

DEVELOPMENT OF BIOTIC TOOLS FOR THE ECOLOGICAL ASSESSMENT OF COASTAL LAGOON ECOSYSTEMS

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NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

DAVID
CABANA
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DEPARTMENT OF ZOOLOGY –MARINE BIOLOGY-

ATHENS 2016

David Cabana Permuy

SUPERVISING COMMITTEE

Prof. Artemis Nicolaidou

Dr Sofia Reizopoulou

Prof. Alberto Basset

EXAMINING COMMITTEE

Prof. Artemis Nicolaidou

Dr Sofia Reizopoulou

Prof. Alberto Basset

Prof. Persefoni Megalophonou

Prof. Hera Karayanni

Dr Nomiki Simboura

Dr Salvatrice Vizzini

ΑΝΑΠΤΥΞΗ ΒΙΟΛΟΓΙΚΩΝ ΕΡΓΑΛΕΙΩΝ ΓΙΑ ΤΗΝ
ΑΞΙΟΛΟΓΗΣΗ ΤΗΣ ΟΙΚΟΛΟΓΙΚΗΣ ΚΑΤΑΣΤΑΣΗΣ
ΠΑΡΑΚΤΙΩΝ ΛΙΜΝΟΘΑΛΑΣΣΩΝ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ
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ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ
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David Cabana Permuy

ΣΥΜΒΟΥΛΕΥΤΙΚΗ ΕΠΙΤΡΟΠΗ

Καθ. Α. Νικολαΐδου, ΕΚΠΑ

Δρ Σ. Ρειζοπούλου ΕΛΚΕΘΕ

Prof A. Basset Παν. Salento

ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ

Καθ. Α. Νικολαΐδου ΕΚΠΑ

Δρ Σ. Ρειζοπούλου ΕΛΚΕΘΕ

Prof. A. Basset Παν. Salento

Αν. Καθ.Π. Μεγαλοφώνου, ΕΚΠΑ

Επ. Καθ. Η. Καραγιάννη, Παν Ιωαννίνων

Δρ Ν. Σύμπουρα ΕΛΚΕΘΕ

Dr S.Vizzini Παν. Palermo

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1 Introduction

It is well known that human population size and the rate of growth are important factors in the generation of environmental disruption and biodiversity loss (Vitousek et al. 1997). Focusing on the oceans a wide array of environmental problems can be lined up at the worldwide scale. Habitat loss, nutrient loading, reduction of biodiversity, trophic collapse, invasion of alien species, decline of ecosystems services, are amongst the current critical threats to oceans and seas in planet earth.

Humankind interferes in the ecology of the main marine realms, from estuaries, to rocky and sandy shores, moving across the pelagic ecosystem, deep sea, mangrove forest, seagrass meadows, coral reefs and polar regions (Kaiser 2011). There is not a single ecosystem in our oceans that could be categorized as “impact free”, from the poles to the tropics the marine realms are being threaten by our civilisation (Halpern et al. 2008).

Within the Mediterranean Sea things do not differ. Moreover, the Mediterranean Sea has been strongly influenced by human activities for millennia. The Sea of the ancient cultures, the cradle of the modern societies, the crossroad of the traders, the diffusor of humankind DNA is also under threat (Freeman 2014; Lacan et al. 2011). An estimation done by Bianchi and Morri (2000) predicts that more than 8500 macroscopic marine species should be living in the Mediterranean Sea. Nonetheless the Mediterranean marine biodiversity is undergoing rapid alteration. According to Bianchi (2007) the increased occurrence of warmer-water biota indicates that the Mediterranean Sea is under a process of “tropicalization” and as consequence more new species are able to live within the basin (Bianchi 2007). The Suez Canal and the Strait of Gibraltar are the main freeways for the migration and introduction of subtropical and tropical species. A total of 986 alien species have been already listed within the Mediterranean Sea (Zenetos et al. 2012), nonetheless the introduction of alien species is just part of the problem. The growth of the human population over the Mediterranean coastal area is accompanied by a wide array of activities, e.g. shipping, tourism development, urban and industrial pollution, fisheries overexploitation which alter the marine and coastal ecosystems.

Coastal lagoons together with *Posidonia oceanic* meadows are amongst the most productive and at the same time threatened coastal ecosystems in the Mediterranean (Moreno et al. 2001; Pérez-Ruzafa et al. 2011). Alterations in these ecosystems drive to shifts in the coastal and marine food webs leading to biodiversity declines, making them major conservation challenges.

Recognizing the importance of coastal lagoon ecosystems the present work focuses on the aspects of their ecology, hoping that it may contribute to their management and conservation.

1.1 Mediterranean lagoonal ecosystems

“Transitional water ecosystems” refers to the water bodies located on the coast where continental drainage waters (fluvial and phreatic waters) meet and mix with saline marine waters. Common transitional water ecosystems are: estuaries, deltas, rias, and lagoons.

In geomorphological terms, coastal lagoons have usually formed where valley mouths have been submerged by the sea during the world-wide Late Quaternary marine transgression, which on tectonically stable coasts brought the sea up to circa its current level about 6000 years ago (Mörner 2005). The Late Quaternary amelioration of global climate precipitated the melting of snowfields, glaciers and ice sheets in polar and mountain regions; as a result the valley mouths submerged to form transitional water ecosystems. The resultant coastal lagoons have a variety of shapes and sizes, related to the configuration of the former coastline and the enclosing spits, as modified by internal erosion and deposition (Bird 1994).

In the Mediterranean, coastal lagoon ecosystems are semienclosed and sheltered shallow water bodies mostly with muddy bottoms. These ecosystems are naturally organically enriched areas, both as a result of river input and the recycling of materials within the system. In defining the typology of Mediterranean coastal lagoons, diverse aspects as size, salinity, tidal range, exposure, mixing characteristics and mean water depth have been used (Basset et al. 2006b; Guelorget et al. 1983; Kjerfve 1994)

1.1.1 The physical environment

Most lagoon properties arise from their geomorphology and configuration (Pérez-Ruzafa et al. 2011; Pérez-Ruzafa et al. 2007b). From a physical perspective, these ecosystems are characterized by the presence of boundaries and transitions between land and water, between the water column and the sediment and the atmosphere, between the lagoon, the sea and freshwater inputs. Due to this condition Mediterranean coastal lagoons suffer a wide variation of abiotic factors, which are attributed to both the geomorphological configuration and climatic influences.

The depth of a lagoon seldom exceeds a few meters (Kjerfve, 1994). Because of their shallowness, lagoons are highly susceptible to changes in precipitation, evaporation and wind. This results in varied changes in salinity and temperature. Furthermore, the tidal exchange and freshwater inflows also alter salinity (Guelorget et al. 1983). Therefore, lagoons could have water ranging from fresh to brackish to hypersaline. Typically within the Mediterranean salinities are lower during the cold wet seasons, while they tend to increase during the warm dry season due to the high rate of evaporation and diminished of fresh water input. Depending on the size of the barrier which impedes the flow of water in and out of the lagoon, coastal lagoons may be partially or entirely enclosed (Kennish and Paerl, 2010). Lagoons can be classified into choked, restricted and leaky according to how water is exchanged with the ocean. (Kjerfve, 1994).

- Choked lagoons usually have a narrow channel to the sea and are formed in areas where the energy of waves in the sea is high. The narrow inlet mostly prevents the tides from entering, and also prevents much mixing of water.
- Restricted lagoons usually have more than one channel to the sea, temporarily regulating water exchange, but usually there is good water exchange, and a net transport of water to the sea. Wind plays a role in restricted lagoons, as surface currents may develop because of the wind and result in mixing of water. The flushing time is very much shorter than in choked lagoons.
- Leaky or open lagoons have wide channel(s) to the sea, unhindered interchange of water and fast water currents.

1.1.2 The biotic environment

The lagoonal ecosystem is formed by a wide array of landscapes; barrier islands, coastal spits and contiguous wetlands and forests provide a set of highly diversified habitats which support a rich biodiversity. Primary producers, fish, birds and benthic communities form the main groups of lagoonal organism.

Primary producers

Seagrass meadows are at the basis of the ecosystem functioning and essential in the production of oxygen, incorporation of C14 and assimilation of CO₂, (Knoppers 1994). In the Mediterranean region, there are three species of euryhaline seagrasses: *Zostera noltii*, *Z. marina* and *Cymodocea nodosa*. Seagrass species provide physical habitat and play a key role in biogeochemical processes contributing to lagoons water quality. By contrast, the massive presence of opportunistic seaweeds such as *Gracilaria*, *Ulva* and *Cladophora* spp. along with cyanobacteria indicate a degraded eutrophic state of the lagoon environment (Kennish and Paerl 2010).

With regard to photosynthetic organisms, besides seagrasses and seaweed, a large number of phytoplanktonic organism can grow in the water column. The role of phytoplankton only becomes relevant in periods when macrophytes are absent (Bazzoni et al. 2013). High phytoplankton biomass decreases light availability favoring the community of primary producers that is most competitive for light, i.e., phytoplankton to the detriment of macrophytes (Cebrian et al. 2014). The increase of phytoplankton biomass, on account of the input of nutrients, leads to a suite of adverse conditions as eutrophication. This phenomena harmfully impact ecosystem health, result in greater vulnerability to disturbances and loss of ecosystem services (Bullock et al. 2011). Thus, phytoplankton is a powerful indicator of trophic conditions due to their ability to respond to environmental changes.

Fish communities

Coastal lagoons are nursery areas, feeding grounds and pathways for the diadromous migration of many fish species (Koutrakis et al. 2005). The structure of fish assemblages can be attributed to the dominance of those groups that better tolerate the fluctuations of abiotic and biotic factors, as well as to the nature of food webs that are established in the lagoon systems (Franco et al. 2008). Species richness and composition of fish communities can be explained by the rate of water exchange between the sea and the lagoon. Therefore, fish species richness in coastal lagoons is mostly determined by colonization rates from the adjacent coastal zone (Pérez-Ruzafa et al. 2007b). Species such as *Anguilla anguilla*, *Atherina boyeri*, *Dicentrarchus labrax*, *Liza ramada*, *Mugil cephalus* and *Sparus auratus*, are common species reported in Mediterranean coastal lagoons (Perez- Ruzafa et al., 2007).

Birds

Mediterranean lagoons are an important refuge of birds migrating from southern and northern latitudes. The richness and population abundance of avian species indicates that the aquatic bird fauna is extremely important in terms of ecological relevance and conservation in all the lagoons of the Mediterranean (Bijlsma 1987). Birds are in fact critical components of the lagoon ecological processes (e.g. food webs) and, at the same time, coastal lagoons are essential for all wetland birds as they provide habitats and feeding grounds (Peñuelas et al. 2002). Lagoon ecosystems can sustain both herbivorous (e.g. waterfowl of the Anseriformes order, ducks, geese, swans, etc.) and carnivorous species that feed on small benthic invertebrates and on fish (e.g. cormorants) (Covas and Blondel 1998).

Benthic fauna

Lagoon benthos includes a wide variety of mobile or sessile aquatic organisms living in or on the substrate and represents a cornerstone for the cycle of nutrients in the lagoon environment. Zoobenthos consists of macroinvertebrates that live in the sediment or on the bottom amongst the vegetation. In addition to molluscs and crustaceans, which are very important for lagoon fisheries, lagoon zoobenthos includes polychaetes and insect larvae. The surface area, the water salinity and the outlet width and length of lagoons can actually be considered as key parameters limiting and defining the environmental niche space of benthic macroinvertebrates in lagoon ecosystems (Barbone and Basset 2010)

1.2 Humans and Mediterranean coastal lagoons

Mediterranean coastal lagoons have historically been of great interest to humans because they offer high biological productivity and provide harbor and navigation facilities (Pérez-Ruzafa et al. 2011). However, human use of these ecosystems, which includes altering species composition and distribution as well as shifting ecosystem function, has led to an unprecedented degradation and damage of natural habitats since the industrial revolution (Lotze et al. 2006). Within the Mediterranean region human exploitation (e.g., overpopulation, tourism, agriculture, and aquaculture) and natural factors, such as climate have severely undermined their ecological resilience causing irreversible and long-term reversible damage (Brinson and Malvárez 2002).

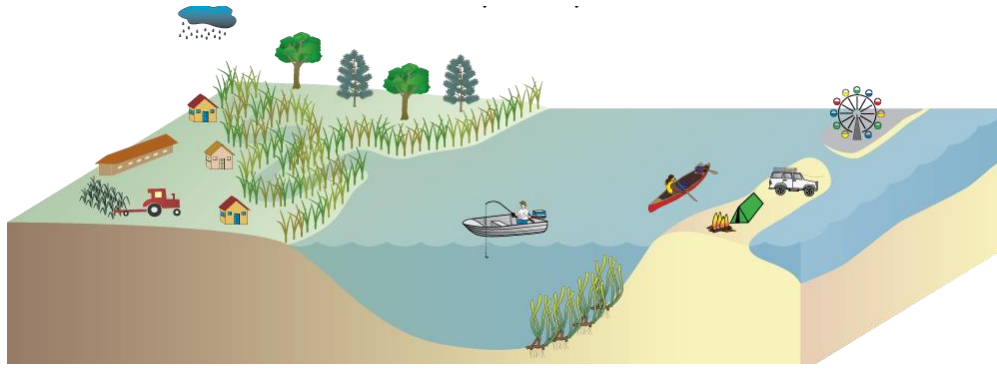


Fig 1.1 Typical human uses in a low impacted Mediterranean coastal lagoon. Illustration created by Jane Thomas, Integration and Application Network, University of Maryland Center for Environmental.

The human activities developed around the Mediterranean coastal lagoons includes: salt works, agriculture, aquaculture, fishery, industry, tourism, recreation and urbanisation Fig.1.1. This conjunction of many interests over such restricted areas leads to the reduction in the ecological quality status of this waterbodies.

Being aware of the threats over the coastal and marine ecosystems, the European Commission, has launched a set of Directives to start coping with the wildlife and nature conservation. The first directive targeting the coastal waters was the Water Framework Directive (WFD) (60/2000EC), followed by the Marine Strategy Framework Directive (MSFD) (CD/2008/56/EC). Both directives set a baseline in the sense that they prescribe steps to reach a common European goal for the good ecological status of the coastal and marine waters respectively.

1.3 Thesis Outline

Within this project “Development of biotic tools for the ecological assessment of coastal lagoon ecosystems” we wanted to fulfill a set of works to both provide the ecological quality status for the benthic habitats and to generate scientific knowledge. Results of both might be potentially used by the decision makers and managers to improve the management at the lagoonal part at the Marine Protected Area of Messolonghi.

Chapter 2

Considering the lines marked by the WFD (Commission 2000), we did a bibliography research to better understand the present state of the evaluation of the Ecological Quality Status EQS in the Mediterranean transitional waters bodies. This part of the work has been peer-reviewed and published with the title “Towards the implementation of the Water Framework Directive in Mediterranean transitional waters: the use of macroinvertebrates as biological quality elements”. (Cabana et al. 2013)

Chapter 3

The next step was to describe the macroinvertebrate communities and assess the current ecological quality status of the main benthic habitats of Messolonghi lagoon. This part of the work was based on a seasonal sampling which took place in in 2013, and formed an essential baseline for further research. The characterization of the taxonomic groups, together with the environmental variables gave us a holistic knowledge of the ecology of the benthic communities and allowed us to define the typology of the lagoon. Lastly and, maybe, most importantly we applied indicators that permit us to define the EQS of the benthic habitats. This was crucial not only to guide our steps in the present work; it may also serve as reference for

future EQS evaluations. A manuscript with the title “*Macrobenthic community structure and Ecological Quality status assessment of the Marine Protected Area of Messolonghi lagoon, Greece*” is in preparation.

Chapter 4

Knowing the main structure of the benthic communities, we wanted to account for the natural variability of both their functional and their taxonomic features. In order to achieve this task we made a multiscale, spatial and temporal, study of the β -diversity of the macroinvertebrate communities. This work is *in press* under the title “*Multiscale functional and taxonomic β -diversity of the macroinvertebrate communities in a Mediterranean coastal lagoon*” (Cabana et al. 2016)

Chapter 5

The study of the macroinvertebrate communities and the environmental factors which shape them disclosed that habitat was playing a key role in the distribution of the macroinvertebrate communities. Thus, we wanted to follow further this line and try to understand the role of the habitat complexity. By means of fractal dimension we determined the complexity of the main groups of vegetation forming the lagoonal benthic habitats. The findings may be potentially used to take management decisions especially to define the zonation of uses within the lagoonal ecosystem. Manuscript in preparation under the title, “*Habitat complexity modulates macroinvertebrates body length and community structure in a lagoonal ecosystem*”.

Chapter 6

Our last work within this project was the study of the macroinvertebrate food web. Being aware of the main anthropogenic sources of pollution and of the role played by the habitat structuring the macroinvertebrate communities we tried to evaluate the role of both in the food web of the macroinvertebrates. For this work we sample in 3 different habitats in the central part of the lagoon and we did an analysis of the principal sources and macroinvertebrate organisms within each habitat. Manuscript in preparation under the title, “*Anthropogenic impact in a Mediterranean coastal lagoon traced by means of nitrogen isotopic enrichment in the main benthic macroinvertebrate groups*”

2 Towards the implementation of the Water Framework Directive in Mediterranean transitional waters. The use of macroinvertebrates as biological quality element

Abstract

During the last decade the Water Framework Directive (WFD) has driven scientific community endeavors towards the development of assessment tools to determine the Ecological Quality Status (EQS) for all surface waters, including transitional waters (TWs). Macroinvertebrates being used as Biological Quality Elements encouraged the development of distinct multimetric and multivariate indices, initially based on taxonomic approaches. Those indices were mostly developed for the marine environment and applied extensively on TWs. The main discrepancies in the ecological quality status assessment arise on TWs, partially due to the difficulty in discriminating the effects of natural stress from anthropogenic impact. As a response, indices following functional approaches are being developed and applied in assessing the EQS in these environments. Next, the validation and intercalibration of the metrics as well as the settlement of reference conditions are additional sources of variability inherent to any assessment. This paper aims at briefly presenting the different steps needed for the implementation of WFD on Mediterranean TWs. It highlights existing difficulties and possible research lines to be explored in order to reduce sources of variability and better assess the status of such water bodies.

Keywords: Water Framework Directive; Mediterranean coastal lagoon; transitional waters; assessment; macroinvertebrate indices; body size; biological traits; reference conditions; intercalibration.

2.1 Introduction

Since ancient times human population grows rapidly along coastal areas. This intensification is mainly prompted by the welfare that seas offer to human kind and the amount of recreational, cultural and economic activities that they enable. As a result, coastal ecosystems have become the most pressured ecosystems in a worldwide scale (Halpern et al. 2008). Thus, agri- culture, aquaculture, dredging, domestic and industrial discharges, land reclamation and tourism, among other anthropogenic activities, alter the natural condition of coastal eco- systems, frequently decreasing their health state.

Occurring at the edge of land and sea transitional waters (TWs) (e.g. coastal lagoons, estuaries, rias) play an essential ecological role. Due to their high productivity (Levin 2001; Viaroli et al. 1996) and wide variety of habitat types they are able to host high biodiversity (Pérez-Ruzafa et al. 2010) and provide specific conditions (e.g. ecological isolation) that permit the housing of endemic species (Basset et al. 2006b; Sabetta et al. 2007). In the Mediterranean basin, coastal lagoons are well-studied transitional water bodies (“Mediterranean Lagoon” has 1017 entrances by title, abstract and key words in Scopus for the period 2000-2012) and are still the object of scientific debate in terms of definition, classification, functioning, ecological quality assessment and management.

Besides the necessity on deeply research hydrological and ecological principles acting on transitional waters, the implementation of the Water Framework Directive (WFD) (Commission 2000) encouraged the scientific community to develop indices as a tool for impact assessment. Thus, WFD compliant indices, depending on richness and diversity parameters, have been developed for each of the biological quality elements (BQE), i.e., fish, macroalgae, macroinvertebrates, phytoplankton and seagrasses. The main target of the WFD is the classification of the ecological quality status (EQS) of the water bodies and the achievement of good status for the European surface waters by 2015. Herein Mediterranean coastal lagoons are included and macroinvertebrates is one of the BQE to be studied.

Thirteen years after the launch of WFD, many macroinvertebrate based indices were developed (Borja et al. 2000; Dauvin and Ruellet 2007; Fano et al. 2003; Simboura and Zenetos 2002; Teixeira et al. 2009). But, the extent of their applicability and reliance is still a matter of scientific discussion. One of the main characteristics for most of these indices is their reliance in the classic model of benthic ecology of (Pearson and Rosenberg 1978). This model describes the changes in the composition and structure of benthic communities along a gradient of organic enrichment, where high levels of organic material cause a decrease in species diversity and biomass of the benthic communities.

In various cases biotic indices were found less efficient in assessing pollution in Mediterranean TWs (Munari and Mistri 2010; Ponti et al. 2008; Simboura and Reizopoulou 2008), since a number of species inhabiting these environments are adapted to wide ranges of environmental parameters (natural disturbance) and anthropogenic impact. On the other hand, taxonomy free indices based on a functional approach as body size, feeding groups and biological traits, even if not fully WFD compliant, can be proved efficient and applicable on these ecosystems (Basset et al. 2012a; Mouillot et al. 2006; Reizopoulou and Nicolaidou 2007b).

Moreover the implementation of the WFD presents additional challenges to the development and validation of efficient metrics. The establishment of reference conditions (RC), by finding pristine or slightly disturbed reference sites is challenging where landscapes have been substantially altered. In these cases, other approaches, such as best professional judgment (BPJ), are currently used but still with various

degrees of uncertainty. Another challenging task, still incomplete, is the establishment of quality threshold values in the eco-regional intercalibration exercise. Thus the scientific debate is open and moving forward in terms of WFD implementation on Mediterranean TWs.

This review aims at providing a comprehensive evaluation of the different steps necessary for the WFD implementation on Mediterranean TWs. Thus advances, gaps of knowledge and sources of uncertainties are identified here in terms of macroinvertebrates ecological quality status (EQS) assessment on Mediterranean TWs. The current situation in terms of TWs typology, ecological quality indices, indices validation, establishment of RC, and intercalibration process is considered.

2.2 Lagoon typology

Under the increasing pressure in demand for sufficient quantities of good water quality the WFD establishes a basis for the protection of the surface waters. Its principal purpose is to promote a sustainable water use, aiming at the improvement of the aquatic environment. For the clear, reliable and efficient application of its objectives the WFD assigns surface waters into one of six surface water categories (i.e. rivers, lakes, transitional waters, coastal waters, artificial and heavily modified water bodies) that may be additionally sub-divided into ecologically relevant surface water body types (Commission 2000). The definition of further sub-types is also accepted.

Water body type constitutes the management unit for the WFD, where reference conditions and environmental objectives may be outlined. Additionally the WFD defines TWs as “bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows”. Mediterranean coastal lagoons, little influenced by fresh water input, may also lay under the WFD definition of coastal waters. This is questioned by Tagliapietra and Ghirardini (Tagliapietra and Ghirardini 2006), who proposed to assign coastal lagoons in a category of their own.

The WFD leaves the Member States (MSs) the identification and location of the water body type boundaries and gives two possible approaches. If approach A is followed, water bodies within each ecoregion (i.e., Atlantic Ocean, Norwegian Sea, Barents Sea, North Sea, Baltic Sea, and Mediterranean Sea) shall be differentiated according to descriptors based on mean annual salinity and mean tidal range. Otherwise, if approach B is followed, a set of obligatory descriptors (i.e., latitude, longitude, salinity and tidal range) and other optional ones (i.e., depth, current velocity, wave exposure, mean temperature, mixing currents, turbidity, substratum composition, shape, water temperature range) or combinations of these, are required to ensure that type specific biological RC can be reliably derived.

Over the years, coastal lagoons have been categorized according to:

- Salinity ranges, where the Venice system (Battaglia 1959) is the most extensively used
- Geomorphology and water exchange (Kjerfve 1994), and
- Degree of Confinement (Guelorget et al. 1983).

Within the Mediterranean ecoregion, in response to the WFD demands, the scientific community started to define possible approaches to determine a typological scheme that will provide the highest possible biological homogeneity within few types. Thus, this scheme might account for the most meaningful sources of variability and being ecologically relevant.

In accordance with recent publications, salinity is a key parameter in the definition of coastal lagoon typology. Mean annual salinity and maximum salinity that account for variations of major biological data are proposed for the definition of lagoon typology in (Basset et al. 2006a; Basset et al. 2006b). Likewise the WFD recommends the salinity categories of Venice System (oligohaline, from 0.5 to <5psu; mesohaline, from 5 to <18psu; polyhaline, from 18 to <30psu; euhaline ≥ 30 psu) that combined with surface area (small < 2.5 km² ; large > 2.5 km²) account for significant variations in macroinvertebrate density (n/m²) (Barbone et al. 2012). Similar to the Venice System a 3 levels salinity typology was defined for small Balearic TWs (Lucena-Moya et al. 2009) based on the invertebrate taxa variability. That system sets the threshold between oligohaline and mesohaline at 5psu while the mesohaline level comprises a range of salinities between 6psu and 26psu, where the threshold for euhaline is set. In addition, a lagoon sub-typology (i.e., stable salinity vs. variable salinity) was proven, in terms of invertebrate richness and composition, taking also in consideration the temporal scale variability. Results on this research point out the significance of the temporal scale variation in small Balearic coastal lagoons. Moreover, a large spatio-temporal scale study revealed salinity, combined with either openness or tidal range, as critical explanatory variables in terms of taxonomic richness (Barbone and Basset 2010).

Although, salinity is the most tested factor to define a Mediterranean lagoon typology, a combination of assorted factors is the prevailing approach. The analysis of salinity and tidal range, (i.e., WFD approaches A and B), in combination with lagoon shape is considered effective when establishing analogies between TWs typology and ecosystem niche in (Basset et al. 2006b). Following a tidal/climatic approach (Tagliapietra and Ghirardini 2006) defined North Adriatic lagoons as having microtidal regime (i.e., 0.5–1.0 m) and positive hydric balance and the rest of Italian and Mediterranean lagoons as having nanotidal regime (i.e., < 0.5m) and negative hydric balance. A further sub-type, correspondent with the lagoon system inlet, is also suggested in (Tagliapietra and Ghirardini 2006) for the large North Adriatic lagoonal systems, in order to reduce the ample internal variability. A more complex definition of typology is presented in (Barbone et al. 2012; Basset et al. 2012b). This method, a mixed-model procedure, permits a metric specific compilation to derivate the optimal lagoon typology from the analysis of a set of environmental factors (i.e., salinity (oligohaline, mesohaline, polyhaline and euhaline), lagoon confinement (two levels: choked and restricted), surface (large and small), sediment granulometry (mud and sand), vegetation (presence/absence and submerged/emerged) and season (fall and spring)). The novelty here lies in the multifactor consideration and the specificity with the metric applied for the final assessment. However the inclusion of a biological factor (i.e., vegetation) for the definition of the lagoon typology does not follow WFD guideline and may be controversial. (Tagliapietra and Ghirardini 2006) consider that biological aspects should not be included in the characterization of typologies to avoid circular reasoning. (Basset et al. 2006b) also notes that only abiotic characteristics are relatively independent of anthropogenic pressures. Furthermore the WFD does not incorporate the vegetation as factor prone to be comprised in the typology definition. Thus the vegetation aspect might be included in the settlement of the reference conditions where the habitat type plays an important role (Diaz et al. 2004).

Besides, lagoon size not specifically quoted in the WFD, may constitute a determinative factor to outline a lagoon typology. The Problem regarding comparability of data among different transitional water bodies is raised in (Lucena-Moya et al. 2012; Pardo et al. 2011), where the lagoon size and driving forces are considered to have a strong effect in nutrient accumulation and fauna composition. A significant macroinvertebrate species–area relationship was detected elaborating data from 26 Italian lagoons (Sabetta et al. 2007). Moreover, a study (Guilhaumon et al. 2012) comparing the relationship of benthic species richness to the surface area in 18 Italian lagoons, indicated that c. 25-30% of species richness could be explained by differences in the surface area, confirming the necessity of this kind of standardization to enhance comparability among water bodies with different size.

Much effort has been expended in describing the “typology” of the transitional waters in each member country of the European Community. After all, the Mediterranean lagoon typology drawn up by the Mediterranean Geographical Intercalibration Group (MED-GIG) (Pardo et al. 2011) adopted a two-factor classification system combining the degree of water exchange, classifying lagoons into leaky, restricted and choked (after (Kjerfve 1994)), with water salinity (Basset et al. 2012b).

Identifying the type of each water body is the first step, before a baseline of current conditions can be determined (the so-called “reference conditions”) and an appropriate monitoring regime can be developed, against which future improvements can be made.

2.3 Macroinvertebrate indices

Due to their sessile, sedentary and relatively long life, benthic macroinvertebrates are considered as important elements to be suitable and sensitive indicators of natural and anthropogenic variations (Pearson and Rosenberg 1978). It has been proved that they represent a good indicator of both temporal and chronic disturbances (Dauer 1993). Their combination with other BQE and hydro-morphological, chemical and physico-chemical supporting factors may lead to a more detailed picture of the ecosystem health state.

Indices based on the ratio of sensitive/opportunistic species are an essential part of the WFD metrics for benthic macroinvertebrates. In addition, WFD compliant indices in most cases contain parameters accounting for diversity and abundance. With the compel of the WFD the MSs have either developed or adopted one or few benthic indices for the EQS assessment of their coastal and TWs benthic ecosystems (Borja et al. 2009a; Diaz et al. 2004; Occhipinti-Ambrogi and Forni 2004; Pinto et al. 2009). Thus, an array of different metrics was recently applied for the assessment of the benthic ecosystem in Mediterranean TWs (Table 2.1).

Apart from other feasible classifications e.g. (Dauvin et al. 2010; Diaz et al. 2004), indices could be divided in taxonomy based indices and functional and body size based indices. Even though not fully compliant with the WFD requirements, few recent publications reinforce the utilization and validation of the two last metrics types (Basset et al. 2012a; Marchini et al. 2008; Quintana et al. 2006; Reizopoulou and Nicolaidou 2007b; Sigala et al. 2012; Teixeira et al. 2009)

2.3.1 Taxonomy based indices

Full compliance with the WFD recommendations in terms of indices construction is scarce. The fulfillment of the Directive requires the implementation of multimetric indices accounting for diversity, abundance and sensitive/tolerant species.

Multivariate indices as M-AMBI (Borja et al. 2004; Muxika et al. 2007), BAT (Teixeira et al. 2009) and MIBIIN (Lucena-Moya and Pardo 2012) are few of the metrics applied on Mediterranean TWs in full agreement with the Directive (Table 2.1). M-AMBI, is based on a factor analysis of species richness (S), Shannon diversity index (H') and AMBI (Borja et al. 2000). Even if not originally designed for TWs it is the most applied index and it is implemented by the MED-GIG to utilize in Spanish, Greek and French TWs (Table 2.2). Recently the multivariability of the index has been questioned in (Sigovini et al. 2013). BAT selects Margalef (d) and Shannon (H') diversity indices to provide diversity measures and includes AMBI to assess the composition of macrobenthos. Next, MIBIIN was exclusively developed for Mediterranean TWs. Its main characteristic is the specificity of the metrics in agreement with 3 ranges of salinity (i.e., oligohaline, mesohaline and euhaline). Thus, 3 formulae result from this method, each of them

relies on different sensitive/tolerant taxa (i.e., frequency of; *Cyprideis torosa* and *Polychaeta* for the oligo-MIBIIN, Amphipoda+Gastropoda+Isopoda for the meso-MIBIIN and *Artemia salina* for the eu-MIBIIN) (Table 2.1). The MIBIIN is being implemented for further use on Spanish Mediterranean TWs (Pardo et al. 2011). Through multivariate analysis more information is evaluated simultaneously and the relationship between variables is understood better.

Univariate indices, based on a set of sensitive/tolerant species applied on Mediterranean TWs are AMBI (Borja et al. 2000), BENTIX (Simboura and Zenetos 2002) and I2EC (Grall and Glemarec 2003), (Table 2.1). Hence a biotic index is derived from the proportions of individual abundance in few ecological groups (i.e., AMBI considers five groups, I2EC four and BENTIX three) related to the degree of sensitivity/tolerance to an environmental stress gradient. None of these indices were originally developed for Mediterranean TWs and only BENTIX was constructed based on data from Mediterranean coastal waters. Lately the MEDOCC (Pinedo and Jordana 2007) resulted as a Mediterranean adaptation of AMBI, to be further applied on coastal waters. The novelty of these indicators was their capability to rank the degree of environmental degradation in a given scale.

Table 2.1. Indices applied in Mediterranean lagoons and their formulation. (*) Indices based on taxonomic sufficiency, (underline) multifactorial indices, (italic) functional based indices.

Index	Formulation	References
AZTI Marine Biotic Index (AMBI)	$AMBI = \{(0 \times \%G1) + (1,5 \times \%G2) + (3 \times \%G3) + (4,5 \times \%G4) + (6 \times \%G5)\} / 100$	Borja et al., 2000 (Borja et al. 2000) http://www.azti.es/
Benthic Opportunistic Polychaetes Amphipod Index BOPA*	$BOPA = \log(f_p/f_s + 1) + 1$	Gesteira and Dauvin, 2000 (Gesteira and Dauvin 2000)
Benthic Index (BENTIX)	$BENTIX = [6 \times \%GS + 2 \times \%GT] / 100$	Simboura and Zenetos, 2002 (Simboura and Zenetos 2002)
Coastal Endofaunal Evaluation Index (I2EC)	Based on the threshold percentages of 5 different EGs in their respective proportions	Grall and Glémarec, 2003 (Grall and Glemarec 2003) http://envlit.ifremer.fr/var/envlit/storage/documents/dossiers/bioevaluation/site/bioev_c22_gb.htm
Ecofunctional Quality Index (EQI)	The sum of weights given to 8 biological attributes, each transformed onto a dimensionless of 0-100 quality scale	Fano et al., 2003 (Fano et al. 2003)
Multivariate AMBI (M-AMBI)	Factorial analysis of 3 indices (AMBI, Shannon and Species richness)	Muxika et al., 2007 (Muxika et al. 2007)
Benthic Quality Index (BQI)	$BQI = \sum \{(A_i / TotA) * ES_{0,05}^{10} \log(S+1)\}$	Rosenberg et al., 2004 (Rosenberg et al. 2004)
Benthic Ecosystem Quality Index (BEQI)	$EQR = \text{average}(EQR \text{ species} + EQR \text{ density} + EQR \text{ similarity})$	Van Hoey et al., 2007 (Van Hoey 2007) http://www.beqi.eu/
Infaunal Quality Index (IQI)	$IQI = (((0,38 \text{ AMBI}^{10}) + (0,08 (1 - 2^{-\text{AMBI}}) + (0,54 S^{100,1})) - 0,4) / 0,6$	Prior et al., 2004 (Prior et al. 2004)
Norwegian Quality Index (NQI)	$NQI = 0,5 (1 - \text{AMBI}/7) + 0,5 (SN/2,7)(N/(N+5)) / NQI_{ref}$	Rygg 2006 and Borja et al., 2007 (Borja et al. 2007; Rygg 2006)
Benthic Index on Taxonomic Sufficiency (BITS) *	$BITS = \log\{(6fI + fII) / (fIII + 1) + 1\} + \log\{nI / (nII + 1) + nI / (nIII + 1) + 0,5nII / (nIII + 1) + 1\}$	Mistri & Munari, 2008 (Mistri and Munari 2008) http://www.bits.unife.it
Fuzzy Index of Ecosystem Integrity (FINE)	Fuzzy-based, developed with a complex system of 7 metrics and 768 logic rules	Mistri et al., 2008 (Mistri et al. 2008) http://web.unife.it/progetti/FINE/
Benthic Assessment Tool (BAT)	Factorial analysis of 3 indices (Margalef Index, Shannon-Wiener and AMBI)	Teixeira et al., 2009 (Teixeira et al. 2009)
Multimetric Index of the Balearic Island based on Invertebrate Communities (MIBIIN)*	According to salinity: oligo-MIBIIN = \sum Frequency of sensitive genus + Genus richness + Frequency of (<i>Cyprideis torosa</i> + <i>Polychaeta</i>) meso-MIBIIN = \sum Sensitive genus richness + Bray-Curtis Dissimilarity + Frequency of (Amphipoda+Gastropoda+Isopoda). euh-MIBIIN = \sum Sensitive genus richness + Frequency of <i>Artemia salina</i>	Lucena-Moya and Pardo, 2012 (Lucena-Moya and Pardo 2012)
Infaunal Trophic Index (ITI)	$100 - 33,3(TG_2 + 2TG_3 + 3TG_4) / TG_{1,2,3,4}$	Word, 1978 (Word 1978)
Biological Trait Analysis (BTA)	Incorporates information on species distribution and their biological characteristics	Statzner, 1994 (Statzner et al. 1994)
Index of Size Distribution (ISD)	Skewness of the distribution of individuals in geometric size classes	Reizopoulou and Nicolaidou, 2007 (Reizopoulou and Nicolaidou 2007b)
Index of Size Spectra Sensitivity (ISS)	$ISS = \sum p(CL_i) * \omega_i * S$	Basset et al., 2012 (Basset et al. 2012a)

The taxonomic sufficiency approach in Mediterranean TWs is followed in metrics like BOPA (Dauvin and Ruellet 2007; Gesteira and Dauvin 2000), QAELS (Boix et al. 2005) and BITS (Mistri and Munari 2008) (Table 2.1). BOPA, originally developed to study the impact of oil spills on the Atlantic coastal and TWs, was lately applied on Mediterranean TWs (Table 2.2). The metric combines total abundance, the frequency of amphipods and opportunistic polychaetes. On the other hand QAELS is obtained by means of

two measurements: taxon sensitivity to water quality by abundance of Cladocera, Copepoda and Ostracoda and taxon richness of insects and crustaceans. This metric is being implemented for Spanish lentic and oligohaline TWs. Furthermore BITS, developed for microtidal lagoons from the western Adriatic Sea, is based on a ratio formulation of sensitive, tolerant and opportunistic families. This index was included on the validation process for Italian TWs by the MED-GIG(Pardo et al. 2011). The main advantages of this approach are the reduction of identification effort and the error derivate from species level identification (Dauvin et al. 2010)

A Multifactorial approach is used on Mediterranean TWs in two indices, EQI (Fano et al. 2003) and FINE (Mistri et al. 2008) (Table 2.1), both developed on a set of Italian lagoons (Table 2.2). EQI combines a complex suite of attributes, (i.e., total biomass of primary and secondary producers, abundance of secondary producers, macrofauna richness and diversity, together with trophic measures) into an ecologically meaningful index. FINE is based on the selection of biological attributes that are fundamental for lagoon ecosystem function. Thus, composed of seven ecosystem factors (i.e. number of species, number of individuals, taxonomic diversity, functional diversity, macrofauna biomass, macroalgae biomass, and seagrass) each of them with different levels (Table 2.3).

