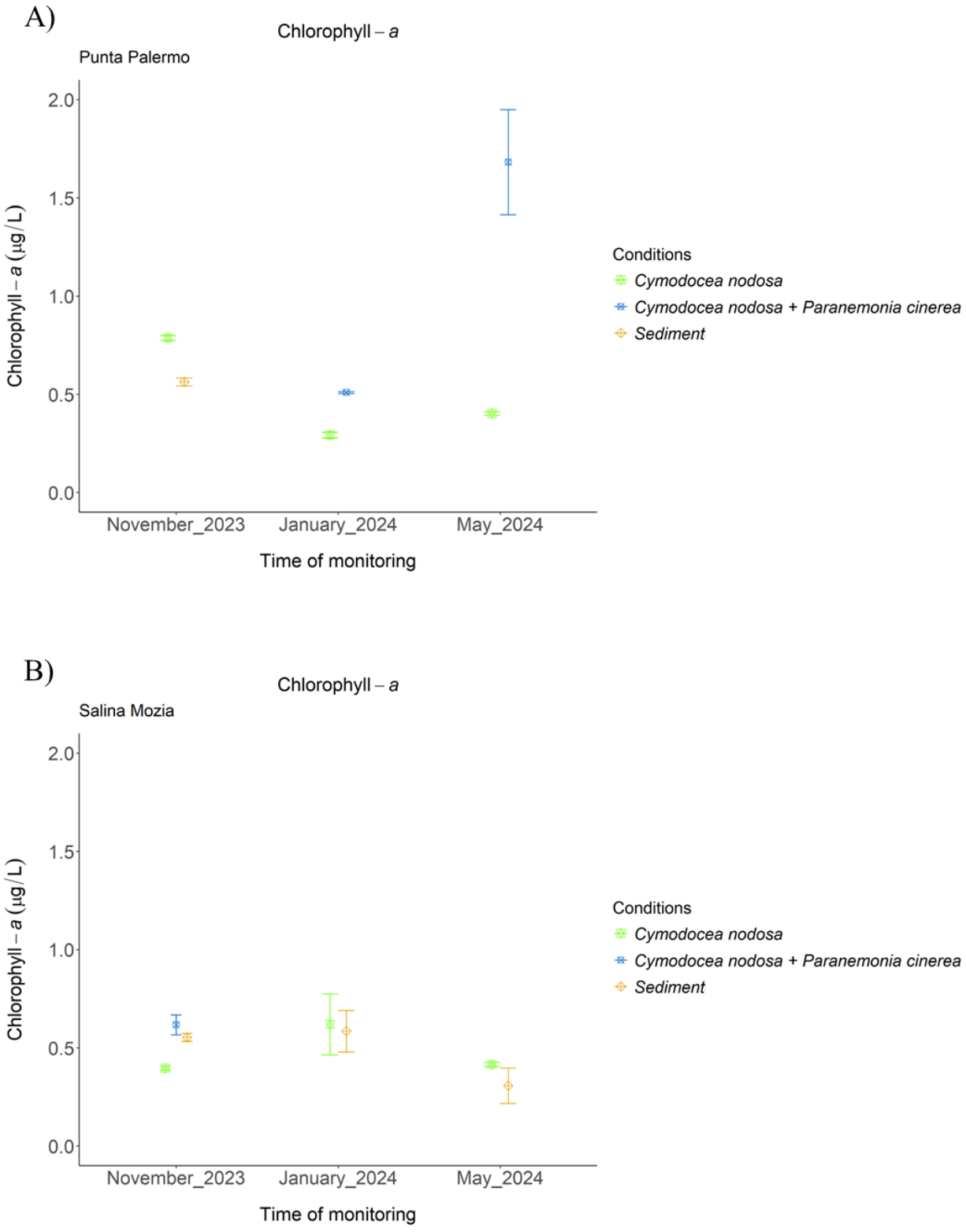

Figure 1: Temporal changes in Chlorophyll-*a* (µg/L) in the water column in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

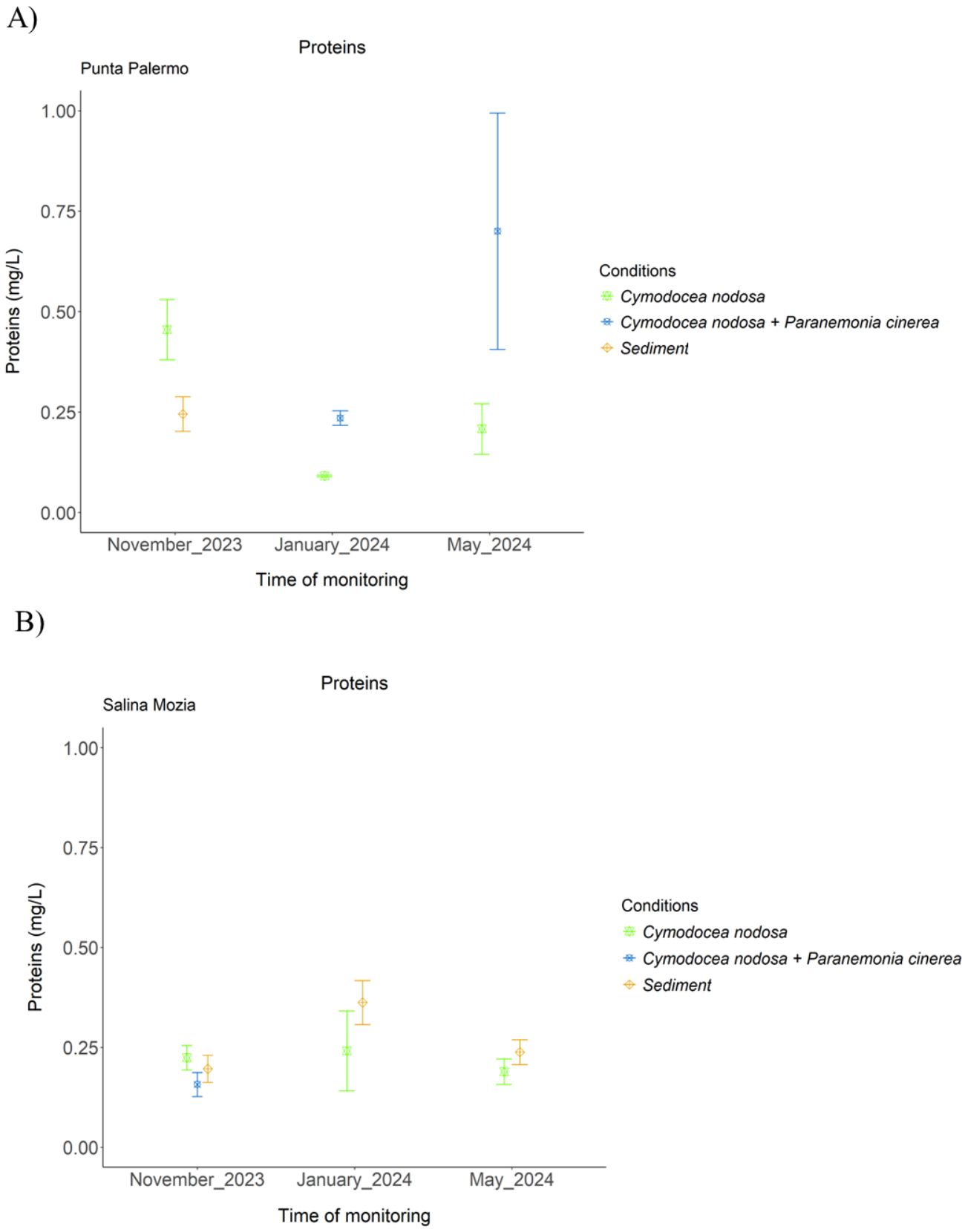

Figure 2: Temporal changes in Proteins (mg/L) in the water column in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea,* and Sediment, in Punta Palermo (A) and Salina Mozia (B).

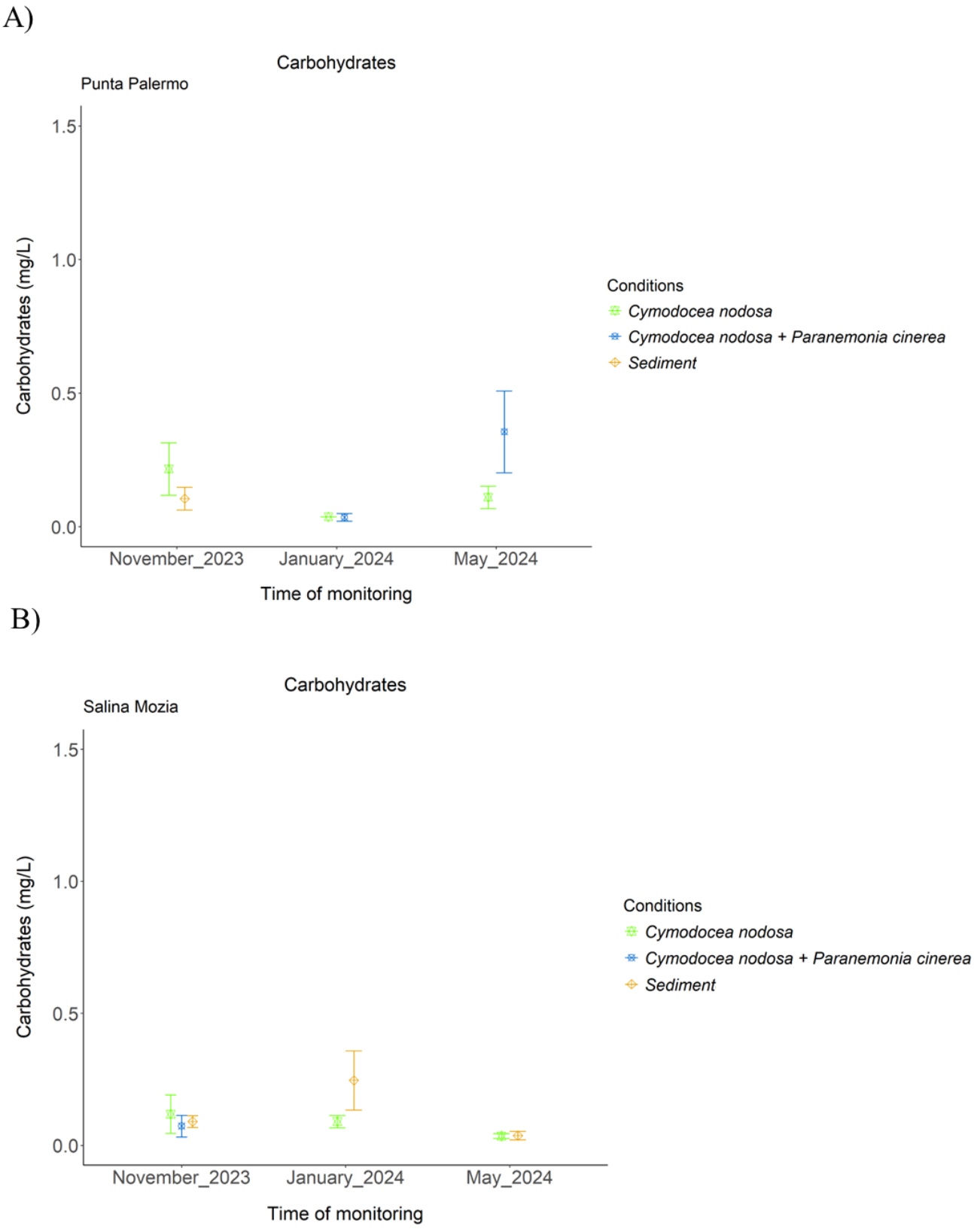

Figure 3: Temporal changes in Carbohydrates (mg/L) in the water column in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