Table 2.2. Taxonomy based indices in peer-review EQS assessment studies on Mediterranean transitional waters. (CW) coastal waters, (FW) fresh waters, (TW) transitional waters, (*) taxonomic sufficiency, (underline) multifactorial indices. Country: (AL) Albania, (GR) Greece, (IT) Italy, (SP) Spain, (TN) Tunisia

Index	Designed	Country	Lagoon/s	Publications		
AMBI (Borja et al. 2000) (Borja et al. 2000)	CW	AL	Karavasta	Munari et al., 2010 (Munari et al. 2010)		
			Karavasta, Patok	Ponti et al., 2008 (Ponti et al. 2008)		
		GR	Papas, Tsopeli, Vivari	Simboura and Reizopoulou, 2008 (Simboura and Reizopoulou 2008)		
			Almini Grande	Ponti et al., 2008 (Ponti et al. 2008)		
		Caprolace lake, Fogliano lake	Prato et al., 2009(Prato et al. 2009)			
		Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2008; Munari and Mistri, 2010 (Munari and Mistri 2008b; Munari and Mistri 2010)			
		IT	Comacchio, Goro, Lesina, Olbia, Orbetelo, Scardovari, Tortoli Venice	Marchini et al., 2008 (Marchini et al. 2008)		
			Comacchio, Goro, Lido delle Nazioni, Scardovari	Abbiati et al., 2010 (Abbiati et al. 2010)		
			Lesina	Pinna et al., 2013(Pinna et al. 2013)		
			Orbetelo, Padrogiano, San Teodoro, Tortoli	Munari and Mistri, 2007 (Munari and Mistri 2007)		
			Pialassa Baiona	Ponti et al., 2007(Ponti et al. 2007b)		
		SP	Venice	Pranovi et al., 2007 (Pranovi et al. 2007)		
			Mar Menor	Marin-Guirao et al., 2005 (Marin-Guirao et al. 2005)		
		TN	Bizerte	Afli et al., 2008 (Afli et al. 2008)		
BOPA* (Gesteira and Dauvin, 2000) (Gesteira and Dauvin 2000)	TW	IT	Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2008 (Munari and Mistri 2008b)		
			Orbetelo, Padrogiano, San Teodoro, Tortoli	Munari and Mistri, 2007 (Munari and Mistri 2007)		
		Venice	Pranovi et al., 2007 (Pranovi et al. 2007)			
		TN	Bizerte	Afli et al., 2008 (Afli et al. 2008)		
BENTIX (Simboura and Zenetos, 2002) (Simboura and Zenetos 2002)	CW	AL	Karavasta, Patok	Ponti et al., 2008 (Ponti et al. 2008)		
			GR	Tsopeli, Papas, Vivari	Simboura & Reizopoulou, 2008 (Simboura and Reizopoulou 2008)	
		IT	Almini Grande	Ponti et al., 2008 (Ponti et al. 2008)		
			Caprolace lake, Fogliano lake	Prato et al., 2009 (Prato et al. 2009)		
		Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2010 (Munari and Mistri 2010)			
		Comacchio, Goro, Lesina, Olbia, Orbetelo, Scardovari, Tortoli, Venice	Marchini et al., 2008 (Marchini et al. 2008)			
		IT	Comacchio, Goro, Lido delle Nazioni, Scardovari	Abbiati et al., 2010 (Abbiati et al. 2010)		
			Lesina	Pinna et al., 2013 (Pinna et al. 2013)		
			Pialassa Baiona	Ponti et al., 2007 (Ponti et al. 2007b)		
			Venice	Pranovi et al., 2007 (Pranovi et al. 2007)		
			SP	Mar Menor	Marin-Guirao et al., 2005 (Marin-Guirao et al. 2005)	
		TN	Bizerte	Afli et al., 2008 (Afli et al. 2008)		
		I2EC (Grall and Glémarec, 2003) (Grall and Glémarec 2003)	CW	TN	Bizerte	Afli et al., 2008 (Afli et al. 2008)
		EQI (Fano et al., 2003) (Fano et al. 2003)	TW	IT	Comacchio and Goro	Fano et al., 2003 (Fano et al. 2003)
M-AMBI (Borja et al., 2004; Muxika et al., 2007) (Borja et al. 2004; Muxika et al. 2007)	CW	AL	Karavasta, Patok	Ponti et al., 2008 (Ponti et al. 2008)		
			Karavasta	Munari et al., 2010 (Munari et al. 2010)		
		Karavasta, Narta, Patok	Barbone et al., 2012; Basset et al., 2012b (Barbone et al. 2012; Basset et al. 2012b)			
		GR	Agiasma, Logarou	Barbone et al., 2012; Basset et al., 2012b (Barbone et al. 2012; Basset et al. 2012b)		

			Papas, Tsopeli, Vivari	Simboura and Reizopoulou, 2008 (Simboura and Reizopoulou 2008)
			Alimini Grande	Ponti et al., 2008 (Ponti et al. 2008)
			Alimini, Cesine, Grado Marano, Grado Valle Cavanata, Grado Valli da Pesca, Margherita di Savoia, Torre Guaceto	Barbone et al., 2012; Basset et al., 2012b (Barbone et al. 2012; Basset et al. 2012b)
		IT	Caprolace lake, Fogliano lake	Prato et al., 2009 (Prato et al. 2009)
			Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2010 (Munari and Mistri 2010)
			Comacchio, Goro, Lido delle Nazioni, Scardovari	Abbiati et al., 2010 (Abbiati et al. 2010)
			Lesina	Pinna et al., 2013; Borja et al., 2011 (Borja et al. 2011; Pinna et al. 2013);
BQI (Rosenberg et al., 2004) (Rosenberg et al. 2004)	CW	IT	Lesina	Borja et al., 2011 (Borja et al. 2011)
QAELS (Boix et al., 2005) (Boix et al. 2005)	FW	SP	99 lentic ecosystems in Catalunya	Boix et al., 2005 (Boix et al. 2005)
			Ter Vell	Badosa et al., 2008 (Badosa et al. 2008)
BEQI (Van Hoey et al., 2007) (Van Hoey 2007)	CW	IT	Lesina	Borja et al., 2011 (Borja et al. 2011)
IQI (Prior et al., 2004) (Prior et al. 2004)	CW	IT	Lesina	Borja et al., 2011 (Borja et al. 2011)
NQI (Rygg 2006 and Borja et al., 2007) (Borja et al. 2007; Rygg 2006)	CW	IT	Lesina	Borja et al., 2011 (Borja et al. 2011)
BITS * (Mistri & Munari, 2008) (Mistri and Munari 2008)	TW	AL	Karavasta	Munari et al., 2010 (Munari et al. 2010)
			Karavasta, Narta, Patok	Basset et al., 2012b (Basset et al. 2012b)
		GR	Agiasma, Logarou	Basset et al., 2012b (Basset et al. 2012b)
			Papas, Tsopeli, Vivari	Simboura and Reizopoulou, 2008 (Simboura and Reizopoulou 2008)
			Alimini, Cesine, Grado Marano, Grado Valle Cavanata, Grado Valli da Pesca, Margherita di Savoia, Torre Guaceto	Basset et al., 2012b (Basset et al. 2012b)
		IT	Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2010 (Munari and Mistri 2010)
			Comacchio, Goro, Lido delle Nazioni, Scardovari	Abbiati et al., 2010 (Abbiati et al. 2010)
			Comacchio, Goro, Venice	Mistri and Munari, 2008 (Mistri and Munari 2008)
			Lesina	Pinna et al., 2013; Borja et al., 2011 (Borja et al. 2011; Pinna et al. 2013)
FINE (Mistri et al., 2008) (Mistri et al. 2008)	TW	IT	Comacchio, Goro, Lesina	Mistri et al., 2008 (Mistri et al. 2008)
			Comacchio, Gorino, Goro, Lesina, Scardovari, Venice	Munari and Mistri, 2008 (Munari and Mistri 2008b)
			Orbetelo, Padrogiano, San Teodoro, Tortoli	Munari and Mistri, 2007 (Munari and Mistri 2007)
BAT (Teixeira et al., 2009) (Teixeira et al. 2009)	TW	AL	Karavasta, Narta, Patok	Basset et al., 2012b (Basset et al. 2012b)
		GR	Agiasma, Logarou	Basset et al., 2012b (Basset et al. 2012b)
		IT	Alimini, Cesine, Grado Marano, Grado Valle Cavanata, Grado Valli da Pesca, Lesina, Margherita di Savoia, Torre Guaceto	Basset et al., 2012b; Borja et al., 2011 (Basset et al. 2012b; Borja et al. 2011)
MIBIIN * (Lucena-Moya and Pardo, 2012) (Lucena-Moya and Pardo 2012)	TW	SP	34 lagoons at the Balearic Archipelago	Lucena-Moya and Pardo, 2012 (Lucena-Moya and Pardo 2012)

Apart from the above, other multimetric indices are being implemented for coastal and TWs within each ecoregion and MSs; BQI (Rosenberg et al. 2004) in Sweden, NQI (Rygg 2006) in Norway, IQI (Borja et al. 2007) in UK, BEQI (Van Hoey 2007) in Belgium and Netherland, DKI (Borja et al. 2007) in Denmark, BBI (Perus et al. 2007) in Finland, and the ZKI (Kotta et al. 2012) in Estonia. Lately (Borja et al. 2011) has also applied BQI, BEQI, IQI and NQI in the Mediterranean and other European transitional waters, which were proved to respond differently to diverse anthropic pressures.

In spite of the wide array of metrics, only few have been designed for Mediterranean TWs. Due to the above and to the high natural variability of such environments (Dauvin 2007; Dauvin and Ruellet 2009), the application of those metrics produces discordant results (Gamito and Furtado 2009; Munari and Mistri 2010; Ponti et al. 2008; Simboura and Reizopoulou 2008). It should be noted that community diversity cannot be successfully used in lagoons as a sole index of environmental quality, given that variations of biodiversity and species richness are mainly related to the degree of marine influence, reflecting the natural stress conditions. Therefore, community diversity alone is inappropriate to discriminate anthropogenic from natural stress (Reizopoulou and Nicolaidou 2004; Reizopoulou and Nicolaidou 2007b). The “Estuarine Quality Paradox” suggested by (Dauvin 2007; Elliott et al. 2007) posed a criticism on the biotic indices performance in cases of naturally stressed ecosystems as TWs, where the disturbance tolerant species naturally dominate. Due to the natural dominance of tolerant and opportunistic species, transitional waters present strong analogies with coastal waters affected by organic pollution (Reizopoulou and Nicolaidou 2007b; Simboura and Reizopoulou 2008). Furthermore many of these indices mainly detect organic enrichment and/or indicate generic pressures and they may fail with other sources of stress (Marin-Guirao et al. 2005).

The use of multi-metric methods could be more consistent in the detection of quality than single indices (Borja et al. 2011), whereas the combination of hydromorphological and physicochemical descriptors might contribute in discriminating other pressure types. The underestimation or overestimation of ecological quality status given by biotic indices in coastal lagoons demonstrate the weakness of such indices in discriminate among the anthropogenic and natural stress in lagoonal ecosystems as reported in (Simboura and Reizopoulou 2008).

Flaws related to the use of richness or indicator species in order to assess the ecological status of transitional water body masses highlight the needs for indicators integrating alternative community features. (Mouillot et al. 2006). To overcome the situation, metrics based on a single biological trait as feeding groups (Gamito and Furtado 2009; Gamito et al. 2012b; Word 1978) and body size (Basset et al. 2012a; Reizopoulou and Nicolaidou 2007b) are being considered and applied in TWs. In this line, a step forward is the compilation of few biological traits in the same equation through BTA (Marchini et al. 2008; Paganelli et al. 2012; Sigala et al. 2012).

2.3.2 Functional and body size based indices

An index based on feeding groups as a proxy of anthropogenic disturbance in coastal waters is presented in (Word 1978) by computing four feeding groups (i.e., suspension feeders, carrion feeders, surface deposit feeders and subsurface deposit feeders) into the Infauna Trophic Index (ITI) (Table 2.1). Next, another approach based on the Shannon diversity index and Pielou evenness index is applied on Atlantic TWs to study the feeding diversity (Gamito and Furtado 2009; Gamito et al. 2012b). Here main trophic groups (i.e., suspension feeders, deposit feeders, suspension/deposit feeders and grazers) are combined into the same diversity index. The basic principle is that stressed environments may experience decreased diversity of feeding groups. The main advantage of these indices lays on their robustness with small samples and the low taxonomic identification effort, since only the most abundant species need to be identified and their feeding habits known. This tool is claimed to bring complementary information to that brought by common WFD compliant indices (Gamito and Furtado 2009; Gamito et al. 2012b). Yet, only ITI was applied on Mediterranean TWs (Afli et al. 2008), and an adaptation to Mediterranean or other TWs on the species lists and feeding guilds might be necessary.

Recently, body-size based approaches are taking special consideration on Mediterranean TWs (Basset et al. 2012a; Gascón et al. 2009; Lardicci and Rossi 1998; Reizopoulou and Nicolaidou 2007b; Reizopoulou et al. 1996) (Table 2.3). On this basis the Index of Body-Size Distribution (ISD) (Reizopoulou and Nicolaidou 2007b) and the Index of Size Spectra Sensitivity (ISS) (Basset et al. 2012a) have been specifically developed for Mediterranean TWs. The ISD is the only index applied on Mediterranean TWs totally taxonomy free. This index represents the skewness of the distribution of individuals of a benthic community in geometric size (biomass) classes, which is used as a measure of disturbance (Table 2.1). Being a taxonomy free index is less time- consuming and it does not require expert knowledge, hence may be a simple and effective tool for the ecological quality assessment. However, an intense seasonal variation due to reproduction patterns, for example recruitment, would tend to increase the skewness of the biomass distribution. The ISS incorporates measures in size structure, in relation with anthropogenic disturbance, and species richness (Table 2.1). It assumes an asymmetric model of size class sensitivity, considering higher sensitivity of larger body size classes and incorporates taxonomic richness as a correction factor. The main strength of this index lays on the habitat approach design. First results and validations have shown robust consistency between anthropogenic pressures and the EQS (Basset et al. 2012a). Nonetheless chemical pollutants may still represent sources of uncertainty. Other drawback may be the semi-dependence on the time consuming taxonomic analysis if the correction factor needs to be applied.

The easiness of measure, the independence of taxonomic compositions, the comparability and possibility of intercalibration are the main advantages identified for body-size indices in (Basset et al. 2004). Besides these assets, the sensitivity of small taxa to particular anthropogenic disturbance, the sampling probability of larger sizes affected by sampling effort, the size selective predation pressures, the time and costs derived from measuring the body size are the main drawbacks derived from the use of body size spectra indices (Basset et al. 2012a).

A further step in the use of biological traits is their combination in the same analysis via the Biological Traits Analysis (BTA). Biological traits (BT) are components of an organism's phenotype that influence ecosystem processes and can comprise different levels (Reiss et al. 2009). Thus, morphological (body size, feeding type), reproductive (propagule dispersal, fecundity), behavioural (degree of mobility, attachment) traits are few of those considered as indicators of key features on marine ecosystem functioning (Bremner et al. 2006b; Frid et al. 1999; Frid et al. 2008). Therefore, functional diversity, i.e. the diversity of biological traits, is an important property of a community as it measures the role that organisms have in ecosystems (Schleuter et al. 2010).

Anthropogenic impact, apart from causing a decline in taxonomical diversity, can also induce functional shifts by replacing functional and biological traits. BTA study potential links between functional diversity and ecosystem functioning enclosing in the same statistical analysis selection of traits and categories (Bremner et al. 2006b). Originally applied to freshwaters macroinvertebrates (Statzner et al. 1994), it is now being implemented on marine and TWs (Frid et al. 2008; Oug et al. 2012; Tillin et al. 2006).

Table 2.3. Functional and body size based indices in peer-review EQS assessment studies on Mediterranean transitional waters. (CW) coastal waters, (FW) fresh waters, (TW) transitional waters, (*) index with taxonomy based correction factor. Country: (AL) Albania, (GR) Greece, (IT) Italy, (SP) Spain, (TN) Tunisia.

Index	Designed	Country	Lagoon/s	Publications
ITI (Word, 1978) (Word 1978)	CW	TN	Bizerte	Afli et al., 2008 (Afli et al. 2008)
BTA (Statzner, 1994) (Statzner et al. 1994)	FW	IT	Comacchio, Goro, Lesina, Oibia, Orbetelo, Scardovari, Tortoli, Venice	Marchini et al., 2008 (Marchini et al. 2008)
ISD (Reizopoulou and Nicolaidou, 2007) (Reizopoulou and Nicolaidou 2007b)	TW	GR	Papas, Tsopeli, Vivari	Simbora and Reizopoulou, 2008; Reizopoulou and Nicolaidou, 2007 (Reizopoulou and Nicolaidou 2007b; Simbora and Reizopoulou 2008)
ISS* (Basset et al., 2012a) (Basset et al. 2012a)	TWs	AL	Karavasta, Narta, Patok	Basset et al., 2012a; Barbone et al., 2012 (Barbone et al. 2012; Basset et al. 2012a)
		GR	Agiasma, Logarou	Basset et al., 2012a; Barbone et al., 2012 (Barbone et al. 2012; Basset et al. 2012a)
		IT	Alimini, Cesine, Grado Marano, Grado Valle Cavanata, Grado Valli da Pesca, Lesina, Margherita di Savoia, Torre Guaceto	Barbone et al., 2012; Basset et al., 2012a; Borja et al., 2011 (Barbone et al. 2012; Basset et al. 2012b; Borja et al. 2011)

Likewise BTA implementation occurs on Mediterranean TWs (Table 2.3); (Marchini et al. 2008) studying the function and EQS on a set of Italian lagoons stated the importance of traits linked to resources consumption over those linked to life cycle and (Sigala et al. 2012) used BTA for investigating community structure across a natural stress gradient in three Mediterranean lagoons.

Hence, through BTA, diverse forces structuring the benthic community have been determined. For example, elevated fishing pressure was found to boost borrowers, infauna and scavengers in (Tillin et al. 2006); fresh water influence was found to derive in less complex functional macroinvertebrate assemblages in (Paganelli et al. 2012); and sediment pollution with cadmium was detected to arouse trait features as small size, shallow sediment dwelling and subsurface deposit feeders dominance in (Oug et al. 2012).

The approach requires a precise selection of traits that correlate better with health state, however, connections between functional diversity and environmental alteration are still undefined (Petchey and Gaston 2006). Besides, gaps in the knowledge of species ecology and biology may also limit the power of these types of analyses (Bremner et al. 2003). Now the question is which traits should be included in a given BTA and how the traits have to be weighted. Also unexplored is the establishment of reference conditions and intercalibration in order to fulfil the WFD recommendations in matters of EQS.

Given these results, it seems appropriate to further investigate the relationship among functional traits and ecosystem health. (Basset 2010; Pacheco et al. 2010) already suggested the importance of including BTA together with taxonomical approaches in order to determine ecosystems stress. However, the unclear relationship between many BT and the ecological functioning complicates the interpretation of the analysis.

2.4 Metrics validation. Coping with human impact sources

The goal of developing an index rests in its further capacity to discriminate and scale the EQS (i.e. High-Good-Moderate-Poor-Bad) of a given management unit. Determining the performance and sensitivity of those indices is crucial, especially if they are used to assess types of stressors other than those for which they were developed (Quintino et al. 2006). An index may discern natural from anthropogenic pressures and identify how the last might affect the condition of a particular water body (e.g. coastal lagoon).

To test the response of a given index a validation is needed. Ideally the validation should be done with different datasets than the ones used for the index development. Thus, following best professional judgment (BPJ) supported by statistical analyses, the validation dataset may undergo a priori status classification and a posteriori justification may befall (Basset et al. 2012a; Borja and Dauer 2008; Weisberg et al. 2008). Furthermore the independent validation, by scientists other than those suggesting the metric, is a commitment (Borja et al. 2009a).

The unequal application and validation of the WFD metrics among seas, ecoregions and environments indicate TWs as the least explored ecosystems in these terms (Birk et al. 2012). However, metrics validated in surface waters other than TWs, where the natural stress sources are dissimilar, might not respond in the same way (Basset et al. 2012b). In addition, human activities in TWs are numerous and their combined effects over the aquatic ecosystem are still poorly known. Therefore, multi-impact, ecosystem-linked and independent validations would bring more realistic results, which might enable more comprehensive management decisions.

2.4.1 Sources of human impact and indices validation on Mediterranean transitional waters

A metric validation against different sources of human impact is necessary (Borja et al. 2009a), particularly when many indices are still dependent on the Pearson-Rosenberg model for organic enrichment (Quintino et al. 2006). In the Mediterranean TWs human pressures mainly derive from activities such as agriculture, aquaculture, damming, dredging, domestic and industrial discharges, land reclamation, harbour and industrial development, recreational and tourism growth. Most of the above activities may result on organic and nutrient loading, acidification, introduction of alien species, hydrological and physico-chemical alterations and physical modification (Table 2.4). Hence, a validation for physical disturbance and for non organic and chemical pollution is essential but scarce yet (Quintino et al. 2006). As a fact macroinvertebrate based indices often indicate very generic pressures and they are not able to discern the source. The problems derived from the generic application of indicators to both transitional and marine environments affected by both natural and human pressures are highlighted in (Borja et al. 2011; García et al. 2010; Lucena-Moya et

al. 2009; Neto et al. 2010). In Mediterranean TWs the comparison of metrics among water bodies with different pressures is the common procedure, due to both, the lack of data and the high natural variability of these environments (Birk et al. 2012).

Lately a pressure index method based on BPJ (Aubry and Elliott 2006) is being applied in Mediterranean and other waters to quantify human pressure factors and gradients (Aubry and Elliott 2006; Barbone et al. 2012; Basset et al. 2012a; Borja et al. 2011; Borja et al. 2009b; Van Hoey et al. 2013). This pressure index scale is defined from (high) to (low), providing values for each impact within the correspondent area. Based on this a pressure index (average pressure intensity) is calculated. Lately, it has been applied to determine the response of M-AMBI, BQI, BITS, BEQI, NQI, IQI, BAT and ISS metric in Mediterranean and other European TWs in (Basset et al. 2012a; Borja et al. 2011). The definition of a pressure-impact relationship is useful to validate the dose of response of the metric and the establishment of reference conditions. Criticism might overcome due to its reliance on BPJ (Teixeira et al. 2010; Thompson et al. 2012; Weisberg et al. 2008).

Table 2.4. Main characterized human activities, pressures and effects on Mediterranean transitional waters.

Human activities	Pressure	Effects	References
Agriculture	Nutrient, pesticide and fertilizer load	Primary production, oxygen, food chain, biocommunity changes, algal blooms	(Camacho et al. 2012; Conesa and Jiménez-Cárceles 2007; García-Pintado et al. 2007; García-Sánchez et al. 2012; Karageorgis et al. 2011)
Aquaculture	Organic load, hazard substances	Primary production, oxygen, food chain, alien species, biocommunity changes	(Chapelle et al. 2000; Orfanidis et al. 2005; Ponti et al. 2007a)
Domestic discharges	Organic load, hazard substances	Primary production, oxygen, food chain, biocommunity changes, pollutants	(Bernardello et al. 2006; Camacho et al. 2012; Conesa and Jiménez-Cárceles 2007; García-Pintado et al. 2007; García-Sánchez et al. 2012; Karageorgis et al. 2011; Lardicci et al. 2001; Lardicci and Rossi 1998)
Industrial discharges	Hazard substances, organic load, acidification	Primary production, oxygen, food chain, biocommunity changes	(Bellucci et al. 2002; Bernardello et al. 2006; Covelli et al. 2011; Rigaud et al. 2011)
Navigation	Hazard substances, physical modification	Sediment resuspension, alien species, oil spills, biocommunity changes	(Koutsoubas et al. 2000; Pérez-Ruzafa et al. 2007a; Rapaglia et al. 2011)
Fishing	Removal of individuals, physical modification	Biodiversity, community and food chain modification, sediment resuspension	(Dimitriou et al. 2007; Pérez-Ruzafa and Marcos 2012)
Physical modification (dredging, dumping, harbour and industrial development)	Water level and water flow alteration, artificial habitats, salinity variation	Biodiversity shifts, habitat loss, sediment resuspension, change in sediment properties, biocommunity changes	(Delpy et al. 2012; Fontolan et al. 2012; Gianni et al. 2011; Panda et al. 2013; Ponti et al. 2009)

2.5 Settling Reference Conditions. Coping with natural stress sources

An essential step in WFD implementation is the establishment of RC, which is a description of the biological quality elements that would exist at high environmental status. Optimally, RC define and provide an estimation of the biological communities variability due to ecological, physical and climatic factors (Borja et al. 2012a). The establishment of RC would permit to derive an ecological quality ratio (EQR) of 5 levels with values between 0 and 1 comprising boundaries for (High-Good-Moderate-Poor-Bad). Thus, RC can be defined with biological datasets from water bodies in natural baseline conditions, by modelling or combination of both.

Defining RC is a particular challenge in TWs due to their (i) high natural spatio-temporal variability. This triggers shifts in community composition (Gravina et al. 1989; Mogias and Kevrekidis 2005; Pérez-Ruzafa et al. 2007a; Stora and Arnoux 1983) and favours the dominance of certain highly selective species (Cognetti and Maltagliati 2000; Elliott and Whitfield 2011) able to recolonize rapidly, (Reizopoulou and Nicolaidou 2004), (ii) complex mosaic of habitats. Different habitats can be defined within a Mediterranean TW body type with distinct benthic assemblages (Basset et al. 2012b). The combination of physical factors, sediment type (Fresi et al. 1983; Nicolaidou 1983) and presence of vegetation (Arocena 2007; Bachelet et

al. 2000; Mistri et al. 2000) are major factors structuring benthic communities within habitat types, (iii) natural eutrophication and organic enrichment are especially common in Northern Adriatic TWs (Viaroli et al. 2001; Zaldívar et al. 2008). Increases in temperature facilitate phytobenthos decomposition causing oxygen depletion followed by mortality events that may drive shifts on benthic assemblages (Basset et al. 2013; Mistri 2002; Zaldívar et al. 2008), and (iv) biological interactions. Top-down and bottom up processes, benthic-pelagic coupling and larvae recruitment influence benthic community structure (Pérez-Ruzafa et al. 2005; Reiss and Kröncke 2005; Rosenberg 2001; Selleslagh et al. 2012; Tagliapietra et al. 1998). Last but not least there is the difficulty to *obtain datasets representing pristine conditions*.

To account the numerous sources of variability in Mediterranean TWs is a challenge for the scientific community (Barbone and Basset 2010; Barbone et al. 2012; Basset et al. 2012b; Borja et al. 2012b; Borja et al. 2009a; Lucena-Moya et al. 2012). Thus, two main variability components are identified in (Basset et al. 2012b); an inter-lagoon component, which might be partially reduced by establishing a lagoon typology classification and an intra-lagoon component which results from habitat patchiness and seasonality. A different habitat assignation of samples can lead to differences in RC values and ecological quality status scores (Chainho et al. 2007; Van Hoey et al. 2013). A water body EQS misclassification is prone to occur given the high habitat diversity that those environments sustain. Efforts to incorporate the natural variability have been made in various studies (Barbone et al. 2012; Basset et al. 2012b; Borja et al. 2012b; Lucena-Moya et al. 2012). In coastal lagoons different approaches have been used to reduce the deviation caused by natural effects: the water body approach, where the main physical factors structuring community are considered appropriate to classify the EQS (Basset et al. 2006b; Lucena-Moya et al. 2009), the habitat approach, where a physical and biological factors are considered to define benthic assemblages (Blanchet et al. 2008; Gamito et al. 2012a) and finally, a combination of both, where a basin specific RC reflecting benthic assemblages at each habitat type are described (Barbone et al. 2012; Basset et al. 2012a; Lucena-Moya and Pardo 2012). This last option seems to be more far-reaching, since the major variability sources are considered.

Modelling historical datasets (Andersen et al. 2004; Kotta et al. 2012; Nielsen et al. 2003), best professional judgement (Barbone et al. 2012; Borja et al. 2012a; Van Hoey et al. 2013) or paleoecology principles (Schönfeld et al. 2012) are some approaches none of which appear to be definitive. In lack of historical data a combination of expert judgement on scoring pressure indices and ecosystem modelling may be appropriate to set RC (Borja et al. 2012a; Borja et al. 2012b; Muxika et al. 2007; Van Hoey et al. 2013). Moreover, even when RC are defined, some degree of uncertainty still occurs in the EQS assessment, since all metrics used for the classification purposes are affected by the abiotic and habitat components of the ecosystems (Barbone et al. 2012). Given these conditions a step forward in any assessment is the evaluation of uncertainty derived from any assumption and source of variability (Clarke 2012; Hering et al. 2010).

2.6 Metrics Intercalibration. Coping with the consistency among member states

The comparability of monitoring and assessment results among MSs is a crucial part of the ecological status classification (Commission 2000). This process requires an eco-regional intercalibration (IC) network consisting of monitoring sites in each MS. Each ecoregion is represented by a GIG involving MSs that share common intercalibration body types and can thus compare their monitoring results. The final goal of any GIG is to set class boundaries for the High/Good and the Good/Moderate status of a given BQE, making the different national metrics comparable among MSs (Commission 2011).

In the thirteen years from the initiation of the WFD two publications in the Official Journal of the European Commission have addressed the intercalibration issue. The first, published in 2005, sets a register of sites forming the intercalibration network (Commission 2005). During this first IC transitional waters were not intercalibrated (Carletti and Heiskanen 2009), and the only TW body listed among the Mediterranean countries was Laguna di Venezia -bacino meridionale. Three years later a second publication presented the first results of the intercalibration exercise for coastal waters (Commission 2008). The MED-GIG working on benthic macroinvertebrates incorporated the threshold values (High/Good and Good/Moderate) to apply on soft sediment communities, whereas thresholds for BENTIX (Cyprus and Greece), M-AMBI (Slovenia) and MEDOCC (Spain) were defined just for coastal waters. These publications show how the intercalibration task for macroinvertebrates is mostly accomplished for Mediterranean coastal waters but still incipient in terms of TWs. After the Phase II MEDGIG IC for TWs none of the proposed indices was fully intercalibrated (Pardo et al. 2011). A new intercalibration exercise may close gaps assessed in the first phases (Commission 2011).

The dimension of the intercalibration exercise in an eco-regional basis combining water body types, biological quality elements and specific pressures complicates the WFD implementation in TWs. Moreover, the issue of natural variability needs to be further addressed, as it has important implications in the use of the National Methods, establishment of reference conditions as well as in the intercalibration (Duarte 2008)

2.7 Future research

The WFD implementation challenges the scientific community to determine a series of multidisciplinary techniques to evaluate the health state of European surface waters. Thirteen years after its publication, advances had been made but the complete implementation is still pending. The different ecoregions, water body types and biological quality elements remain in different phases of WFD implementation (Birk et al. 2012; Birk et al. 2013).

EQS assessments comprise various steps that can affect the comparability among same water body types. First, sources of uncertainty can arise from sampling methodologies, laboratory processing, index development, data analysis and interpretation (Cao and Hawkins 2011). In Mediterranean TWs major differences in sampling methods exist among countries, research centres and projects. In the field the surface of the grab, sieve mesh size (Aarnio et al. 2011; Pinna et al. 2013) and sampling effort (Fleischer et al. 2007) may potentially affect the comparability. In the laboratory the sample processing and the taxonomic accuracy are also sources of uncertainty (Dauvin et al. 2010). Thus, the standardization of base protocols would minimize basic sources of uncertainty (Cao and Hawkins 2011).

Apart from fundamental differences in sampling and data elaboration, the definition of lagoon typology, the use of different metrics and their applicability are potential sources of variability.

Despite the efforts, subjective choices exist in the construction of the indices (species lists and mathematical approach), validation, definition of threshold values and intercalibration process (Borja et al. 2009a). The use of best professional judgment (BPJ) on any of these may also raise technical and legal problems in the WFD implementation. Disagreement among benthic ecologists over the use of BPJ in determining the EQS of the benthic ecosystem exists (Teixeira et al. 2010; Thompson et al. 2012; Weisberg et al. 2008), making the assessment task more complex for TWs.

The establishment of a water body typology scheme is necessary to implement the WFD. Salinity (maximum, average) and complementary factors as openness and tidal range are included in few studies (Basset et al. 2006a; Basset et al. 2006b; Lucena-Moya et al. 2009). Still, the problems deriving from the

definition of a typology are faced on the MED-GIG (Pardo et al. 2011). Other factors such as water body size can play a fundamental role in the capacity of a lagoon to tolerate abrupt changes. In addition, from a climatological perspective, parameters, such as precipitation, evaporative processes, surface wind, river run-off and sea water intrusion, that define lagoon's water budget and further regulate the biological and other environmental characteristics should be taken into account.

As presented herein, a wide range of metrics has been developed and applied during the last decade. Totally compliance with the WFD seems to be still scarce, and the recommendation in (Diaz et al. 2004) to normalize and intercalibrate existing metrics rather than developing new ones, seems not to be appropriated for these highly variable and patchy ecosystems. The necessary steps to be followed in developing an index according to (Borja and Dauer 2008) are: (i) selection of candidate metrics; (ii) metric combination; (iii) index validation; (iv) index application to diverse anthropogenic pressures; (v) index interpretation and (vi) index intercalibration. So far, only few follow this outline (Borja et al. 2012b).

Although the use of taxonomy based metrics is stressed in the WFD (Commission 2000), those may be inappropriate to discriminate anthropogenic from natural stress on TWs where strong analogies with organic polluted coastal waters exist (Reizopoulou and Nicolaidou 2007b; Simboura and Reizopoulou 2008). Thus, the development of novel metrics taking into account the high natural variability of these particular environments is necessary. Approaches, based on taxonomic sufficiency (Dauvin et al. 2010) and phenotypic characteristics as body-size (Basset et al. 2012a; Reizopoulou and Nicolaidou 2007b) lately applied in Mediterranean TWs, appear to be worthy to further explore. These indices are friendlier to apply for technicians and managers due to the simplification either elimination of taxonomical identification. Furthermore, the use of BTA is incipient on Mediterranean TWs, however results entail their use (Bremner et al. 2006a; Frid et al. 2008; Marchini et al. 2008; Paganelli et al. 2012; Sanders et al. 2007; Sigala et al. 2012). Given the variety of pressures on Mediterranean TWs and the response of dissimilar traits to these pressures, (Bremner et al. 2006a) more research in this line might be pertinent. Nonetheless, the complexity derived in applying this approach is the main drawback for its implementation on a WFD basis. BTA based studies might be facilitated with the construction of a collaborative database among Mediterranean research groups working on this research line.

New indices have to be validated against different sources of anthropogenic disturbance and their ecological behaviour should be statistically tested (Borja et al. 2012b). Overall, a lack of accurate and wide metrics validation is pending. Metric validations mainly occur against organic and nutrient loading ((Zaldívar et al. 2008) and references there in), since these are major pressures acting on Mediterranean TWs. New approaches in the validation procedures may be explored, whereas a combination of the response of other BQEs and physicochemical parameters may enhance the validation process. A validation approach, based on BPJ, scaling natural and anthropogenic pressure gradients has been applied in Mediterranean and other European TWs in (Basset et al. 2012a; Borja et al. 2011). Still the BPJ dependence might be controversial in some cases (Teixeira et al. 2010; Thompson et al. 2012; Weisberg et al. 2008).

Due to the lack of pristine undisturbed areas (Halpern et al. 2008), different methods *as* modelling (Andersen et al. 2004; Nielsen et al. 2003), defining pressure indices (Barbone et al. 2012; Borja et al. 2012a; Van Hoey et al. 2013) or paleoecology principles (Schönfeld et al. 2012) partially dependent on BPJ have being applied in order to assess RC. But, uncertainty also occurs in the abiotic and biotic component of the ecosystem (Gamito et al. 2012a). Given these conditions we need to identify and evaluate the degree of uncertainty derived from any assumption and source of variability (Clarke 2012; Hering et al. 2010). Furthermore, future research on the settlement of RCs might try to comprehend the natural variability and patchiness existent in Mediterranean TWs. Hence, habitat mappings, by combination of techniques as

remote sensing, side-scan sonar, video and photography analysis might bring positive results on the settlement of RCs and EQS assessment.

One of the last steps is the intercalibration of the WFD compliant metrics. An accurate intercalibration would enhance the harmonization of the threshold values for High/Good and Good/Moderate, and by extension this would enable to take more consistent management actions at a MS level and further in the whole of Europe. The WFD intercalibration process, has being described in (Duarte 2008) as one of the vastest and widest intercalibration procedures in the world. Thus the intercalibration of macroinvertebrate metrics on the Mediterranean basin is still in progress after the first steps in 2005 (Commission 2005).

Scientific, management and legal challenges are still present in any of the different steps necessities to implement the WFD in Mediterranean TWs.

3 Macrobenthic community structure and Ecological Quality status assessment of the Marine Protected Area of Messolonghi lagoon, Greece

Abstract

It is well known that the macroinvertebrate organisms are good indicators of the environmental condition of benthic habitats. Within the lagoon of Messolonghi the last study on the macroinvertebrate communities dates from 1984. The aim of the present study was twofold: First to identify the main environmental variables structuring the macrobenthic communities and to describe their taxonomic composition and seasonal shifts. Second, to evaluate the ecological quality status of the benthic habitats and communities, by means of a set of Water Framework Directive intercalibrated benthic indicators for Mediterranean transitional waters, i.e. AMBI, M-AMBI and BENTIX.

The study reports the highest species richness of benthic fauna for a lagoonal environment in Greece, while the habitat type is determinative of the benthic communities. Four habitat types were described, i.e. marine, vegetated, unvegetated and unvegetated next to a sewage outflow. The presence of vegetation had the primary control in determining species richness and community diversity in Messolonghi lagoon. Neither confinement nor seasonal pattern was disclosed. Results on the Ecological Quality Status give an overall Good status for the main lagoon of Messolonghi, yet differences amongst the used indicators are reported.

Key words; macroinvertebrate communities, benthic habitats, Ecological Quality Status, Mediterranean lagoon.

3.1 Introduction

Mediterranean coastal lagoons are transitional water bodies functioning as buffer ecosystems, where fresh inland waters from rivers and runoff meet with the saline coast waters. Within these ecosystem different environmental factors, as salinity and sediment composition, have been widely described to play a key role structuring the communities of macroinvertebrates living in the soft bottom (Galuppo et al. 2007; Nicolaidou et al. 2006; Ponti et al. 2007a; Reizopoulou and Nicolaidou 2004). Seasonal patterns of benthic diversity have been recorded in Mediterranean lagoons, with the highest values mostly occurring during spring and autumn, when environmental conditions are more favorable (Gravina et al. 1989). Diversity indices in lagoons are often affected by fluctuations of the abundance of the most dominant species (Arias and Drake 1994; Mistri et al. 2000). Besides the daily and seasonal variations, other stochastic processes as storms or anoxia episodes also play an important role in structuring the benthic and other lagoonal communities (Cladas et al. 2016; Gianni et al. 2011; Koutsodendris et al. 2015) (Guelorget et al. 1983; Nicolaidou and Reizopoulou 2005; Reizopoulou et al. 2014a). Thus, brackish water benthic assemblages include euryhaline species rather adapted to a shallow, sheltered environment, and opportunistic species with a high tolerance to stress and to disturbance (Cognetti and Maltagliati 2000)

A common descriptor applied for studying the assemblages of the benthic communities is the degree of confinement, which is marked by the salinity ranges that eventually will determine the species composition of a given lagoonal habitat or section.

Over the last several decades anthropogenic activities within the coastal areas has increased. Coastal lagoons in this area frequently show physical and chemical disturbances and fluctuations, sometimes being considered as sinks for waste, which causes their accelerated environmental degradation (Newton et al. 2013). Due to the amount of natural and economic goods and services that Mediterranean lagoons yield make them a rather anthropogenic ecosystem. Common activities altering the natural status of the Mediterranean lagoons are fisheries, salt works, agriculture, tourism development and residual waters among others.

Being conscious of these threats and in the absence of a specific European regulation, an umbrella directive for the protection of the coastal water bodies was launched in the last decade: the European Water Framework Directive (2000/60/EC), (Commission 2000). The WFD targets the conservation and sustainable use of the marine ecosystem, reaching at least a Good Ecological Status (GES) for all European coastal water bodies. where GES is the state where the distortion resulting from human activity is only slightly deviating from undisturbed conditions. Therefore, the accomplishment of these objectives needs to integrate conservation objectives, management measures (e.g. Marine Protected Areas), monitoring and assessment actions, but also to follow an ecosystem-based approach (Granek et al. 2010). One of the important ecosystem components is benthic invertebrate fauna. Due to their sessile, sedentary and relatively long life macroinvertebrate species are considered sensitive indicators of natural and anthropogenic variations in the marine ecosystem (Pearson and Rosenberg 1978) and they are proved to be good indicators of temporal and chronic disturbances also (Dauer 1993). Thus, the assessment of the condition of the benthic habitats is one of the evaluation criteria in the WFD. An assessment procedure for determining the condition of soft-sediment benthic habitats is required.

The aim of this study is twofold: first to identify and describe the macroinvertebrate communities and second to assess the ecological quality status of the main benthic habitats.

3.2 Material and Methods

3.2.1 Study area

The lagoon system of Messolonghi is situated on the Greek Ionian coast, on the northern side of the Patraikos Gulf, between the Acheloos and Evinos rivers. It is the largest lagoon complex in Greece covering approximately 15.000 ha and consisting of 6 different basins. The lagoonal complex, part of the National Park of Messolonghi, is also a Marine Protected Area, a Ramsar Site, an Important Bird Area (IBA), and part of the Natura 2000 network. The region is characterized by a typical Mediterranean climate. During 2013, the sampling year, the annual precipitation was 1016 mm, of which a percentage of 73% (741.6 mm) was registered in 3 months (January, February and November). The mean annual air temperature was 18.4C, oscillating between the mean monthly minimum of 10.3C in January, and the mean monthly maximum, 28.5C in August (<http://www.meteo.gr>).

The present study was carried out in the main lagoon of Messolonghi, which is an open lagoon communicating with the sea through a shallow and wide frontal area. Throughout the year the lagoon presents a range of salinity and temperature, as a result of the shallowness (mean depth about 1 m). Climatic factors such as rainfall and wind rapidly affect the temporal variations of abiotic parameters of the water masses. (Gianni et al. 2011). According to the presence or absence of vegetation we can distinguish vegetated habitats, characterized by the dominant vegetation (*Cymodocea nodosa*, *Rytiphlaea tinctoria* and *Valonia aegagropila*) and unvegetated habitats. To characterize the macroinvertebrate communities and to assess their Ecological Quality Status (EQS) a set of sampling stations were selected as to represent all: *Cymodocea nodosa* in marine environment (M1), *Valonia aegagropila* (M3), *Rytiphlaea tinctoria* and *Cymodocea nodosa* (M5), *Rytiphlaea tinctoria* (M6), and bare sediment (M4, M7 and M8) (Fig.3.1). The non-vegetated site M8 is located next to a sewage treatment plant outflow.

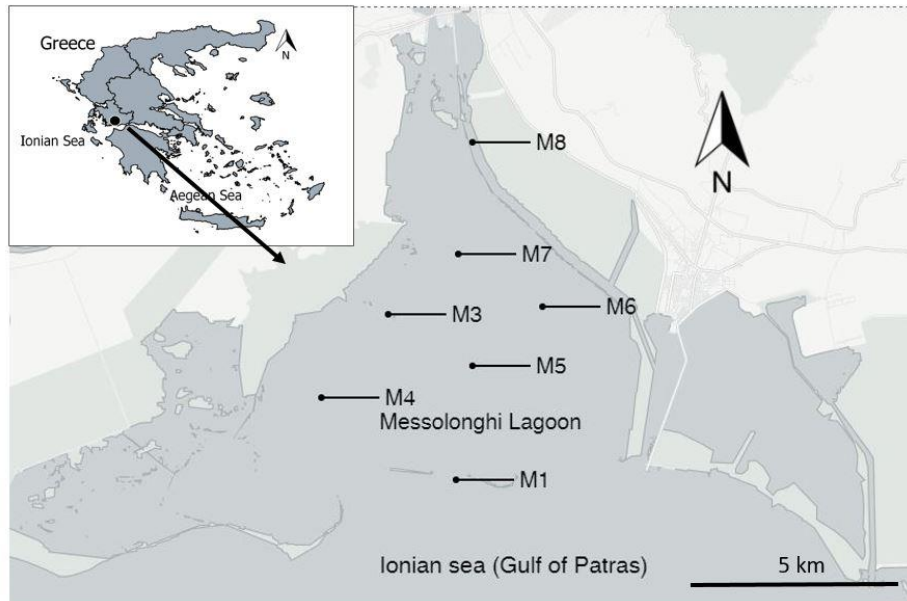


Fig. 3.1: Lagoon of Messolonghi (Greece). Seven sampling sites across the main lagoon covering the main habitats, sampled 4 times in 2013

3.2.2 Data collection

Data collection took place four times during 2013 (January, April, July and November). For the analysis of benthic macroinvertebrates, sediment samples (3 replicates) were collected with a box corer (0.023m²surface area) at each sampling site. The samples were sieved through a 0.5-mm mesh sieve and stored in 4% formalin solution with Rose Bengal. In the laboratory, the collected organisms were sorted, identified to the lowest possible taxonomic level (generally species) and counted.