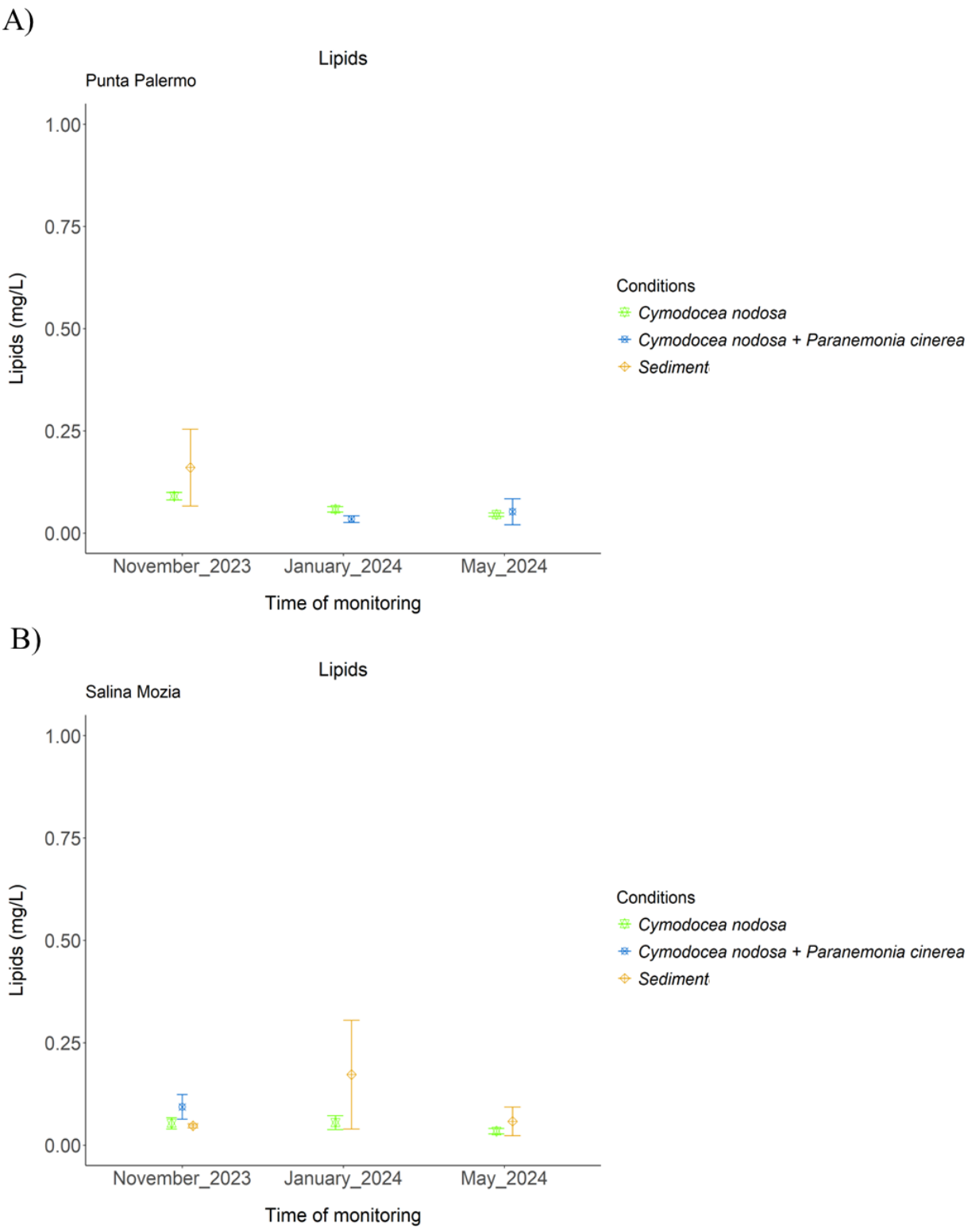

Figure 4: Temporal changes in Lipids (mg/L) in the water column in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

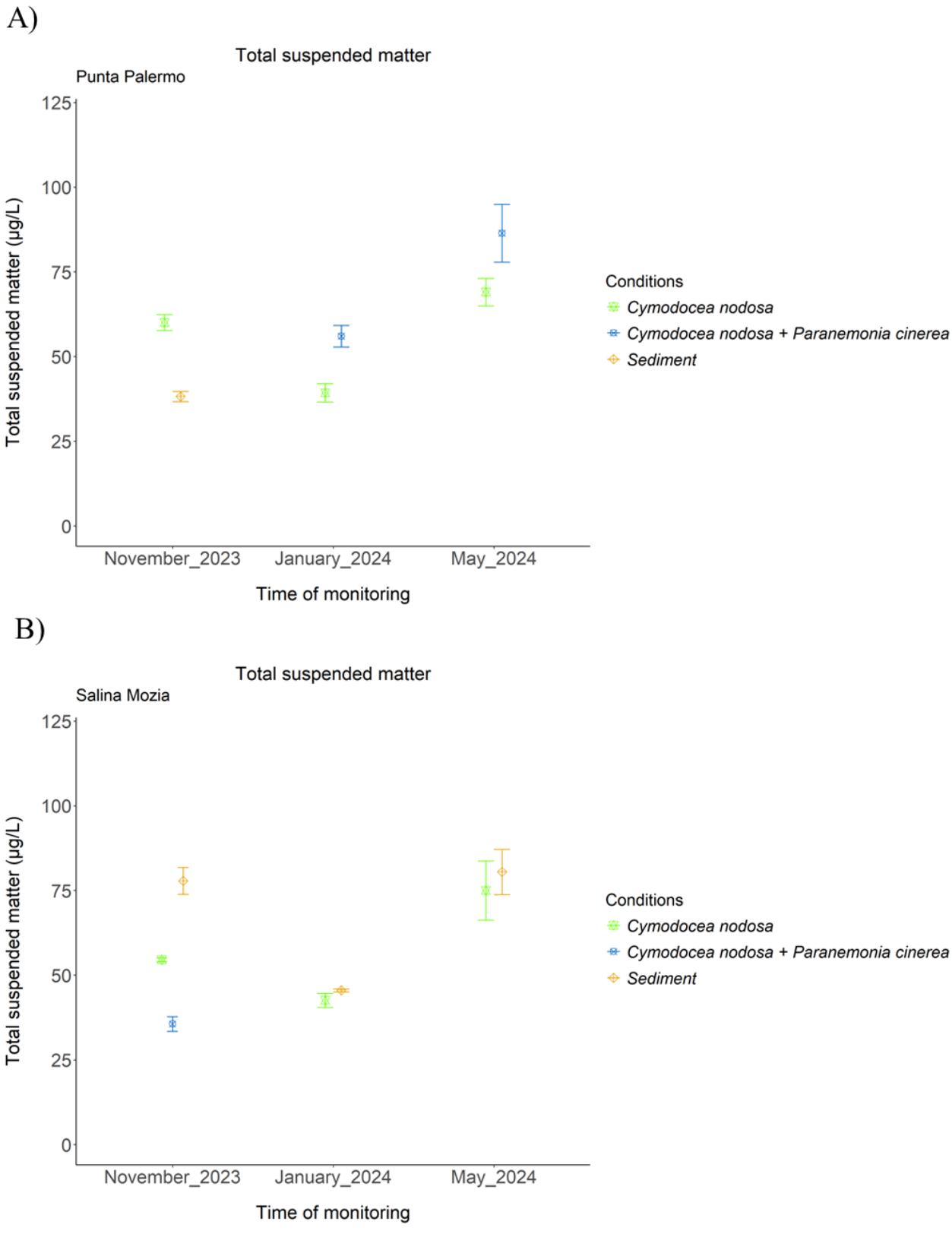

Figure 5: Temporal changes in TSM (mg/L) in the water column in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

Table 1: ANOVA results on biochemical analysis in water column.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Conditions	2	0.4558428	0.2279214	2.4126494	1.847e-01	Chl-a ~ Conditions + Sites * Time
Sites	1	0.0566080	0.0566080	0.5992213	4.739e-01	Chl-a ~ Conditions + Sites * Time
Time	2	0.0800540	0.0400270	0.4237035	6.761e-01	Chl-a ~ Conditions + Sites * Time
Sites:Time	2	0.4478153	0.2239077	2.3701624	1.888e-01	Chl-a ~ Conditions + Sites * Time
Residuals	5	0.4723467	0.0944693	NA	NA	Chl-a ~ Conditions + Sites * Time
Conditions	2	0.0538802	0.0269401	1.5049059	3.079e-01	Proteins ~ Conditions * Sites* Time
Sites	1	0.0182293	0.0182293	1.0183088	3.592e-01	Proteins ~ Conditions * Sites* Time
Time	2	0.0219595	0.0109798	0.6133436	5.778e-01	Proteins ~ Conditions * Sites* Time
Sites:Time	2	0.1104606	0.0552303	3.0852319	1.340e-01	Proteins ~ Conditions * Sites* Time
Residuals	5	0.0895075	0.0179015	NA	NA	Proteins ~ Conditions * Sites* Time
Conditions	2	0.0115028	0.0057514	0.8106543	4.955e-01	Carbohydrates ~ Conditions + Sites * Time
Sites	1	0.0048824	0.0048824	0.6881730	4.446e-01	Carbohydrates ~ Conditions + Sites * Time
Time	2	0.0021409	0.0010705	0.1508813	8.637e-01	Carbohydrates ~ Conditions + Sites * Time
Sites:Time	2	0.0588699	0.0294349	4.1488140	8.669e-02	Carbohydrates ~ Conditions + Sites * Time
Residuals	5	0.0354739	0.0070948	NA	NA	Carbohydrates ~ Conditions + Sites * Time
Conditions	2	0.0080508	0.0040254	2.6716120	1.625e-01	Lipids ~ Conditions + Sites * Time
Sites	1	0.0008429	0.0008429	0.5594359	4.882e-01	Lipids ~ Conditions + Sites * Time
Time	2	0.0031612	0.0015806	1.0490169	4.165e-01	Lipids ~ Conditions + Sites * Time
Sites:Time	2	0.0045872	0.0022936	1.5222429	3.046e-01	Lipids ~ Conditions + Sites * Time
Residuals	5	0.0075336	0.0015067	NA	NA	Lipids ~ Conditions + Sites * Time
Conditions	2	420.6838810	210.3419405	1.3087784	3.490e-01	TSM ~ Conditions + Sites * Time
Sites	1	7.8165386	7.8165386	0.0486357	8.342e-01	TSM ~ Conditions + Sites * Time
Time	**2**	**2351.1723203**	**1175.5861602**	**7.3146697**	****3.275e-02****	**TSM ~ Conditions + Sites * Time**
Sites:Time	2	184.9447238	92.4723619	0.5753766	5.958e-01	TSM ~ Conditions + Sites * Time
Residuals	5	803.5811655	160.7162331	NA	NA	TSM ~ Conditions + Sites * Time