An additional sediment sample was collected at each site, the uppermost 2 cm of which were kept for granulometry and total carbon analysis. For the granulometric analysis, the samples were originally separated to coarse-grained (N63 μ m) and fine g-grained (v63 μ m) fractions by wet sieving. Further classification of the sand and mud fractions was accomplished with standard sieves and the grain size analyzer Sedigraph 5100. At each sampling site salinity, temperature, pH and dissolved oxygen were monitored close to the bottom using a multi-probe meter (YSI 600QS).

3.2.3 Statistical analysis

3.2.3.1 Environmental variables

For identifying water and sediment physicochemical characteristics with the highest discriminatory power across the studied sites and seasons we run the Canonical Analysis of Principal Coordinates routine (CAP). CAP analysis based on normalized Euclidian distances was used to express the physicochemical similarity across the studied sites (water and sediment variables) as well as to identify how the water variables correlated with seasons.

3.2.3.2 Macroinvertebrate community analysis

The different studied sites and Messolonghi habitats, *sensu* (Cabana et al. 2016), were characterized per season by a set of multivariate techniques. With SIMPER analysis we identified the species with higher contribution to each site to describe the macroinvertebrate communities of each studied site and habitat. We used PERMANOVA analysis (Anderson et al. 2008) to test for Shannon diversity differences across the sites and seasons. We performed the distance based redundancy analysis ordination method (dbRDA), 9999 permutations, to test the relationship of the species composition with a set of continuous biological (algae weights) and physicochemical variables measured in the sediment (sand%, total carbon) and water column (temperature, salinity and dissolved oxygen). The standard community indices, species richness, abundance and Shannon diversity were also calculated.

3.2.3.3 Ecological Quality Status

For the EQS assessment we use the set of indices intercalibrated for the Mediterranean basin for the implementation of the WFD (Commission 2000); i.e., AMBI (Borja et al. 2000)M-AMBI (Muxika et al. 2007) ,BENTIX (Simboura and Zenetos 2002). Two biotic indicators (AMBI and BENTIX) and one multivariate (M-AMBI).

AMBI method is based on the assignment of species to five ecological groups, according to the sensitivity to an increasing stress gradient (Borja et al. 2000). Group I – species very sensitive to organic enrichment. Group II – species indifferent to enrichment. Groups III – species tolerant to excess organic matter enrichment. Group IV and V – Second and first order of opportunistic species. The software for calculating AMBI is available at <http://www.azti.es/>

$$\text{AMBI} = (0) \times (\%GI) + (1,5) \times (\%GII) + (3) \times (\%GIII) + (4,5) \times (\%GIV) + (6) \times (\%GV) / 100$$

M-AMBI is calculated by factor analysis of AMBI, species richness (as number of taxa) and Shannon diversity (H' on \log_2 base) values. The use of this method requires the setting of reference conditions (Muxika et al. 2007), specific for each type or habitat.

Factor analysis: S, AMBI, Shannon diversity index

BENTIX index classifies the benthic macroinvertebrates into two general ecological groups and assigns a score 1 or 2 according to their sensitivity or tolerance to disturbance. GS: includes species sensitive or indifferent to disturbance or general stress. GT: includes species tolerant to disturbance and the opportunistic ones. The BENTIX index was validated with data from Greek marine ecosystems and appears to work successfully (different ecological quality classes corresponding to different stress). (Simboura and Zenetos 2002). The software for calculating BENTIX is available at <http://www.hcmr.gr/en/the-BENTIX-index>

$$\text{BENTIX} = (6 \times \%GS + 2 \times \%GT) / 100$$

Reference Conditions. Although the lagoon of Messolonghi is a MPA it has been subject to increased human pressures especially in recent years. Besides, there are no historical data available concerning its ecological conditions. Thus, here we use the reference conditions and Ecological Quality Ratios proposed in Simboura and Reizopoulou (2008) for the eastern Mediterranean transitional waters, i.e.; $H=4$, $S=50$, $\text{AMBI}=0$. Also, EQR boundaries: High; 0.83, Good; 0.53, Moderate; 0.39, Poor; 0.21.

3.3 Results

3.3.1 Environmental conditions

The set of stations included in this work covers the main habitats across the whole main lagoon of Messolonghi, with average depth of 1 meter. (Fi. 1). On the basis of the yearly average cross year average salinity (33.5 psu), Messolonghi can be characterized as a typical euhaline open Mediterranean lagoon with no marked confinement factor (Battaglia 1959; Guelorget and Perthuisot 1983a).

Amongst the studied physicochemical variables (Table 3.1) temperature and salinity follow a rather similar pattern: Salinity values range from a minimum of 17.3 psu in the inner-most site M8 (January) and a maximum 48.5psu (July) at the south-west of the main lagoon, siteM4 (Table 3.1, Fig 3.2). The

temperature values go from a minimum of 11.5C (January) in M8 and a maximum of 30.4 (July) in M7. Overall, a general salinity and temperature trend is characterized for an increase from January to July to come back to April values in November. The dissolved oxygen registered from average minimum of 7.6 mg/l (July) in M4 and a maximum average of 9.4mg/l (January) in M8.

Lagoonal sediments were sandy in M1, M8 and M6 and mostly muddy, with variation in silt and clay in the rest of the studied sites. (Table 3.1). The average content in percentage of total carbon within the sediment are highly variable from minimum values of 1.6±0.1 in M8 to a maximums of 10.5±0.5 in M6. The weight of the vegetation within the habitat identified higher proportions of vegetation in M3, mostly characterized by *V. aegagropitla* followed by M5 and M6 composed by a combination of *R.tinctoria* and *C.nodosa* and last M1 which is composed solely by *C.nodosa*. The other habitats are unvegetated.

Table 3.1. Main water and sediment physical and chemical variables. Water column next to the bottom; Temperature (°C) and salinity (PSU) cross seasonal (max-min).DO; dissolved oxygen (mg/l) and pH. Sediment composition; % of sand, silt and clay, TC (total carbon) and vegetation dry biomass. ± Standard deviation; explains the seasonal variability.

Site	Temperature	Salinity	DO	pH	Sand	Silt	Clay	TC	Depth(m)	Vegetation
M1	27.1-13.1	40.8-27.11	8.4±0.8	8.1±0.2	77.6±10.7	14.3±4.3	8.1±8.7	4.6±0.2	0.5	1.1±0.8
M3	29.2-12.2	39.5-25.5	9.1±1.4	8.4±0.3	25.4±5.5	35.9±8.4	38.7±3.6	9.1±0.2	1	6.1±2.1
M4	28.8-15.5	48.5-31.9	7.6±0.7	8.1±0.3	13.2±6.1	47.3±8.8	39.5±7.2	7.9±0.6	1	0
M5	29.5-11.9	43.2-32.5	8.4±0.4	8.2±0.2	47.6±9.6	37.8±16	14.5±7.3	8.7±0.2	1	3±1.8
M6	30.2-11.9	44.4-31.6	7.7±0.8	8.2±0.3	55.3±5.0	26.1±1.3	18.6±5.8	10.5±0.5	1.6	5.6±2.9
M7	30.4-12.5	39.8-25.6	8.5±1.8	8.6±0.5	24.2±10.1	45.6±8.8	34.7±7.5	9.9±0.8	1.7	0
M8	31.7-11.5	38.8-17.3	9.4±1.4	8.6±0.4	63.1±7.8	17.4±4.1	19.4±8.3	1.6±0.1	1.5	0

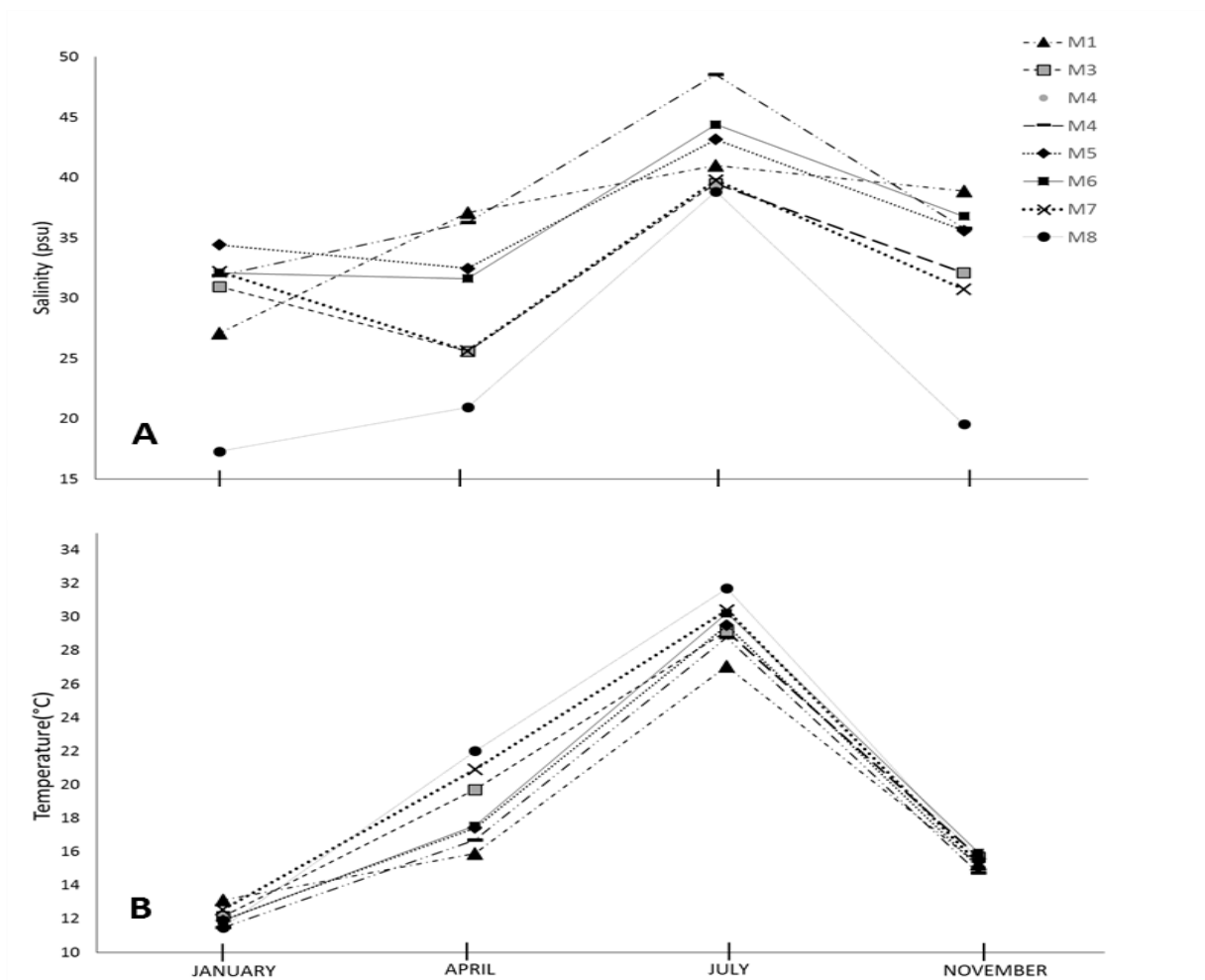


Fig. 3.2 Salinity during the sampling period, across the 7 studied sites.

The Canonical analysis of principal coordinates (CAP) routine for the studied physicochemical variables, in the water and sediment, suggest a separation of two main groups over the eigenvalue 1 (R^2 0.95) and another two groups less discriminated over the eigenvalue 2 (R^2 0.54) (Fig. 3.3 A). CAP 1 discriminates M1 and M8 at the right of the plot and at the left the rest of the sites (M3, M4, M5, M6 and M7). The CAP2 separates M7 and M8 in the upper part of the plot and the rest of the sites (M1, M3, M4, M5 and M6) at the bottom of the plot.

The CAP for water variables seasonally obtained (Fig 3.3B) separates along the CAP1 axis (R^2 0.98) the July samples, from all the rest. CAP1 axis correlates positively with temperature and salinity and negatively with dissolved oxygen. Samples plotted over CAP2 axis, present a seasonal gradient along the CAP2 (R^2 0.69) which correlates better with PH presenting the lowest values in April and the highest in January.

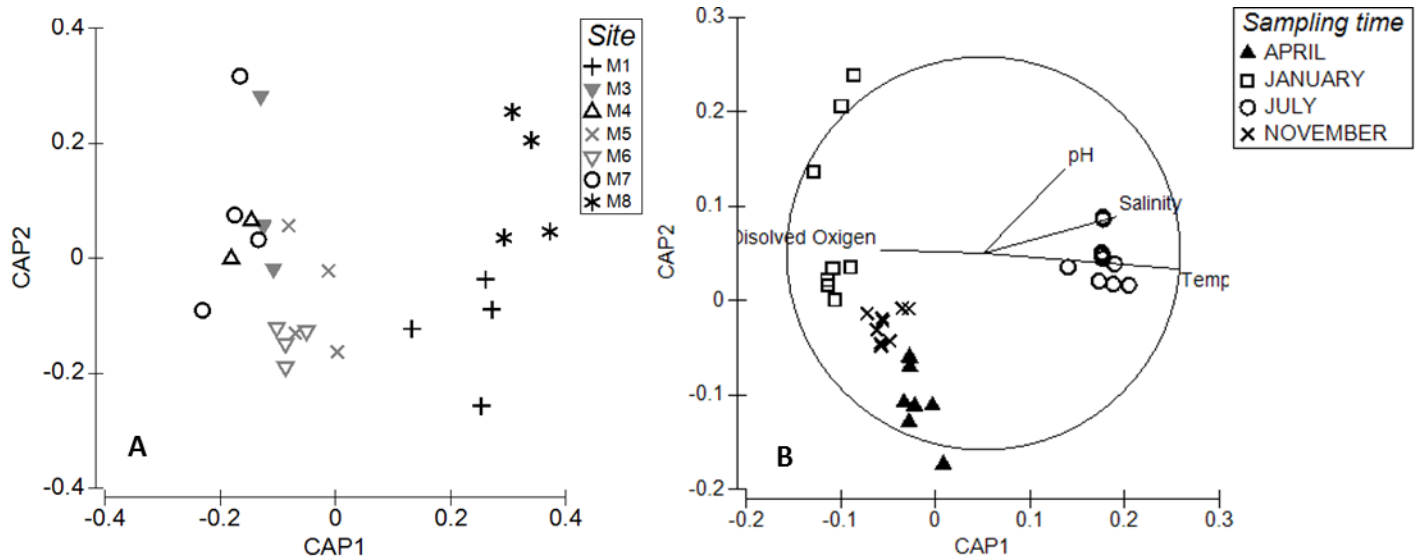


Fig 3.3. Canonical analysis of principal coordinates. (A) Main discriminating water and sediment physicochemical variables. (B) Main discriminating water physicochemical variables for the 4 sampling times.

3.3.2 General characterization of the macroinvertebrate communities

In total 24046 macroinvertebrate individuals, belonging to 194 taxonomic groups, were identified in this study. Overall, the percentage of identified taxa across the set of 7 studied sites was dominated by Polychaeta 55%, Crustacea 28%, Mollusca 11%, Echinodermata 4% and others 2%.

The total macroinvertebrate abundance ranged from 1223 to 14 organisms per sample (0.0023m^2). The sites with highest abundance of macroinvertebrates were located across the whole lagoon, corresponding to vegetated habitats.

3.3.3 General distribution patterns

The distribution of the benthic macroinvertebrate species richness (S) and abundance (N) and Shannon (H') follows a variable pattern across the studied sites and across the seasons (Fig 3.4). The S distribution presents two main groups, one with the sites hosting less than 15 species including M4, M7 and M8 and another with sites hosting from 30 to 40 species which comprises the vegetated sites M1, M3, M5 and M6 (Fig 3.4A). The N pattern of distribution indicates M4 as the poorest site followed by M7 and then M8. Maximum N was recorded in M3 with an average of 120 individuals. M1, M5 and M6 correspond to average abundance values between 65 and 90 (Fig. 3.4 B). The last community descriptor, $H'\text{Log}_2$ indicates M3 as the most diverse site followed by M5, M6 and M1, all of them with values over 4.5. Lastly, the sites M4, M7 and M8 range in a narrow strip from 3.15 to 3.25 (Fig. 3.4 C).

The taxonomic groups with the highest contribution varied depending on the study site. Overall, along the seasons and stations were the polychaetes; *Janua pagenstecheri* (7.7%), *Fabricia stellaris* (5.9%), the bivalve: *Abra segmentum* (6.18%), and the crustacean *Microdeotopus gryllotalpa* (4.8%).

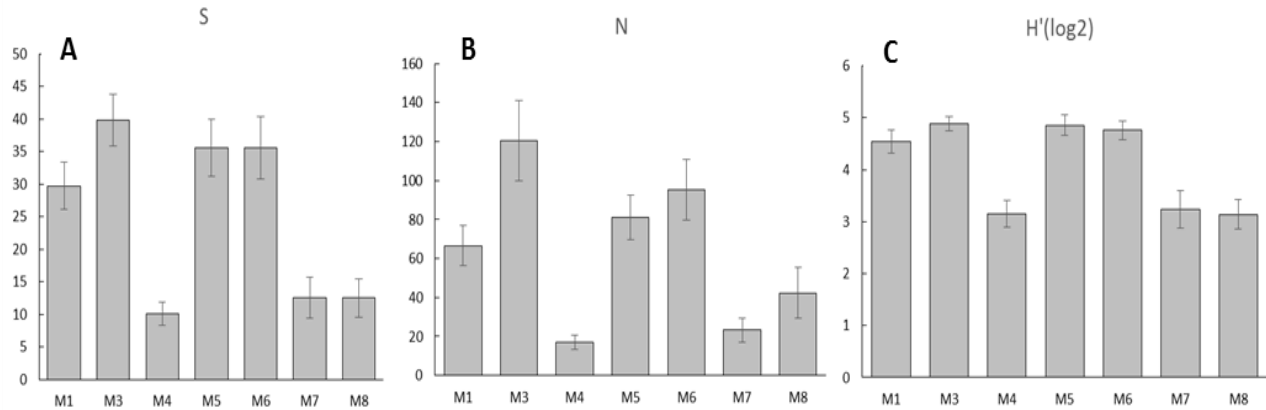


Fig 3.4. Biological descriptors for the 7 sampling sites. Average species richness for the 4 sampling times; (A), Average abundance (B), Average Shannon $H' \log_2$ (C). Vertical bars; standard deviation for the replicates.

3.3.4 General diversity seasonal patterns

The variation of the macroinvertebrate diversity across the set of sites and seasons identifies a set of highly variable patterns (Fig.3.5). The test for the Shannon diversity similarities across the studied sites and seasons (PERMANOVA, Table 3.2) presented significant dissimilarities for the factor site but not for the factor seasons. Further pairwise test differentiated two groups of sites with no significant difference among (M4, M7 and M8) and (M3, M5, M6), M1 was significant different to the rest of the sites $P > 0.05$.

Overall, two main patterns were identified: one with diversity ranging between 2.9 and 3.8 and another with diversity ranging from 4 to 5.3. The first group brings together those sites with unvegetated bottom, contrarily the second group brings together those sites with vegetation. Within the unvegetated, sites M4 and M7 follow a similar pattern along the sampling period, with a drop of diversity in April and July, contrarily M8 registers an increase of diversity during July and a drop in November. Within the vegetated sites, we can identify two main groups: sites M1 and M5 follow a similar pattern, which registered an increase of diversity from January to July and a consequent drop in July similar to that of April; and another group including sites M3 and M5, where the diversity is quite stable with a slight drop from January to July, when there is an increase up to November.

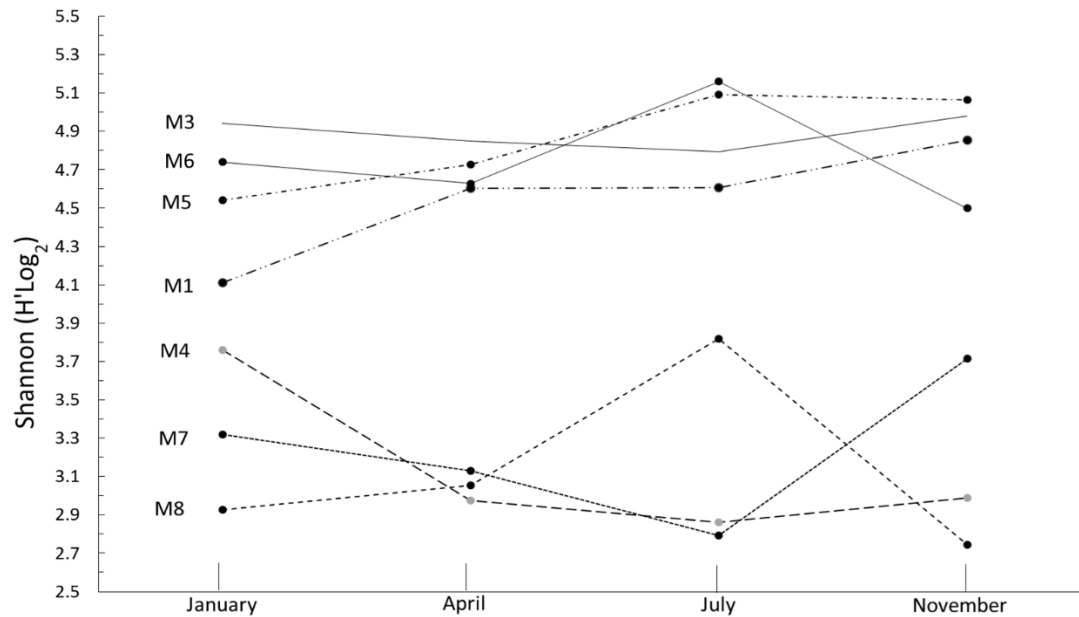


Fig 3.5. Shannon diversity for each sampling site and season; January, April, July and November.

Table 3.2. PERMANOVA to test the hypothesis of no differences in Shannon diversity across the sites and seasons.

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Season	3	82.982	27.661	0.61587	0.6439	9950
Site	6	8201.6	1366.9	30.435	0.0001	9959
Season*Site	18	1273.8	70.768	1.5756	0.0819	9911
Residual	55	2470.2	44.913			
Total	82	11987				

For the macroinvertebrate community assemblages the two RDA axes significantly explained 26 % of the total variation, ($P < 0.001$). The 16.6% of the variation expressed by axis 1 identified a gradient that contrasted with vegetated sites occurring in the right side of the ordination and unvegetated sites that occupied the left side of the ordination. The 9.5% of the variation expressed by axis 2 identified a gradient that contrasted with sandy bottom occurring in the upper side of the ordination, (Fig 3.6).

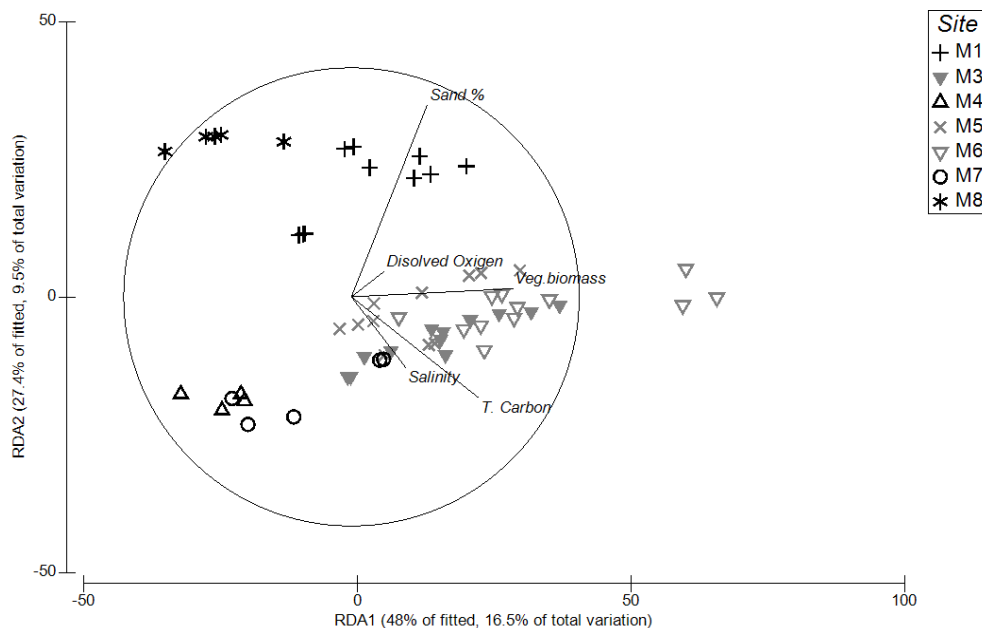


Fig 3.6. Redundancy analysis (RDA) biplot for the macroinvertebrate community global model (vegetation biomass and physicochemical explanatory variables). RDA's integrates the four sampling periods; January (J), April (A), July (X) and November (N). Grey symbols for vegetated sites and black symbols for unvegetated sites.

3.3.5 Macroinvertebrate assemblages; structure and distribution.

Marine habitat assemblage (site M1); is characterized by a high species richness (avg: 30 Spp. per sample) and macroinvertebrate abundance (avg. 275 ind per sample). The community is dominated by the polychaetes *Capitella minima* and *Capitella capitata* with an average of 45 and 15 individuals per sample respectively, contributing both up to 21% to the community (Fig.3.7A). The site M1 is characterized by an average cross annual salinity corresponding to a rather coastal Mediterranean condition (Fig.3.2A). The average depth is 50cm and the main vegetation cover is *Cymodocea nodosa* with an average dry weight per sample of 1.13g.

Vegetated habitat assemblage (M3, M5, and M6); is characterized by a highest species richness (avg; 36 Spp per sample) and macroinvertebrate abundance (300 ind. per sample). The community is dominated by the polychaetes *Janua pagaestechery* (avg 50 ind. per sample), *Fabricia sp.* (avg. 16 ind per sample) and *Exegone dispar* (avg. 16 ind per sample).

-M3 is characterized by *Janua pagaestechery*, *Microdeotopus grylotalpa*, with a cumulative contribution to the community of 17%. (Fig.3.7B). The main vegetation is *Valonia aegagropila* and *Cladophora sp.* with an average weight per sample of 2.5g and 2.4g respectively.

-M5 is characterized by *Exegone dispar*, *Janaua pagaestechery* and *Microdeotopus bifidus*, with a cumulative contribution to the community of 21%. (Fig.3.7D). The main vegetation is *Cymodocea nodosa* and *Rytiphlaea tinctoria* with an average weight per sample of 5.8g and 1.6g respectively.

-M6 is characterized for *Fabricia sp.*, *Janua pagaestechery* and *Microdeotopus bifidus*, with a cumulative contribution to the community of 24%. (Fig.3.7E). The main vegetation is *Cymodocea nodosa* and *Rytiphlaea tinctoria* with an average weight per sample of 0.3g and 5.3g respectively.

Unvegetated habitat assemblage (M4, M7); is characterized by the lowest species richness (avg; 12 Spp per sample) and macroinvertebrate abundance (50 ind. per sample). The community is dominated by the polychaetae *Armandia cirrhosa* (avg 50 ind. per sample) and the bivalve *Abra segmentum* (56 avg ind. per sample).

-M4 is characterized by *Nephtys hombergii*, *Abra segmentum* and *Armandia cirrhosa* with a cumulative contribution to the community of 62%. (Fig.3.7C).

-M7 is characterized by *Iphinoe serrata*, *Ostracoda*, *Abra segmentum* and *Armandia cirrhosa* with a cumulative contribution to the community of 68%. (Fig.3.7F).

Unvegetated Sewage habitat assemblage (M8); is characterized by the lowest species richness (avg; 12 Spp per sample) and has the highest macroinvertebrate abundance (277 ind. per sample). The community is dominated by the polychaetes *Spio decoratus* (avg 82 ind. per sample), and *Capitella capitata* (avg. 19 ind per sample) and the bivalve *Abra segmetum* (avg. 101 ind per sample), contributing up to 64% to the community (Fig.3.7G).

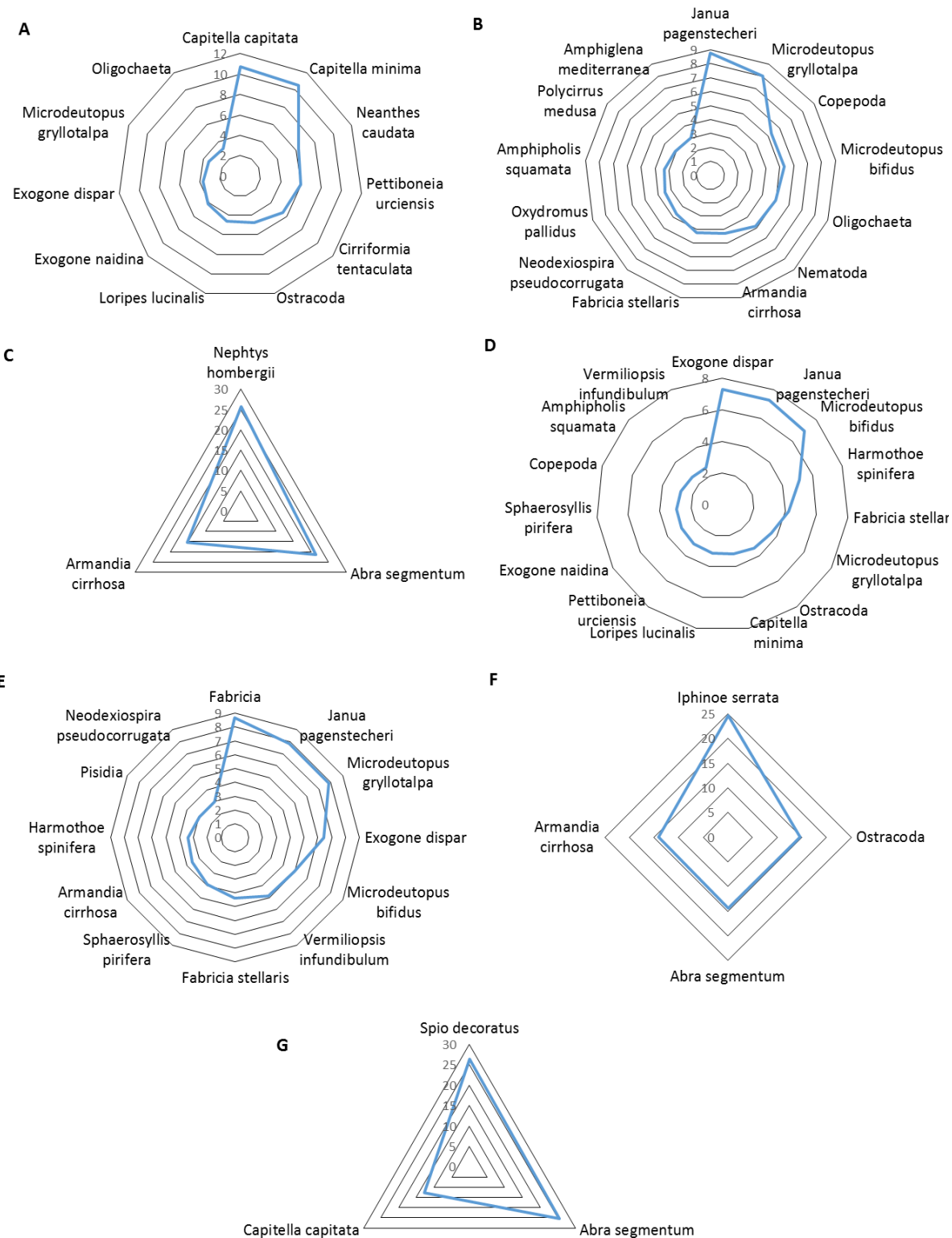


Fig 3.7. SIMPER analysis for the 7 sites (showing the taxonomic groups contributing up to 60% of the community). M1 (A), M3 (B), M4 (C), M5 (D), M6 (E), M7 (F) and M8 (G). Axis numbering indicates the percent contribution of each taxa. Read clockwise from higher to smaller contribution.

3.3.6 Ecological Quality Status

3.3.6.1 Messolonghi EQS

The Ecological Quality Status was assessed by means of AMBI, M-AMBI and BENTIX. Overall, no seasonal pattern in the EQS was identified by means of any of the indices.

The mean EQS classification obtained by means of AMBI evaluates Messolonghi lagoon as good (Fig 3.8, 3.11). AMBI overall is the most homogeneous of the three indices, and few deviations across seasons or sites exist. By sites, the lowest evaluation was recorded in M1 and M8. M1 gets an evaluation of poor EQS in January, moderate in April and November and good in July. Besides, M8 is overall classified as good but in January AMBI a moderate status for this site. The sites with highest classification were M4, M6 and M7, in July for M4 and M7 and in January for M6. The index classified these sites as good at the rest of the seasons.

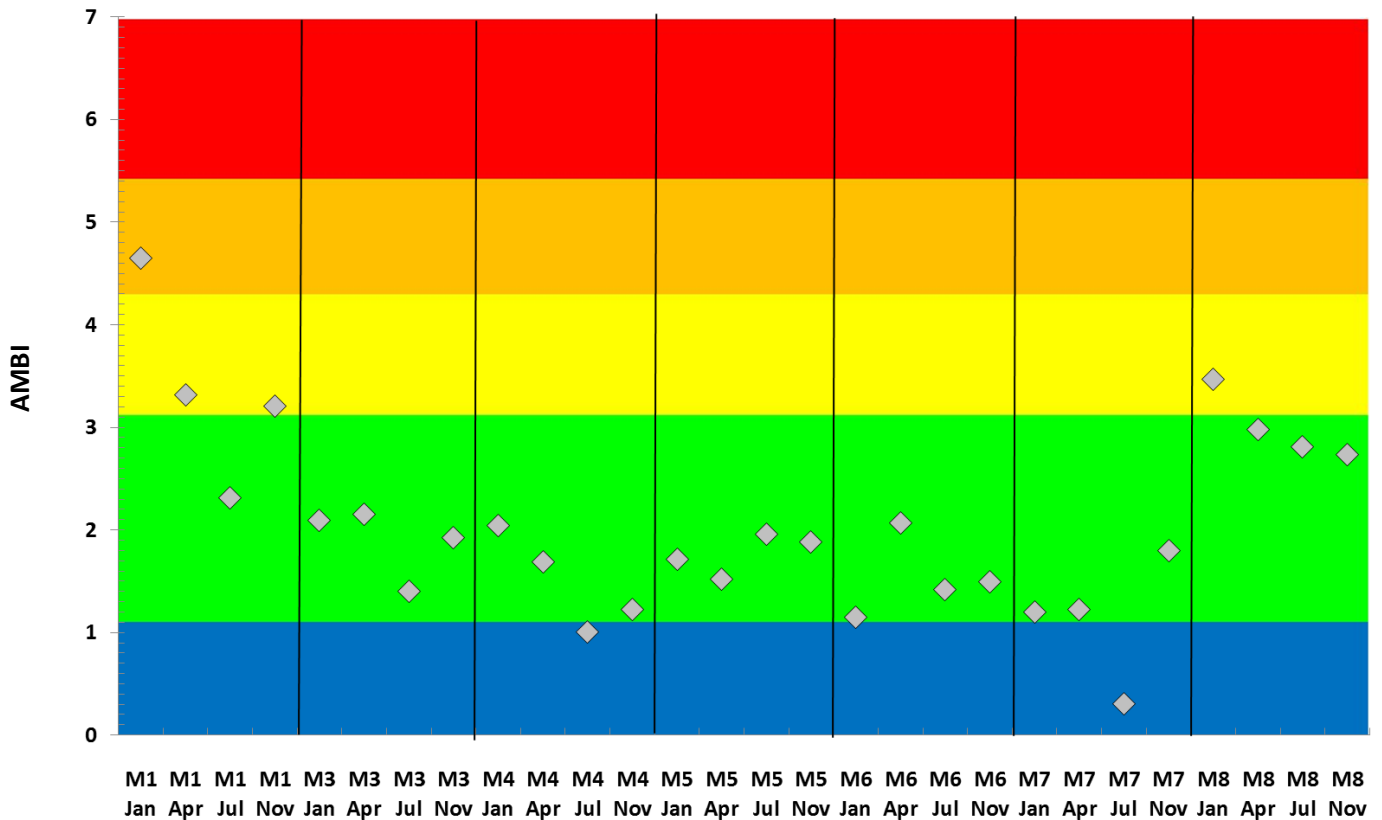


Fig 3.8. Ecological Quality Status for the AMBI metric, for the seven studied sites and sampling season. For the EQS color coding; orange (Poor), yellow (Moderated), green (Good), blue (High).

The EQS classification obtained by means of M-AMBI, determines a good EQS for the set of studied sites within the main lagoon of Messolonghi (Fig.3.9, 11). M-AMBI is the index giving the highest percentage of “high” ecological status to the benthic habitats among benthic indices. By sites, M8 obtains the lowest status classified in January as poor and at the rest of the seasons as moderate. On the other hand,

the sites M5, M3 and M4 were assigned to the highest EQS. M5 was classified across the 4 seasons as high followed by M3 which was classified as high in January, April and July, but as good in November. M6 ranged between high (January and July) and good (April and November).

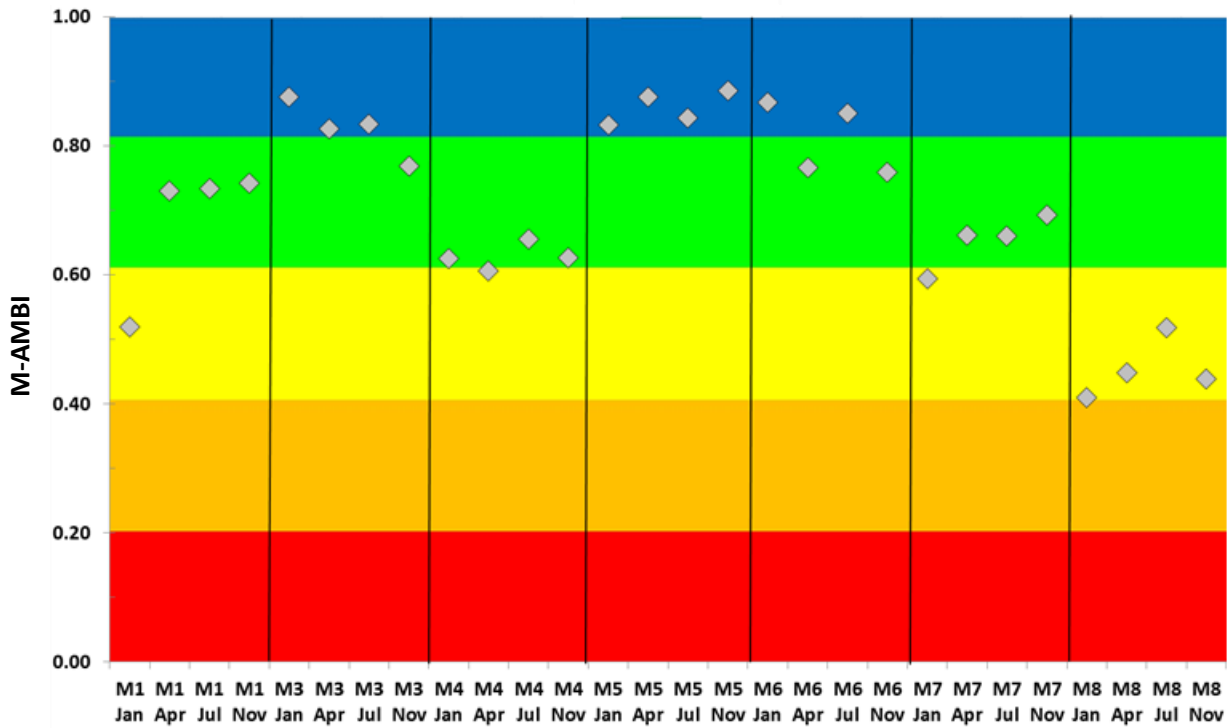


Fig 3.9. Ecological Quality Status for the M-AMBI metric, for the seven studied sites and sampling season. For the EQS color coding; orange (Poor), yellow (Moderated), green (Good), blue (High).

Overall, BENTIX assigned the highest percentage of moderate class to Messolonghi lagoon sites (Fig. 3.10, 3.11). By sites, the lowest classification (poor) was assigned to M8 and M3. M8 was classified consistently in poor class in January, April and July, and only in November was classified as moderate. M3, was classified as poor in July and November and as good and moderate in January and April respectively. On the other hand, the best EQS was determined in M7 and M5. BENTIX classified M7 in January as high, in July and November as good and in April as moderate. On the other hand, M5 were classified as in good status in January and April and as in moderate in July and November. The rest of the sites (M1, M3 and M6) are mostly classified as in moderate EQS with slight variations.

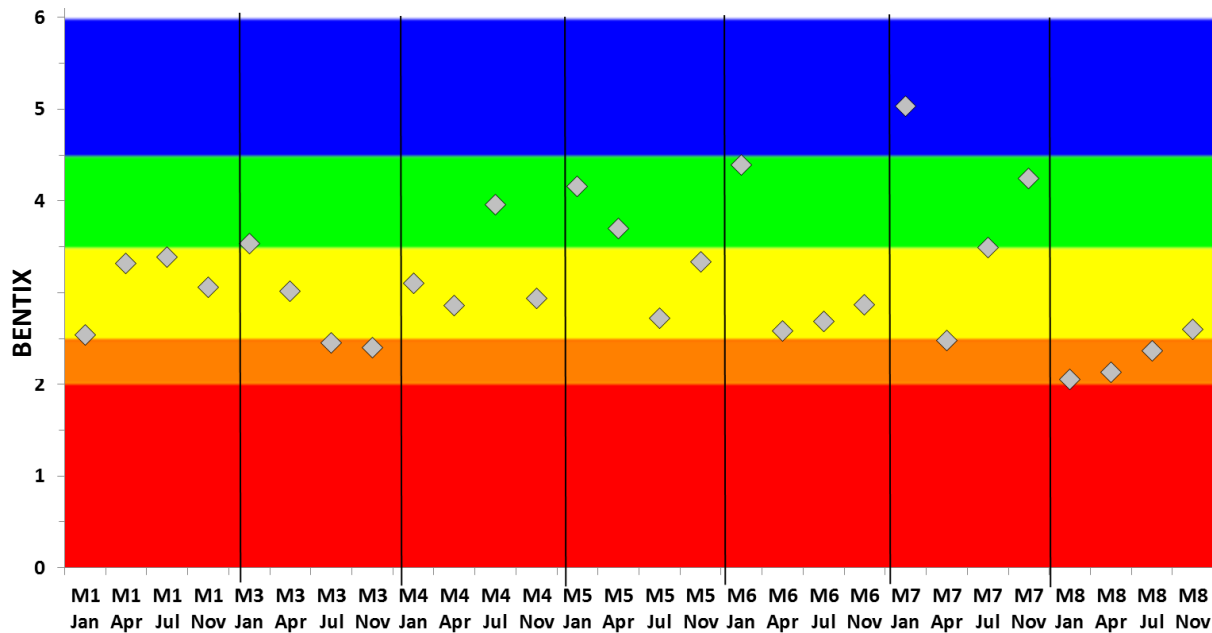


Fig 3.10. Ecological Quality Status for the BENTIX metric, for the seven studied sites and sampling season. For the EQS color coding; orange (Poor), yellow (Moderated), green (Good), blue (High).

3.3.6.2 Comparison of metrics

The percentage of matching EQS across the 3 studied indices is rather dissimilar. Overall, the percentage of total matching, thus the 3 indices agree in the EQS, is rather low (3.5%) and contrary the rate of mismatch is (32.1%). The M-AMBI with AMBI present the higher percentage of agreement (35.7%), followed by AMBI with BENTIX (17.8%) and M-AMBI with BENTIX (10.7%). Overall, M-AMBI is the less conservative, thus gives mostly higher values than the other indices. Contrary BENTIX is the most conservative giving the lower EQS values overall (Table 3.3).

Table 3.3. Percentage of EQS determination for each state across the 4 seasons and 7 studied sites.

	High	Good	Moderate	Poor
AMBI	10.7%	82.1%	3.6%	3.6%
M-AMBI	32.1%	46.4%	17.9%	3.6%
BENTIX	3.6%	25%	53.6%	17.9%

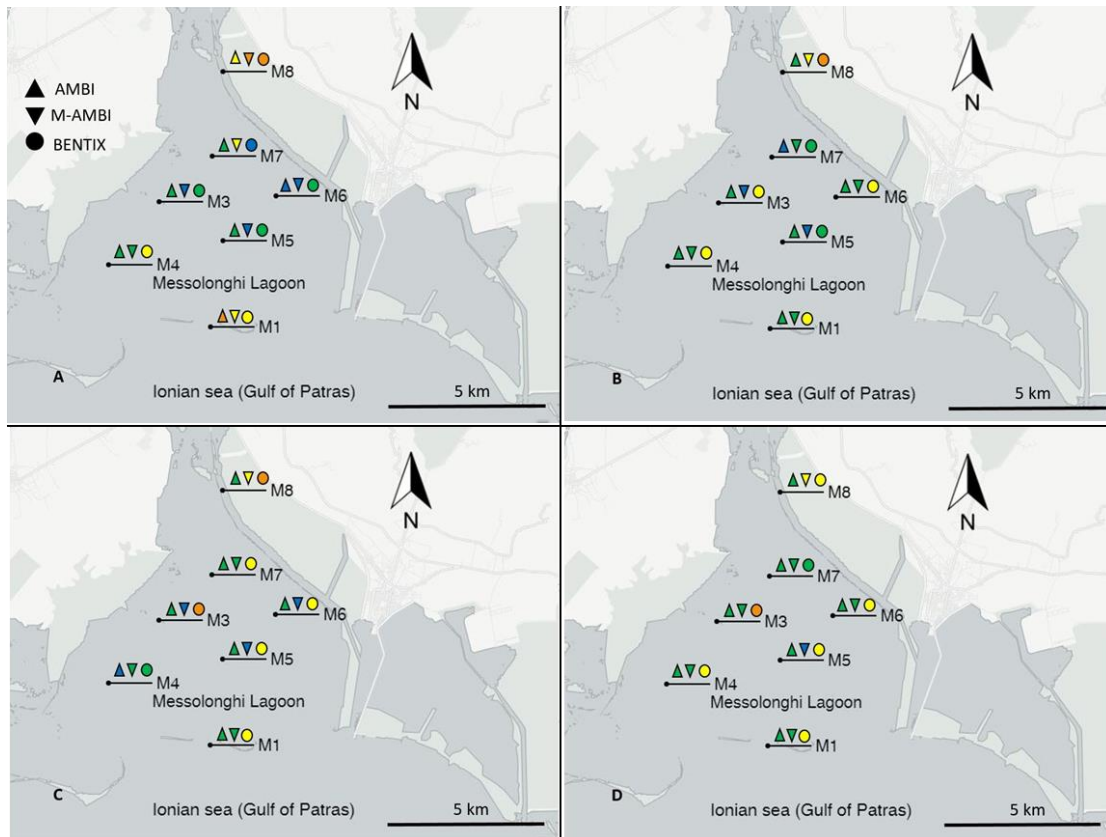


Fig 3.11. Ecological Quality status assessment of the main lagoon of Messolonghi in 2013, by means of AMBI, M-AMBI and BENTIX. A; January, B; April, C; July, D; November. For the EQS color coding; orange (Poor), yellow (Moderated), green (Good), blue (High).

3.4 Discussion

3.4.1 Physicochemical characteristics

The main lagoon of Messolonghi is a typical open and Euhaline Mediterranean lagoon (avg.33.5 psu) with no confinement pattern, which is in contrast with the common confinement pattern described in numerous Mediterranean and Greek lagoons (Battaglia 1959; Guelorget and Perthuisot 1983a; Nicolaidou et al. 1988; Reizopoulou and Nicolaidou 2004). The confinement pattern marks a scale of environmental stress determining the spatial distribution and species richness of the macroinvertebrate communities (Reizopoulou et al. 2014a). In the year of study the salinity and temperature presented pronounced increase in July, accounting for a high evaporation rate, prompted by the relatively shallowness of the water body and the low tidal influences (Fig 3.2).

The lowest salinity, recorded in the norther-most part of the lagoon might be attributed to the contribution of Aetoliko lagoon, which is a less saline water reservoir. Aetoliko, receives fresh waters from runoff and small tributary streams, and communicates with Messolonghi main lagoon and the sea through

a canal where station M8 is located. During the year of the study the meteorological station of Aetoliko registered the 53% of the rains during January and November thus explaining the lowest cross-year salinity at this time in M8. (<http://www.meteo.gr>). On the other hand, M1, located in the border line between the lagoon and the sea presents salinity values similar to the neighboring coastal water (K. Sigala, personal communication, July 2016).

The pH was rather stable, apart from a slight increase during January (Fig 3.3B). The oxygen presented an opposite response to the salinity and temperature, thus a substantial decrease was observed in July, but no anoxic season or location was recorded (Table 3.1, Fig 3.2B). The highest cross annual dissolved oxygen was measured in the innermost and next to the sewage outflow site M8, apparently not being affected by the high organic matter content (N and C) discharged from the sewage treatment plant (E. Arevalo, personal communication, February 2014). This might be due to the strong currents observed in the rather narrow canal (20m aprox.) between Messolonghi main lagoon and the smaller lagoon of Aetoliko (Gianni et al. 2011). The lagoon of Messolonghi, as most of the Mediterranean lagoons, amplifies the salinity and temperature changes compared with those occurring in the neighboring coastal waters (Ferrarin et al. 2014).

The sediment type there shows a clear differentiation between sandy and muddy bottoms with scatter distribution across the lagoon. The sandy sites are found in areas characterized by strong water movements, corresponding to both the meeting of lagoonal and coastal water (M1) and canal between Messolonghi and Aitoliko (M8). Contrarily the muddiest sites, with higher content in silt and clay, are recorded at the vegetated sites and central part of the lagoon. The vegetation acts as a physical barrier for the water, slowing down the currents and enhancing the sedimentation of fine sediment particles (Madsen et al. 2001). Thus, the general physicochemical characteristics of the studied sites are mostly differentiated by the sediment type and the vegetation; the sandy M1 and M8 stations are discriminated along the axis (CAP1) from the muddiest sites, and the vegetated M1, M5 and M6 discriminated from the unvegetated M8, M4 and M7 by the (CAP 2), (Fig 3.3A).

3.4.2 Macroinvertebrate communities

The macroinvertebrate community in Messolonghi lagoon is characterized by euhaline species over the major studied sites. To our knowledge, Messolonghi is the most diverse coastal lagoon in Greece and one of the richest in the Mediterranean with 194 macroinvertebrate taxonomic groups identified in this work. A previous study in the same lagoon had identified 139 species of polychaetes and mollusca (Nicolaidou et al. 1988). Across the published bibliography common species richness in Greek and Mediterranean lagoons stay below 75 Spp. per lagoon (Koutsoubas et al. 2000; Mistri et al. 2000; Munari and Mistri 2008a; Nicolaidou 2007; Nicolaidou et al. 2006; Reizopoulou and Nicolaidou 2004). Messolonghi, being one of the widest transitional basins within the Mediterranean, the species area relationship could partially explain the high diversity (Basset et al. 2006b). Besides, other factors as, wide open area for communication with the sea, not marked salinity variation and high variety of habitats, and might contribute to the increase of the abundance and diversity of macroinvertebrate groups. It should be noted, however, that in the comparison with other lagoons the sampling effort across the main habitats and seasons could contribute to misrepresentation (Marini et al. 2013).

The community descriptors (S, N and H') outlined three main groups with no significant differences for Shannon diversity amongst the clustered sites (PERMANOVA, table 3.2). First, M3, M5 and M6 are vegetated sites where the highest species richness, abundance and Shannon diversity was recorded. Second, M1 is characterized by being a marine environment in terms of salinity ranges, presents also high Shannon

diversity but less species richness and abundance. Third, M4, M7 and M8 are unvegetated sites and hold the lowest values for the set of community descriptors, (Fig 3.4, 3.5).

In this work we identified the key macroinvertebrate communities within the main lagoon of Messolonghi. A macroinvertebrate assemblage or community refers to a set of species that interact and are found in a specific place (Morin 2009). In this work we delineated the main taxonomic structure and diversity and abundance patterns in the lagoon of Messolonghi across the main structuring component, the habitat type. Thus overall we can differentiate 4 main communities, all of them characterized by marine and estuarine species. The previous detailed work in the lagoonal complex of Messolonghi had classified the species composition of the main lagoon in a confinement scheme as “intermediate”(Nicolaidou et al. 1988). Besides, in the work of Nicolaidou et al. (1988) the species described to be dominant are mostly present in our study but now do not rank as the overall key groups. This result show a possible shift in the key species within the main lagoon during the last 35 years.

The main macroinvertebrate communities identified in this work four;

The Marine habitat assemblage (*Mar*); is one of the highest in species richness similar to the vegetated habitat also with one of the highest number of organism per sample. The community is dominated by the cosmopolitan group of polychaetes belonging to the family Capitellidae; *Capitella minima* and *Capitella capitata* (Fig.3.7A). This family is very common in marine and estuarine soft bottom sediment. Generally classified as tolerant to organic matter enrichment(Reish 1979). Other groups with lower contribution as the bivalve *Loripes lucinalis* appear within the groups with relatively contribution to the community also described in (Nicolaidou et al. 1988) in a neighboring location to our site M1.

The Vegetated habitat assemblage (*Veg*); presents the highest densities, species richness and Shannon diversity (Fig. 3.5). The main groups structuring this community are also very cosmopolitan groups recorded in the whole Mediterranean and the rest of the world. Among those predominate tube building polychaetes as the Serpulidae, *Janua pagaestechery* and the Fabriciinae, *Fabricia sp.*, very common in the Mediterranean coastal waters (Giangrande et al. 1995). The presence of vegetation, overall as a habitat former increases the disposal of habitat and niches for a widest variety of taxonomic groups if compared with unvegetated bottoms, thus the further understanding of how this habitats increase the complexity of the habitat might contribute to better understanding the communities here described (Arocena 2007; Bartholomew 2000; Schlacher et al. 1998). (Fig. 3.7 B, D, E)

The Unvegetated habitat assemblage (*Unv*); presents the lowest species richness and abundance. This community presents a higher similarity with the common description for other Mediterranean and Greek lagoonal benthic communities (Evangelopoulos et al. 2008; Nicolaidou 2007; Nicolaidou et al. 2006; Reizopoulou and Nicolaidou 2007a). Besides, the main taxonomic groups representing this assemblage, i.e. *Abra sp.* and *Armandia cirrhosa* had been reported as dominant groups in (Nicolaidou et al. 1988) (Fig. 3.7C,F)

The Unvegetated Sewage habitat assemblage (*UnvSw*); is rather similar to the Unv habitat in species richness, but presents a higher abundance and dissimilar taxonomic groups. This community is typical at organically polluted places and the contributing groups is reduced to *Spio decoratus*, *Capitella capitata* and *Abra segmentum*. (Fig. 3.7G).

Cabana et al. (2016) *in press*, described the vegetation, sand content and total organic carbon as the variables better explaining the structuring of the described benthic communities in Messolonghi. Besides, variables as salinity or dissolved oxygen did not explain significantly the community patterns. In this work, RDA analysis (Fig.3.7) supported these results by providing a clear discrimination of the communities along the RDA1 axis, which is mostly explained by the vegetation biomass, and the RDA2 axis explained by the sand content.

Therefore, our results support the key role played by the vegetation in structuring the main community descriptors. Habitats holding higher complexity increase both the surface area available to colonizers and the three dimensional space for protection against predation, thus leading to increased macroinvertebrate abundance and diversity (Ferreiro et al. 2014; Fuchs 2013; Heck Jr and Wetstone 1977; St Pierre and Kovalenko 2014; Taniguchi et al. 2003). Besides, the presence of vegetation enhances the retention of nutrients (da Silva et al. 2009).

Seasonal variability did not prove significant for the Shannon diversity across the studied sites (PERMANOVA, table 3.2). This lack of seasonal variability in the benthic fauna was also reported in (Nicolaidou 2007), a phenomenon attributed to the continuous reproduction of abundant species and the species interaction. Besides, the fact of Messolonghi being an open lagoon, with lack of anoxic periods, also contributes to maintain the benthic populations stable across the year (Lardicci et al. 2001).

Overall the openness of the lagoon permits a high rate of exchange with the coastal waters thus increasing the biodiversity and reducing the probability of populations' isolation.

3.4.3 Ecological Quality Status

Within the framework of the WFD, different classification systems have been implemented for Mediterranean transitional waters (Cabana et al. 2013). In this work the ecological quality status of the Lagoon of Messolonghi by means of AMBI and M-AMBI, is mainly classified as good, and as moderate by BENTIX. Benthic specialists recommend the use of several indicators with complementary properties to offer more strength and better support for management decisions (Borja 2004; Reizopoulou et al. 2014b). Consequently we chose for this study the 3 commonly used multimeric (AMBI and BENTIX) and multivariate (M-AMBI) indicators, based on different parameters; diversity measures, different species sensitivity/tolerance scoring and different mathematical algorithms, i.e factor analysis for M-AMBI, different weighting coefficients, boundaries and formula design for AMBI and BENTIX. The comparability of these indicators has been already widely tested (Ponti et al. 2008; Ponti et al. 2007b; Reizopoulou et al. 2014b).

In general, the differences in EQS outcome by the different benthic indicators is lower between AMBI and M-AMBI, and BENTIX is generally more conservative, thus giving lower EQS classification. The sites with overall lower quality and with higher degree of agreement for the 3 used indicators are M1 and M8, both sites characterized by the presence of a relative high quantity of tolerant species as the polychaetae belonging to the Capitellidae family, included in the list of tolerant/opportunistic species by the 3 used indicators. Thus amongst the used indicators only AMBI is not able to detect the effect of the sewage organic load on the benthic community at the M8 site, classifying it as of a good status. At this site BENTIX appears to be the most sensitive/severe classifying the site as poor state and M-AMBI as moderate. (Fig.3. 8, 3.9, 3.10)

Amongst sites classified as non-impacted M3, M5 and M6, are classified by M-AMBI as high EQS being overall the less conservative for these locations where AMBI determines an overall good status while BENTIX is again the most conservative rating these sites as moderate.

This differences in final EQS amongst the studied indicators and sites, states the importance behind the intercalibration exercises in order to improve the correspondence between indicators, and to enhance the applicability in a wider scale (Ruellet and Dauvin 2007). Despite the use of intercalibrated indicators with different indicators still respond differently to the various natural stress sources and anthropogenic pressures (Elliott and Quintino 2007; Elliott and Whitfield 2011). Therefore, studies like the present one are valuable because they indicate the weaknesses and strengths of the different indicators in a local region and show that the use of different indicator types provide a better insight in the ecological status of a habitat. In this sense, it is advisable to put more effort and weight in intercalibration studies to weight assessment results against qualitative pressure data. For the WFD purpose, it is necessary to select indicators that adequately detect all anthropogenic impact types and to use appropriate combinations to permit an adequate global assessment.

However, there is still a gap in data and knowledge in terms of measuring the function of the ecosystem using benthic indicators specially in transitional waters where the sources of natural stress contribute to make more difficult the task of asses the EQS (Elliott and Quintino 2007).

Overall, the patterns of overestimation and underestimation were already described in the same fashion in previous works and agree with the results in these works; (Reizopoulou et al. 2014b; Simboura and Reizopoulou 2008)

3.5 Conclusion

The lagoon of Messolonghi is an open eualine Mediterranean lagoon with no confinement pattern identified.

The lagoon of Messolonghi to our knowledge presents the benthic community more diverse studied yet in Mediterranean context, this condition might be explained by the high surface and variety of habitats.

The main variable structuring the benthic communities is the habitat type being more rich the vegetated habitats.

The EQS overall is good, yet there are 2 sites, M1 and M8 which present lower EQS rating form moderate to poor depending on the used indicator.

4 Multi-scale functional and taxonomic β -diversity of the macroinvertebrate communities in a Mediterranean coastal lagoon

Abstract

Benthic macroinvertebrate communities form the basis of the intricate lagoonal food web. Understanding their functional and taxonomic response, from a β -diversity perspective, is essential to disclose underlying patterns with potential applicability in conservation and management actions. Within the central lagoon of Messolonghi we studied the main environmental components structuring the macroinvertebrate community. We analyzed the β -taxonomic and β -functional diversity across the main habitats and seasons, over a year time frame. Our results outline habitat type and vegetation biomass as the major factors structuring the communities. We found environmental variability to have a positive correlation with functional β -diversity, however no correlation was found with taxonomic β -diversity.

Across the seasons an asynchronous response of the functional and taxonomic β -diversity was identified. The taxonomic composition displayed significant heterogeneity during the driest period and the functional during the rainy season. Across the habitats the unvegetated presented higher taxonomic homogeneity and functionally heterogeneity, contrary the vegetated habitats present higher taxonomic variability and functional homogeneity. Across the seasons and habitats a pattern of functional redundancy and taxonomic replacement was identified. Besides high functional turnover versus low taxonomic turnover was documented in an anthropogenic organically enriched habitat

We conclude that habitats display independent functional and taxonomic seasonal patterns, thus different processes may contribute to their variability. The framework presented here highlights the importance of studying both β -diversity components framed in a multiscale approach to better understand ecological processes and variability patterns. These results are important to understand macroinvertebrate community assembly processes and are valuable for conservation purposes.

Key words; Beta diversity, functional diversity, benthic macroinvertebrates, habitats, multiscale, coastal lagoon.

4.1 Introduction

The benthic macroinvertebrate communities respond to the natural variability of the environment and to the interference of human activities. The study of biological diversity is necessary as species richness cannot account in detail for the levels of community variation.

The extent of change in community composition in relation to an environmental gradient or pattern of environments was defined as β -diversity (Whittaker 1960; Whittaker 1972). The β -diversity is the effective number of distinct compositional units in the area of study and informs about the degree of differentiation among biological communities (Tuomisto 2010). Patterns of β -diversity are the consequence of multiple processes operating at different spatial and temporal scales. Understanding the variation in species composition permits a better view on the processes which drive biodiversity (Tuomisto and Ruokolainen 2006). Communities holding different species composition are likely to have different trait diversity. At which extent the diversity influences ecosystem function depends on the traits and niches covered by species. Thus, the study of the functional diversity assemblages may bring additional information beyond what species richness or diversity can explain (Cadotte et al. 2011). Therefore, the combination of both functional and taxonomic diversity may contribute to a better understanding of the ecological processes governing both functional and taxonomic β -diversity patterns.

The variation in environmental conditions, the habitat heterogeneity and the degree of isolation are the main processes which allow species with different functional and ecological requirements to occur across a set of sites, thus increasing the β -diversity (Anderson et al. 2011; Fitzpatrick et al. 2013). The understanding of the processes and their operative scale structuring the benthic communities is crucial for marine conservation and resources management (Hewitt et al. 2005)

The coastal lagoon ecosystems are sheltered and shallow transitional water bodies, where continental and coastal waters meet (Kjerfve 1994). Mediterranean coastal lagoons differ from each other according to their size, salinity and tidal ranges, exposure, mixing characteristics and depth, (Guelorget and Perthuisot 1983b). Due to their geomorphological conditions these ecosystems are very susceptible to morphological changes along the year. Both, temporal and spatial variability is a fact due to seasonal or stochastic process (Ghionis et al. 2015). Thus, due to the high natural variability and diversity of habitats, the study of spatial and temporal scales is essential in order to understand the main sources of communities' variability.

Researchers studying Mediterranean coastal lagoons have identified different main drivers for the macroinvertebrate community. In restricted lagoons, with small communication with the sea, temperature, dystrophic events and salinity have been described to play key roles (Basset et al. 2013; Cladas et al. 2016; Reizopoulou and Nicolaidou 2004; Vignes et al. 2010). Contrarily, in more open lagoons other factors, such as sediment and habitat type may be important drivers of macroinvertebrate communities. Yet, studies that make comparisons across the main lagoonal habitat types, trying to account for the degree of variability of the macroinvertebrate communities from a β -diversity perspective, are rather rare. Since the different habitats present different spatial, structural and physicochemical components related with the level of nutrients and the sediment type, it could be expected that ecological patterns and processes would differ. Besides, if a particular habitat is structuring and regulating the variability of the macroinvertebrate community we might expect also some type of functional traits organization and variability at this level.

Yet, little is known about how those two ecological components perform framed in a β -diversity perspective. Thus, in this study we aim to identify, in a set of spatial and temporal scales, how these two components, functional and taxonomic β -diversity, evolve and respond to a set of physicochemical and habitat components. We hypothesized that both components may respond similarly if framed in the same spatial and temporal scale. Furthermore, we hypothesized that both taxonomic and functional β -diversity respond similarly to environmental heterogeneity.

4.2 Methods

4.2.1 Study area

The lagoon system of Messolonghi is situated on the Greek Ionian coast, on the northern side of the Patraikos Gulf, between the Acheloos and Evinos rivers. It is the largest lagoon complex in Greece covering approximately 15.000 ha and consisting of 6 different basins. The lagoonal complex, part of the National Park of Messolonghi, is also a Marine Protected Area, a Ramsar Site, an Important Bird Area (IBA), and part of the Natura 2000 network. The region is characterized by a typical Mediterranean climate. During 2013, the sampling year, the annual precipitation was 1016 mm, of which the 73% (741.6 mm) were registered in 3 months (January, February and November). The mean annual temperature was 18.4C, oscillating between the mean monthly minimum of 10.3C in January, and the mean monthly maximum, 28.5C in August (<http://www.meteo.gr>).

The present study was carried out in the main lagoon of Messolonghi, which is an open lagoon communicating with the sea through a shallow and wide frontal area. Throughout the year the lagoon presents a range of salinity and temperature, as a result of its shallowness (mean depth about 1 m). Climatic factors such as rainfall and wind rapidly affect the temporal variations of abiotic parameters of the water masses (Gianni et al. 2011). According to the presence or absence of vegetation we can distinguish vegetated habitats, characterized by the dominant vegetation (*Cymodocea nodosa*, *Rytiphlaea tinctoria* and *Valonia aegagropila*) and unvegetated habitats. To assess the β -diversity of the lagoon, sampling stations were selected as to represent all habitats: *Cymodocea nodosa* in marine environment (M1), *Valonia aegagropila* (M3), *Rytiphlaea tinctoria* and *Cymodocea nodosa* (M5), *Rytiphlaea tinctoria* (M6), and bare sediment (M4, M7 and M8) (Fig.4.1). The non-vegetated site M8 is located next to a sewage treatment plant outflow.

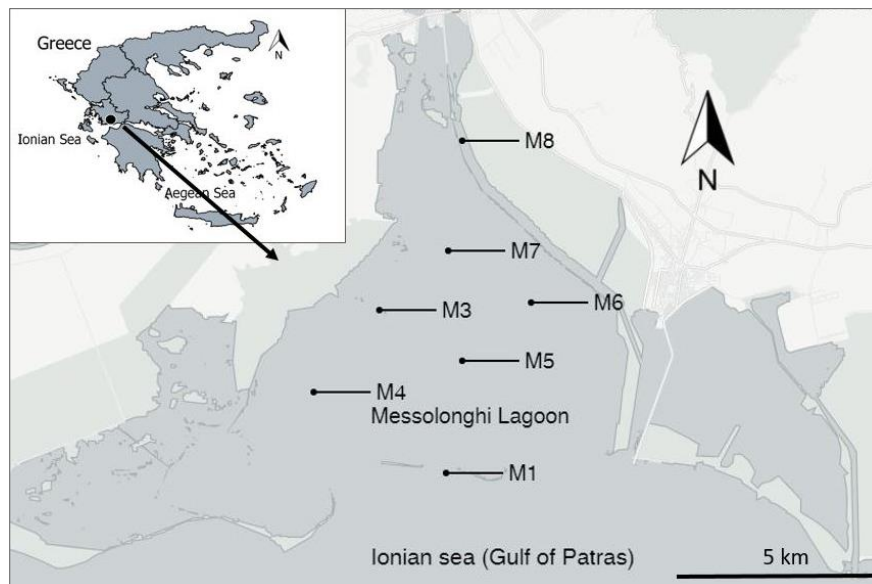


Fig. 4.1: Lagoon of Messolonghi (Greece). Seven sampling sites across the main lagoon covering the main habitats, sampled 4 times in 2013.

4.2.2 Data collection

Data collection took place four times during 2013 (January, April, July and November). For the analysis of benthic macroinvertebrates, sediment samples (3 replicates) were collected with a box corer (0.023m² surface area) at each sampling site. The samples were sieved through a 0.5-mm mesh sieve and stored in 4% formalin solution with Rose Bengal. In the laboratory, the collected organisms were sorted, identified to the lowest possible taxonomic level (generally species) and counted. Their maximal length was measured under the stereoscope with a stage micrometre.

An additional sediment sample was collected at each site, the uppermost 2 cm of which were kept for granulometry and total carbon analysis. For the granulometric analysis, the samples were originally separated to coarse-grained (N63 μ m) and fine g-grained (v63 μ m) fractions by wet sieving. Further classification of the sand and mud fractions was accomplished with standard sieves and the grain size analyzer Sedigraph 5100.

At each sampling site salinity, temperature, pH and dissolved oxygen were monitored close to the bottom using a multi-probe meter (YSI 600QS).

4.2.3 Analysis of biological traits

We selected seven traits (19 states), which are associated with biological adaptations to habitats and to physicochemical conditions and which describe the functional composition of the benthic macroinvertebrate communities. They are: mobility, position in the sediment or water column, trophic mode, exoskeleton material, development mode, reproduction mode, and average body size (Table 4.1). The traits for each taxon, except for body length, were derived from literature sources, such as publications (Fauchald and Jumars 1979; Pearson and Rosenberg 1978) and databases as <http://polytraits.lifewatchgreece.eu/>. The body length was obtained directly by measuring each organism. Individual taxa were coded according to the extent to which they display each category using a fuzzy-coding procedure (Chevenet et al. 1994), which allows assessment of affinity of a taxon to multiple categories, using discrete scores from 0 (no affinity) to 3 (total affinity). Trait category scores for each taxon present at a station were weighted (multiplied) by their abundance at that station. These abundance-weighted trait category scores were then summed over all taxa presenting that code at the station, to provide a measure of the frequency of occurrence of trait categories over the whole assemblage (Charvet et al. 2000). This weighting procedure was repeated for each station in the dataset, resulting to a station-by-trait table. This matrix was subjected to multivariate analysis. Biological Trait Analysis (BTA) uses multivariate ordination to describe patterns of functional composition over entire assemblages. Several ordination tools are available for this purpose. The choice of analytical tool is a balance between the powers of the tool to describe changes in trait composition and the ease with which results can be interpreted (Bremner et al. 2006a).

Table 4.1. Main selected traits and correspondent states for the functional composition by means of biological traits analysis (BTA).

Traits	States
Mobility. Describes the general locomotion of the organisms	Sessile/tube building Semimobile/Crawling Mobile/free swimming
Water column/sediment position. Describes whether the organism spends most of its time at the bottom or in the water column or amongst the vegetation.	Epifauna Surface Subsurface
Trophic mode. General description of the method through which resources are acquired.	Suspension feeder Deposit feeder Predator Scavenger Grazer
Exoskeleton material. The primary material in the exoskeleton matrix	Calcium Chitine
Reproduction mode. Describes the state of having just one of at least two distinct sexes in any one individual organism.	Gonochoristic Hermaphrodite
Development mode. Describes whether offspring have or not larval form and the type of form.	No Planktotrophic Lecitotrophic
Average body length	Body length (mm)

4.3 Data analysis

All the multivariate analyses were carried out using the packages PRIMER V6 (Clarke and Gorley 2006) with PERMANOVA+ (Anderson et al. 2008).

Initially, we performed non metric Multidimensional Scaling (nMDS), using Bray-Curtis similarity, to visualize the relative dispersion of the sample units in relation to site, habitat and season. The ordinations of both macroinvertebrate abundance and functional diversity were based on whole set of samples.

To test the relationship between a set of environmental variables (i.e. sand content and total carbon in sediment, temperature, salinity and dissolved oxygen in the water column and weight of vegetation) with both taxonomic composition and functional assemblages, we performed the distance-based linear model (DISTLM) (McArdle and Anderson 2001). The skewness of the physicochemical measures was checked by means of draftsman plots and the data was square root transformed accordingly. AIC (An Information Criterion) routine was used as a selection criterion, and the contribution of each independent variable was described by the amount of explained variation.

Differences in taxonomic and functional assemblages composition was tested using permutational multivariate analysis of variance, PERMANOVA (Anderson 2001). We analyzed the differences in taxonomic and functional assemblages across all 7 sample sites, and between any distinct cluster identified by the MDS: unvegetated (*Unv*), Vegetated (*Veg*), Unvegetated-Sewage (*UnvSw*) and Vegetated-Marine (*Mar*).

Due to existing significant variability we estimated and compared the sizes of each component of variation which correspond to the different spatial scales. The interaction among the different factors was tested with PERMDISP routine, which was used in the determination of the β -diversity (Anderson et al. 2006)

In analyzing the differences in β -diversity, we considered the definition given by Anderson et al. (2006), considering β -diversity as the variability in species and traits composition among sample units at a given scale (Anderson et al. 2006). The scales used were both spatial (distinct habitats identified by the MDS plot), and temporal (month). By means of Jaccard compositional similarity (based on presence absence) and PERMDISP routine, for testing the homogeneity of the multivariate dispersion (Euclidean distances), we tested for differences in functional and taxonomic β -diversity among the studied habitats (see above) at three levels.

-First level (spatial): β -diversity of each habitat within a given season (explains the spatial variability of the samples within a given habitat).

-Second level (temporal): β -diversity of each habitat across the 4 seasons (explains the temporal variability of a given habitat along the four sampling periods)

-Third level (spatial-temporal): β -diversity of a given habitat in reference to the other habitats along the four seasons.

For testing the correlation between the environmental variability and the β -diversity variability, we tested the null hypothesis of homogeneity in the multivariate dispersions among the sampling sites and habitats. PERMDISP routine based on Euclidean distances was applied. We also tested the null hypothesis of homogeneity in the multivariate dispersions among the sites using the Jaccard dissimilarity measure for the taxonomic and functional diversity matrices. We then related by linear regression directly the distances to the centroids of environmental to the biological measures.

The contribution of each species to the sites was investigated using SIMPER analysis for both the taxonomic and functional matrices (Clarke and Warwick 1994).

4.4 Results

4.4.1 Physicochemical descriptors

The DISTLM marginal test for the studied variables showed no statistical significance for temperature ($P=0.056$) in the taxonomic composition and temperature and salinity ($P=0.125$ and $P=0.058$ respectively) for the biological traits distribution. The non-significant variables were then excluded from the final model to determine the best predictor variables. The DISTLM models significantly explained the 35% of the macroinvertebrate distribution and the 50% of the biological traits distribution. (Table 4.1). The macroinvertebrate community is explained by the Total Carbon>Sand content>Vegetation biomass>Salinity>Dissolved Oxygen and the biological traits by Vegetation biomass>Sand content>Total Carbon (Table 4.2).

Table 4.1. Best DISTLM fitting models for the studied physicochemical variables and the taxonomic and the functional assemblages. AIC= Log-likelihood associate with the model. R^2 = coefficient of determination. RSS= residual sum of squares. Salinity (S), Dissolved Oxygen (DO), Sand content (S %), Total Carbon (TC), Vegetation biomass (VB). An Information Criterion (AIC), Residual Sum of Squares (RSS).

BEST fitting model	AIC	R^2	RSS	# Variables	Variables
Macroinvertebrate assemblages	655.13	0.345	177570	5	S, DO, S%, TC, VB
Functional assemblages	501.14	0.498	29780	3	S%, TC, VB

Table 4.2. Set of studied variables which significantly contribute to the model ($p < 0.01$) to better explain the assemblages of the macroinvertebrate assemblages and the biological traits. Salinity (S), Dissolved Oxygen (DO), Sand content (S %), Total Carbon (TC), Vegetation biomass (VB). An Information Criterion (AIC), Sum of Squares (SS).

	Variable	AIC	SS(trace)	Pseudo-F	Prop. Contribution.
Macroinvertebrate assemblages	Salinity	677.74	15406	4.942	0.057
	D.	677.39	7029	2.2902	0.026
	Sand %	670.67	24517	8.7529	0.090
	T.	661.03	28997	11.742	0.107
	Veg.	655.13	17516	7.6943	0.065
Functional assemblages	Sand %	542.46	8229.5	13.213	0.139
	T.	530.15	7997.7	15.039	0.135
	Veg.	501.14	13295	35.717	0.225

4.4.2 Benthic macroinvertebrate communities

In total 24046 macroinvertebrate individuals, belonging to 194 taxonomic groups (94% to Spp. level), were identified in this study. Anellida, mainly Polychaetes were the most diverse group followed by Crustacea and Mollusca. The structure of the macroinvertebrate assemblages varied amongst habitats.

The MDS analysis for the visualization of the macroinvertebrate community assemblages suggests a fair separation amongst the different habitat groups (Fig.4.2 A). A neat separation in the bottom of the plot occurs between M8 (*Unvegetated Sewage*) and M1 (*Marine*) and in the top of the plot between the M7 and M4 (*Unvegetated*) and the M3, M5 and M6 (*Vegetated*). Additionally, a clear separation of the unvegetated locations in the left of the plot occurs versus the vegetated on the right. The effect of the season added little differentiation in the two-dimensional representations.

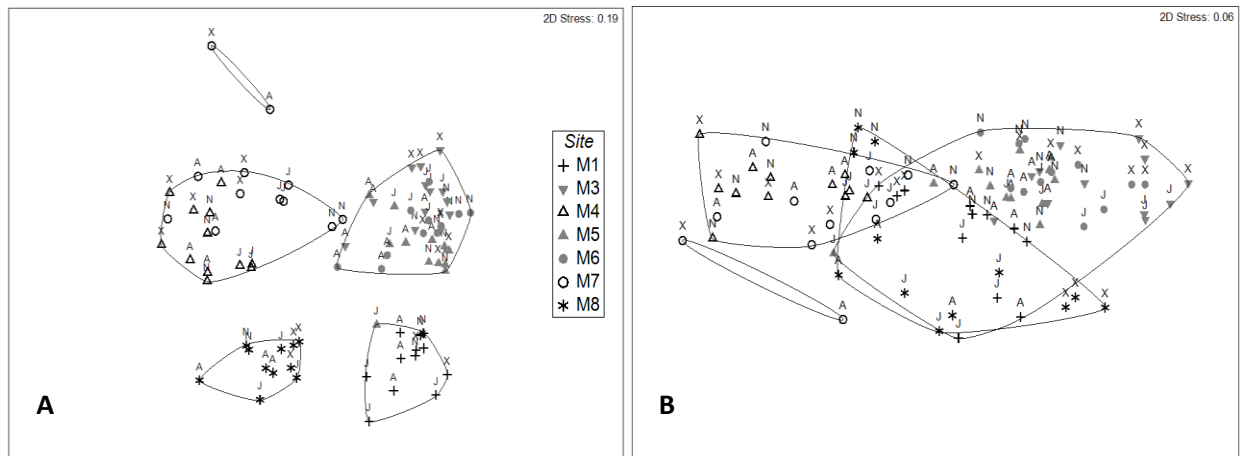


Fig. 4.2: (A) MDS scaling for the macroinvertebrate communities. (B) MDS scaling for the functional diversity assemblages. MDS integrates the four seasons; January (J), April (A), July (X) and November (N). Grey symbols for vegetated sites and black symbols for unvegetated sites. Contour lines enclose samples with 20% Bray-Curtis similarity.

The MDS visualization for the functional diversity assemblages is less differentiated than the macroinvertebrate community assemblages. The maximum distinction occurs along one dimension and runs from the left with the group of the unvegetated (M7 and M4), to the right with the group of the vegetated (M3, M5 and M6). In between and overlapping with both groups lies the marine station (M1) and the unvegetated next to the sewage outflow (M8).

Table 4.4. PERMANOVA to test the hypothesis of no differences in macroinvertebrates across habitats and seasons.

Source	df	S.S.	MS	Pseudo-f	P(perm)	Unique. Perms.	P(MC)
Seasons	3	1956000	4651900	11.145	0.0316	9985	0.0348
Habitat	3	8644300	41018000	14.695	0.009	9966	0.0117
Site(Habitat)	3	-2509900	2881400	34.391	0.0001	9979	0.0036
Season*Habitat	9	-2509900	-278880	-0.661	0.6619	9983	0.6919
Season*Site(Habitat)	9	3878300	430920	5.1432	0.0933	9989	0.0932
Residual	55	4608100	83784				
Total	82	158340000					

The test for the taxonomic similarities of the macroinvertebrate community assemblages, sites across the seasons (PERMANOVA, Table 4.3), and habitats across seasons (PERMANOVA, Table 4.4) presented significant dissimilarities for each factor. The components of variation determined by PERMANOVA routine shows that the greatest variation occurs at the habitat level, followed by season and site. Thus, the lowest component of variation occurred at the replicate level

Table 4.3. PERMANOVA to test the hypothesis of no differences in community across sites and seasons

Source	df	S.S.	MS	Pseudo-f	P(perm)	Unique. Perms.	P(MC)
Seasons	3	1.9642E7	6.5473E6	78.145	0.0001	9969	0.0001
Site	6	1.317E8	2.195E7	261.98	0.0001	9925	0.0001
Season*Site	18	1.3684E6	76022	0.907	0.4127	9990	0.3933
Residual	55	4.6081E6	83784				
Total	82	1.5834E8					

The test for the functional similarities of the macroinvertebrate community assemblages, sites across seasons (PERMANOVA, table 4.4) and habitats across seasons (PERMANOVA table 4.5) presented significant dissimilarities for each of the studied factors. Regarding the components of variation determined by PERMANOVA routine, the greatest variation occurs at the habitat level, followed by the sample level (Residual) and finally season, the variation of which is comparable in size with the interactions.

Table 4.5. PERMANOVA to test the hypothesis of no differences in functional diversity assemblages across sites and sampling times.

Source	df	S.S.	MS	Pseudo-f	P(perm)	Unique. Perms.	P(MC)
Season	3	3089.1	1029.7	7.0929	0.0001	9921	0.0001
Site	6	36360	6059.9	41.743	0.0001	9933	0.0001
Season*Site	18	11724	651.33	4.4865	0.0001	9885	0.0001
Residual	55	8129.8	145.17				
Total	82	59302					

Table 4.6. PERMANOVA to test the hypothesis of no differences in functional assemblages across habitats groups (from MDS) and seasons.