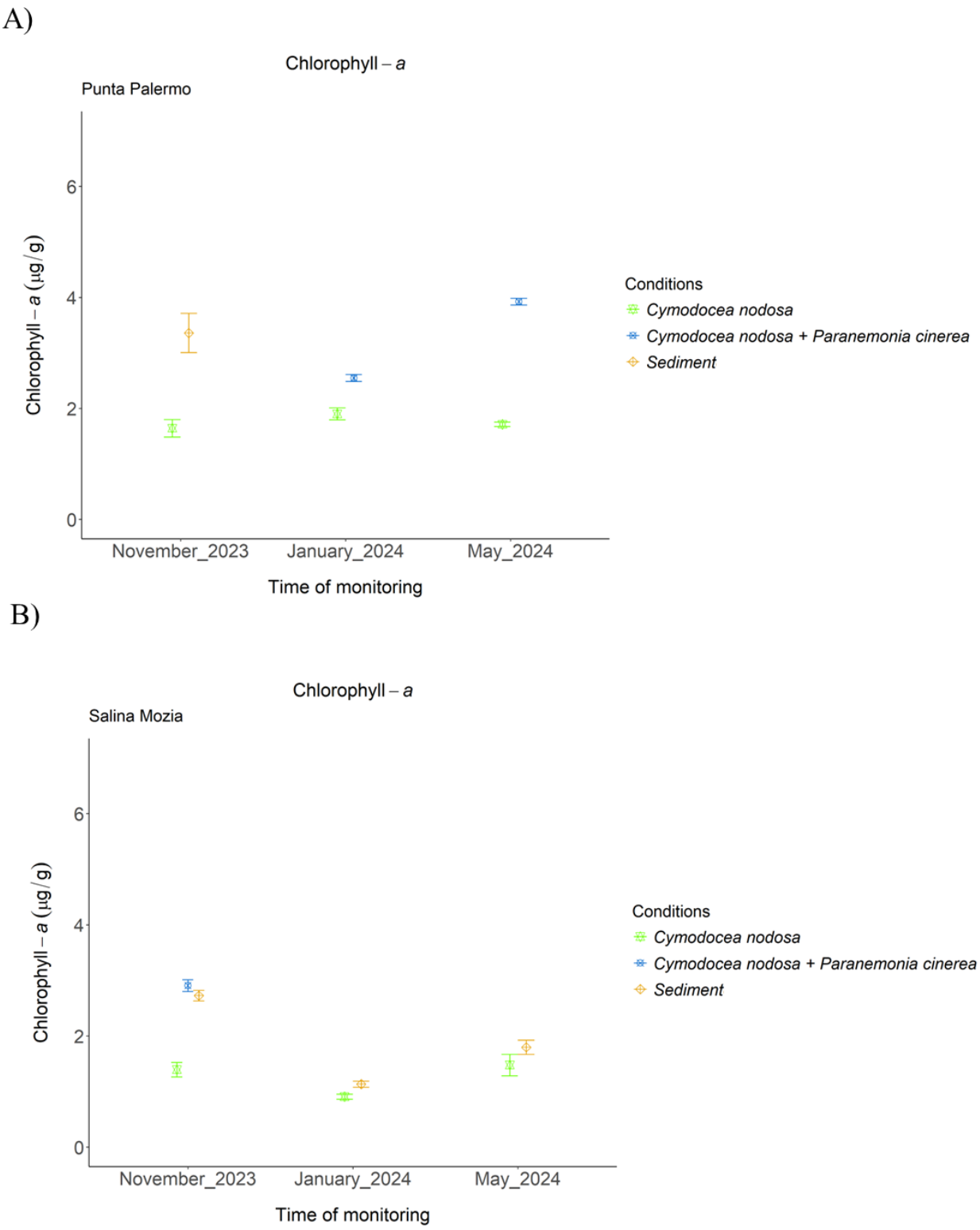

Figure 6: Temporal changes in Chlorophyll-*a* (µg/g) in the sediment in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