Source	df	SS	MS	Pseudo-F	P(perm)	Uniq.perms	P(MC)
Season	3	2895.5	965.16	2.2177	0.0181	9929	0.0286
Habitat	3	33090	11030	10.12	0.004	210	0.0042
Site(Habitat)	3	3269.9	1090	7.5079	0.0001	9939	0.0001
Season*Habitat	9	7807	867.44	1.9931	0.0348	9925	0.0313
Season*Site(Habitat)	9	3917	435.22	2.9979	0.0001	9899	0.0002
Residual	56	8129.8	145.17				
Total	83	59302					

4.4.3 Beta diversity

Habitat heterogeneity and β -diversity

There were no significant differences of environmental heterogeneity along the seasons, as deviation to the centroid, among habitats along the 4 sampling periods (*PERMDISP*; F : 1.604, $P(\text{perm})$: 0.372) was not statistically significant. The correlation of the environmental heterogeneity with the taxonomic diversity was not significant (Fig.4.3 A), while a weak but significant ($P < 0.05$) positive correlation was found with the functional diversity. (Fig.4.3 B).

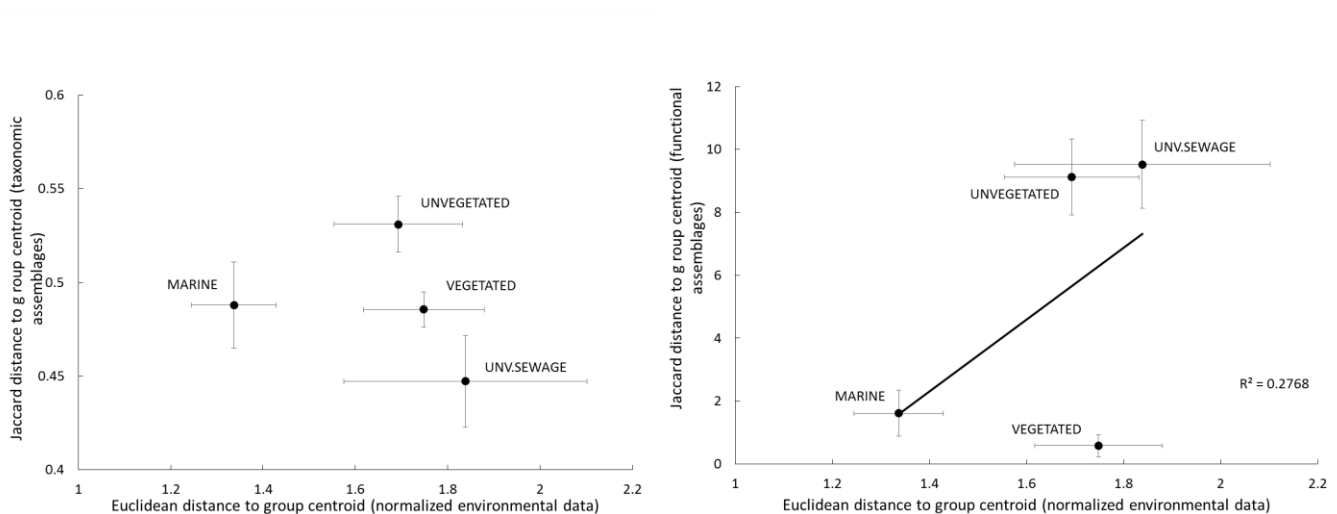


Fig. 4.3: Jaccard distance to group centroids on taxonomic (A) and functional (B) data vs. Euclidean distances to group centroids on normalized environmental data for habitats. Regression line for significant correlations ($P < 0.05$).

Multiscale β -diversity

The variation of the β -diversity, as variance of the deviation to the centroid, of the macroinvertebrate assemblages varied across the habitats; Vegetated (*Veg*), Unvegetated (*Unv*), Vegetated Marine (*Mar*), Unvegetated next to sewage outflow (*UnvSw*) and sampling period to different extents.

1st Level. Spatial variability of β diversity within habitats at the same season.

At this level the taxonomic β -diversity varied significantly in July (*PERMDISP*, F : 64.136, $df1$: 3, $df2$: 17, $P(\text{perm})$: 0.0001) and November (*PERMDISP*, F : 32.59, $df1$: 3, $df2$: 17, $P(\text{perm})$: 0.0002). No significant β -diversity variation was found in January and April. Conversely, the functional β -diversity varied significantly in January (*PERMDISP*, F : 7.3151, $df1$: 3, $df2$: 17, $P(\text{perm})$: 0.017), April (*PERMDISP*, F : 18.598, $df1$: 3, $df2$: 17, $P(\text{perm})$: 0.001) and November (*PERMDISP*, F : 10.106, $df1$: 3, $df2$: 17, $P(\text{perm})$: 0.043). No significant variation in functional β -diversity was found in July.

The habitat which presented the highest taxonomic β -diversity was the *Unv*, while *Mar* and *UnvSw* held the lowest, with no significant difference between them (Pairwise comparisons; July, $P(\text{perm})$: 0.101 and November, $P(\text{perm})$: 0.123.) (Fig.4.4).

The highest functional β -diversity was shown in the *UnvSw* habitat which presented higher values in January and April and the *Unv* in November (Fig.4.5). Lowest functional β -diversity was found in *Veg* in January and November and in the *Mar* in April. Pairwise comparisons indicate as significant the differences between maximum and minimum β -diversity ($P < 0.05$)

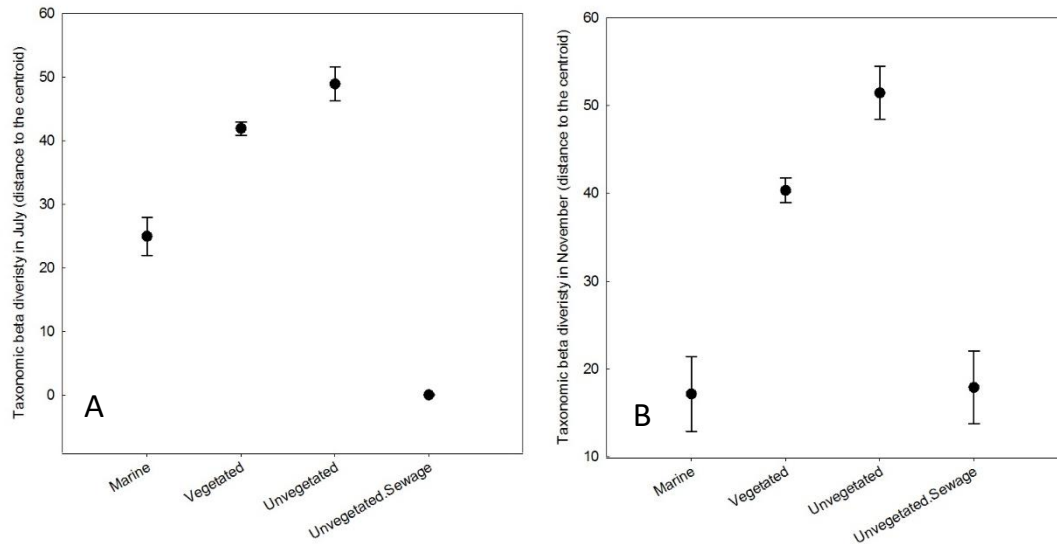


Fig. 4.4: β -diversity (as distance to the centroid) of each habitat in July (A) and November (B), explaining the spatial variability of the samples within a given habitat. Only significant differences are shown. July, $P < 0.05$ (*) and November, $P < 0.001$ (***)

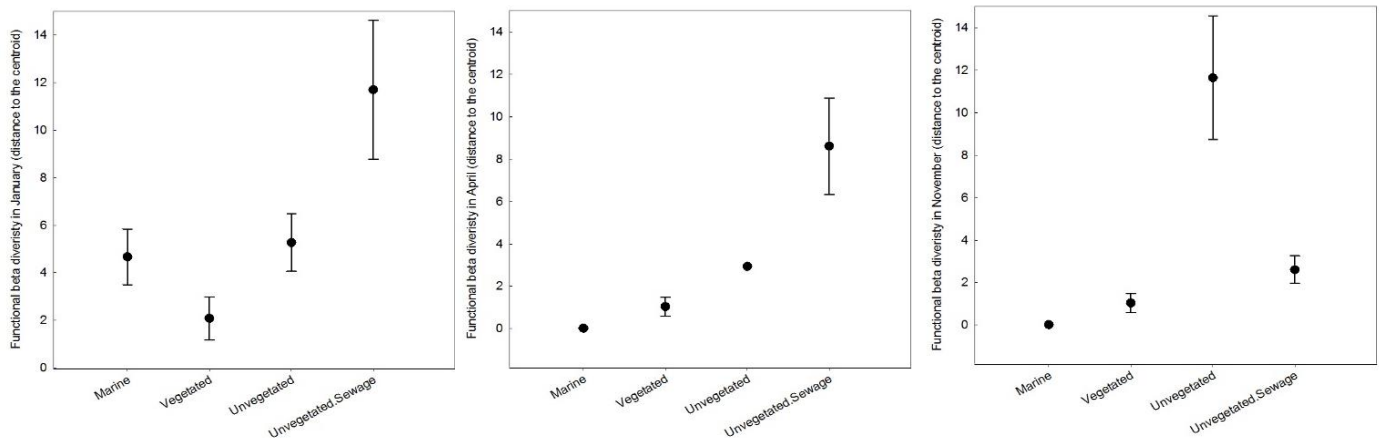


Fig. 4.5: Significantly different β -diversity (as distance to the centroid) of each habitat within a given season (explains the spatial variability of the samples within a given habitat). January and November $P < 0.05$ (*), April $P < 0.001$ (***)

2nd Level. Temporal variability of β diversity within habitats.

Variation of taxonomic β -diversity (distance to centroid) varied significantly among the four seasons within the *Veg* (*PERMDISP*, F: 5.0756, df1: 3, df2: 32, P(perm): 0.010) *Mar* (*PERMDISP*, F: 18.845, df1: 3, df2: 8, P(perm): 0.015), and *UnvSw* (*PERMDISP*, F: 18.702, df1: 3, df2: 7, P(perm): 0.046) habitats. No significant variation in terms of functional β -diversity was found at this level.

Overall, significantly higher taxonomic β -diversity is registered in April across the *Veg* and *UnvSw* habitat types, and in July across the *Mar* habitat type (Fig.4.6). The lowest taxonomic β -diversity was recorded in November for every habitat, nonetheless no significant difference was found among November and July (P: 0.3421) in the *Veg* habitat and between November and January (P: 0.1002) in the *Mar* habitat, (Fig.4.6).

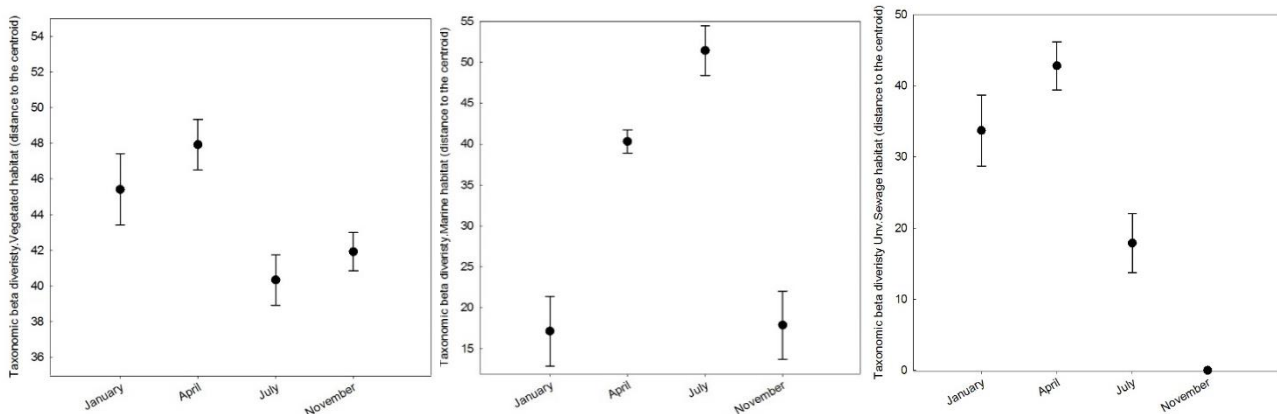


Fig. 4.6: Significant different β -diversity of a given habitat type among the 4 seasons (explains the internal variability of a given habitat over the four seasons). Vegetated and Marine habitat P<0.01, Sewage-Unvegetated P<0.001.

3rd Level. Spatial-temporal variability of β diversity within habitats in 2013.

Taxonomic and functional β -diversity varied significantly across the habitats and sampling months (*PERMDISP*, F: 4.224, df1: 3, df2: 80, P(perm): 0.024) and (*PERMDISP*, F: 30.247, df1: 3, df2: 80, P(perm): 0.0001) respectively.

For the taxonomic β -diversity, *UnvSw* holds the lowest and *Unv* the highest variation (Fig.4.7 A). The pairwise comparison indicated no significant differences between *Veg* and *Mar* (P: 0.9309).

For the functional β -diversity, *Veg* holds the lowest variation and the *UnvSw* the highest variation (Fig.4.7 B). The pairwise comparison indicated no significant differences in *Mar* vs *Veg* habitats (P: 0.460) and *Unv* vs *UnvSw*, (P: 0.861)

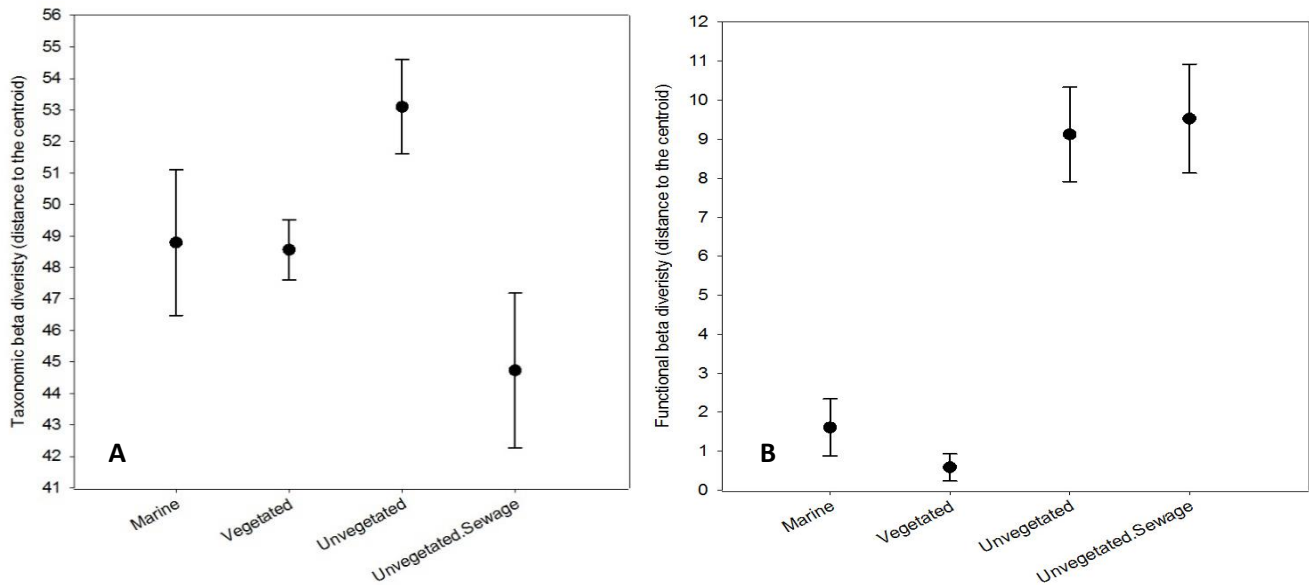


Fig. 4.7: Variation in the distribution of distance to centroid (Spatial-temporal variation of β -diversity) among habitats within 2013 (A); taxonomic diversity (B) functional diversity.

4.5 Discussion

Ecological requirements and stochastic processes are the main β -diversity drivers, and both act contemporarily shaping the benthic communities (Chase and Leibold 2003). Among the ecological requirements, the habitat type is a key environmental filter shaping macroinvertebrate communities, determining both functional and taxonomic composition (Galuppo et al. 2007; St Pierre and Kovalenko 2014; Verdonschot et al. 2012). Other factors as salinity, and temperature have been also proved to structure macroinvertebrate, plankton and algae communities in transitional waters (Remane 1934; Schubert et al. 2011; Telesh and Khlebovich 2010). Significant differences in the benthic assemblages across space and time may indicate different levels of variability with reference to ecological patterns and processes. Better understanding of β -biodiversity patterns will help in the effective conservation plans of Mediterranean coastal lagoons.

In this paper we studied the benthic macroinvertebrate communities with special focus in identifying the degree of variation by means of both functional and taxonomic β -diversity across the main benthic habitats and seasons during 2013. Besides, we studied the main physicochemical factors structuring the communities and whether these factors and their variability contribute, or not, to draw β -diversity patterns.

4.5.1 Benthic macroinvertebrate communities

We tested for significant differences in the functional diversity and taxonomic diversity across seasons, habitats and sites and each factor presented significant dissimilarities (PERMANOVA, tables 4.3, 4.4, 4.5 and 4.6). Indeed, the factor 'habitat' ranked as the highest component of variation (S. 1,2), revealing the strong effect that the habitat has in structuring both the functional and taxonomic assemblages in Messolonghi lagoon (Fig.4.2 A, B). Conversely, lower contribution to the structure of the community was allocated to the season, being also differentiated, to a lesser degree though, in the two dimensional MDS.

We tested a set of physicochemical variables to identify the one which best explains the functional and taxonomic assemblages (Table 4.1). Total carbon, vegetation biomass and sand content in sediment, contributed significantly to the structure of both taxonomic and functional diversity. The vegetation acting as an ecosystem engineer and habitat former (Jones et al. 1997), plays a key role in structuring the functional (22.5%) and the taxonomic (6.5%) assemblages in Messolonghi lagoon. Benthic vegetation slows down the currents, facilitating the sedimentation of fine sediment and organic particles (de Boer 2007; Ginsburg and Lowenstam 1958). Besides, the presence of vegetation is related with the provision of food, habitat and refuge (Ferreiro et al. 2014). The filtering capacity of vegetated habitats leads to increased species richness and more significantly to clusters of biological traits, as demonstrated by the functional redundancy. In vegetated habitats we found a high contribution of traits accounting for sessile and tube building (9.5%), suspension feeders (6.7%), deposit feeders (6.7%) and epifauna (6.3%). Conversely, the unvegetated habitats hold individuals of bigger size (30.2%), deposit feeders (6%), subsurface organisms (5.6%) and surface organisms (5.5%) (S. 3). Yet, the length of the macroinvertebrates would possibly introduce bias, due to the different shapes and morphologies should be added, even though availability of the length per weight equations for transitional water macroinvertebrates is too limited yet across the available literature (Rosati et al. 2012).

4.5.2 Beta diversity

Generally, major variability was associated to the taxonomic β -diversity; contrarily, the functional β -diversity was overall more homogeneous across studied scales. Overall, our results suggest a set of patterns within each studied level in relation to both the functional and the taxonomic β -diversity across the studied habitats and seasons.

Beta diversity and environmental heterogeneity

Higher environmental variation among sites within a region leads to an increased number of niches (Leibold et al. 2004). Our results indicate that the environmental heterogeneity predicted significantly (but moderately) the functional β -diversity, but did not explain the taxonomic β -diversity, (Fig.4.3 A, C). Higher functional β -diversity was registered with the *Unv* and *UnvSw*, both unvegetated habitats which suffer from high physicochemical changes along the four seasons (Fig.4.7 B). This type of response has been similarly described by Dimitriadis et al. (2012) who found environmental variation to induce a large amount of variability in functional trait assemblages at local and regional scale. Other factors as habitat heterogeneity was the predominant driver of beta diversity of stream macroinvertebrates (Astorga et al. 2014). Besides, habitats with higher physicochemical variability and less refuge available, due to the lack of vegetation, maintain a higher variance in traits composition (Townsend et al. 1997). Thus, the variation in the environment and the heterogeneity of the habitat allow species with different ecological requirements to occur, thus increasing the functional β -diversity. In this work, temporal variability is determined within a yearly term and covering the main seasons. Yet, even though our results correlate, associating habitat heterogeneity with higher functional β -diversity, these results need to be interpreted with caution and a more intense sampling effort covering the main physicochemical variables (increasing sampling frequency within each season) would help to better understand the temporal scale and intensity of the variability and thus to better support the hypothesis that major environmental variability holds a higher functional β -diversity.

1st Level

Functional and taxonomic β -diversity asynchronicity

Firstly, we tested whether the relationship between the functional and taxonomic β -diversity across the different habitats differ in a given season. Larger spatial distances among habitats within the lagoon are likely to lead to stronger variation in species composition due to dispersal constraint (Leibold et al. 2004). Due to the relatively short distances, across the whole lagoon, we expected to find support to the hypothesis that both functional and taxonomic β -diversity variability may respond similarly across the habitats and seasons. Nonetheless, our findings describe an asynchronous pattern: as the taxonomic β -diversity increases, the functional β -diversity decreases.

Across the whole set of habitats, July and November presented higher taxonomic heterogeneity, where *Veg* and *Unv* host the major variability. Major heterogeneity corresponds to a more dissimilar species composition across the habitats for the whole lagoon (Fig.4.4). Contrarily, the functional β -diversity, presented higher degree of heterogeneity during January, April and November, the *UnvSw* and *Unv* being the habitats with higher β -diversity. (Fig.4.5).

In what the habitats are concerned, the higher heterogeneity is displayed in both: habitats with higher environmental heterogeneity (Fig.4.3), and the unvegetated habitats (*UnvSw*, *Unv*). *UnvSw* and *Unv* have a reduced number of species, and in such habitats a change in a restricted number of species may result in a high impact for the functional β -diversity (Lake 2011). Thus, in habitats holding high environmental heterogeneity and additionally lower number of species, a change in the environmental conditions can eliminate some species and as a consequence some unique traits will be lost (Faulwetter et al. 2015; Schriever et al. 2015).

Overall, taxonomic β -diversity presents higher homogeneity during the rainy months (January and April) while the functional β -diversity during the dry period (July). This pattern describes high levels of species similarity across the studied habitats but high functional dissimilarity during January and April. The homogenization of the taxonomic groups may be explained by the input of fresh water during the cold/rainy season (Cañedo-Argüelles and Rieradevall 2010). Conversely, the homogeneity of the cross-lagoonal functional diversity in July corresponds with the higher taxonomic heterogeneity. Our study provides evidence of an asynchronous response of the functional and taxonomic diversity to environmental factors across Messolonghi. To our knowledge this kind of pattern was not described previously in benthic macroinvertebrate communities. Both spatial and temporal scales are inherently linked and exploring both patterns and processes in ecological studies provides the benefit of testing new ecology theories (White et al. 2010).

2nd Level

Functional redundancy (functional stability vs taxonomic replacement)

Our work identified a set of patterns across the main lagoonal habitats. The combination of both spatial and temporal scales permitted us to identify patterns that are not observable in a single scale.

At this level, none of the studied habitats presented significant variation in the functional β -diversity. The lack of variability in every studied habitat describes a pattern of functional redundancy, commonly described for the macroinvertebrate communities (Bogan et al. 2013; Sigala et al. 2012). The functional redundancy may be a result of environmental filtering which restricts the trait diversity (Heino 2005). A high functional redundancy is an indicator of the relative resilience of the traits, which may help to maintain the ecosystem functions after disturbance of the system (Schmera et al. 2012). Thus, the loss or gain of

taxonomic groups do not cause significant variability in the functional diversity. Contrarily, at this scale we found a great taxonomic variability, which supports the idea of the functional redundancy. Therefore we have to reject the hypothesis that both components i.e. taxonomic and functional β -diversity might respond similarly if framed in the same spatial and temporal dimension. Here, heterogeneous patterns across the seasons for *Veg*, *Mar* and *UnvSw* were outlined (Fig.4.6). Overall, April holds the first rank in taxonomic β -diversity and the unvegetated habitat (*Unv*) holds the higher level of homogeneity across the year. On the other hand, the vegetated habitats (*Veg* and *Mar*) present a higher structural complexity and heterogeneity which contribute to taxonomic richness (St Pierre and Kovalenko 2014) and trigger the β -diversity (Astorga et al. 2014). Besides, the dominant lagoonal vegetation i.e. *C.nodosa*, *R.tinctoria*, *V.aegagropila*, undergo seasonal cycles of growth and decay that may contribute to increase the availability of habitat, thus ecological niches (Leibold et al. 2004).

3rd level

Low taxonomic replacement vs High functional replacement. Year based resilience.

Framing the β -diversity within a whole study period gives a more holistic perspective where annual β -diversity patterns can be outlined across the studied habitats. Overall, no significant differences, in both functional and taxonomic β -diversity, were outlined between the *Mar* and *Veg* habitats (Fig.4.7 A, B). Both *Mar* and *Veg* present the lowest values in functional β -diversity, thus being more resilient along the year. The loss or gain of one species will not impact the functional β -diversity on the basis of the aforementioned functional redundancy (Fig.4.7 B). Contrarily, higher taxonomic β -diversity was recorded in the *Unv* and *UnvSw*. The functions of these habitats, reduced in species richness compared with the vegetated ones, are performed by a small set of organisms, consequently the loss or gain of one species can drastically modify the functional diversity (O'Gorman et al. 2010).

Concerning the annual variability of the taxonomic β -diversity, *Unv* shows the highest and *UnvSw* neighbouring the Aitoliko sewage treatment plant outflow shows the lowest variability (Fig.4.7 A). *UnvSw* composed of tolerant organisms such as *Spio decoratus* (26.4%), *Abra segmentun* (25.5%), *Capitella capitata* (12.75%) and *Microdeotopus gryllotalpa* (6.67%) form a very resilient community able to tolerate high levels of organic load as those found in a sewage treatment plant outflow (S3). Conversely, as aforementioned, the replacement of functional diversity is high, because one single species may result in a high impact for the functional β -diversity (Lake 2011). Nevertheless, even though a homogeneous community across the year may indicate some degree of resilience, from a conservational perspective the weight of this community to the lagoonal biodiversity is rather low. Thus, for conservation purposes other characteristics than resilience need to be considered. The information retrieved shows that studying every habitat across the spatial and temporal scale is imperative for conservation purposes.

4.6 Conclusions

In the lagoon of Messolonghi habitat type and the vegetation biomass are the major elements contributing to structure both the functional and taxonomic composition of the macroinvertebrate communities. Despite the anticipated similar response of the functional and taxonomic β -diversity this work determines different patterns. Functional and taxonomic β -diversity respond asynchronously and dissimilarly variably was also identified across the habitats in relatively short distances. We conclude that different processes may contribute to shape the composition of the community. This study underlines the importance and complementarity of studying both, taxonomic and functional diversity to better understand the ecological processes. It highlights the importance to study different spatial and temporal scales which adds perspective

when framing the variability of macroinvertebrate communities in naturally stressed ecosystems as Mediterranean coastal lagoons. These results are important to understand macroinvertebrate community assembly processes and are valuable for conservation purposes.

5 Habitat complexity modulates macroinvertebrates body length and community structure in a lagoonal ecosystem

Abstract

Habitat complexity in marine benthic ecosystems plays an important role modulating macroinvertebrate communities; however its influence in transitional waters has been poorly studied. After McAbendroth et al. (2005) fractal indices, we investigate how habitat complexity structures the macroinvertebrate communities in a lagoonal ecosystem. We aim to disclose to which extent habitat complexity determines the benthic community and to validate the effectiveness of the fractal indices in transitional waters.

At a phytal level, surface and perimeter fractal dimension revealed significant differences. Fractal measurements based on the surface denoted limitations across scales, conversely for measurements based in the perimeter the degree of self-similarity is key element. At a sample level, the fractal index weighting methods and scale factor did not differed, indicating some methodological weakness.

At the community level macroinvertebrate species richness, abundance and diversity, highly correlated with the complexity of the samples. Moreover more complex samples host organisms with a wider range of sizes. Results denote the relevance of habitat complexity in modulating the macroinvertebrates community, which forms the basis of the lagoonal ecosystem food web.

Key words; habitat complexity; fractal dimension; benthic habitats; macroinvertebrates; body length; phytal elements

5.1 Introduction

Coastal lagoons in the Mediterranean Sea are usually shallow water bodies receiving variable amounts of fresh water. Due to their geomorphological and hydrological characteristics, environmental conditions in the lagoons frequently undergo hydrodynamic and physicochemical fluctuations on a daily and seasonal basis (Nicolaidou et al. 2006). Within these transitional ecosystems, macroinvertebrate studies addressing community patterns and biological traits as body size have paid most of their attention to the governing physicochemical conditions (Pearson and Rosenberg 1978; Renne 1934; Tagliapietra et al. 2012) while less attention has been paid to the structure of the habitat. The rather patchy distribution, which macroinvertebrate organisms follow, is linked to their gradient of physicochemical tolerance and life history of their populations (Giangrande et al. 1995). The organisms also respond to a set of extrinsic factors which have been reported to a different extent: habitat complexity, human pressures, food availability, prey stress and larvae dispersal among others.

In transitional ecosystems phytal components, as algae and marine phanerogam meadows, are common keystone structures and ecosystem engineers. Phytal components modulate resources as refuge for fish, larval recruitment, food source, and habitats for other species, by adding three dimensional heterogeneity to the habitat and determining species diversity at different scales (Hemminga and Duarte. 2000; Jones et al. 1996; Tews et al. 2004).

Within these ecosystems, habitat complexity plays a key role in the regulation of macroinvertebrate species abundance and richness as well as body size of organisms (Hansen et al. 2010; Lee 2006; Taniguchi and Tokeshi 2004). This aspect has been addressed from different perspectives, following a wide array of approaches, indices and scales aiming to account for the complexity of a given unit, structure or site (e.g. sediment, leaf, plant, algae, mix of plants, patch or habitat (Frost et al. 2005; Kovalenko et al. 2012; Tokeshi and Arakaki 2012). Thus, researches addressing habitat complexity explained differently its effects on the macroinvertebrate organisms, i.e. increase of refuge spaces (Bartholomew and Shine 2008), food sources (Taniguchi et al. 2003), microhabitats (Matias et al. 2011).

To investigate the effect of the habitat over macroinvertebrate communities fundamental approaches account for the weight, volume and surface of phytal elements, as well as for the number and density of structures. Within seagrass meadows aboveground plant biomass strongly correlated with both invertebrate biodiversity and abundance (Heck Jr and Wetstone 1977). The size and composition of the macroinvertebrate community was determined by the plant availability, phytal biomass and volume, pointing out the possible effect of species-area relationship as a sampling artefact (Attrill et al. 2000; Hill et al. 1994; Lomolino 2001). Contrary, Matias et al. (2010) found that larger areas were colonized by more species but not by more individuals. These fundamental approaches, however, do not account for habitat complexity but for the amount of accessible habitat. Thus, indices of habitat complexity accounting for structural aspects were further developed, aiming also to reduce the effect caused by the confounding effect that may be linked to the surface area of the studied structure.

Frequently, the indices used to define the habitat complexity formed by phytal components retrieve a set of two dimensional measurements. Some proposed indices rely on the interstices created among the branches and leaves as sources of microhabitats or refuge spaces; the interstitial volume relative to plant volume in Hacker and Steneck (1990) and the interstitial space index (ISI) in Dibble et al. (1996) and in Dibble and Thomaz (2006). Other indices rely on the interaction of predator-prey and habitat; a set of two dimensionless indices investigating the interference structural complexity/predator foraging ability (Bartholomew 2000) and the fish-free volume index (FFV) which accounts for the absolute amount of space unavailable to a fish predator but available to invertebrate prey (Warfe et al. 2008)

Likewise, complexity approaches structured by means of the fractal dimension of a phytal element, or set of them, have also been implemented. One of the first attempts to use fractal dimension to describe the architecture of submerged macrophytes was made with imitation plants in Jeffries (1993). A fractal is a geometric figure characterized by great complexity in its boundaries and fractal dimension quantifies the degree of complexity. A higher fractal dimension indicates greater complexity, (Mandelbrot and Blumen 1989). Accounting for habitat complexity by means of fractal dimension needs a distinct focus over the scale due to the dependency that fractal dimension has over it (Halley et al. 2004; Schmid 1999; Tokeshi and Arakaki 2012). Across publications, setting a number of scales considered to cover a meaningful ecological window for the studied organisms, is a common course. The aim is to retrieve information enough to explain the extent to which the studied organisms interact with the studied structure.

In transitional waters the fractal dimension was commonly measured at the extent of a single plant. Attrill et al. (2000) applied a monospecific complexity index including; epiphyte biomass, the fractal dimension and the ratio leaves/shoot in *Zostera marina* seagrass. A similar approach was also applied for macrophyte habitats in Thomaz et al. (2008) and Dibble and Thomaz (2009). In aquatic ecosystems, however, the ecological succession of the phytal elements tends to the aggregation elements with different degree of contribution to the structural complexity of a given site. Being aware of this natural situation McAbendroth et al. (2005) described a fractal dimension based index, the main strength of which is the ability to integrate the complexity of each species of plant included within a set of stands. A similar cumulative fractal index was also applied recently invasive aquatic plants in lakes (Kovalenko et al. 2009) and marine macroalgal species (Torres et al. 2015; Veiga et al. 2014).

Researches in Mediterranean transitional ecosystems, addressing macroinvertebrate community distribution and body size, had mostly focused on physicochemical aspects and human pressures linked to the water column and sediment (Reizopoulou and Nicolaidou 2004; Zaldívar et al. 2008). To our understanding, the identification of key structural elements, that might co-regulate macroinvertebrate populations and organism body size, is imperative for both ecological and conservational interest (Tews et al. 2004).

Thus, the main goal of this work is to understand at which extent habitat complexity structures the macroinvertebrate body length distribution and community features.

Here we fetch the validation of McAbendroth et al. (2005) fractal index in a transitional water ecosystem with physiologically and morphological distinct phytal components. Modifications to the original fractal index were made; firstly a set of micro scales that might be relevant for epiphytic organisms which rely on microstructures is considered. Secondly, an alternative weighting method based on the volume of phytal components is tested

Then, several hypothesis were investigated. Firstly, whether the fractal dimensions, of a phytal individual, differs in relation to the surface area and perimeter, at four different magnification scales. Secondly, whether a reliable proxy for sample complexity, can be estimated via a method of weighting the fractal dimension of phytal individuals within a sample, according to their biomass or volume. And finally, whether sample complexity correlates with species richness, abundance, and diversity, as well as with macroinvertebrate body length and whether habitat and seasonality interferes in this context.

5.2 Material and methods

5.2.1 Study area

The lagoon system of Messolonghi is situated on the Greek Ionian coast, on the northern side of the Patraikos Gulf, between the Acheloos and Evinos rivers. It is the largest lagoon complex in Greece covering about 15.000 ha and consisting of 6 different basins. The lagoonal complex, part of the National Park of Messolonghi, is also a Marine Protected Area, a Ramsar Site, an Important Bird Area (IBA) and part of the Natura 2000 network.

The region is characterized by a typical Mediterranean climate. During 2013, the sampling year, the mean annual precipitation was 1016 mm, of which 741.6 mm were registered in 3 months (January, February and November). The mean annual temperature was 18.4C, oscillating between the mean monthly minimum of 10.3C in January, and the mean monthly maximum, 28.5C in August (<http://www.meteo.gr>). The network of fresh water tributaries is largely anthropogenic as a result of streams diversion and channeling of irrigation runoffs into canals that discharge in the lagoonal complex. The Aetoliko sewage treatment plant discharges into the inner and northern most part of Messolonghi lagoon proper.

The present study was carried out in the central part of Messolonghi main lagoon, which is an open lagoon communicating with the sea through a shallow and wide frontal area. On the Western side there is a sand spit that runs West-East and covers approximately one half of the frontal area. The other half is open, except for two small islands which act as barriers. The lagoon presents a wide range of salinity and temperature as a result of the lagoon shallowness (mean depth about 1 m), whereas climatic factors such as rainfall and wind rapidly affect the temporal variations of abiotic parameters of the water masses. (Gianni et al. 2011). The principal habitats in the main water body are patchy, characterised by dominant algae such as *Rytiphlaea tinctoria*, *Valonia aegagropila*, *Cladophora spp*, and the seagrass *Cymodocea nodosa*.

5.2.2 Sampling plan and data collection

To investigate the effects of habitat complexity on the benthic macroinvertebrate community composition and body length, three sampling sites (M3, M5, and M7) were established corresponding to three different habitats at the central part of the lagoon. The proximity of the sites (average distance among sites 800 m) was intended to reduce the variability of other physicochemical factors, whilst encompassing a set of different habitats with different phytal compositions to guarantee a complexity gradient among the samples. Each sampling site is composed by different algae and seagrass; M3 was dominated by *Valonia aegagropila*, M5 by a combination of *Rytiphlaea tinctoria* and *Cymodocea nodosa* and M7 was a very sparse and undeveloped *Cymodocea nodosa* meadow which presented some areas of bare sediment. The sampling was carried out four times in 2013 (January, April, July and November).

At each site three samples of surface sediments and vegetation were collected with a box corer of (0.023m²) and washed in a 0.5 mm square mesh sieve. The retained material was fixed with 4% buffered formalin, stained with Rose Bengal, for further benthic macroinvertebrate and phytal composition analysis. The benthic macroinvertebrates in each replicate were sorted, identified to the lowest possible taxonomic level (mostly species level) and counted. Their maximal length was measured under the stereoscope.

To account for the effects of habitat complexity on the macroinvertebrate community distribution, and body length structure the fraction of subsurface organisms were discarded. These live within the sediment and have no direct physical dependence on the aboveground phytal elements. Thus, based on peer review and grey bibliography the 11216 organisms belonging to 142 species were classified into three categories according to their habitat affinity i.e. epifauna, surface, and subsurface. By means of biological trait analysis subsurface organism were removed, and a data matrix of the remaining individuals was produced (Statzner et al. 1994).

5.2.3 Complexity measurements

McAbendroth et al. (2005) assessed the complexity of macrophyte stands following a fractal dimension approach. Based on this methodology, we assessed the complexity at two levels: at the level of the phytal species and at the level of sample units which encompass different phytal species and degrees of complexity.

At the phytal species level the fractal dimension of the surface (da) and the perimeter (dp) was measured at four magnification scales (Figure 5.1). For scale up to the sample unit level, the fractal dimension for each sample was weighted by means of the wet volume (Dv) and dry biomass (Dm) of the phytal components. The result is a combination of different approaches to calculate the fractal dimension of a given sample unit. The novelty introduced in this work is the introduction a set of micro-scales, and the use of the phytal volume to weight the complexity index.

5.2.4 Complexity at phytal species level (dp and da)

The scale at which the structural variable is measured may depend on what is perceived as a habitat by the studied organisms (Tews et al. 2004). Thus, for each phytal species (*Cladophora sp.*, *Cymodocea nodosa*, *Rytiphlaea tinctoria* and *Valonia aegagropila*) fractal dimension (d) was measured at four magnification scales biologically meaningful for the studied organisms; one macro-scale image taken from a 25 cm distance sized (60*45mm) and three micro-scale taken under the stereoscope, i.e. *0.8 (32*23 mm), *1.6 (13.5*11.5 mm), and *2.5 (10*4 mm), (Figure 5.1). For each phytal species at each studied magnification scale three replicate images from 3 randomly selected individuals were taken to account for the average fractal dimension. Each image was converted to binary, and then by the box-counting method the fractal dimension for the perimeter (dp) and surface (da) was estimated with FracLac (Karperien 1999) for ImageJ(Schneider et al. 2012), (Figure 5.1). This resulted in 8 mean fractal dimension values for each of the phytal species arising from 4 magnification scales (macro, *0.8, *1.6 and *2.5) and two approaches (dp and da). Hence, da and dp account for a different complexity aspect: da describes the area occupancy and indicates how the perception of the surface area might change with the scale, dp explains the complexity of the plant profile, which is related with the nature of the gaps between plants parts.

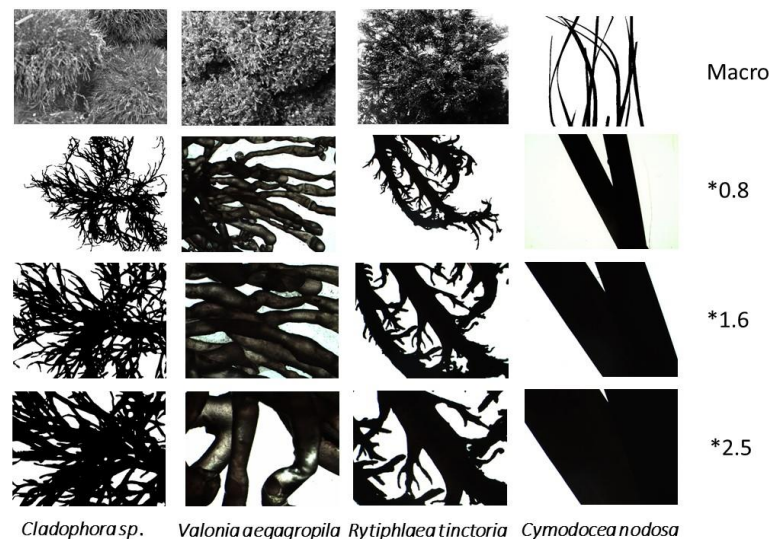


Fig. 5.1 Set of scales used for the measurement of the fractal dimension under the da and dp approaches of the four phytal components.

5.2.5 Complexity at sample unit level

The complexity was scaled up from phytal species to sample unit level. For each sample unit the fresh volume and dry biomass of each phytal component was measured, by immersion in a measuring cylinder, and weighing after 48 hours drying at 60°C, respectively. The method used to weight the fractal dimension for each sample relies on the mean fractal dimension of each phytal species at each studied magnification scale within a given sample. In this way, volume (Dv) and dry biomass (Dm) weighted geometric mean is used to estimate the fractal value in a given sample. The weighted geometric mean reduces the effect of very high or low values which might bias the arithmetic mean (Seixas et al. 1988). The use of both approaches, dry biomass and volume, aims to evaluate if the complexity of a given sample might be conditioned by the weighting process. For instance, a set of samples with dissimilar occurrence of *Valonia aegagropila*, which presents a high volume/biomass ratio, might be more sensible to the weighting procedure due to the high wet volume and low dry mass of this specific alga.

$$Dm = b \frac{db}{\sum dz} + c \frac{dc}{\sum dz}$$

b : dry biomass of sp. b

c : dry biomass of sp. c

db : average fractal dimension spp. b; dc : average fractal dimension spp. c; dz : average fractal of the phytal species within the sample.

$$Dv = b \frac{db}{\sum dz} + c \frac{dc}{\sum dz}$$

b : wet volume of sp. b

c : wet volume of sp. c

5.3 Data Analysis

5.3.1 Complexity assessment

The approaches for complexity assessment, at the level of phytal species ($da-dp$) and sample unit ($Dv-Dm$), were tested with factorial ANOVA fixed effects across the four magnification scales (i.e. macro, 0.8*, 1.6* and 2.5*), after the normality of the residuals (Kolmogorov Smirnov) and homogeneity of the variances (Levene's test) was ascertained. The degree of correlation between the fractal indices and their weighting methods was proved with linear regression analysis. The set of complexity indices (i.e. Dam ; fractal dimension index for the surface weighted with phytal biomass, Dpm ; fractal dimension index for the perimeter weighted with phytal biomass, Dav ; fractal dimension index for the surface weighted with phytal volume, Dpv ; fractal dimension index for the perimeter weighted with phytal volume) were standardized and tested with factorial ANOVA.

5.3.2 Macroinvertebrate body length and community structure

The body length distribution parameters: mean, mode, skewness, minimum and maximum, percentile 10 and percentile 90 as well as the community indices; species richness, abundance and Shannon diversity were tested with Spearman rank to test correlation with the Dpm fractal complexity index.

5.3.3 Body length

The slope of the regression of macroinvertebrate abundance against body length was plotted to find a relation with the Dpm fractal index across the studied habitats. Significant Spearman rank correlated parameters were tested with analysis of covariance (ANCOVA) and linear regression.

The relationships between the macroinvertebrate body length distributions across the sampling sites was tested. The body length distribution data were Log (x+1) transformed and the Bray Curtis resemblance matrix was calculated PERMANOVA analysis.

5.3.4 Community structure

Significant Spearman rank correlations between the *Dpm* fractal complexity index and macroinvertebrates abundance, species richness and Shannon diversity (Hlog_e) were further tested with analysis of covariance (ANCOVA) and linear regression. Macroinvertebrates abundance data were log+1 transformed to fulfil Levene's test.

Multi-Dimensional Scaling (MDS) was applied to present the similarity of the community structure amongst the 3 sampled locations which represent a different habitat (i.e. M3; *Valonia aegagropila*, M5; *Cymodocea nodosa-Rytiphlaea tinctoria*, and M7; Sparse *Cymodocea nodosa*-bare sediment). Abundance data were square root transformed to reduce the effect of the zero counts (absence) on the Bray-Curtis similarity. PERMANOVA analysis was used to test statistical differences in a seasonal and habitat base. The contribution of each species across habitats was disclosed with SIMPER analysis.

The SPSS 20 statistical package was used for non-parametrical statistics as for the Spearman rank correlations, regression ANOVA and ANCOVA analysis. PRIMER 7 was used for the calculation of the clustering and classification methods, community indices and the PERMANOVA

5.4 Results

In total 9992 epifauna and surface macroinvertebrate individuals, belonging to 122 species, were included in this study. Polychaetes were the most diverse group followed by Crustacean and Mollusca. The structure of the macroinvertebrate assemblages varied amongst habitats. Throughout the studied sites, the species contributing the most (i.e. 50%) to the community were obtained with the SIMPER analysis. In *Valonia aegagropila* habitat (M3); *Microdeutopus gryllotalpa* 9.28%, *Janua pagenstecheri* 7.85%, *Microdeutopus bifidus* 7.15%, Ostracods 6.16% and *Schistomeringos rudolphi* 4.6% reached a contribution of the 50% to the given macroinvertebrate community. Conversely; *Janua pagenstecheri* 7.73%, *Microdeutopus bifidus* 7.66%, *Fabricia stellaris* 7.35%, *Exogone dispar* 6.39%, *Microdeutopus gryllotalpa* 6.25%, *Harmothoe spinifera* 5.76%, *Pettiboneia urciensis* 4.05% and *Sphaerosyllis pirifera* 3.97% in *Cymodocea nodosa-Rytiphlaea tinctoria* habitat (M5), and the sparse *Cymodocea nodosa*-bare sediment habitat (M7) was dominated by *Iphinoe serrata* 29.38% and *Abra segmentum* 21.34%.

5.4.1 Complexity at phytal species level (dp and da)

The fractal dimension measurements across the different phytal species varied significantly across the studied approaches and magnification scales.

The fractal dimension on perimeter measurements (*dp*) varied from a maximum of 1.46 in *Cladophora sp.* at macro scale to a minimum of 1.09 at the highest magnification (*2.5) in *Cymodocea nodosa*. The general trend in *dp* measurements at any magnification scale is: *Cladophora sp.* > *Valonia aegagropila* > *Rytiphlaea tinctoria* > *Cymodocea nodosa*. Thus, among the measured phytal species *Cladophora sp.* presented the most complex and *Cymodocea nodosa* the least. The *dp* values are highly scale dependant and mostly tend to decrease from the macro scale to the *2.5 magnification scale (Table 5.1). The inter-scale variation for *dp* is higher for *Valonia aegagropila* followed by *Rytiphlaea tinctoria*, *Cymodocea nodosa* and *Cladophora sp.* which shows more stable *dp* values (Table 5.1).

The fractal dimension values on surface measurements (*da*) varied from 1.97 at *2.5 magnification scale to a minimum of 1.63 at macro scale, both in *Cymodocea nodosa*. The general trend in *da* measurements is: *Cymodocea nodosa* > *Valonia aegagropila* > *Rytiphlaea tinctoria* > *Cladophora sp.* at the 3 micro scales (i.e. *0.8, *1.6, *2.5) and *Rytiphlaea tinctoria* > *Valonia aegagropila* > *Cladophora sp.* > *Cymodocea nodosa* at the macro scale. The *da* values are scale dependant and increases from macro scale to *2.5 magnification scale. Generally, *Cymodocea nodosa* presented the higher inter-scale variability

followed by *Cladophora sp.*, *Valonia aegagropila* and *Rytiphlaea tinctoria* where da values are more stable (Table 5.1).

Comparing the two fractal approaches da complexity values are higher than dp at any studied scale and the differences between them increase as the magnification scale does for any of the studied phytal species (Table 5.1). Thus, the effect of the thallus/leaf surface measurements over the final complexity index increases as the magnification scale does.

Factorial ANOVA over the complexity of the studied phytal species showed significant differences across the magnification scales, approaches dp and da for the four studied phytal elements. (Table 5.2).

Table 5.1 Mean fractal dimension (\pm sd) based on three measurements on the perimeter (dp) and surface (da). For each phytal species four scales were measured in images at different magnification scales; macro (60x450mm), 0.8 (32X23mm), 1.6 (13.5x11, 5mm) and 2.5 (10x4mm).

Phytal species	scale	dp	da	$da-dp$
<i>Cladophora sp.</i>	macro	1.46 \pm 0.056	1.78 \pm 0.036	0.319 \pm 0.091
	0.8	1.39 \pm 0.030	1.78 \pm 0.034	0.387 \pm 0.060
	1.6	1.33 \pm 0.025	1.84 \pm 0.023	0.613 \pm 0.067
	2.5	1.22 \pm 0.031	1.90 \pm 0.031	0.702 \pm 0.045
<i>Cymodocea nodosa</i>	macro	1.34 \pm 0.014	1.63 \pm 0.009	0.289 \pm 0.022
	0.8	1.10 \pm 0.002	1.90 \pm 0.045	0.798 \pm 0.044
	1.6	1.10 \pm 0.011	1.92 \pm 0.039	0.859 \pm 0.016
	2.5	1.09 \pm 0.010	1.97 \pm 0.021	0.862 \pm 0.039
<i>Valonia aegagropila</i>	macro	1.45 \pm 0.007	1.86 \pm 0.003	0.408 \pm 0.010
	0.8	1.18 \pm 0.031	1.91 \pm 0.027	0.723 \pm 0.042
	1.6	1.20 \pm 0.019	1.92 \pm 0.021	0.726 \pm 0.049
	2.5	1.16 \pm 0.046	1.93 \pm 0.060	0.914 \pm 0.108
<i>Rytiphlaea tinctoria</i>	macro	1.42 \pm 0.032	1.93 \pm 0.044	0.513 \pm 0.068
	0.8	1.21 \pm 0.016	1.87 \pm 0.022	0.655 \pm 0.035
	1.6	1.19 \pm 0.014	1.88 \pm 0.014	0.746 \pm 0.026
	2.5	1.15 \pm 0.007	1.92 \pm 0.015	0.794 \pm 0.026

5.4.2 Complexity at sample unit level (Dm and Dv)

The degree of correlation of the fractal complexity indices with their relative complexity weighting approach went from 0.397 in Dav at macro scale to 0.544 under Dam *2.4 scale (Figure 5.2). Throughout the complexity up scaling process (from phytal level to sample level) the differences in magnification scale (i.e. macro, *0.8, *1.6 and *2.5) fractal measurement (da and dp) and the weighting approaches become no significant, factorial ANOVA (Table 5.3). Due to non-significant differences amongst the array of approaches we focused all further analysis exclusively in the Dpm fractal index at macro scale.

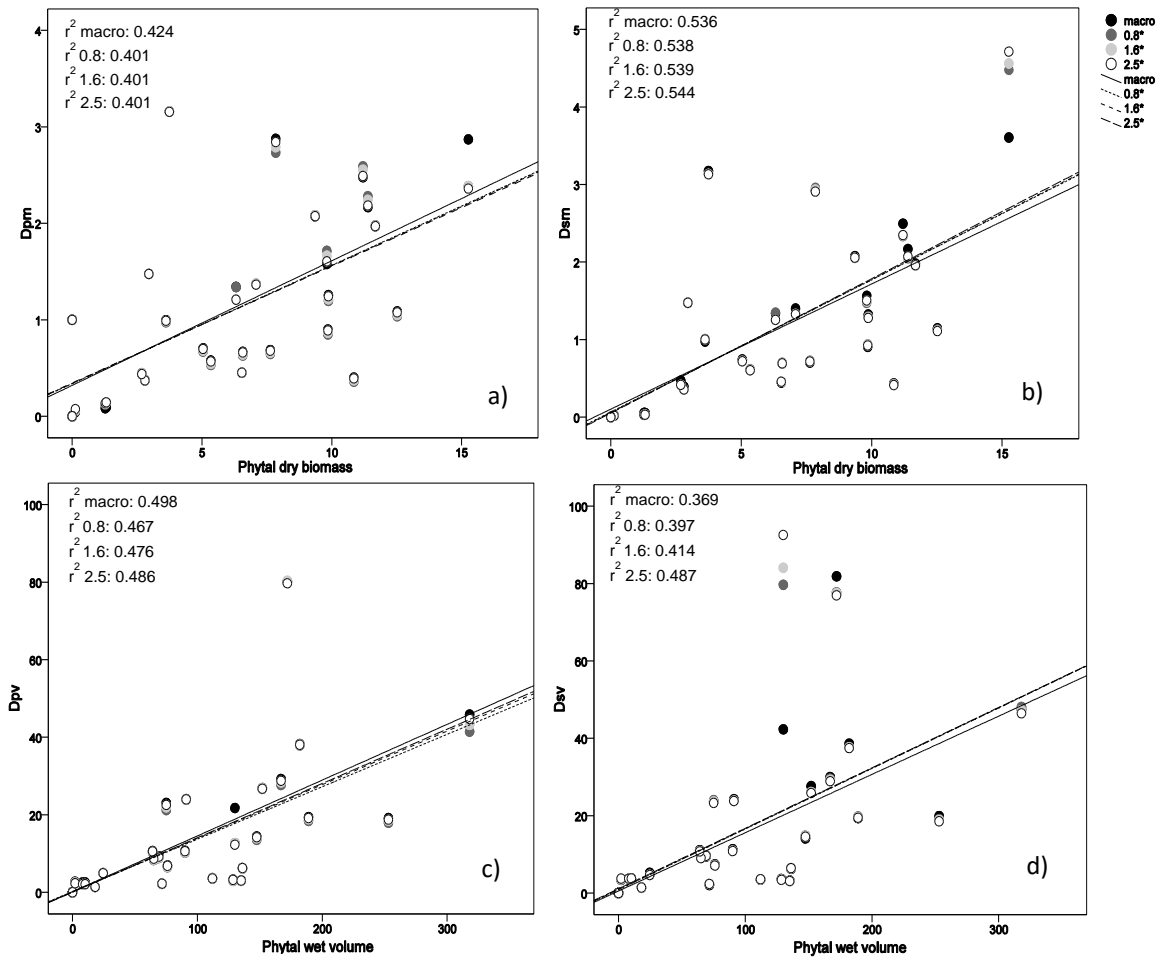


Fig. 5.2 Degree of correlation of the fractal complexity indices at the four magnification scales with the relative weighting approach.

5.4.3 Macroinvertebrate body length

The relationships between the fractal index at the macro magnification scale and the slopes of the normalized macroinvertebrates abundance/body length distributions did not indicate any significant relation (Figure 5.3), thus the relative abundance of small or big animals did not covary with the complexity of the sample.

Spearman rank correlations presented significant correlations with the minimum and maximum body length as with the percentiles (Table 5.4). P10 and minimum body length presented negative correlation, -0.569ρ and -0.483ρ respectively. Conversely, P90 and maximum length presented positive correlations, 0.376ρ and 0.334ρ respectively. The additional studied body length parameters as mean, mode and skewness, did not present any significant correlation with the complexity index (Table 5.4).

The highest correlations, P10 and P90, were further tested with ANCOVA analysis; the factor site did not show significant effect, (Table 5.5). By excluding the factor (site), P10 reveals a significant negative

linear correlation with *Dpm* ($r^2 = 0.131$) and P90 presents positive correlation ($r^2 = 0.226$) (Figure 5.4). For the P90, the site M7 ($r = 0.317$) differs from the general trend presenting a negative slope, (Figure 5.4b). As a result, the variation on the minimum body length, percentile 10 and percentile 90 weakly correlates with changes in the sample complexity.

Multivariate analysis, PERMANOVA was performed to disclose macroinvertebrate community body length distribution patterns in terms of seasonality and site. Body length showed significant differences among sites and not among seasons (PERMANOVA, Table 5.6)

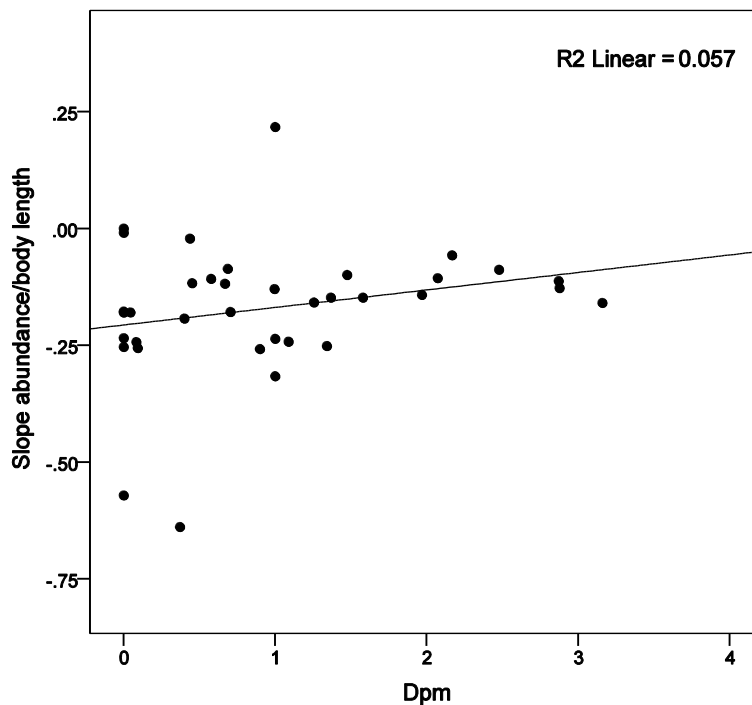


Fig 5.3 Regression relationships between *Dpm* fractal index at the macro magnification scale and the slopes of the normalized macroinvertebrates abundance / body length distributions for each of the 34 samples.

5.4.4 Macroinvertebrate community distribution

The fractal complexity index presented a significant positive correlation with the macroinvertebrates abundance (0.716 rho), species richness (0.669 rho), and Shannon's diversity (0.496 rho); $p < 0.05$.

In the analysis of covariance (ANCOVA), the factor site revealed significant effect for the macroinvertebrate species richness, Shannon's diversity and abundance (Table 5.5). The complexity index better correlated with the macroinvertebrates abundance ($r = 0.500$) followed by the species richness ($r = 0.473$) and the Shannon diversity ($r = 0.262$), (Table 5.5, Figure 5.6). Across the sites; Species richness correlation is highest M7 ($r = 0.289$) followed by M3 ($r = 0.289$), the site M5 presents the lowest inter-site correlation ($r = 0.080$), (Figure 5.6a). For the Shannon's diversity M7 presents the highest correlation ($r = 0.27$), then M5 and M3 ($r = 0.024$) for both, (Figure 5.6b). Last the interstice correlation for the macroinvertebrates abundance reach the highest correlation at M3 ($r = 0.245$) followed by M7 ($r = 0.191$) and M5 ($r = 0.061$), (Figure 5.6c). Across habitats differences in correlation also indicate the existence of a degree of complexity within the sample

On the assumption that the sites are independent, no correlation was found within any of them and the correlation only appears when all the locations are grouped. The highest positive correlations, being driven by the site M3, ($r=0.500$)

Multivariate analyses, PERMANOVA and MDS, on the species abundance data indicated significant differences across site and season (PERMANOVA, table 5.7). MDS shows a clear discrimination at a site level and less clear in a seasonal base. (Figure 5.7).

5.5 Discussion

This case study is carried out in a transitional ecosystem, Messolonghi Lagoon in western Greece, which is a typical open lagoon (Guelorget and Perthuisot 1983b). In these naturally stressed ecosystems the governing physicochemical factors as confinement, salinity, temperature, sediment granulometry and organic carbon in the sediment are typically studied to explain aspects of macroinvertebrate body length and community structure. Even though habitat complexity has been proved to play a key role in the structuring of those communities, there is a lack of validated methods to account for it. The use of methodological standard methods may provide more consistent comparison in this field (Thomaz and Cunha 2010). In the present study the habitat complexity of three neighboring sites, located in the central part of the lagoon, where the physicochemical characteristics of the water and sediment are largely similar was investigated. This gave the opportunity to focus on habitat complexity and to describe body length patterns and community structure across habitats within this diverse ecosystem.

5.5.1 Complexity at the phytal level

The phytal elements studied across three sites in Messolonghi lagoon presented significant differences by means of the fractal measurements and scales, (Table 5.2). The significant discriminatory condition for the fractal surface (da) and fractal perimeter (dp) enable them to quantify the complexity, so making them a suitable tool to study the effects of the habitat complexity (Halley et al. 2004; Schmid 1999). Yet, a set of artefacts, which need to be considered, were detected across the complexity measurements.

Table 5.2 Factorial ANOVA for the fractal dimension values at the phytal specie level between fractal methods dp and da , scales and phytal species.

Factor	SS	df	MS	F	p
dp vs da	9.673	1	9.673	9250.1	0.0001
Scale	0.069	3	0.023	22	0.0001
Phytal species	0.0864	3	0.0288	27.5	0.0001
dp vs da *scale	0.5687	3	0.1896	181.3	0.0001
dp vs da *phytal species	0.1409	3	0.0470	44.9	0.0001
Scale*phytal species	0.0889	9	0.0099	9.4	0.0001
dp vs da *scale*phytal species	0.1153	9	0.0128	12.3	0.0001
Error	0.0669	64	0.0010		

dp describes the structural fractal dimension and characterizes the complexity of the thallus edges (plant architecture in one dimension). The high degree of boundary fragmentation ranked *Cladophora sp.* as the most complex. Alternatively, *Cymodocea nodosa* is denoted as the least complex, distinguished by the un-fragmented structure of the leaf (Table 5.1). Across the set of magnification scales *Cladophora sp.* presents the lowest dp inter-scale variations, explained by the greater structural auto-replicability (Corbit and Garbary 1995; McAbendroth et al. 2005). Conversely, the un-fragmented and quasi linear *Cymodocea nodosa* leaves presented the lowest dp variation across the micro scales. This condition reflects the artefact derivate from the lack of self-similarity in *Cymodocea nodosa* leaves, which are not true fractals. Also, demonstrates that the use of a different scale might interact differently depending on the phytal element.

Thus, a high degree of auto-replicability, but also the cross scale stability of the thallus/leaf contour shape can moderate the inter scale variations for dp . Special attention may be paid when bulking, in the same fractal context, elements that are far of being a true fractal with those that are close to the self-similarity as occurs here with *Cymodocea nodosa*.

Besides, the two dimensional da fractal measures, depending on the scale may describe the textural fractal dimension of the thallus/leaf in high magnification scales (Schmid 1999), and the dimension of the interspaces between the thallus/leaves in lower magnification scales (McAbendroth et al. 2005). Greater da variation across the set of magnification scales occurs in *Cymodocea nodosa*, (Table 5.1). At the micro scales the increase of da values with the magnification scale denotes the sensitivity to the amount of area occupied in a grid under the box counting method. This characteristic constrains the maximum magnification scale for which fractal analyses are appropriate under this method (Halley et al. 2004). Thus, da measures in wide flat leaves of which there are not ramifications (such as *Cymodocea nodosa*) do not account for structural patterns but mostly reflect the surface availability, thus acting as a confounded variable (Johnson et al. 2003). Thus, as the magnification scale increases the complexity does due to a confounded effect.

The establishment of a sized scale to account for the aimed process within a study is a key subject in terms of habitat complexity. The micro magnification scales, intended to account for those processes occurring over the surface of the leaf/thallus presented limitations. Thus, photo resolution, software restrictions or both may reach the detection limit of the method when the information obtained across the magnification scales is constant. This occurs when higher magnification does not account for greater thallus/leaf contour detail (Figure 5.1). Due to the observed limitations, the use of transversal slices from the thallus/leaf by means of dp , might better describe the surface roughness at these micro scales. Likewise, the use of micro scales, even if technically feasible by microscopy techniques, might not account for processes occurring in a wider scale depending on the organism's body length and its habitat perception (Dibble et al. 2006).

5.5.2 Complexity at the sample unit level

At this level from 36.9% to 54.4% of the complexity variation is explained by the weighting method across the set of scales (Figure 5.2). Conversely, at a sample level the lack of significant differences across the magnification scales and weighting methods reveals methodological weakness to keep the information retrieved from the inter-scale variability at a phytal level (Table 5.3). Thus, the final complexity value of a given sample is being driven by the weighting method and the complexity of phytal element appear to be masked (Table 5.3). Moreover, methodological limitations make difficult to account for the complexity originated from the interspaces between neighbor phytal elements, also pointed out in (McAbendroth et al. 2005).

Table 5.3 Factorial ANOVA for complexity at the sample level between weighting approaches, type of fractal measurement surface based and perimeter based, habitat type and scale at a sample level.

Factor	SS	df	MS	F	p
Intercept	4444.4444	1	4444.4444	607.246	0.001
<i>Dv vs Dm</i>	0.000	1	0.000	0.000	1.000
<i>Da vs Dp</i>	0.000	1	0.000	0.000	1.000
Habitat type	3059.594	2	1529.797	209.017	0.001
Scale	0.000	1	0.000	0.000	1.000
Error	3864.445	528	7.319		

Dv fractal dimension weighed by the total volume, *Dm*; fractal dimension weighed by the total biomass, *Da*; weighted fractal index calculated by means of surface measurements, *Dp* weighted fractal index calculated by means of fractal perimeter measurements

5.5.3 Body length

The relative abundance of small or big animals did not increase or decrease with the complexity of the sample (Figure 5.3), concurring with Ferreiro et al. (2014) where macroinvertebrate body length distribution was not associated to the fractal dimension. Conversely, McAbendroth et al. (2005) found a negative relationship between macrophytes stand complexity and the slope of the biomass/body size scaling relationship, thus the presence of smaller organisms in more complex samples. Also, Taniguchi and Tokeshi (2004) found a negative relationship in the mean body size of riverine invertebrates with habitat complexity.

By using the percentiles, P10 to account for the smaller macroinvertebrate body lengths and P90 for the larger, the effect of outlier values was reduced and a more balanced representation was achieved (Table 5.4, Figure 5.4). P10 negative correlations, indicate that in more complex samples macroinvertebrates reach smaller sizes. On the other hand, P90 and maximum body length positive correlation indicate that in more complex samples macroinvertebrates reach larger body sizes.

Overall, this type of response supports the hypothesis; higher the sample complexity, wider the range of body sizes of organisms found in the sample. Complex habitats support a greater microhabitat availability, with greater heterogeneity of structural elements, reducing predation (Michael Gee and Warwick 1994; Tokeshi and Arakaki 2012). Besides, the large variation in space sizes may provide habitable space and refuge to macroinvertebrates with a wider variety of body sizes. Thus, complex habitats may act as a microhabitats for smaller organisms and likewise acting as refuge against predation, thus macroinvertebrates could reach larger sizes.

Table 5.4 Spearman rho correlation for the *Dpm* fractal index at macro scale with the studied body length parameters. Bold marked correlations are significant at $p < 0.0500$

	Mean	Mediana	Moda	Skewness	Minimum length	Maximum length	Percentile 10	Percentile 90
<i>Dpm</i>	-0.154	-0.233	-0.306	0.247	-0.483	0.334	-0.569	0.376

Dpm; fractal of the perimeter weighed by the total phythal biomass

Apart from habitat complexity, other factors, may play a key role in the body size patterns and distribution (MacArthur and Wilson 1967). Seasonal factors as; macroinvertebrate life cycles (Giangrande et al. 1995), the cross seasonal colonization patters among different levels of habitat complexity (Taniguchi

and Tokeshi 2004), the *Cymodocea nodosa* seasonal growth, and the differences in fractal complexity across the development stage algae (Corbit and Garbary 1995) among others were expected to influence the macroinvertebrate body length. However not significant differences were detected across the 4 sampling periods (Table 5.6). The body length distribution presented significant different across the three studied sites habitats which responds to the range of complexity across the sites.

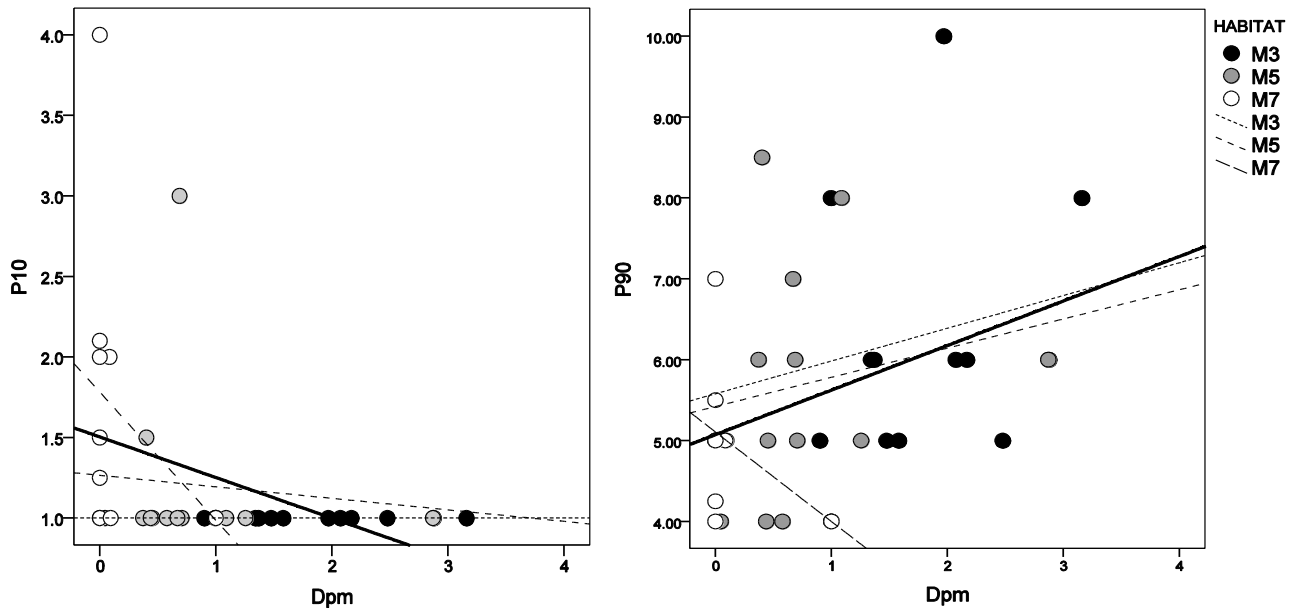


Fig. 5.4 Linear correlation within and across sampling sites for the body-length percentile 10 (P10) and percentile 90 (P90) with *Dpm* fractal complexity index. Percentile 10; M3, $r=0.000$; M5, $r=0.008$; M7, $r=0.160$; total, $r=0.131$. Percentile 90; M3, $r=0.035$; M5, $r=0.030$; M7, $r=0.317$; total, $r=0.121$

Table 5.5 ANCOVA for the variations in Percentile 10 and 90 for macroinvertebrates body length vs the *Dpm* fractal complexity index. Fixed factors; habitat and season. Covariate; *Dpm* complexity index at macro scale. Significant effects in bold.

Source	Sum of Squares					Sum of Squares				
	df	F	Sig.	P. Eta ²	df	F	Sig.	P. Eta ²		
<i>Dpm</i>						NO fixed factor				
Fixed factor HABITAT										
Percentile 10	0.337	1	0.89	0.350	0.027	1.881	1	5.13	0.030	0.131
Fixed fact.	0.459	2	0.61	0.549	0.037					
Intercept	20.498	1	54.64	0.00	0.631	37.832	1	103.20	0.000	0.752
Error	12.004	32				12.463	34			
Percentile 90	0.290	1	0.15	0.697	0.005	8.976	1	4.67	0.038	0.121
Fixed fact.	5.338	2	1.42	0.256	0.082					
Intercept	307.868	1	164.21	0.000	0.837	430.852	1	224.21	0.000	0.868
Error	59.997	32				65.335	34			
Spp. richness	277.897	1	7.39	0.011	0.188	2522.881	1	30.55	0.000	0.473
Fixed fact.	1,604.515	2	21.33	0.000	0.571					
Intercept	3,584.353	1	95.31	0.000	0.749	3307.901	1	40.05	0.000	0.541
Error	1,203.353	32				2807.869	34			
H loge	0.281	1	2.10	0.156	0.062	3.563	1	12.04	0.001	0.262
Fixed fact.	5.793	2	21.71	0.000	0.576					
Intercept	41.085	1	308.06	0.000	0.906	54.191	1	183.14	0.000	0.843
Error	4.268	32				10.061	34			
Abundance	1004.803	1	5.64	0.024	0.150	11399.84	1	34.06	0.000	0.500
Fixed fact.	5680.011	2	15.94	0.000	0.499					
Intercept	10566.180	1	59.30	0.000	0.650	7573.864	1	22.63	0.000	0.400
Error	5701.030	32				11381.04	2			

5.5.4 Community distribution

Macroinvertebrates species richness and abundance was found to have significant positive correlation with plant complexity, measured by means of fractal dimension, across lagoonal, lake and riverine ecosystems (Dibble and Thomaz 2009; Ferreira et al. 2014; Thomaz et al. 2008). In coastal waters Veiga et al. (2014) found correlation for the algae complexity and epifauna. Likewise, we found a strong positive correlation of the species richness, abundance and Shannon diversity at different levels across the studied fractal complexity index, differing from results obtained in (McAbendroth et al. 2005) by means of the same complexity index. On the other hand, other indices of complexity involving algae interspaces complexity (Hicks 1980) and plants complexity and heterogeneity (St Pierre and Kovalenko 2014) were significantly related with the abundance and diversity of harpacticoid and the macroinvertebrate taxonomic richness respectively.

Table 5.6 PERMANOVA for the macroinvertebrates body length distribution across seasons and sites. Significant different tests in bold.

Source	df	SS	MS	Pseudo-F	P(perm)
Season	3	6364.8	2121.6	1.348	0.14
Site	2	31049	15524	9.8635	0.001
Season*Site	6	15497	2582.8	1.641	0.011
Residuals	24	37774	1573.9		
Total	35	90684			

Table 5.7 PERMANOVA for the macroinvertebrate population distribution across seasons and sites. Significant different tests in bold.

Source	df	SS	MS	Pseudo-F	P(perm)
Season	3	17073	5690.9	3.6658	0.001
Site	2	31343	15672	10.095	0.001
Season*Site	6	22232	3705.4	2.3868	0.001
Residuals	24	37259	1552.4		
Total	35	1.0791E5			

The significant correlations of the habitat complexity and the abundance of organisms, can be explained by the presence of more loci for the settlement of small invertebrates which might be partially supported also by the P10 correlation. However, this correlation does not exclude other factors which may play a role in structuring this communities, i.e. mutualism of the studied organism with the specific studied plants, complementary use of different plants by different animals, and toxicity of metabolic products that may attract or repel specific organisms (Davenport et al. 1999; Matsuda et al. 2015; Torres et al. 2015)

The level of interaction of surface availability and complexity per se, and how the sampling effort acts as a confounding variable in this type of studies acquires different impact across studies (Ferreiro et al. 2013; Thomaz et al. 2008; Torres et al. 2015). A general trend indicates a noticeable level of interaction, although some studies as Verdonschot et al. (2012) did not find any effect of the surface area over species richness and abundance from field experiments which manipulated complexity and surface aspects.

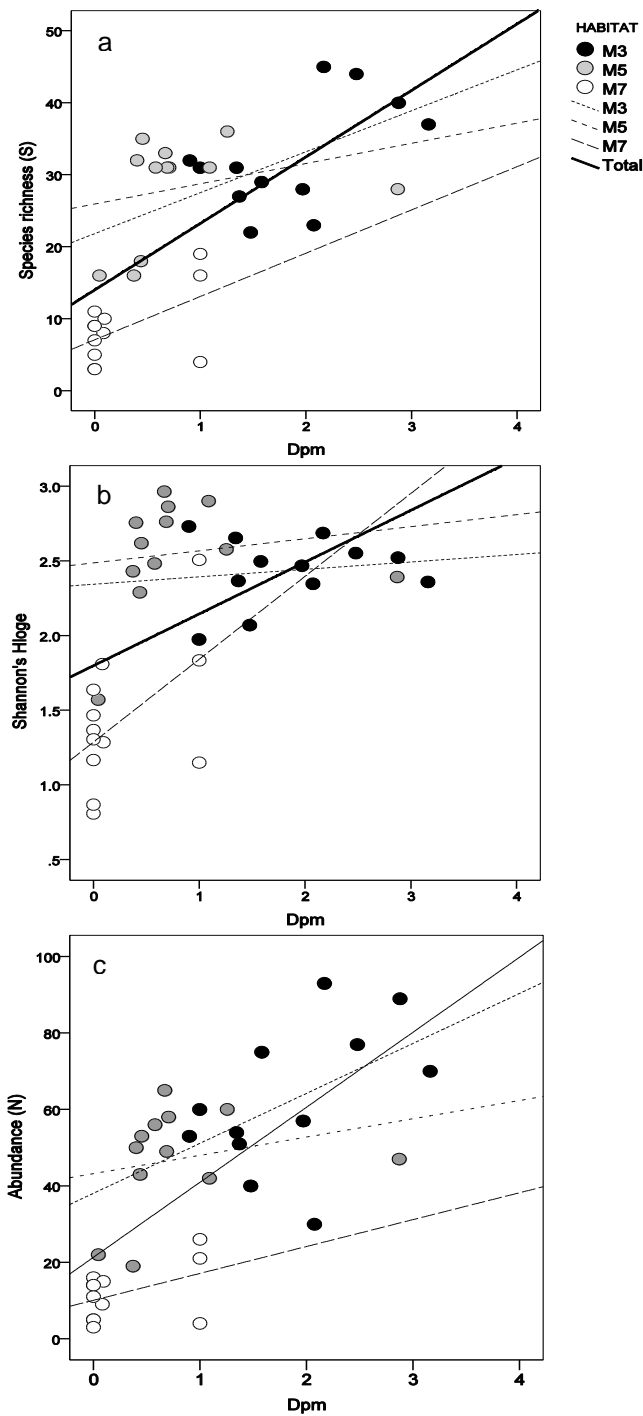


Fig. 5.5 Linear correlation within and across sampling sites for the species richness, Shannon's diversity (Hloge) and the abundance (N) with Dpm fractal complexity index. Species richness; M3, $r=0.289$; M5, $r=0.080$; M7, $r=0.290$; total, $r=0.473$. Shannon's diversity; M3, $r=0.024$; M5, $r=0.024$; M7, $r=0.274$; total, $r=0.262$. Abundance; M3, $r=0.245$; M5, $r=0.061$; M7, $r=0.191$; total, $r=0.500$.

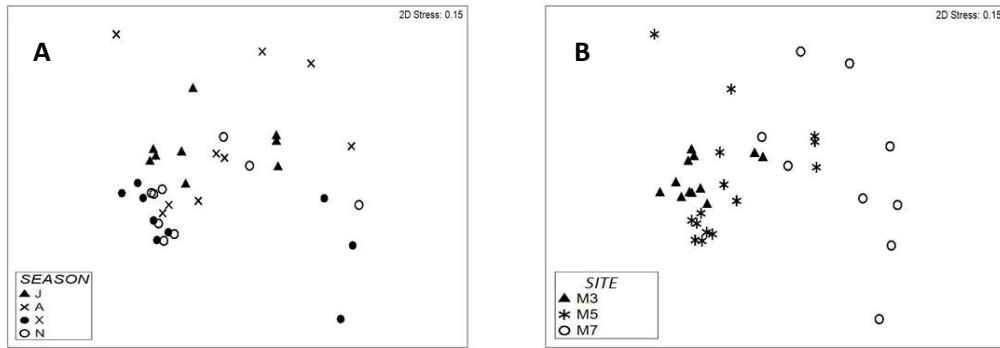


Fig.5.6 MDS performed on the macroinvertebrates species composition along the four sampling seasons(J, January; A, April; X, July; N, November) (A), and the three sites (B), included in this work.

Analysis of covariance revealed that variations in species richness, Shannon diversity and abundance depend on the complexity of the sample and the sampling site (Fig 5.6a; Table 5.5). This kind of response might be explained by the low complexity variability within any of the sites, disclosing a complexity gradient across the sites. Thus, the M3 location, with the best correlation, might be linked to the major complexity intervariability within this specific site. The study of the community distribution patterns with PEMANOVA (Table 5.7) and MDS (Figure 5.6) showed significant differences across sites and seasons. The factor site has a clear discrimination effect and appears to mainly lead the community distribution. Thus the strong correlations in species richness, Shannon diversity and abundance and complexity, might be also co-drive by other intrinsic ecological aspects linked to a due habitat.

5.6 Conclusions

This study exposed the usefulness of fractal dimension indices as complexity proxies, revealing strengths but also presenting methodological limitations. This points at the need of validating existing approaches across field and laboratory experiments, where the number of confounding variables can be better monitored. The fractal indices after (McAbendroth et al. 2005) show the ability to discriminate degrees of complexity across the studied phytal elements, but discrimination power across magnifications scales and weighting methods was ineffective after the up scaling to sample complexity. Besides, methodological limitations at the micro scales, that might be ecologically relevant for the studied organisms, are especially noticeable for *da* approach. Fractal dimension measured under the box counting methodology is especially sensitive to the amount of area occupied in the grid at the micro scales. Besides, the lack of self-similarity is a source of artefacts in this kind of analysis, and the inclusion of extensively diverse morphologies of phytal elements may draw erroneous results in terms of fractal complexity comparability.

The proposal of a complexity index and the scale of work has to be ecologically relevant, to the addressed habitat, structures and organisms (Dibble et al. 2006). The use of diverse magnification scales intended to account for a meaningful ecological window for the studied organisms is desirable. Still this is a rather inexact subject where most of the studies, based on best professional judgement, consider a single given scale or set of scales to determine a meaningful ecological space for a group of organisms (e.g. macroinvertebrates). We do understand that there are needs to explore different scales but this option may be narrowed by analysis of the biological traits of the specific studied organism. Organisms belonging to the same group, e.g. meiofauna, macrofauna, megafauna, cover a wide variety of habitat preferences, body size, motility, reproduction, larval dispersal. The aggregation of organisms with different traits within the same scale may lead to imprecise answers.

In conclusion, the P10 and P90 body length distribution allow us to support the hypothesis; higher the sample complexity, wider the range of body sizes of organisms found in the sample. This outcome points at the importance of considering habitat complexity in addition to the traditional physicochemical approaches when defining macroinvertebrate body length trends and community distribution. More research is needed in terms of detection limits for fractal indices.

Recently, first attempts to account for the habitat complexity following 3-dimensional methods have arisen. Orland et al. (2016) applied computer aided tomography techniques to visualize kelp holdfast structures, and Kamal et al. (2014) estimated the habitat complexity of mangrove roots by using a RGB_D scanning sensor. 3D methods may account with more spatial perspective for habitat complexity and may increase the capability of describing the complexity of a whole sample without defragmenting each phytal species so also accounting the interspaces among components.