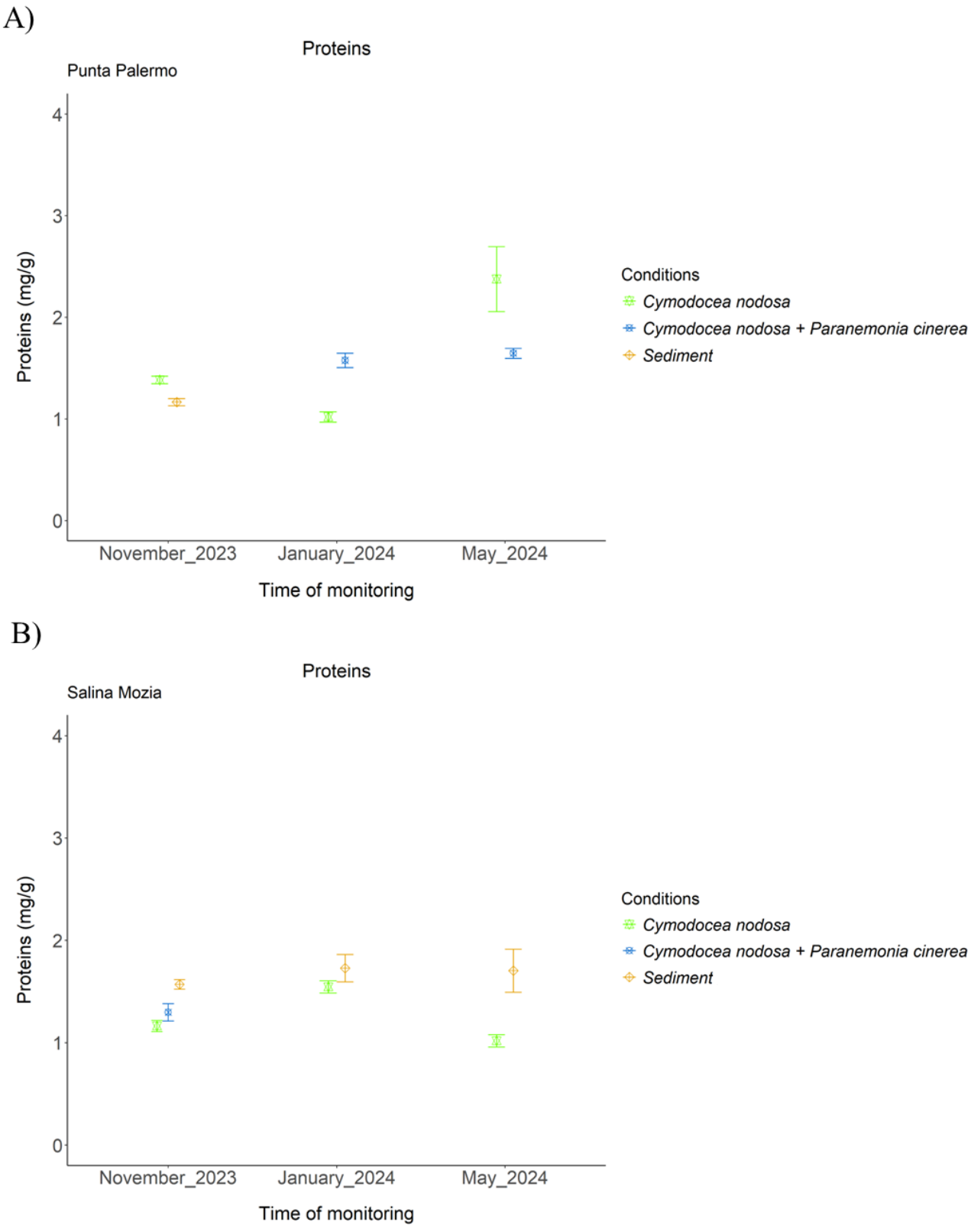

Figure 7: Temporal changes in Proteins (mg/g) in the sediment in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

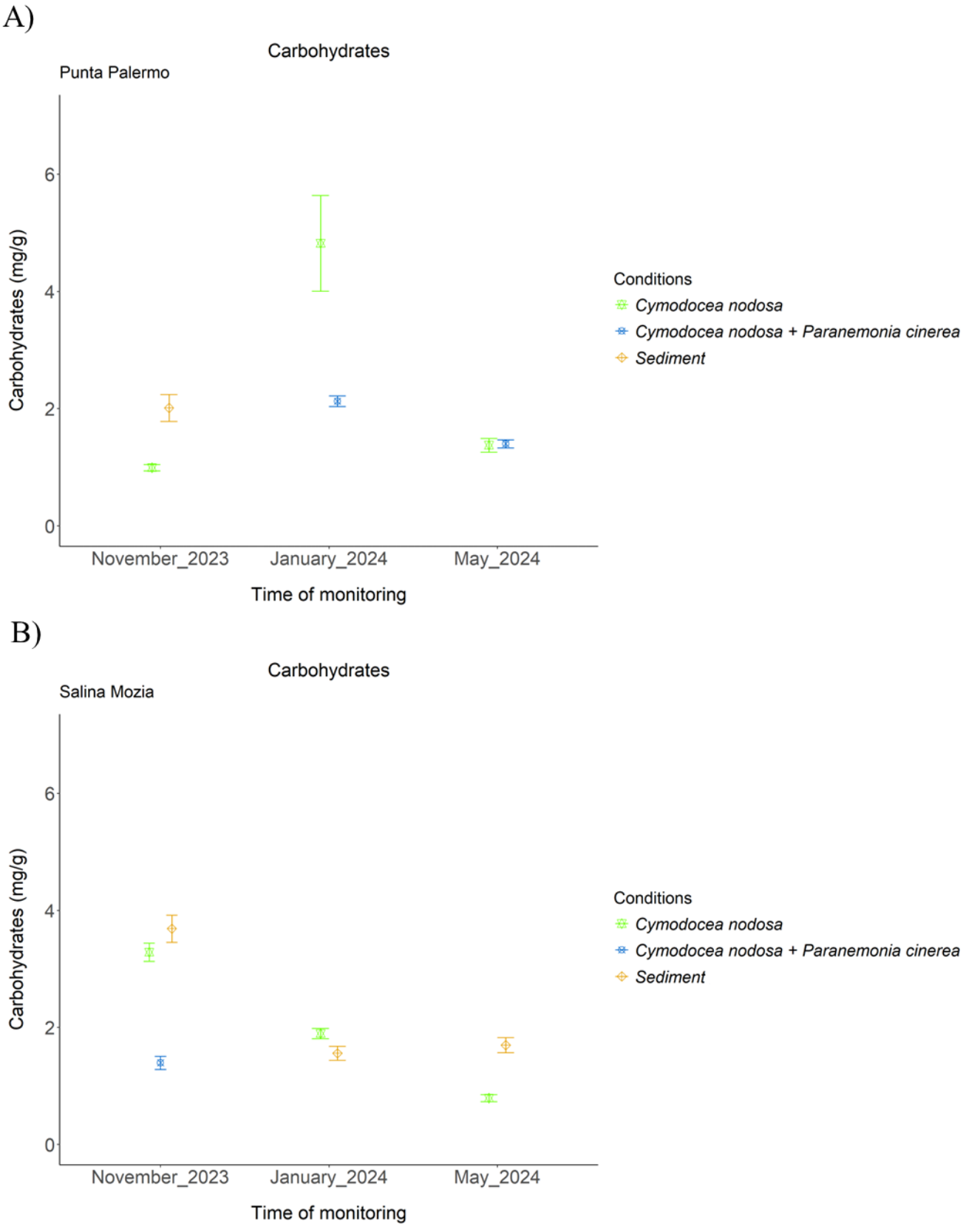

Figure 8: Temporal changes in Carbohydrates (mg/g) in the sediment in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea,* and Sediment, in Punta Palermo (A) and Salina Mozia (B).