Due to the high correlation found between macroinvertebrates community distribution and sample complexity, we outline the need of considering habitat complexity to project transitional waters management plans. New challenges may lead to upscale this type of studies to be cost/effective for monitoring plans.

6 Anthropogenic impact in a Mediterranean coastal lagoon traced by means of nitrogen isotopic enrichment in the main benthic macroinvertebrate groups

Abstract

Autochthonous organic matter and allochthonous inputs, from coastal waters or anthropogenic activities, are key sources of organic matter in lagoonal ecosystems. It is yet uncertain at which extent those diversified organic matter resources can affect the benthic macroinvertebrate food-web. The benthic macroinvertebrate food web in Messolonghi lagoon was studied across the main benthic habitats; by means of the carbon and nitrogen isotopic composition. Across the studied habitats, the autochthonous food resources were not significantly different in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition. Anthropogenic terrestrial inputs in the form of POM, from a sewage treatment plant outflow and two irrigation canals, presented reduced $\delta^{13}\text{C}$ signatures and enriched $\delta^{15}\text{N}$ signatures in comparison with the POM signatures across the studied habitats. However, the $\delta^{15}\text{N}$ signatures across the studied sources and organisms ranked as one of the lowest in any Mediterranean coastal lagoon, which might be explained by the cycle of chemical fertilizers. A total of 14 macroinvertebrate groups matched across the studied habitats. Benthic macroinvertebrates in the *V.aegagropila* habitat presented a significant enrichment in $\delta^{15}\text{N}$ composition. However, no significant differences in the $\delta^{13}\text{C}$ signature were found. The results outline the impact that anthropogenic originated food resources have over the $\delta^{15}\text{N}$ signature in the benthic macroinvertebrate food web. Anthropogenic enrichment may lead to primary production and macroinvertebrate dietary shifts across an enrichment gradient. No significant impact of the habitat itself could be traced.

Our study provides an insight of how the benthic macroinvertebrates food web can be N enriched by anthropogenic inputs and how these can be detected in short distances.

6.1 Introduction

The geomorphology of Mediterranean coastal lagoons, shallow and with restricted communication with the sea, form a specific ecosystem with low tidal range, reduced currents and wide fluctuation of salinity. Among others, these conditions enhance the supply of a broad array of economic and ecological services; extensive aquaculture, fishing, tourism, urban development and agriculture principally (Costanza et al. 1997; Rova et al. 2015). As a result, these activities lead to morphological modifications, nutrient and pollutant loads and loss of biodiversity.

Coastal lagoons form transitional ecosystems where allochthonous and autochthonous organic matter couple. A high rate of both sources sink and are introduced in the lagoonal food web by different groups (Vizzini et al. 2005).

The shallowness and semi-closed condition of these ecosystems contribute to rise the temperature that jointly with the high concentration of nutrients promotes the development of algae, phanaerogams and phytoplankton blooms. The benthic habitat, relative to the phanerogams, seagrass and algae may conform to the autochthonous resources.

On the other hand, the external material that forms the allochthonous resources, can derive from both coastal and terrestrial origins; rivers, drainages, anthropogenic effluents, terrestrial vegetation and marine coastal supply. The extent to which one or other resource impacts the lagoonal food web is rather uncertain and different geomorphological and ecological factors may be interrelated (Carlier et al. 2007; Obrador and Pretus 2012).

In transitional ecosystems as coastal lagoons and estuaries, the contribution that autochthonous sources have over the benthic food web is dissimilar and depends on the source itself. Vizzini et al. (2002) reported low significance of the contribution of seagrass to the diet of macroinvertebrates, and Zheng et al. (2015) reported different levels of contribution from algae and phytobenthos.

Besides, the allochthonous food sources from river discharges and runoffs have been traced in coastal waters across different groups of organisms (Careddu et al. 2015; Darnaude et al. 2004). Those are traceable in the lagoonal food web due to their significantly reduced $\delta^{13}\text{C}$ signature and enriched $\delta^{15}\text{N}$ compared with the neighboring marine waters (Blair and Aller 2012).

Moreover, allochthonous organic matter from anthropogenic activities; sewage, agriculture, irrigation and industrial discharges are more $\delta^{15}\text{N}$ enriched if compared to the naturally occurring inflows (Cabana and Rasmussen 1996). This enrichment has been traced across different groups of organisms in aquatic and lagoonal ecosystems; seagrasses (Fernandes et al. 2009), macrophytes and algae (Cole et al. 2004), macroinvertebrates (Aberson et al. 2016; Waldron et al. 2001), crustacean (Connolly et al. 2013) and fish (Vizzini and Mazzola 2006). Negative effects of anthropogenic $\delta^{15}\text{N}$ on aquatic systems include shifts in food webs, disturbance of ecosystem functioning, and reduction of biodiversity (Rabalais 2002).

Stable carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis has been extensively used to trace the course of food sources and the trophic interactions across marine, coastal, transitional and inland waters ecosystems in a worldwide context (Middelburg 2014). $\delta^{13}\text{C}$ signatures are useful for identifying the relative contribution of potential food resources, based on the principle that animals are 1‰ $\delta^{13}\text{C}$ enriched in relation to their diet (B J Peterson and Fry 1987). Additionally, $\delta^{15}\text{N}$ signatures are useful for estimating the relative trophic levels of organisms within aquatic food webs (Cabana and Rasmussen 1996).

In the lagoonal food web the benthic macroinvertebrate community forms one of the first trophic levels (Carlier et al. 2007). Within this group of organisms, different feeding strategies (e.g. suspension feeders, deposit feeders, grazers, predators) and food preferences concur. The main source of energy for benthic macroinvertebrate communities is sedimentary particulate organic matter, consisting of phytoplankton and detrital particles, sediment particulate organic matter, and microphytobenthos at different rates. For instance, some of the benthic macroinvertebrates utilize more the sedimentary organic matter (SOM) and others rely more in particulate organic matter (POM). Besides, particular groups of Polychaeta, Crustacea and Mollusca rely differently on other organisms to meet their energy requirements. Besides, higher benthic biodiversity and abundance leads to the diversification of feeding traits and both contribute to the widening of food chain length (FCL) within the food web (Armitage and Fourqurean 2009; Emmett Duffy et al. 2005; Harmelin–Vivien et al. 2009). Benthic macroinvertebrate food webs include usually from two to three trophic levels. Most species are assigned to the second trophic level (primary consumers), fewer species are grouped in the third trophic level (secondary consumers), and benthic top predators are the least numerous. (Sokołowski et al. 2012; Vizzini et al. 2013).

Thus, allochthonous sources tends to increase the level of nutrients within the lagoonal ecosystems. As a result of nutrient enrichment, shifts in ecosystem productivity, species composition, and primary producer biomass have been reported (Armitage and Fourqurean 2009). Besides, these ecosystems are particularly susceptible to trophic alterations in response to anthropogenic nutrient input due to the close physical links to terrestrial habitats to the input of terrestrial matter into estuarine food webs (Martinetto et al. 2006). Depending on the degree of confinement, the marine or terrestrial source may have more impact in the lagoonal benthic food web and the resource availability may drive feeding shifts.

This work aims at identifying the effect of anthropogenic outflows (irrigation canal and sewage treatment plant) on the macroinvertebrate food web across the main benthic habitats in a Mediterranean costal lagoon. Natural benthic habitats are diverse in these ecosystems, within which non-indigenous species as *Valonia aegagropila* are also evolving. Still there is little knowledge in terms of how the shift of natural habitats can affect the benthic food web length, organism's relative trophic level or cause shifts in trophic traits. Assuming a $\delta^{15}\text{N}$ -enrichment factor of 3.4% between the lower and upper ranges of two successive trophic levels, we estimated the number of trophic levels within each studied habitat. Elucidating the impacts that particular organic matter sources, autochthonous (benthic habitats) and allochthonous (irrigation canals and sewage treatment plant) can provide important information from the first consumer's trophic levels in the lagoonal food web. Shifts at this first level may lead to changes in upper levels and be thus relevant for ecosystem management and conservation matters.

We examined the effects of a set freshwater outflow, result of different anthropogenic activities, on the macroinvertebrates C and N isotopic composition across the main lagoonal benthic habitats.

6.2 Material and methods

6.2.1 Study area

The lagoon system of Messolonghi is located at the northern side of the Patraikos Gulf (Ionian Sea, Greece). It is the largest lagoon complex in Greece covering about 15.000 ha and consisting of 6 different basins. The lagoonal complex, part of the National Park of Messolonghi, is also a Marine Protected Area, a Ramsar Site, an Important Bird Area (IBA) and part of the Natura 2000 network.

The present study was carried out in the central part of Messolonghi main lagoon, which has an average depth of one meter and communicates with the sea through a shallow and wide frontal area. The principal benthic habitats are rather patchy, characterized by dominant macroalgae as *Rytiphlaea tinctoria*, *Valonia aegagropila*, *Cladophora* spp., and the seagrass *Cymodocea nodosa*. The network of fresh water tributaries discharging in the main lagoon is influenced by anthropogenic activities. A system of canals, used to divert water from the Achelous River to irrigate neighboring crops, discharges into the western shore of the main lagoon through two canals. By means of pumping stations, the two principal canals discharge irrigation runoffs in the North-west and South-west shore of the main lagoon. In the southern canal, waters from a saltwort and lagoonal ones joint. Else, in the inner and northern most part of the lagoon is placed a sewage treatment plant outflow form the town of Aetoliko (4500 inh. approx.)

6.2.2 Sampling

Three sites were established in the central part of the lagoon to sample macroinvertebrates, primary producers, SOM and POM. Each sampling site represents a different benthic habitat: M3 *Valonia aegagropila*, M4 is a bare sediment site, and M5 *Rytiphlaea tinctoria* and *Cymodocea nodosa*. The proximity of the sampling sites was intended to reduce the variability of other physicochemical factors, whilst encompassing the main lagoonal habitats. At each main site, five samples of surface sediments and associated vegetation were collected with a box corer (0.023 m²). The samples were carefully washed in a 0.5 mm square mesh sieve to retain the organisms alive and collect the associated vegetation. The macroinvertebrate organisms were identified alive to the lowest possible taxonomic level and let overnight in lagoonal filtered water to release fecal pellets. The vegetal components were carefully cleaned with ultrapure water and frozen. At each main site, surface sediment from the first centimeter layer was collected for SOM analysis and water was collected, filtered over pre-combusted Whatman GF/F filters and frozen for further POM analysis. Additionally, the three main freshwater tributaries were sampled for POM, one directly from the sewage treatment plant pipeline outflow (SW) and two within the main irrigation canals in the proximity of the mouth (IRn and IRs).

In addition, terrestrial plants as *Phragmites* sp., and *Arthrocnemum* sp., very abundant along the shoreline and islets, were collected and cleaned with ultrapure water prior to frozen preservation. The sampling was carried out in November 2014.

6.2.3 Stable Isotope Analysis

Samples were unfrozen at room temperature, oven dried (60°C) and ground with a mortar and pestle. Two sets of samples were prepared from sediment, Whatman GF/F filters and lagoonal and terrestrial primary producers. One set was acidified with HCl to remove carbonates and the second was not acidified to avoid possible interferences on the $\delta^{15}\text{N}$. Gastropods, bivalves, and crabs were dissected prior to drying; only soft tissue was retained for stable isotopic analysis. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were analyzed in an isotope ratio mass spectrometer (ThermoScientific Delta PlusXP) connected to an elemental analyzer (Thermo Scientific Flash EA 1112). Isotopic values were expressed in conventional δ unit notation, as parts per mil deviations from international standards, i.e., Vienna Pee Dee Belemnite carbonate and atmospheric nitrogen (N_2) for carbon and nitrogen, respectively, according to the formula: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N , and R is the corresponding $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio. Analytical precision based on the SD of replicates of internal standards (International Atomic Energy Agency IAEA-NO-3 for $\delta^{15}\text{N}$ and IAEA-CH-6 for $\delta^{13}\text{C}$) was 0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

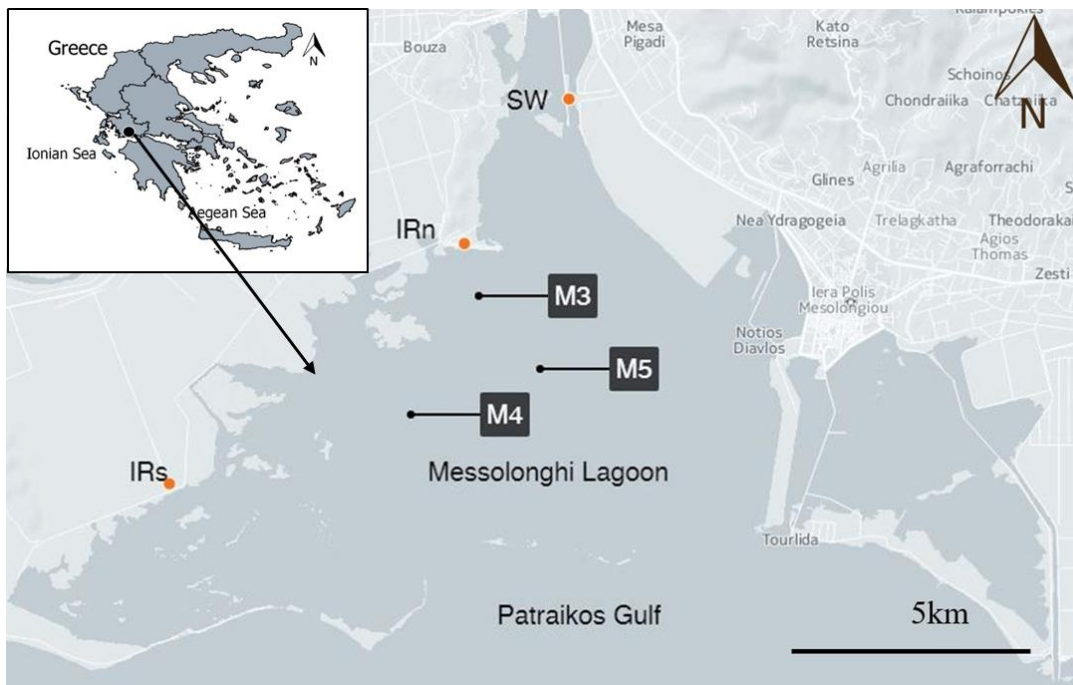


Fig. 6.1. Sampling sites in the main lagoon of the Messolonghi system located in three different habitats (*V.aegagropila*, M3; *C. nodosa-R.tintoria*, M5; and bare sediment, M4) and close to freshwater inflows (sewage treatment outflow, SW; North irrigation canal, IRn; South irrigation canal, IRs).

6.2.4 Data analysis

The POM was tested for differences in isotopic composition between the M3 and M5 and between allochthonous (M3 and M5) and autochthonous (SW and IRn) origin. The IRs was excluded from this analysis due to the difficulty to discriminate the origin of these waters where irrigation, salt works and lagoonal waters joint.

The general relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the relationships between $\delta^{15}\text{N}$ within the main sampled habitats were assessed using linear regression models.

The relative trophic level (RTL) for the consumers was calculated using the following formula $\text{RTL} = (\delta^{15}\text{N}_{\text{cons}} - \delta^{15}\text{N}_{\text{baseline}}) / f + \text{TL}_{\text{baseline}}$. $\delta^{15}\text{N}_{\text{cons}}$ is the average $\delta^{15}\text{N}$ value of a given consumer, $\delta^{15}\text{N}_{\text{baseline}}$ is the average value for a reference species, f is the trophic isotope fractionation values for $\delta^{15}\text{N}$ (here assumed +3.5‰ per each trophic level after Post (2002), $\text{TL}_{\text{baseline}}$ is the trophic level of a baseline, and was equal to 2 as grazers were used. Due to the inter-variability of the $\delta^{15}\text{N}$ across habitats, the baselines were calculated by averaging the three lowest $\delta^{15}\text{N}$ values, belonging to grazers. To reduce among site differences in $\delta^{15}\text{N}_{\text{baseline}}$, the nitrogen isotope baseline has been calculated separately for each site (Jake Vander Zanden and Fetzer 2007). In M3, we used $\delta^{15}\text{N}$ from *Cerithium* sp, Gammaridae and *Idotea baltica*, and for the site M5 we used $\delta^{15}\text{N}$ signatures from Amphipoda pool, Aoridae and *Cymodoce truncata*. Finally, Consumers were classified as primary consumers (TL range 1.6–2.5) and secondary consumers (TL range 2.6–3.5) (Vizzini et al. 2013).

6.3 Results

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for the studied food resources presented large differences among habitats and components (Table 6.1, Fig. 6.2).

$\delta^{13}\text{C}$ signatures registered the overall highest values in sedimentary organic matter (SOM), ranging from $-13 \pm 0.2\text{‰}$ in bare sediment to -7.6‰ in the *V. aegagropila* habitat and the lowest in particulate organic matter (POM) ranging from $-20.3 \pm 0.7\text{‰}$ in bare sediment to $-30.3 \pm 0.2\text{‰}$ in the southern irrigation canal (IRs). Macroalgae had intermediate values, ranging from -18.4‰ in *Cladophora* sp. to $-19.9 \pm 0.1\text{‰}$ in *Valonia aegagropila*. Amongst the vegetation, the lowest $\delta^{13}\text{C}$ values were listed in terrestrial plants (i.e. $-25.8 \pm 0.2\text{‰}$ in *Arthrocnemum* sp. and -24.7‰ in *Phragmites* sp.).

$\delta^{15}\text{N}$ signatures for the food resources were markedly more enriched in terrestrial plants (i.e. 8.7‰ in *Phragmites* sp. and $6.0 \pm 0.5\text{‰}$ in *Arthrocnemum* sp.). The macroalgae ranged from the most enriched *Ulva* sp. 7.9‰ . to the most depleted *V. aegagropila* $2.8\text{‰} \pm 0.0\text{‰}$. The lower $\delta^{15}\text{N}$ across the potential food resources were registered in SOM; $\delta^{15}\text{N}$ between $0.6 \pm 0.0\text{‰}$ in the bare sediment and 0.8‰ in *C.nodosa-R.tinctoria* habitat. The least enriched resource was the *C. nodosa* leaf, $\delta^{15}\text{N}$ 0.1‰ , (Fig. 6.2).

We found significant differences in isotopic composition between the POM in M3 and M5; $\delta^{13}\text{C}$ ($p=0.05$) and $\delta^{15}\text{N}$ ($p=0.05$) and between allochthonous and autochthonous POM; $\delta^{13}\text{C}$ ($p=0.001$) and $\delta^{15}\text{N}$ ($p=0.005$).

The macroinvertebrates associated to the studied habitats belong to a total of 29 groups, 14 of which are present in both the *C.nodosa-R.tinctoria* (M5) and *V.aegagropila* (M3) habitats. Only two groups *Cymodoce truncata* and *Nephtys hombergii* were found and analyzed in the bare sediment habitat. *C.truncata* was the only taxon present across the three habitats. (Table 6.2, Fig. 6.3). The third habitat, M4, bare sediment, in spite of the low $\delta^{15}\text{N}$ signature for the analysed macroinvertebrates, was not considered in the main comparative analysis due to the reduced number of groups and organisms collected.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for the studied macroinvertebrates presented a wide range among habitats.

The $\delta^{13}\text{C}$ values of macroinvertebrates fell within the studied potential food resources. Across the set of habitats the $\delta^{13}\text{C}$ values of macroinvertebrates ranged between $-27.3 \pm 3.6\text{‰}$ (*Loripes lucinalis*) in M3 and $-9.5 \pm 0.1\text{‰}$ (*Amphipholis squamata*) in M5, (Table 6.2, Fig. 6.3). The set of macroinvertebrate matching groups, across M3 and M5, did not present significant differences for $\delta^{13}\text{C}$ signatures Kruskal–Wallis test ($p>0.05$).

The $\delta^{15}\text{N}$ ranged between 1.9‰ (Amphipoda pool) in M5 and 6.6 ‰ (*Syllidae*) and 6.6 ± 0.2 ‰ (*Hippolyte* spp.) in M3, with exception of *Loripes lucinalis* which presented negative $\delta^{15}\text{N}$ values -0.3 ± 0.1 ‰ in M5 (Table 6.2, Fig. 6. 3). The study of the $\delta^{15}\text{N}$ signatures for the matching groups across the habitats showed differences between M5 and M3, being higher in the last (Table 6.2, Fig. 6.4). The habitat M4 presented the most reduced $\delta^{15}\text{N}$ value *Cymodoce truncata*, (Fig.6.4). The highest gap in $\delta^{15}\text{N}$ between the two sites was measured in *Loripes lucinalis* with a difference of 3.9‰ and the lowest by *Amphopolis squamata* which ranked 0.1‰ above in $\delta^{15}\text{N}$, (Fig. 6.4). Only Gammaridea pool had higher signature in site M5.

The linear regression for the $\delta^{15}\text{N}/\delta^{13}\text{C}$ in the two habitats is shown in (Fig. 6.5); the slopes of the regression lines are not significantly different ($P=0.130$), while the Y intercepts are ($P<0.001$). This indicates that macroinvertebrates in M3 have a significantly greater $\delta^{15}\text{N}$ composition than in M5.

Across the studied habitats a total of 7 feeding groups were identified i.e. grazers (G), predators (P), deposit feeders (D), suspension feeders (S) suspension feeders/scavengers (DS), predators/scavengers (PS), and suspension feeders/predators (SP). The general trend shows the predators and scavengers to be more $\delta^{15}\text{N}$ enriched in relation with the grazers, deposit feeders and suspension feeders, (Fig. 6.6 A, B). Thus, the average values of $\delta^{15}\text{N}$ were mostly consistent with proposed trophic groups (Fig. 6.4).

The relative trophic level (RTL) for the consumers calculated by means of the $\delta^{15}\text{N}$ ranged from 1.98 (Gammaridae and *Idotea baltica*) to 2.95 (Syllidea) in M3 (Fig. 6.7A), and from 1.78 (Amphipoda) to 2.97 (Collumbellidae) in M5 (Fig. 6.7B). In M3 the dominant group was the primary consumers (78%) groups and secondary consumers with (22%), on the other hand M5 compiled the 58% within the primary consumers and the 42% within the secondary consumers. The organisms with the highest $\delta^{15}\text{N}$ signatures ranked as secondary consumers. Congruence in RTL is maintained across the habitats for the matching macroinvertebrate groups, except for the *Hippolyte* spp. which ranks as primary consumer in the M5 and secondary consumer in M3. Also the grazers Collumbellidae and *Bittium* sp. ranked as secondary consumers, (Fig.6.7B).

6.4 Discussion

The coastal lagoon of Messolonghi suffers from several anthropogenic impacts, fisheries, extensive aquaculture, salt works, irrigation and sewage inflows as well as from physical modifications. Little is known about the effects of these sum of activities over the macroinvertebrate food web at short distance scales. In this study we attempted to disclose extent at which the main habitats and external inputs contribute as food sources for the benthic macroinvertebrate community. Additionally, we explored how the relative trophic level and feeding guilds may also be impacted by these factors.

The potential autochthonous sources were not possible to compare due to the different vegetation composition across the studies sites and reduced number of replicates. The allochthonous sources are mostly $\delta^{15}\text{N}$ enriched and $\delta^{13}\text{C}$ reduced compared with the lagoonal sources. Besides, compared with other Mediterranean coastal lagoons the $\delta^{13}\text{C}$ signature tends to match and the $\delta^{15}\text{N}$ is generally more depleted, (Carlier et al. 2007; Obrador and Pretus 2012; Vizzini et al. 2013; Vizzini and Mazzola 2003; Vizzini et al. 2005). The macroinvertebrate community was significantly $\delta^{15}\text{N}$ enriched in the *V.aegagropila* habitat, which lies close to the north irrigation canal. Additionally, unexpectedly, grazers were more impacted followed by deposit feeders and predators. The relative trophic levels kept the same structure across the studied habitats.

Potential food sources

The $\delta^{13}\text{C}$

The $\delta^{13}\text{C}$ signatures of potential resources indicated clearly the differences between the allochthonous and autochthonous material. Terrestrial plants are more $\delta^{13}\text{C}$ depleted compared with the algae and seagrass present within the lagoon. *Cymodocea nodosa* had the most enriched $\delta^{13}\text{C}$ composition among the potential sources. Besides, the POM from the irrigation canals and sewage outflow presented $\delta^{13}\text{C}$ significant depleted if compared with lagoonal ones, (Table 6.1, Fig.6.2).

The site M3 presents significant depleted $\delta^{13}\text{C}$ composition compared with the other two sites within the lagoon, indicating direct influence of the irrigation canals plume. Thus, a spatial transition in terms of $\delta^{13}\text{C}$ POM enrichment is observed from terrestrial inflows to M3 and to M4 and M5, (Table 6.1, Fig.6.2). POM isotopic values generally explain the phytoplankton composition, but the terrestrial waters normally also contain aquatic and terrestrial biogenic organic components. Riverine particulates have a combination of three organic carbon components, material from recent primary production, aged soil and fossil carbon from sedimentary rocks. Typically, plant debris dominates the sand-sized and larger fractions of the particulate organic carbon smaller-sized fractions, and especially the clays, have more complicated origins (Blair and Aller 2012). One of the characteristic of these set of materials, transported in suspension by the runoff, is to drive the $\delta^{13}\text{C}$ composition towards depletion, (Fry and Sherr 1989).

Besides, seasonal variations in the lagoonal POM may occur to both the straight link between POM and phytoplankton and also the variability of terrestrial inflows (Mazzola et al. 1999). Moreover, other meteorological factor as the winds sum to the lagoon shallowness (average 1m) will permit the resuspension of sedimentary material to be incorporated to the POM fraction.

The $\delta^{13}\text{C}$ signature in the SOM is highly enriched (12.8‰ to 13‰), similar results were obtained in (Vizzini et al. 2002) in the Stagnone di Marsala, Sicily. Conversely, our $\delta^{13}\text{C}$ SOM values are much enriched compared with other coastal and lagoonal ecosystems in the Mediterranean where $\delta^{13}\text{C}$ values range from 20‰ to 25‰, (Careddu et al. 2015; Vizzini et al. 2005).

This depletion might represent the high contribution of the main habitat *Cymodocea nodosa* that is dominant in the whole lagoon and has the most enriched $\delta^{13}\text{C}$ composition. Besides, the $\delta^{13}\text{C}$ signature for *P.oceanica*, which characterizes the adjacent environment to the lagoon, ranges from -6.19‰ Deudero et al. (2011) to -13.2‰ Michel et al. (2014). Hence, the organic matter from the extensive *P.oceanica* may be imported and sink as litter/detritus into the lagoonal ecosystem, thus contributing also to the enrichment of the $\delta^{13}\text{C}$ signature of the SOM.

We suggest that the terrestrial input in the form of POM might not sink in the sediment and but it is circulated out of the lagoon. Although we are not able to avert the role for other sources, either autochthonous or allochthonous organic matter, that might contribute to a partial depletion compared with the *Cymodocea nodosa*. Thus, the composition of the SOM appears to be significantly impacted by the seagrass $\delta^{13}\text{C}$ and this is reflected in the signature of *Amphipholis squamata* whose diet has been described as omnivorous, including plants fragments and fine particles (Stöhr et al. 2012).

Table 6.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) isotopic composition of the main sampled food sources. In bold the main vegetal habitat former. Particulate organic matter (POM), Sedimentary organic matter (SOM).

SITE	SOURCE	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		mean	SD	mean	SD
PRIMARY PRODUCERS					
M3	<i>Cladophora</i> sp. (n=1)	-18.4		4.8	
	<i>Palisada</i> sp. (n=1)	-20.5		5.4	
	<i>Valonia aegagropila</i> (n=2)	-19.9	0.1	2.8	0.0
M5	<i>Rytiphlaea tinctoria</i> (n=1)	-19.5		3.1	
	<i>Cymodocea nodosa</i> (n=1)	-9.4		0.1	
Terrestrial	<i>Arthrocnemum</i> sp. (n=1)	-25.8	0.2	6.0	0.5
	<i>Phragmites</i> sp. (n=1)	-24.7		8.7	
POM					
M3	POM M3 (n=3)	-24.3	0.3	3.4	0.0
M4	POM M4 (n=3)	-20.3	0.7	1.1	0.1
M5	POM M5 (n=3)	-22.5	0.2	1.7	0.2
SW	POM SW (n=3)	-29.5	0.1	3.3	0.4
IR1	POM IR1 (n=3)	-29.6	0.1	3.6	0.1
IR2	POM IR2 (n=3)	-30.3	0.2	1.5	0.2
SOM					
M3	SOM M3 (n=3)	-7.6		-	-
M4	SOM M4 (n=3)	-13	0.2	0.6	0.0
M5	SOM M5 (n=3)	-12.8		0.8	

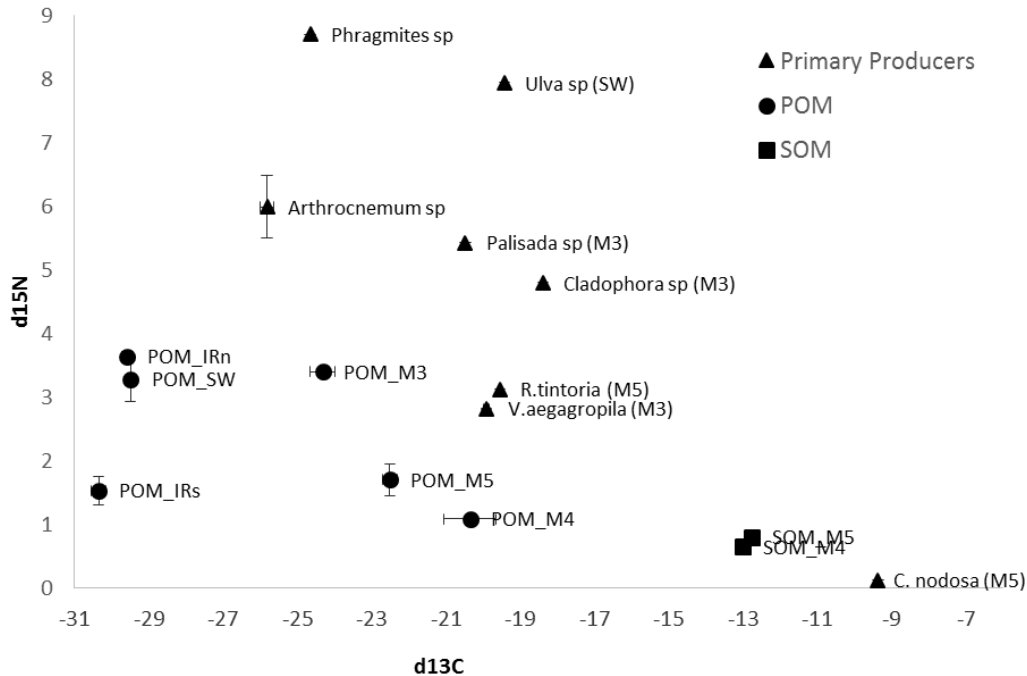


Fig 6.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of organic matter sources across the studied habitats and anthropogenic fresh waters inflows.

The $\delta^{15}\text{N}$

The $\delta^{15}\text{N}$ signature of the potential food sources is considerably reduced compared with other Mediterranean coastal lagoons. Allochthonous sources from terrestrial plants have the highest nitrogen composition as occurs with the algae *Ulva* sp. sampled next to the sewage outflow. The $\delta^{15}\text{N}$ values across the group of Chlorophyta and Phaeophyta algae is very dissimilar. From allochthonous sources the signature of the terrestrial plants *Arthrocnemum* sp. $\delta^{15}\text{N}$ 6‰ is more depleted compared with results in Carlier, Riera et al. 2007 where the average signature was 8.5‰. Besides, among the autochthonous vegetated material, the algae *Cladophora* sp, *Rityphlaea tinctoria* and *Valonia aegagropila*, and the seagrass *Cymodocea nodosa* are distinctly $\delta^{15}\text{N}$ depleted in comparison with the same groups in two Mediterranean lagoons (Vizzini et al. 2013; Vizzini and Mazzola 2003; Vizzini et al. 2002). Only the *Phragmites* sp. ($\delta^{15}\text{N}$ 8.7‰) and *Ulva* sp. ($\delta^{15}\text{N}$ 7.9‰) presented an slight higher values than in Carlier et al. (2007), (6.8‰ and 7.4‰) respectively.

In reference to POM the nitrogen composition is markedly more enriched in those purely anthropogenic sites as the North irrigation canal and the sewage outflow. The proper lagoonal sites (M4 and M5) and the Southern irrigation canal present significantly lower $\delta^{15}\text{N}$ signature. The site M3 is the most enriched, proper lagoonal site, which is affected by the outflow of the neighbor North irrigation canal. $\delta^{15}\text{N}$ from anthropogenic related activities as sewage waters and manure fertilization tends to enrich $\delta^{15}\text{N}$ signature of the runoff waters (Waldron et al. 2001). Conversely the effect of the industrial fertilizers has been described as having a signature close to 0‰, that reaches +8.8‰ due to enrichment after volatilization and denitrification (Anderson and Cabana 2005; Kendall 1998). The low $\delta^{15}\text{N}$ compared with other sewage treatment plants might be explain by the high rainfall registered the days before the sampling, (Table 6.1, Fig.6.2).

Thus we suggest that the clearly lower POM $\delta^{15}\text{N}$ signature, compared with other Mediterranean lagoons could be partly explained by the extensive influence of the waters originated from the irrigation canals where chemical fertilizers did not suffer high rates of volatilization and denitrification. In addition the denitrification processes in the water may increase the $\delta^{15}\text{N}$ values, while assimilation may have the reverse effect. When N is not limiting, plants preferably assimilate the lighter isotope (^{14}N) therefore acquiring a lower isotopic signal. This condition may be reflected in the SOM values (Anderson and Cabana 2005).

Finally, the $\delta^{15}\text{N}$ SOM averaging 0.7‰ is one of the lowest values yet published for a Mediterranean coastal lagoon. Vizzini et al. (2013) reported that values close to zero might be explained by the activity carried out by N_2 -fixing microorganisms, due to the low fractionation during nitrogen fixation (Owens and Law 1989).

The macroinvertebrate community

Across matching groups of macroinvertebrates we found a pattern of $\delta^{15}\text{N}$ enrichment between the two studied sites, yet our levels of $\delta^{15}\text{N}$ fall below the average found in Mediterranean coastal lagoons with similar sources of impact (Vizzini et al. 2013), (Table 6.2, Fig.6.3). They are far less enriched than the food web in Vizzini et al. (2005) where the $\delta^{15}\text{N}$ signatures in primary and secondary consumers were far higher. The equilibrium of denitrification and assimilation processes may lead to an enriched or depleted $\delta^{15}\text{N}$ lagoonal system respectively. The nitrification process may increase the $\delta^{15}\text{N}$ values of aquatic food webs and assimilation may have the reverse effect, since plants preferably assimilate the lighter isotope (^{14}N) when N is not limiting and could therefore acquire a lower isotopic signal (compared with the $\delta^{15}\text{N}$ of inorganic N) in N-enriched rivers (Kendall 1998).

Table 6.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰)macroinvertebrates. Matching groups across at least 2 habitats in bold. According with their feeding traits, organism were classified in> suspension feeder-predator (SP), grazer (G), deposit feeder (D), predator (P), suspension feeder (S), deposit feeder-scavenger (DS) and predator-scavenger (PS). n= number of replicate samples. SD= standard deviation

SITE	fauna	Feeding group	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
			mean	SD	mean	SD
M3	Actiniaria (n=2)	SP	-18.0	0.9	5.0	0.1
	<i>Amphipolis squamata</i> (n=1)	D	-10.7		4.6	
	Aoridae (n=1)	G	-17.6		4.4	
	<i>Cerithium</i> sp (n=1)	G	-16.0		3.5	
	<i>Cyodoce truncata</i> (n=2)	G	-15.1	0.1	4.0	0.1
	Dorvillidae (n=1)	P	-16.7		4.5	
	Gammaridae (n=2)	G	-16.4	0.2	3.3	0.1
	Gibbula sp (n=1)	G	-15.8		4.6	
	<i>Harmothoe spinifera</i> (n=1)	P	-15.4		6.7	
	Hippolyte spp. (n=2)	G	-13.4	0.1	6.6	0.2
	<i>Idotea baltica</i> (n=1)	G	-16.4		3.3	
	<i>Loripes lucinalis</i> (n=1)	S	-27.3		3.6	
	Lumbrineridae (n=2)	DS	-16.6	0.1	6.3	0.0
	Nereididae (n=1)	P	-14.5		3.8	
	Orbinidae (n=2)	D	-16.2	0.0	5.0	0.2
Syllidae (n=1)	P	-15.0		6.6		
Terebellidae (n=1)	D	-15.6		4.7		
M4	<i>Cyodoce truncata</i> (n=1)	G	-13.8		1.6	
	<i>Nephtys hombergii</i> (n=1)	PS	-10.9		3.8	
M5	Amphinomidae (n=1)	P	-13.7		4.0	
	Amphipoda pool (n=1)	G	-15.6		1.9	
	<i>Amphipolis squamata</i> (n=2)	D	-9.5	0.1	4.5	0.0
	Aoridae (n=1)	G	-15.8		2.0	
	<i>Asterina gibosa</i> (n=2)	P	-9.9	0.1	5.1	0.1
	<i>Bittium</i> sp (n=1)	G	-15.1		5.3	
	Collumbellidae (n=1)	G	-20.5		6.0	
	Conidae (n=1)	P	-14.6		5.0	
	<i>Cyodoce truncata</i> (n=2)	G	-13.6	0.1	2.6	0.2
	<i>Fusinus</i> sp (n=1)	P	-16.9		5.3	
	Gammaridae (n=1)	G	-15.7		4.2	
	<i>Harmothoe spinifera</i> (n=1)	P	-14.6		5.8	
	Hippolyte spp. (n=1)	G	-16.1		4.2	
	<i>Idotea baltica</i> (n=1)	G	-16.8		2.7	
	<i>Liocarcinus</i> sp (n=1)	PS	-13.1		5.0	
	<i>Loripes lucinalis</i> (n=2)	S	-25.5	0.1	-0.3	0.2
	Lumbrineridae (n=1)	DS	-15.4		5.2	
	Nereididae (n=2)	P	-16.7	0.0	3.5	0.2
	Orbinidae (n=3)	D	-16.2	0.1	3.7	0.2
	Phyllodoceidae (n=1)	P	-14.3		5.1	
Pussilina sp (n=1)	G	-14.4		3.8		
Syllidae (n=1)	P	-14.9		5.1		
Terebellidae (n=1)	D	-15.0		3.7		
Trochidae (n=1)	D	-16.0		3.6		

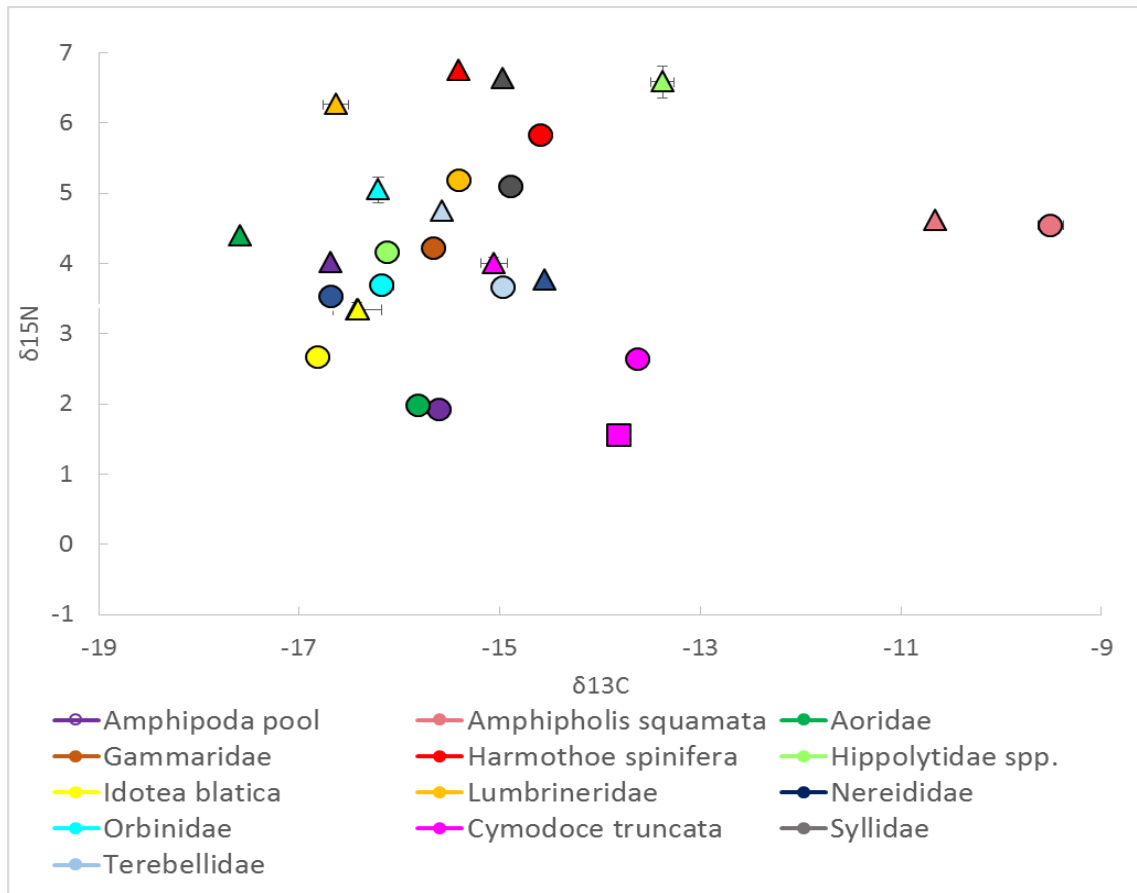


Fig 6.3. Macroinvertebrates $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ signatures for the matching macroinvertebrates groups across the studied sites. Triangles (M3), squares (M4) and circles (M5). *Loripes lucinalis* is out of the graph due to representativeness of the other groups, M3 (3.5 $\delta^{15}\text{N}$ -27.2 $\delta^{13}\text{C}$) and M5 (0.3 $\delta^{15}\text{N}$ -25.5 $\delta^{13}\text{C}$).

$\delta^{15}\text{N}$ enrichment present in the *V.aegagropila* habitat endorse the hypothesis that anthropogenic related enrichment, from fresh water effluents can be discerned by means of isotope organism in the macroinvertebrate community in relatively short distances (Connolly et al. 2013; Vizzini et al. 2013), (Fig.6.4 and Fig.6.5). Other factors apart of distance from the source can contribute to the enrichment rate. Different structural complexity of the algae and seagrass can contribute to different rates of sedimentation and transit of waters from anthropogenic inputs. The depth and the local currents can divert the plume of effluents and impact differently neighbor sites. Else, more seasonal shifts in isotopic composition have been widely described (Carlier et al. 2007; González-Ortegón et al. 2015; Vizzini and Mazzola 2003). Climatological and meteorological aspects as temperatures, rains may affect directly the POM composition, which might be reflected in the benthic food web.

It was not possible to distinguish between the effects of each allochthonous organic matter source (irrigation and sewage). Therefore the overall impact generated by anthropogenic activities over the macroinvertebrate food web was evaluated.

The set of 14 groups of benthic macroinvertebrates, common in the sites M3 and M5, presented a significant different $\delta^{15}\text{N}$ signature between the two main studied habitats. The majority of the groups were

clearly more enriched in M3 than M5, and only Gammaridea pool had higher signature in the site M5, (Fig.6.4 and Fig.6.5).

The highest gap in $\delta^{15}\text{N}$ between the two sites was measured in *Loripes lucinalis* with a difference of 3.9‰. This difference might be enhanced by the presence of diazotroph symbiont organisms in the site M5, explained by the negative values that *L. lucinalis* registered at this site (Kerhereve et al. 2001). It appears that the groups with differences over 2‰ are the grazers; Amphipoda pool, Aoridae and *Hippolyte spp.* Next, the deposit feeders present gains between 1‰ and 2‰, but the *Amphipholis squamata* has the lowest enrichment, 0.1‰. Last, the predators and scavengers range with a gain between 0.3‰ in Nereididae and 1.5‰ in Syllidae (Fig. 6.4).

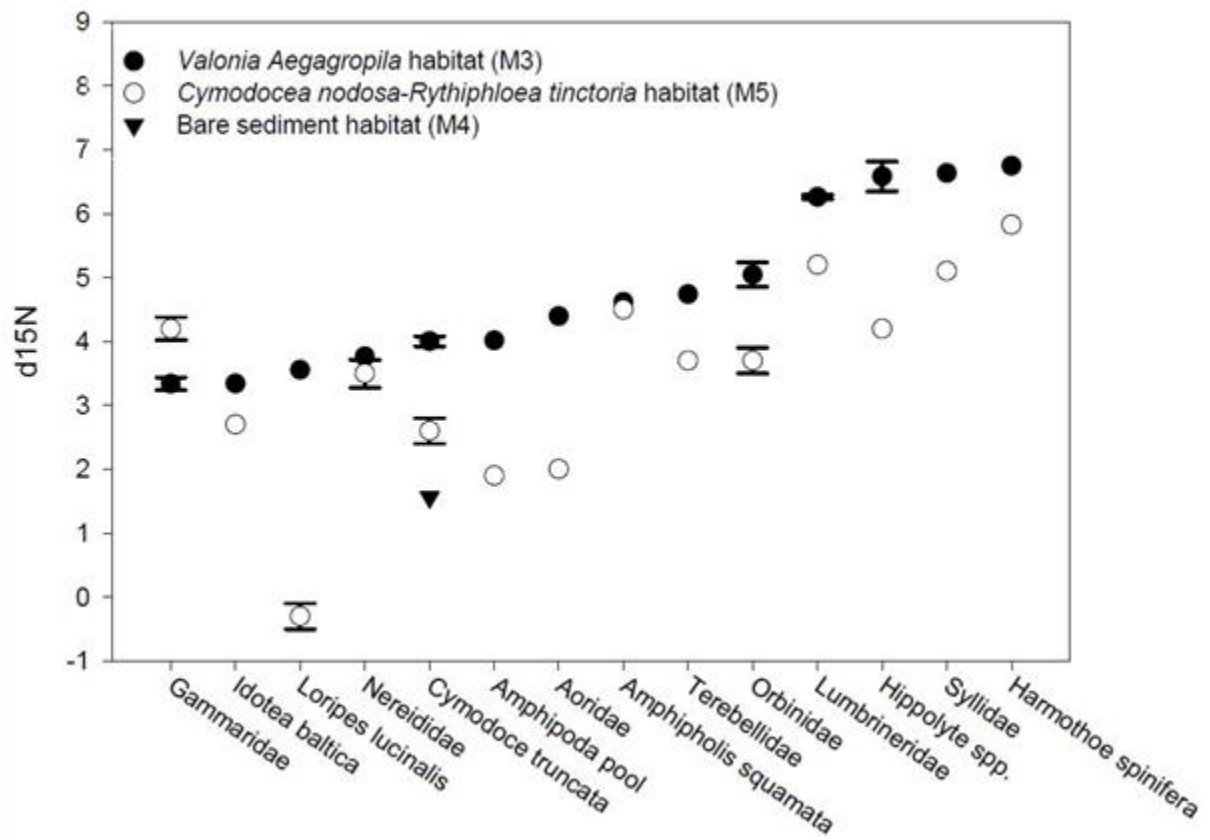


Fig.6.4. $\delta^{15}\text{N}$ values for the feeding groups matching across the three studied habitats.

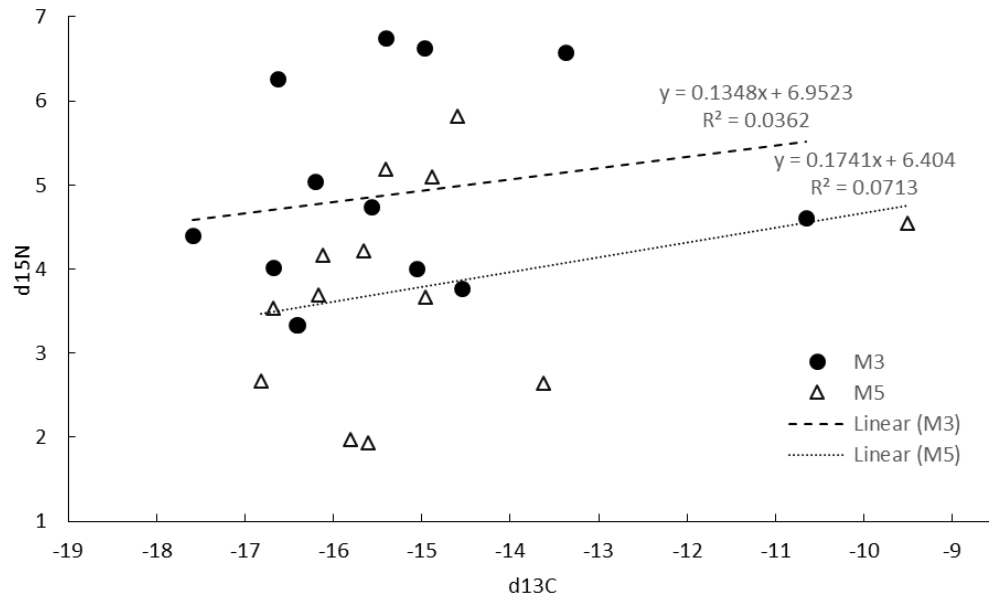


Fig.6.5. Scatterplot of mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of matching species of macroinvertebrate across the *V.aegagropila* and *C.nodosa-R.tinctoria* habitats. The out layer *Loripes lucinalis* was lumped out of the analysis.

In conclusion, between the 2 habitats there is a general trend where grazers reflect the major enrichment followed by the deposit feeders and the last the predators. This results are unexpected because in benthic macroinvertebrates and other organism the $\delta^{15}\text{N}$ composition becomes enriched with the increase of the trophic level (Jennings et al. 1997). Conversely, (Connolly et al. 2013) found the same rate of $\delta^{15}\text{N}$ enrichment across multiple marine taxa. We suggest that $\delta^{15}\text{N}$ enrichment in the environment may drive to diversification in the food sources (Tewfik et al. 2007) and also macroinvertebrates diet diversification (Armitage and Fourqurean 2009; Careddu et al. 2015), thus generating trophic groups differently impacted by the $\delta^{15}\text{N}$ enrichment. Besides, combination of factors as high productivity and low stress has been proved to increase of $\delta^{15}\text{N}$ -value in predators (Anderson and Cabana 2009).

The relative trophic levels, of the groups of common organisms, between two main studied habitats, were similar (Fig. 6.7A, B). Changes in food chain length and trophic structure were related to a variety of factors; body size (Cohen et al. 1993; Jennings et al. 2002), dimensionality of the environment (Briand and Cohen 1987), ecosystem size (Jake Vander Zanden and Fetzer 2007) and environmental perturbations (Pimm 1982; Pimm et al. 1991). In *P.oceanica* meadows shifts in the macroinvertebrate trophic structure were related to the reduction of organic matter in Calizza et al. (2013).

In this study the RTL only changes in the grazer *Hippolyte* spp., which falls within the secondary consumers group in M3 and within the primary consumers in M5, (Fig. 6.7A, B). This shift might be explained by the presence of different species within the group but also by the dynamism of the trophic levels (Jennings et al. 1997; Polis and Strong 1996)

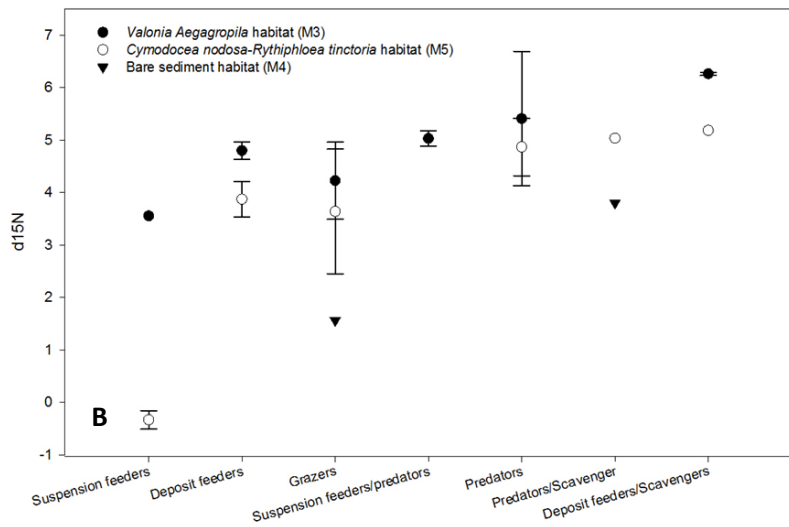
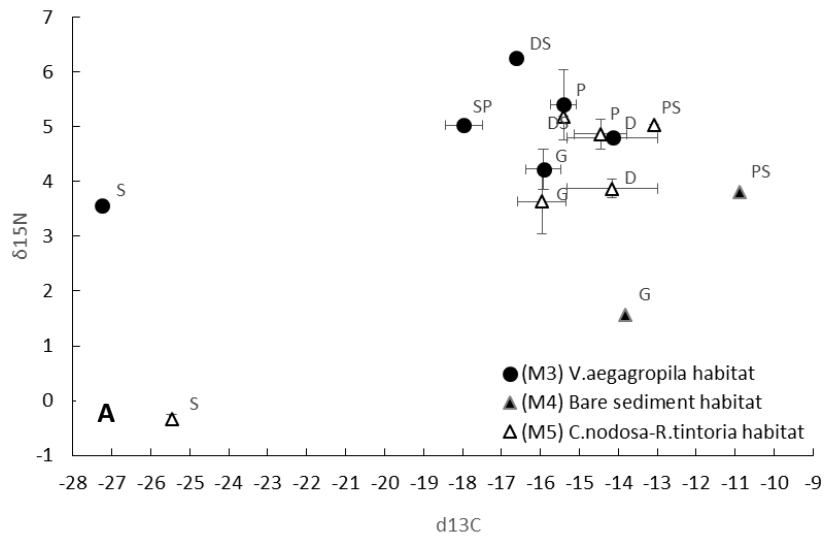


Fig 6.6. (A) Feeding traits distribution across the three studied habitats. G-grazer, P-predator, D -deposit feeder, S-suspension feeder, DS- Deposit feeder/scavenger, PS- Predator/Scavenger and SP-suspension feeder/predator. (B) $\delta^{15}\text{N}$ values for the macroinvertebrates feeding guilds across the three studied habitats.

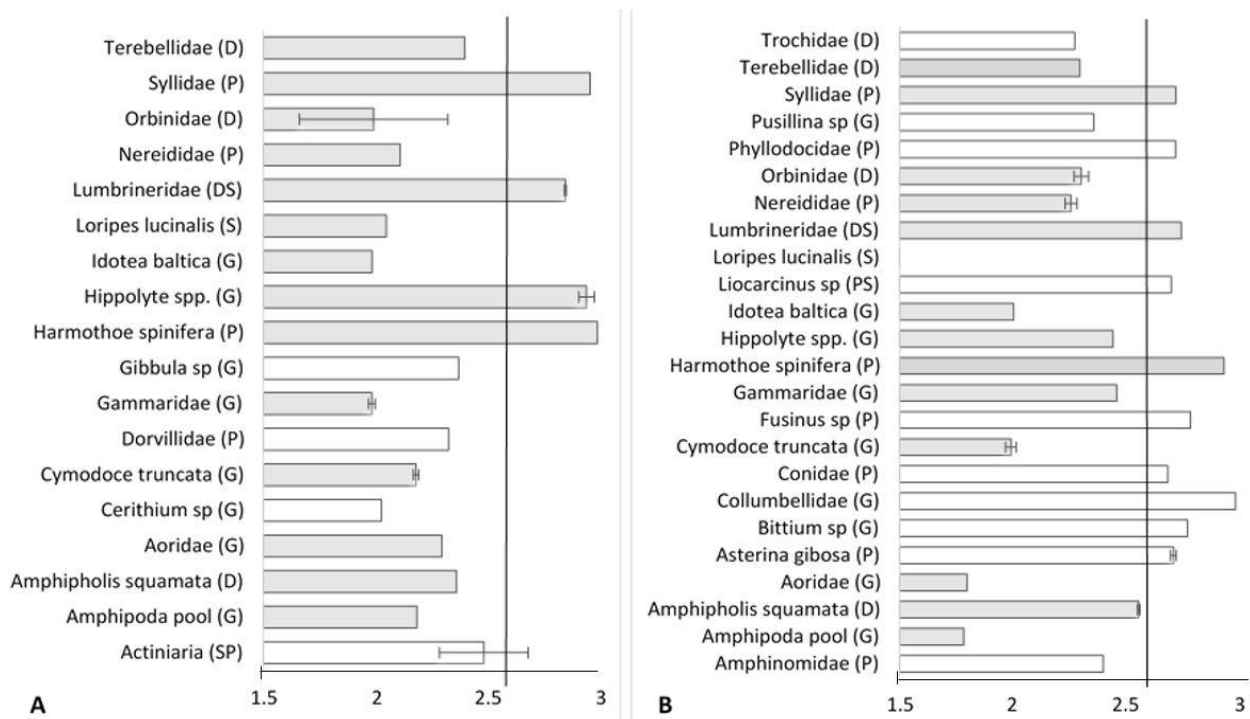


Fig. 6.7. Relative trophic levels (RTL) for the macroinvertebrates in the site M3 (A) and M5 (B). Vertical line; 2.6 threshold value from primary consumers to secondary consumers. Groups present across both habitats in grey column. *Loripes lucinalis* in M5 $\delta^{15}\text{N}$; 1.17‰ (out of graph scale). In brackets feeding guilds; G-grazer, P-predator, D -deposit feeder, S-suspension feeder, DS- Deposit feeder/scavenger, PS- Predator/Scavenger and SP-suspension feeder/predator

Overall, lower levels of $\delta^{15}\text{N}$ in the POM and macroinvertebrate food web support more groups of secondary consumers. Due to the qualitative aspect of this study (i.e. 5 replicates per site, sieved in 0.05mm) we cannot state it in terms of density of organism but taxonomic diversity. M3 presented about a threefold more primary consumers groups than secondary, meanwhile in the M5 the proportion was more balanced and the number of primary consumers was only 10% above the secondary consumers. M3, appears to show a typical pyramidal food web where most species are assigned to the primary consumers, fewer species are grouped in the group of secondary consumers (Sokołowski et al. 2012). Conversely the M5 shows a more balanced food web.

The unbalanced trophic levels and macroinvertebrate food web shifts have been explained; gradients of natural stress caused by the freshwater inflow or salinity gradients (Carlier et al. 2007), nutrients enrichment gradients (Armitage and Fong 2004) and biodiversity (Sokołowski et al. 2012)

However, we found a direct relation of the feeding traits with the relative trophic level. Deposit and suspension feeders dominate the primary consumer level. Conversely, predators, scavengers and few grazers dominate the secondary trophic level. Nonetheless, predators as Amphinomidae, Dorvillidae and Nereididae fall within the first consumer's trophic level. These incongruences might be linked to the basal values for the calculation of the RTL, which may be different for dissimilar environments and organism.

6.5 Conclusions

The $\delta^{15}\text{N}$ signatures of nitrogen at the base of the lagoonal food web are good indicators of the anthropogenic inputs at a small scale. The autochthonous *Cymodocea nodosa*, larger lagoonal habitat former, appears to highly contribute to the formation of the lagoonal SOM; reflected in the high ^{13}C enrichment. Besides, allochthonous sources, from anthropogenic activities appear to highly impact the lagoonal food web in both the short and wider scale.

In the wider scale, the $\delta^{15}\text{N}$ signature Messolonghi lagoon appears to be one of the most depleted in the Mediterranean. This condition may reflect the intensive agriculture and extensive chemical fertilization in the Western drainage area. Besides, in the short distance, the anthropogenic activities, sewage effluent and irrigation runoff, have been traced by means of $\delta^{15}\text{N}$ signature in the POM and macroinvertebrate community. Across the studied locations 14 macroinvertebrate taxonomic groups reflected the $\delta^{15}\text{N}$ enrichment at short distance from the IRn. Variations of nutrient loads may drive to the diversification of food sources and macroinvertebrate diet reflected by the unexpected levels of accumulation in relation with the feeding guilds of the macroinvertebrates. Although, the relative trophic level for the macroinvertebrates remained similar across the studied locations, the two sites presented different structure. Unbalances in the trophic structure can reflect conditions of natural and anthropogenic stress from salinity shifts and nutrient load.

This research due to the lack of material for analysis been done with a limited number of replicaters, this condition makes the data analysis and interpretation rather weak.

More specific research with more replicates and focusing in seasonal changes and accounting for more energy sources may bring more clear results about dietary shifts and levels of impact accumulation over the macroinvertebrate food web and by extension to the lagoonal food web.

7 Summary and Conclusions

The present research was developed in four stages. Originally, we described the benthic invertebrate communities and the environmental variables structuring them, in a coastal lagoon. At the same time, the Ecological Quality Status (EQS) was assessed, using indices fulfilling the Water Framework Directive requirements for the Mediterranean region. Then, the natural variability of the communities was studied, by means of β -diversity, following a multi scale approach. In order to investigate further the role of the habitat complexity we applied fractal dimension indices as complexity proxies. Finally, we investigated the food web of the benthic invertebrates in relation to anthropogenic inputs.

The area of study is the main lagoon of Messolonghi, which is an open lagoon communicating with the sea through a shallow and wide frontal area. To develop this PhD project we set up a network of 7 stations covering the main benthic habitats across the whole lagoon. Collection of benthic samples took place four times during 2013 (January, April, July and November). At the same time additional sediment samples were collected at each site, for granulometry and total carbon analysis. At each sampling site, salinity, temperature, pH and dissolved oxygen were monitored close to the bottom using a multi-probe meter. A separate campaign was carried in November 2014 for the study of the food web. Benthic animals were collected from three different habitats together with their potential food sources (plankton, organic matter in the sediment, and vegetation) for isotope analysis

The lagoon of Messolonghi is a eualine Mediterranean lagoon with no confinement pattern identified. It supports one of the most diverse lagoonal benthic communities in the Mediterranean, a fact which may be explained by the high surface area and the variety of habitats. Habitat type constitutes the major component of variation for both taxonomic and functional macroinvertebrate assemblages. The vegetation biomass, the percentage of sand fraction and the total carbon in sediment are the main variables structuring the benthic macroinvertebrates communities. Four main type of communities were identified: three of them are characterized by natural habit types (i.e. vegetated, marine and unvegetated) and a fourth affected by the sewage treatment outflow from Aetoliko. The Ecological Quality Status of the benthic habitats as estimated by BENTIX, AMBI and M-AMBI is overall good, yet there are 2 sites which present lower EQS rating from moderate to poor depending on the used indicator. There is a slight disagreement amongst the applied indicators, BENTIX being, in general, the most conservative.

Based on the findings that habitat type is the major factor structuring the macroinvertebrate communities we explored the role of habitat complexity. We documented the usefulness of fractal dimension indices as complexity proxies, revealing strengths but also presenting methodological limitations. We pointed out the need of choosing an ecologically meaningful scale to address the habitat complexity according to the organism studied. The biological traits of the specific organism should be taken into account. Organisms belonging to the same group, e.g. meiofauna, macrofauna, megafauna, cover a wide variety of habitat preferences, body size, motility, reproduction, larval dispersal. The aggregation of organisms with different traits within the same scale may lead to imprecise answers. At the community level macroinvertebrate species richness, abundance and diversity, highly correlated with the complexity of the samples. A greater variability in the body size of the macroinvertebrate organisms correlated with the increase of the habitat complexity. These outcomes reveal the significance of considering habitat complexity in addition to the traditional environmental variables for the study of macroinvertebrate communities. Yet, more research is needed to set detection limits for fractal indices.

The variability of the benthic communities in both spatial and temporal scale was also studied. By means of a β -diversity approach, at both functional and taxonomic levels, we found a slight correlation of

the functional β -diversity with environmental heterogeneity. Besides, functional β -diversity appears more redundant than taxonomic β -diversity, even though a low taxonomic replacement but a high functional replacement was recorded in a habitat enriched by anthropogenic organic matter. With this piece of our work we highlight the importance and complementarity of studying both, taxonomic and functional diversity to better understand the ecological processes. Besides, despite the anticipated high connectivity across the macroinvertebrate lagoonal populations, with this work we have demonstrated that in the lagoon of Messolonghi the studied habitats present independent seasonal patterns. Thus, different processes may shape the functional and taxonomic assemblages.

By means of isotope analysis we found that, overall, the studied organisms and food sources had a rather low $\delta^{15}\text{N}$ signature, which is one of the lowest mentioned for similar types in Mediterranean coastal lagoons. Moreover, across the studied locations 14 macroinvertebrate taxonomic groups reflected the $\delta^{15}\text{N}$ enrichment at a relatively short distance from a canal introducing irrigation and runoff waters in the north-west part of the main lagoon of Messolonghi. Although, the relative trophic level for the macroinvertebrates remained similar across the studied locations, the two sites presented different structure.

The results obtained within the framework of this PhD project contribute to 1st, support decision makers in order to improve the management of the lagoon sector at the Marine Protected Area of Messolonghi. 2nd expand the scientific knowledge about Mediterranean lagoonal ecosystems and the benthic macroinvertebrate communities.

Περίληψη και Συμπεράσματα

Η παρούσα διδακτορική διατριβή αναπτύχθηκε σε τέσσερα στάδια. Αρχικώς περιγράψαμε τις βιοκοινωνίες των βενθικών ασπονδύλων σε μια παράκτια λιμνοθάλασσα καθώς και τους περιβαλλοντικούς παράγοντες που τις διαμορφώνουν. Συγχρόνως εκτιμήθηκε η Οικολογική τους ποιότητα σύμφωνα με τους δείκτες που καθορίζονται για τη Μεσόγειο από την Οδηγία Πλαίσιο για τα Υδάτα. Κατόπιν, μέσω της ποικιλότητας- β , μελετήθηκε η μεταβλητότητα των βιοκοινωνιών σύμφωνα με μια πολυμεταβλητή προσέγγιση. Προκειμένου να διερευνηθεί περαιτέρω ο ρόλος της πολυπλοκότητας του ενδιαίτηματος χρησιμοποιήσαμε τη διάσταση των μορφοκλασματικών συνόλων ως εκφραστή της πολυπλοκότητας. Τέλος, διερευνήσαμε το τροφικό πλέγμα των βενθικών ασπονδύλων σε σχέση με ανθρωπογενείς εισροές.

Η περιοχή μελέτης είναι η κυρίως λιμνοθάλασσα του Μεσολογγίου, μια ανοικτή λιμνοθάλασσα που επικοινωνεί με την ανοικτή θάλασσα μέσω ενός ανοικτού και ρηχού μετώπου. Για το σκοπό της διατριβής επιλέχθηκαν 7 σταθμοί που καλύπτουν τα κύρια βενθικά ενδιαίτηματα της λιμνοθάλασσας. Τα βενθικά δείγματα συλλέχθηκαν εποχικά (Ιανουάριο, Απρίλιο, Ιούλιο και Νοέμβριο) το 2013. Συγχρόνως συλλέχθηκαν δείγματα ιζημάτων για κοκκομετρική ανάλυση και ανάλυση του περιεχομένου σε άνθρακα. Σε μία συμπληρωματική δειγματοληψία που έγινε τον Νοέμβριο του 2014, συλλέχθηκαν από τρία ενδιαίτηματα βενθικοί οργανισμοί και η πιθανή τροφή τους (πλαγκτό, οργανικό υλικό από το ίζημα και φυτικοί οργανισμοί) για ανάλυση σταθερών ισοτόπων.

Η λιμνοθάλασσα του Μεσολογγίου είναι μια εύαλη λιμνοθάλασσα που δεν παρουσιάζει πρότυπο περιορισμού. Υποστηρίζει μια από τις υψηλότερες ποικιλότητες αντίστοιχων περιοχών της Μεσογείου, γεγονός που μπορεί να ερμηνευθεί από τη μεγάλη της έκταση και την ποικιλία των ενδιαιτημάτων της. Ο τύπος ενδιαίτηματος είναι η κύρια συνιστώσα τόσο της ταξινομικής όσο και της λειτουργικής σύνθεσης των βιοκοινωνιών. Η βιομάζα των φυτών, το ποσοστό της άμμου και η συνολική ποσότητα του άνθρακα είναι οι κύριες μεταβλητές που διαμορφώνουν τις βιοκοινωνίες των ασπονδύλων. Τρεις από αυτές τις βιοκοινωνίες συνδέονται με φυσικά ενδιαίτηματα, (δηλ. Καλυπτόμενα από βλάστηση, θαλάσσια, χωρίς βλάστηση) και ένα που επηρεάζεται από τη γειτνίαση με την έξοδο του αγωγού επεξεργασίας λυμάτων του Αιτωλικού. Η οικολογική ποιότητα των βενθικών ενδιαιτημάτων που εκτιμήθηκε με τους δείκτες BENTIX,

AMBI and M-AMBI, παρουσιάστηκε γενικώς ως «καλή», αν και δύο σημεία παρουσίασαν χαμηλότερους δείκτες με περιβαλλοντική κατάσταση από «μέτρια» ως «κακή». Υπάρχει μια ελαφρά ασυμφωνία μεταξύ των δεικτών, με τον BENTIX να είναι ο πλέον συντηρητικός.

Γνωρίζοντας ότι ο τύπος του ενδιαιτήματος είναι ο κύριος παράγοντας που διαμορφώνει τις βιοκοινωνίες των βενθικών ασπονδύλων διερευνήσαμε το ρόλο της πολυπλοκότητας του. Τεκμηριώσαμε τη χρησιμότητα των δεικτών της διάστασης fractal ως εκφραστών της πολυπλοκότητας. Οι δείκτες παρουσίασαν τόσο δυνατότητες όσο και μεθοδολογικούς περιορισμούς. Επισημάναμε την ανάγκη επιλογής της κατάλληλης κλίμακας ανάλογα με τον μελετώμενο οργανισμό και τα βιολογικά του χαρακτηριστικά. Οι οργανισμοί, αν και μπορεί να ανήκουν στην ίδια ομάδα, π.χ. μειοπανίδα, μακροπανίδα, μεγαπανίδα, παρουσιάζουν μεγάλη ποικιλία προτιμήσεων, σωματικών διαστάσεων, κινητικότητας, αναπαραγωγής, διασποράς προνυμφών. Η συμπερίληψη οργανισμών με διαφορετικά χαρακτηριστικά στην ίδια κλίμακα μπορεί να δώσουν ανακριβή αποτελέσματα. Στο επίπεδο της βιοκοινωνίας, υπήρξε υψηλή συσχέτιση του αριθμού των ειδών, της αφθονίας και τη ποικιλότητας, με την πολυπλοκότητα των δειγμάτων. Τα αποτελέσματα αυτά δείχνουν πόσο σημαντικό είναι να λαμβάνεται υπ' όψη και η πολυπλοκότητα του ενδιαιτήματος μαζί με τις συνηθισμένες περιβαλλοντικές παραμέτρους κατά τη μελέτη των βιοκοινωνιών των ασπονδύλων. Πάντως, απαιτείται πολλή έρευνα ακόμη, μέχρι να καθοριστούν τα όρια των δεικτών των μορφοκλασματικών συνόλων.

Μελετήθηκε επίσης, η διακύμανση των βενθικών βιοκοινωνιών σε χωρική και χρονική κλίμακα. Η β-ποικιλότητα, μελετήθηκε τόσο σε ταξινομικό επίπεδο όσο και σε επίπεδο λειτουργικών χαρακτηριστικών, και βρέθηκε να συσχετίζεται χαλαρά με το δεύτερο. Επίσης η λειτουργική β-ποικιλότητα εμφανίζεται πιο εύκολο να μεταβληθεί (Redundant) από την ταξινομική β-ποικιλότητα, αν και, στις περιπτώσεις εμπλουτισμού με ανθρωπογενές οργανικό υλικό, η αντικατάσταση ταξινομικών ομάδων ήταν μεγαλύτερη από αυτή των λειτουργικών. Το τμήμα αυτό της διατριβής φωτίζει τη σημασία και την από κοινού μελέτη ταξινομικής και λειτουργικής ποικιλότητας για την καλύτερη κατανόηση των οικολογικών διεργασιών. Βρέθηκε επίσης ότι, παρά την αναμενόμενη σύνδεση μεταξύ των λιμνοθαλάσσιων πληθυσμών, τα ενδιαιτήματα της λιμνοθάλασσας του Μεσολογίου παρουσιάζουν εποχικά πρότυπα ανεξάρτητα μεταξύ τους. Είναι πιθανό, λοιπόν, οι ταξινομικές και λειτουργικές συγκεντρώσεις να καθορίζονται από διαφορετικές διεργασίες.

Μέσω της ανάλυσης των ισοτόπων βρήκαμε ότι, γενικώς, οι μελετούμενοι οργανισμοί και οι πηγές τροφής τους είχαν πολύ χαμηλό αποτύπωμα $\delta^{15}\text{N}$, ευρισκόμενο ανάμεσα στα χαμηλότερα που αναφέρονται για αντίστοιχους Μεσογειακούς τύπους. Επιπλέον, 14 ταξινομικές ομάδες από αυτές που μελετήθηκαν παρουσίασαν εμπλουτισμό σε $\delta^{15}\text{N}$ σε περιοχές κοντά στα αποστραγγιστικά κανάλια στο δυτικό τμήμα της λιμνοθάλασσας Μεσολογίου. Παρ'όλο που το σχετικό τροφικό επίπεδο των ασπονδύλων ήταν παρόμοιο σε όλα τα υπόλοιπα σημεία μελέτης, αυτές οι δύο περιοχές παρουσίασαν διαφορετική σύνθεση.

Τα αποτελέσματα αυτής της διατριβής μπορούν να χρησιμοποιηθούν για την καλύτερη διαχείριση της λιμνοθάλασσας από αυτούς που παίρνουν τις αποφάσεις, ενώ επεκτείνουν την επιστημονική γνώση σχετικά με τις βενθικές βιοκοινωνίες των λιμνοθαλάσσιων οικοσυστημάτων της Μεσογείου.

8 Publications and conferences

PUBLICATIONS

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. (2013) Towards the implementation of the Water Framework Directive in Mediterranean transitional waters: the use of macroinvertebrates as biological quality elements. *Advances in Oceanography and Limnology* 4:212-240

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. (2016) Multiscale functional and taxonomic β -diversity of the macroinvertebrate communities in a Mediterranean coastal lagoon. *Mediterranean Marine Science*. *In press*

CONFERENCES

-Oral presentations

Cabana D, Sigala K, Nicolaidou A, Basset A, Reizopoulou S. Benthic taxonomic and functional diversity patterns across habitats in Messolonghi lagoon, Greece. VI Eurolag & VII Lagunet Conference, Lecce, December 2013.

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. Influences of habitat complexity on benthic macroinvertebrate communities; does biological trait composition responds regularly across seasons? 49th European Marine Biology Symposium. St Petersburg, September 2014.

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. Functional composition of benthic communities across habitat variety in Messolonghi lagoon, Greece. *Littoral* 2014. Klaipeda, September 2014.

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. Habitat complexity by means of fractal dimension; a body length and community structure modulator for benthic macroinvertebrate communities in a lagoonal ecosystem. International Conference on Mediterranean Marine Protected Areas. MMMPA & CIESM Committee VI (Coastal Ecosystems and Marine Policy). Ancona, October 2015.

-Posters

Providakis N, Cabana D, Arevalo E, Sigala K, Ibanhez SP, Nicolaidou A, and Reizopoulou S. Benthic Diversity in Messolonghi Lagoon, Greece. 48th European Marine Biology Symposium. Galway, August 2013.

Cabana D, Sigala K, Nicolaidou A, Reizopoulou S. Management guidelines for Mediterranean coastal lagoons. The use of benthic macroinvertebrate communities as biological quality indicators. International Conference on Mediterranean Marine Protected Areas. MMMPA & CIESM Committee VI (Coastal Ecosystems and Marine Policy). Ancona, October 2015.

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Health

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10 Species list

List of taxonomic groups identified in the frame of this PhD project.

<i>Abarenicola claparedi</i>	<i>Erinaceusyllis cryptica</i>	<i>Monocorophium</i>	<i>Pisidia</i>
<i>Abra alba</i>	<i>Eteone</i> sp.	<i>Monocorophium insidiosum</i>	<i>Pista cristata</i>
<i>Abra prismatica</i>	<i>Eulalia</i> sp.	<i>Monticellina</i> sp.	Platyhelminthes
<i>Abra segmentum</i>	<i>Eulalia clavigera</i>	<i>Myrianida</i> sp.	<i>Platynereis dumerilii</i>
<i>Achelia echinata</i>	<i>Eumida sanguinea</i>	<i>Myrianida brachycephala</i>	<i>Polittapes aureus</i>
Actiniidae	<i>Euphrosine myrtosa</i>	<i>Myrtea spinifera</i>	<i>Polycirrus medusa</i>
<i>Ampelisca</i> sp.	<i>Exogone dispar</i>	<i>Mysidae</i> sp.	<i>Prionospio</i> sp.
<i>Amphiglena mediterranea</i>	<i>Exogone naidina</i>	<i>Mysta picta</i>	<i>Protoaricia</i> sp.
<i>Amphipholis squamata</i>	<i>Fabricia</i> sp.	<i>Mytilaster minimus</i>	<i>Protoaricia oerstedii</i>
<i>Apsudes</i> sp.	<i>Fabricia stellaris</i>	<i>Naineris laevigata</i>	<i>Pseudopolydora antennata</i>
<i>Arabella geniculata</i>	<i>Fusinus rusticulus</i>	<i>Nassarius cuvierii</i>	<i>Pseudopotamilla cerasina</i>
<i>Arenicola</i> sp.	<i>Gammarella</i> sp.	<i>Nassarius reticulatus</i>	Pycnogonidae
<i>Aricidea</i> sp.	<i>Gammarella fucicola</i>	<i>Neanthes</i> sp.	<i>Retusa trunca</i>
<i>Aricidea capensis</i>	<i>Gammarus insensibilis</i>	<i>Neanthes caudata</i>	<i>Rhynchocinetes serratus</i>
<i>Aricidea cerrutii</i>	<i>Gibbula</i> sp.	Nematoda	<i>Ruditapes decussatus</i>
<i>Armandia cirrhosa</i>	<i>Gibbula adansonii</i>	Nemertea	Sagittidae
<i>Asterina</i> sp.	<i>Gibbula pennanti</i>	<i>N. pseudocorrugata</i>	<i>Schistomeringos rudolphi</i>
<i>Asterina gibbosa</i>	<i>Glycera alba</i>	<i>Nephtys hombergii</i>	<i>Scoloplos armiger</i>
<i>Atylus</i> sp.	<i>Glycera tridactyla</i>	Nereididae	<i>Simplaria pseudomilitaris</i>
<i>Bittium reticulatum</i>	<i>Haminoea</i> sp.	<i>Nereiphylla rubiginosa</i>	Sipuncula
<i>Branchiosyllis exilis</i>	<i>Haplosyllis spongicola</i>	<i>Nicomache lumbricalis</i>	<i>Sphaeroma serratum</i>
<i>Brania arminii</i>	<i>Harmothoe spinifera</i>	<i>Nymphon brevirostre</i>	<i>Sphaerosyllis</i> sp.
<i>Capitella capitata</i>	<i>Heteromastus filiformis</i>	Oligochaeta	<i>Sphaerosyllis bulbosa</i>
<i>Capitella minima</i>	<i>Hippolyte leptocerus</i>	Opisthobranchia	<i>Sphaerosyllis glandulata</i>
<i>Caprella acanthifera</i>	<i>Hydroides nigra</i>	<i>Opisthodonta longocirrata</i>	<i>Sphaerosyllis ovigera</i>
<i>Carazziella</i> sp.*	<i>Idotea</i> sp.	<i>Oriopsis eimeri</i>	<i>Sphaerosyllis pirifera</i>
<i>Caulerliella alata</i>	<i>Idotea balthica</i>	Ostracoda	<i>Spio decoratus</i>
<i>Cerastoderma glaucum</i>	<i>Iphinoe serrata</i>	<i>Oxydromus pallidus</i>	<i>Stenosoma acuminatum</i>
Chaetognatha	<i>Iphinoe trispinosa</i>	<i>Paguristes</i>	<i>Stenosoma appendiculatum</i>
Chironomidae	<i>Ischyrocerus inexpectatus</i>	<i>Paguristes syrtensis</i>	<i>Stenosoma wetzeriae</i>
<i>Cirratulus</i> sp.	<i>Janua pagenstecheri</i>	<i>Papillicardium papillosum</i>	<i>Stenothoe monoculoides</i>
<i>Cirriformia tentaculata</i>	<i>Leptochelia</i> sp.	<i>Paradoneis harpagonea</i>	<i>Syllides bansei</i>
<i>Clymenella</i> sp.	<i>Leptochelia savignyi</i>	<i>Paradoneis lyra</i>	<i>Syllides edentatus</i>
Cnidaria	<i>Leucon longirostris</i>	<i>Paraehlersia ferrugina</i>	<i>Syllis armillaris</i>
Copepoda	<i>Limnoria</i> sp.	<i>Parapionosyllis brevicirra</i>	<i>Syllis gerlachi</i>
<i>Ctena decussata</i>	<i>Liocarcinus</i> sp.	<i>Parexogone hebes</i>	<i>Syllis gracilis</i>
<i>Ctenodrilus serratus</i>	<i>Liocarcinus navigator</i>	<i>Parvicardium exiguum</i>	<i>Syllis hyalina</i>
<i>Cumella limicola</i>	<i>Loripes lucinalis</i>	<i>Parvicardium minimum</i>	<i>Syllis pulvinata</i>
<i>Cyclope neritea</i>	<i>Lucinella divaricata</i>	Pectinaria	<i>Syllis westheidei</i>
<i>Cymodoce</i> sp.	<i>Lumbrineris</i> sp.	<i>Perinereis cultrifera</i>	<i>Tachytrypane jeffreysii</i>
<i>Cymodoce truncata</i>	<i>Lumbrineris coccinea</i>	<i>Peringia ulvae</i>	<i>Tharyx multibranchiis</i>
Decapoda juvenile	<i>Lumbrineris latreilli</i>	<i>Perioculodes aequimanus</i>	<i>Timarete</i> sp.
<i>Dexamine spinosa</i>	<i>Lysianassa</i>	<i>Perioculodes longimanus</i>	<i>Tricolia</i> sp.
<i>Ditrupa arietina</i>	<i>Lysianassina longicornis</i>	<i>Perkinsyllis anophthalma</i>	<i>Tricolia tenuis</i>
<i>Dosinia exoleta</i>	<i>Lysidice unicornis</i>	<i>Pettiboneia urciensis</i>	<i>Upogebia</i> sp.
<i>Drilonereis filum</i>	<i>Malacoceros fuliginosus</i>	<i>Phyllodoce</i> sp.	<i>Vermiliopsis infundibulum</i>
<i>Erichthonius</i> sp.	<i>Melita</i> sp.	<i>Pileolaria militaris</i>	<i>Websterinereis glauca</i>
<i>Erichthonius difformis</i>	<i>Microdeutopus bifidus</i>	<i>Pilumnus</i> sp.	
<i>Erinaceusyllis belizensis</i>	<i>Microdeutopus gryllotalpa</i>	<i>Pionosyllis</i> sp.	

*identification not certified.

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