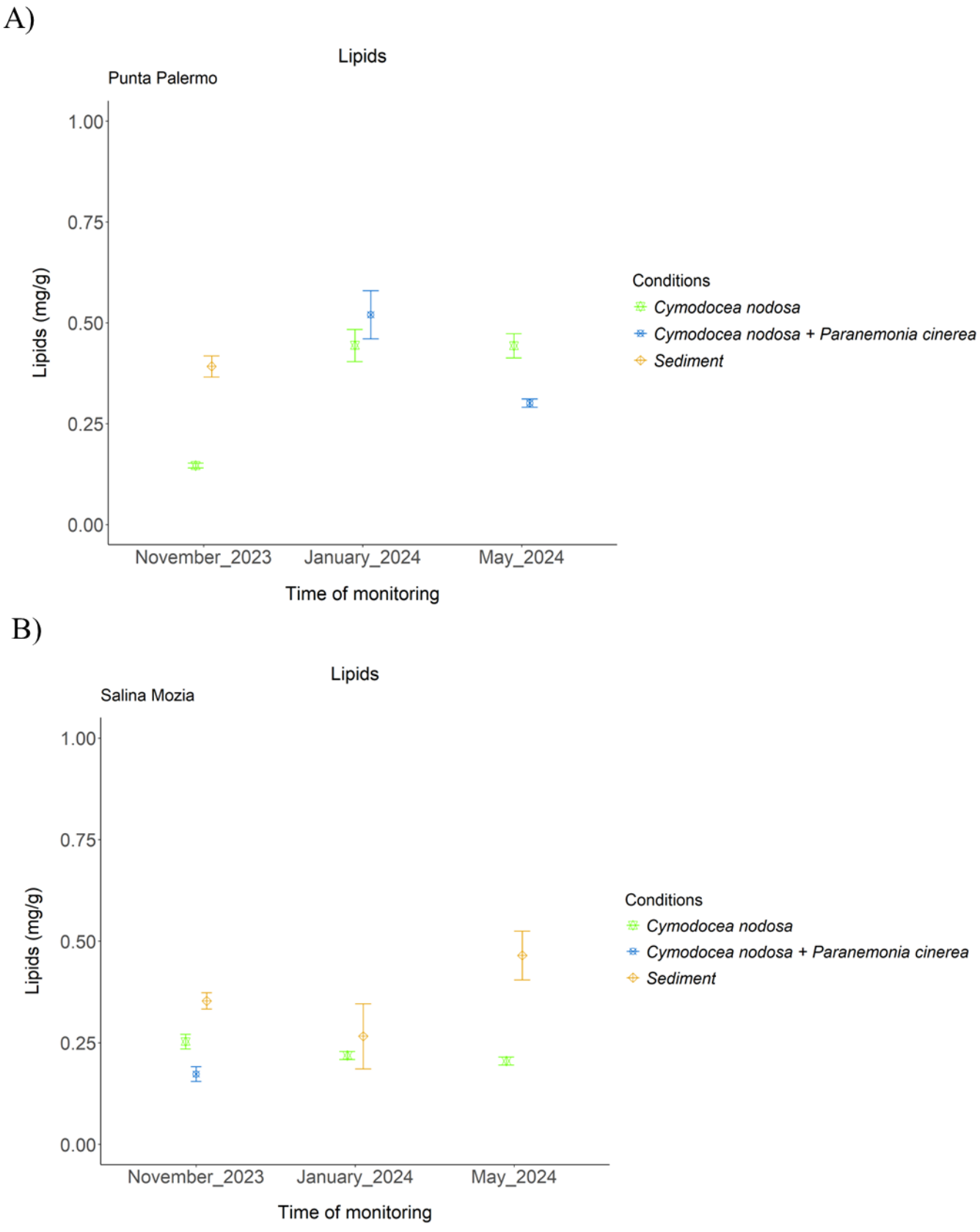

Figure 9: Temporal changes in Lipids (mg/g) in the sediment in three different conditions: *Cymodocea nodosa*, *Cymodocea nodosa* with *Paranemonia cinerea*, and Sediment, in Punta Palermo (A) and Salina Mozia (B).

Table 2: ANOVA results on biochemical analysis in the sediment.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Conditions	**2**	**5.3678412**	**2.6839206**	**9.3157847**	****2.059e-02****	**Chl-a ~ Conditions + Sites * Time**
Sites	1	1.4587179	1.4587179	5.0631533	7.426e-02	Chl-a ~ Conditions + Sites * Time
Time	2	1.4810904	0.7405452	2.5704038	1.707e-01	Chl-a ~ Conditions + Sites * Time
Sites:Time	2	0.1293506	0.0646753	0.2244855	8.066e-01	Chl-a ~ Conditions + Sites * Time
Residuals	5	1.4405231	0.2881046	NA	NA	Chl-a ~ Conditions + Sites * Time
Conditions	2	0.0258153	0.0129077	0.1038990	9.032e-01	Proteins ~ Conditions * Sites* Time
Sites	1	0.0491834	0.0491834	0.3958970	5.568e-01	Proteins ~ Conditions * Sites* Time
Time	2	0.3230380	0.1615190	1.3001317	3.510e-01	Proteins ~ Conditions * Sites* Time
Sites:Time	2	0.6096642	0.3048321	2.4537167	1.809e-01	Proteins ~ Conditions * Sites* Time
Residuals	5	0.6211640	0.1242328	NA	NA	Proteins ~ Conditions * Sites* Time
Conditions	2	0.4530782	0.2265391	0.1860996	8.357e-01	Carbohydrates ~ Conditions + Sites * Time
Sites	1	0.0337864	0.0337864	0.0277552	8.742e-01	Carbohydrates ~ Conditions + Sites * Time
Time	2	3.5576021	1.7788011	1.4612673	3.164e-01	Carbohydrates ~ Conditions + Sites * Time
Sites:Time	2	6.1523750	3.0761875	2.5270573	1.744e-01	Carbohydrates ~ Conditions + Sites * Time
Residuals	5	6.0865012	1.2173002	NA	NA	Carbohydrates ~ Conditions + Sites * Time
Conditions	2	0.0279219	0.0139609	2.2454160	2.014e-01	Lipids ~ Conditions + Sites * Time
Sites	1	0.0410455	0.0410455	6.6015707	5.007e-02	Lipids ~ Conditions + Sites * Time
Time	2	0.0307649	0.0153824	2.4740417	1.791e-01	Lipids ~ Conditions + Sites * Time
Sites:Time	2	0.0500293	0.0250147	4.0232440	9.093e-02	Lipids ~ Conditions + Sites * Time
Residuals	5	0.0310877	0.0062175	NA	NA	Lipids ~ Conditions + Sites * Time

Table 3: ANOVA result on density and cover data of *C. nodosa*.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Sites	1	12224.036	12224.0360	1.441653	2.324e-01	Density ~ Site * Time
Time	**3**	**376921.483**	**125640.4942**	**14.817529**	****3.438e-08****	**Density ~ Site * Time**
Sites:Time	3	40629.890	13543.2966	1.597241	1.941e-01	Density ~ Site * Time
Residuals	112	949668.159	8479.1800	NA	NA	Density ~ Site * Time
Sites	**1**	**1558.081**	**1558.0813**	**6.225245**	****1.405e-02****	**Cover ~ Site * Time**
Time	**3**	**2266.386**	**755.4620**	**3.018415**	****3.286e-02****	**Cover ~ Site * Time**
Sites:Time	**3**	**2163.783**	**721.2611**	**2.881767**	****3.905e-02****	**Cover ~ Site * Time**
Residuals	112	28031.844	250.2843	NA	NA	Cover ~ Site * Time

Table 4: ANOVA result on functional descriptors of *Cymodocea nodosa*.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Sites	**1**	**1.159548e+03**	**1159.5480739**	**45.3639038**	**0.0000000**	**Length ~ Site * Time**
Time	**3**	**1.242533e+04**	**4141.7762748**	**162.0348001**	**0.0000000**	**Length ~ Site * Time**
Sites:Time	**3**	**1.779845e+03**	**593.2815893**	**23.2103951**	**0.0000000**	**Length ~ Site * Time**
Residuals	1282	3.276924e+04	25.5610293	NA	NA	Length ~ Site * Time
Sites	**1**	**5.638660e-02**	**0.0563866**	**12.4558776**	**0.0004314**	**Width leaf ~ Site * Time**
Time	3	2.637340e-02	0.0087911	1.9419743	0.1209666	Width leaf ~ Site * Time
Sites:Time	**3**	**2.303068e-01**	**0.0767689**	**16.9583758**	**0.0000000**	**Width leaf ~ Site * Time**
Residuals	1282	5.803491e+00	0.0045269	NA	NA	Width leaf ~ Site * Time
Site	1	2.571540e-02	0.0257154	1.1783587	0.2800213	Biomass of leaves ~ Site * Time
Time	3	6.208220e-02	0.0206941	0.9482657	0.4199341	Biomass of leaves ~ Site * Time
Site:Time	3	5.855290e-02	0.0195176	0.8943580	0.4465156	Biomass of leaves ~ Site * Time
Residuals	112	2.444185e+00	0.0218231	NA	NA	Biomass of leaves ~ Site * Time
Site	1	2.750300e-03	0.0027503	2.6389167	0.1070857	Biomass epiphytes ~ Site * Time
Time	3	3.159800e-03	0.0010533	1.0105938	0.3908936	Biomass epiphytes ~ Site * Time
Site:Time	3	2.617300e-03	0.0008724	0.8370945	0.4762734	Biomass epiphytes ~ Site * Time
Residuals	112	1.167277e-01	0.0010422	NA	NA	Biomass epiphytes ~ Site * Time

Table S5: ANOVA result on changes in number of sea anemones (n) among sites and time of monitoring.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Sites	**1**	**56.03333**	**56.033333**	**21.04068**	**1.18e-05**	**Number of sea anemone ~ Site * Time**
Time	**3**	**113.16667**	**37.722222**	**14.16480**	**1.00e-07**	**Number of sea anemone ~ Site * Time**
Sites:Time	**3**	**126.50000**	**42.166667**	**15.83371**	**0.00e+00**	**Number of sea anemone ~ Site * Time**
Residuals	112	298.26667	2.663095	NA	NA	Number of sea anemone ~ Site * Time

Table S6: ANOVA test on changes in number of sea anemones (n) among depth (cm) , sites and time of monitoring.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Depth	1	160.8832873	160.8832873	76.1210652	0.0000000	Number sea anemone ~ Depth * Sites * Time
Sites	**1**	**0.4774034**	**0.4774034**	**0.2258808**	**0.6355917**	**Number sea anemone ~ Depth * Sites * Time**
Time	3	88.0391748	29.3463916	13.8850879	0.0000001	Number sea anemone ~ Depth * Sites * Time
Depth:Sites	1	31.9117992	31.9117992	15.0988968	0.0001798	Number sea anemone ~ Depth * Sites * Time
Depth:Time	3	55.4582396	18.4860799	8.7465896	0.0000316	Number sea anemone ~ Depth * Sites * Time
Sites:Time	3	10.2014937	3.4004979	1.6089274	0.1918050	Number sea anemone ~ Depth * Sites * Time
Depth:Sites:Time	3	27.1893339	9.0631113	4.2881625	0.0067711	Number sea anemone ~ Depth * Sites * Time
Residuals	104	219.8059347	2.1135186	NA	NA	Number sea anemone ~ Depth * Sites * Time

Table S7: ANOVA test on changes in number of sea anemones (n) among density of *C. nodosa* (n. shoots/m^2) , sites and time of monitoring.

Factor	Df	Sum Sq	Mean Sq	F Value	p-value	Variables
Density	1	4.888259	4.888259	2.230474	0.1473497	Number sea anemone ~ Density * Sites * Time
Sites	**1**	**59.721795**	**59.721795**	**27.250587**	**0.0000188**	**Number sea anemone ~ Density * Sites * Time**
Time	**55**	**382.391931**	**6.952581**	**3.172408**	**0.0010063**	**Number sea anemone ~ Density * Sites * Time**
Density:Sites	1	2.238819	2.238819	1.021556	0.3214645	Number sea anemone ~ Density * Sites * Time
Density:Time	27	87.744828	3.249808	1.482862	0.1592644	Number sea anemone ~ Density * Sites * Time
Sites:Time	5	0.000000	0.000000	0.000000	1.0000000	Number sea anemone ~ Density * Sites * Time
Density:Sites:Time	3	0.000000	0.000000	0.000000	1.0000000	Number sea anemone ~ Density * Sites * Time
Residuals	26	56.981035	2.191578	NA	NA	Number sea anemone ~ Density * Sites * Time

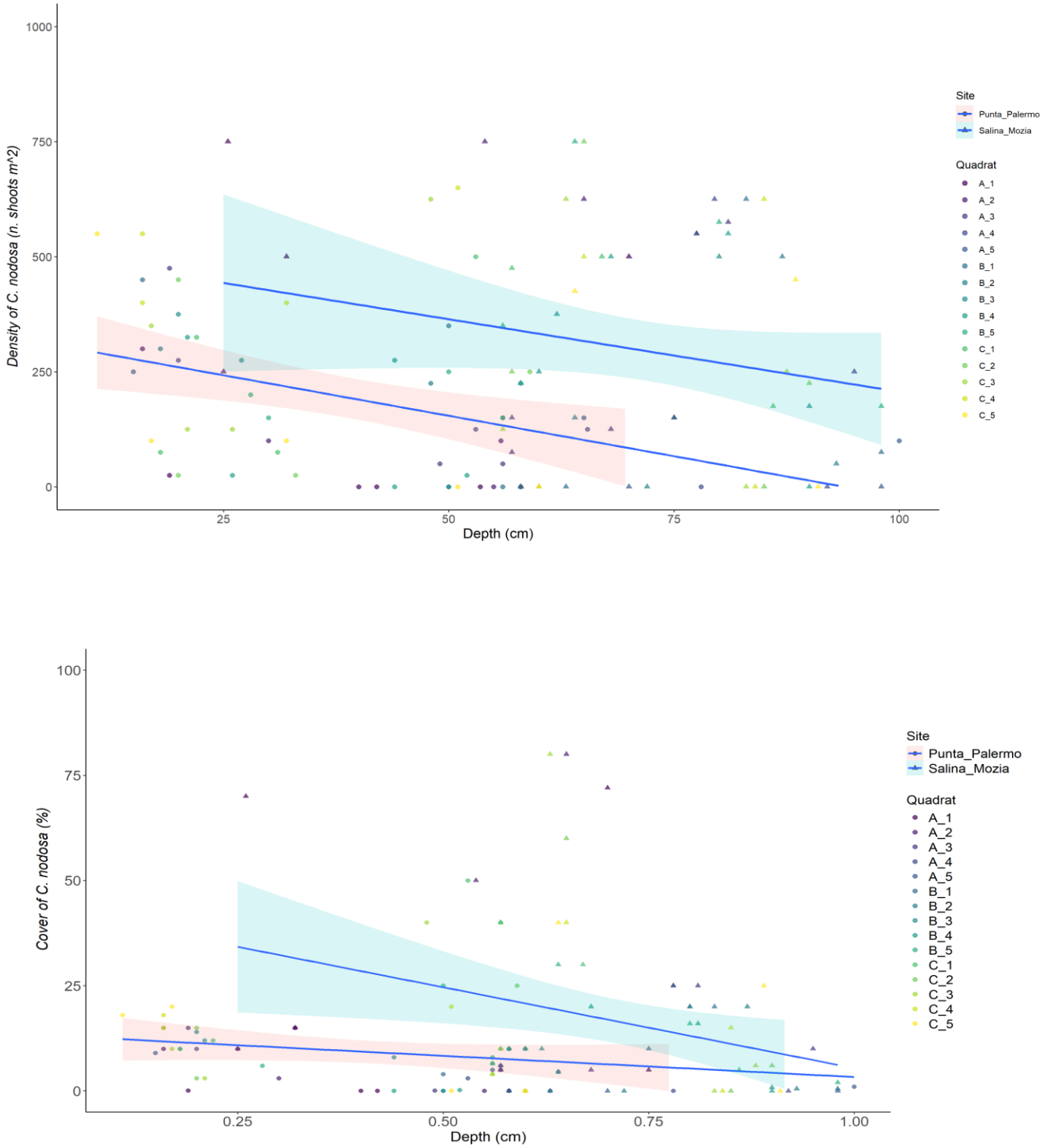

Figure 10: Relationship between density (n. shouts/m^2) (A) and percentage cover (%) (B) of *Cymodocea nodosa* among sites.

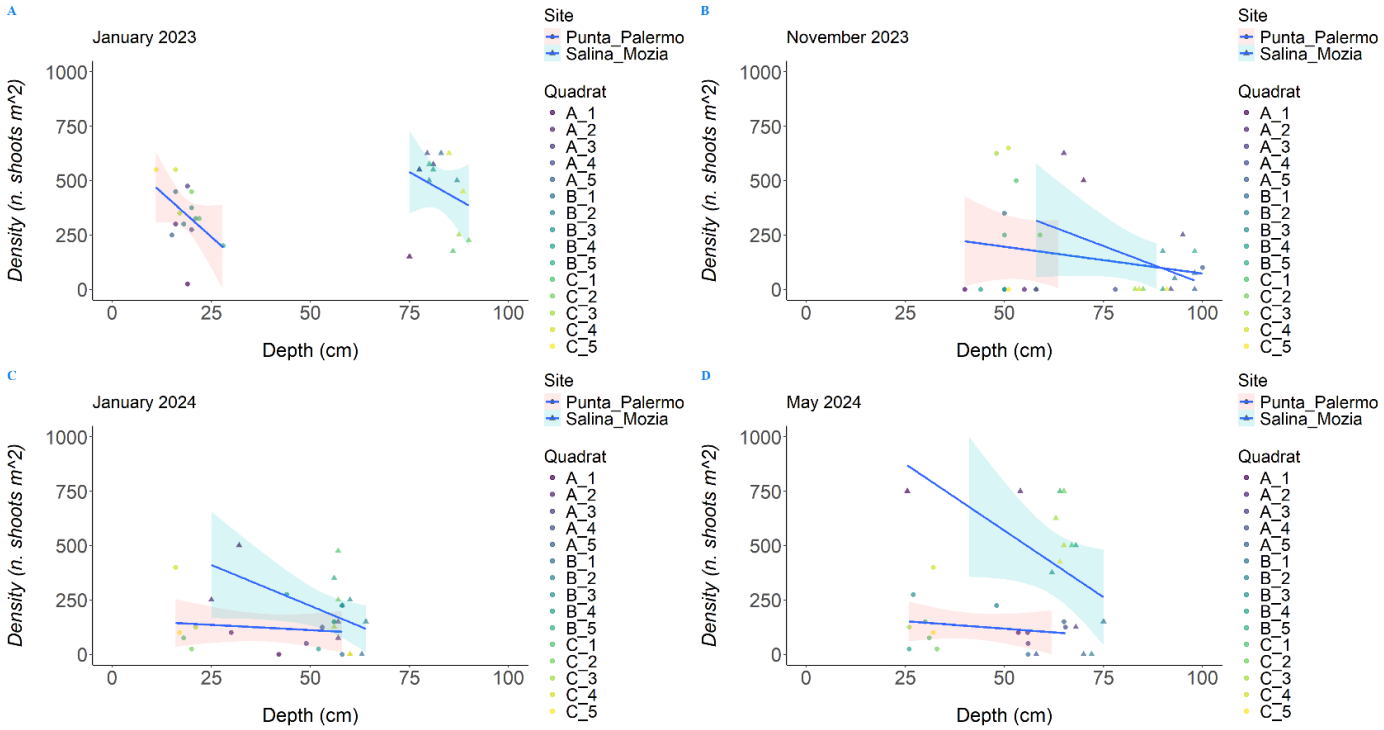

Figure 11: Relationship between density of *Cymodocea nodosa* (n. shouts/m^2) among sites and quadrat in different time of monitoring.

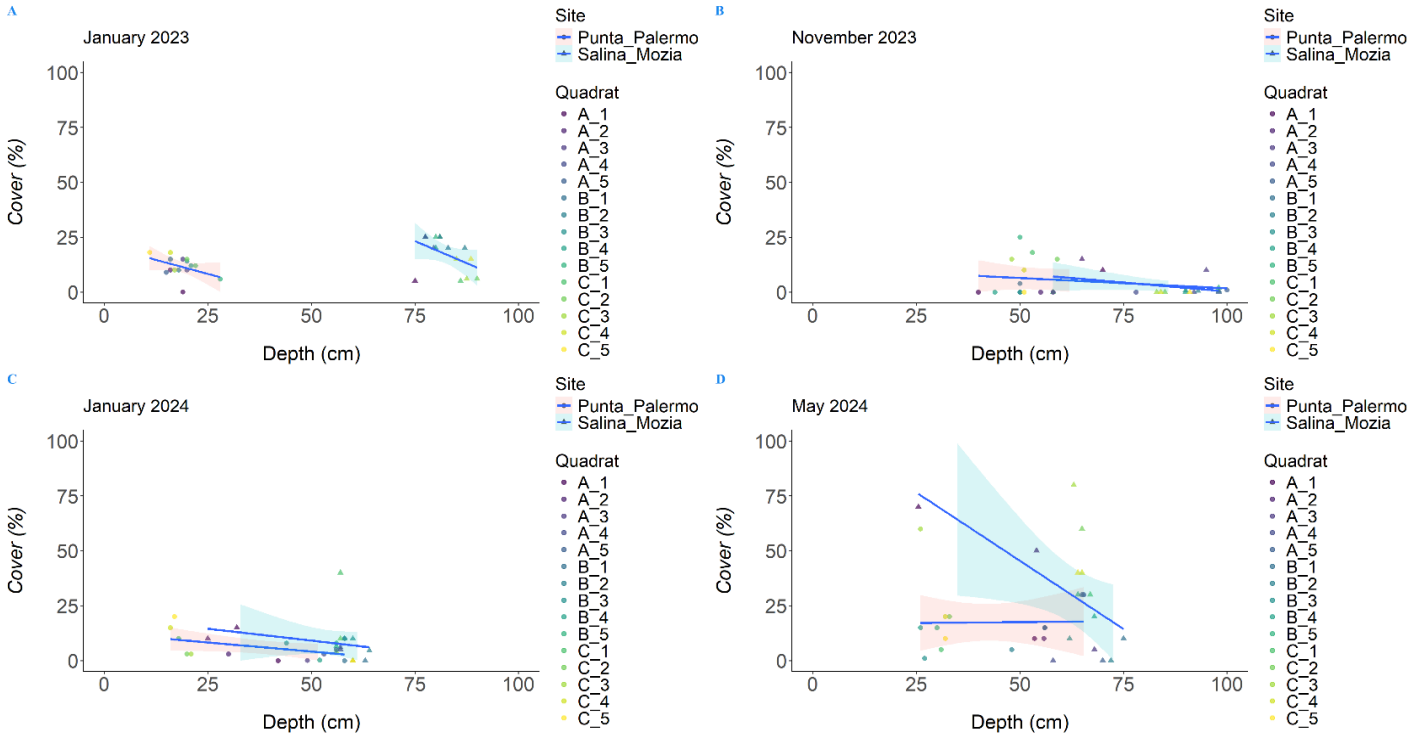

Figure 12: Relationship between cover of *Cymodocea nodosa* (%) among sites and quadrat in different time of monitoring.